Contemporary management of lymph node metastases from an unknown primary to the neck: I. A review of diagnostic approaches

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ABSTRACT: In an era of advanced diagnostics, metastasis to cervical lymph nodes from an occult primary tumor is a rare clinical entity and accounts for approximately 3% of head and neck malignancies. Histologically, two thirds of cases are squamous cell carcinomas (SCCs), with other tissue types less common in the neck. With modern imaging and tissue examinations, a primary tumor initially undetected on physical examination is revealed in >50% of patients and the site of the index primary can be predicted with a high level of probability. In the present review, the range and limitations of diagnostic procedures are summarized and the optimal diagnostic workup is proposed. Initial preferred diagnostic procedures are a fine-needle aspiration biopsy (FNAB) and imaging. This allows directed surgical biopsy (such as tonsillectomy), based on the preliminary findings, and prevents misinterpretation of postsurgical images. When no primary lesion is suggested after imaging and panendoscopy, and for patients without a history of smoking and alcohol abuse, molecular profiling of an FNAB sample for human papillomavirus (HPV) and/or Epstein–Barr virus (EBV) is important. © 2011 Wiley Periodicals, Inc. *Head Neck* **35**: 123–132, 2013

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The term *cancer of unknown primary (CUP) site* represents a heterogeneous disease entity characterized by the presence of lymphatic and/or hematogenous metastases and an inability to identify the primary tumor. After thorough diagnostic workup, including meticulous medical history and clinical examination using modern diagnostic tools, CUP accounts for approximately 3% of all patients with cancer and 3% to 5% of those with solid tumors.[1] Whether it is the small size, hidden location (eg, tonsillar crypt), slow growth rate, or eventual involution of the primary tumor that hinders its recognition is speculative.[2] Subsequent manifestation of the primary site occurs in ≤20% to 30% of cases.[3]

From a prognostic perspective, CUP can be categorized into 2 groups comprising 4 major subtypes, as determined by routine histopathological criteria (Table 1). The vast majority of patients (80% to 90%) falls into the poor-risk

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TABLE 1. Cancer of unknown primary tumor: subset stratification according to prognosis (adapted from References 1, 4, 5).

Group/tumor type	Frequency	Comment
Favorable prognosis	15% to 20%	Treated according to guidelines for one of the major known tumor types
Extragonadal germ cell syndrome		
Adenocarcinoma metastatic to the axillary lymph nodes (in women)		
Squamous cell carcinoma metastatic to the neck nodes		
Unfavorable prognosis	80% to 90%	Median survival of 7–11 months
Adenocarcinoma metastatic to the bone, brain, viscera		

CUP group and present with adenocarcinoma metastatic to bone, brain, and/or viscera. Even with platinum-based combination chemotherapy regimens, with or without new generation cytotoxic drugs, complete responses are seldom observed and median survival typically ranges between 7 to 11 months.[4,5] In the favorable prognostic subset of 15% to 20% of patients with CUPs, the biological behavior of the metastatic disease is similar to other major known tumor types (ie, germ-cell tumors, adenocarcinoma of the breast or ovary, or squamous cell carcinoma [SCC] of head and neck).

Metastatic cancer in cervical lymph nodes from an unknown primary constitutes a favorable-risk CUP group. Histologically, SCC accounts for 53% to 77% of cases,[6–8] particularly when masses are situated in the upper two thirds of the neck, and generally indicates an origin from a hidden primary somewhere in the head and neck region. Tissue types other than SCC should also be considered (ie, adenocarcinoma, undifferentiated carcinoma, lymphoma, melanoma, papillary thyroid carcinoma, and central nervous system tumors).[9,10] Metastases to nodes in the lower neck often arise from cancer below the clavicles, and are mainly adenocarcinomas.[9] In the present review, current literature on CUP in cervical lymph nodes will be considered with an emphasis on contemporary diagnostic procedures for primary tumor identification, including molecular testing and positron-emission tomography (PET) scanning. The discussion will center on SCC, with brief mention of other histological types.

Incidence and presentation

The proportion of patients in whom the primary tumor is ultimately unknown reflects recent advances in imaging and pathology, as well as the accuracy and extent of the diagnostic workup. The reported incidence of metastatic CUP in cervical lymph nodes of SCC histology ranges from 1.5% to 9% of all head and neck malignancies.[11–13] The wide range may be due to institutional variations in diagnostic techniques and approaches implemented over time. On reviewing the data of national registries, metastatic SCC to cervical lymph nodes with unknown primary seems to account for no more than 3% of head and neck malignancies. In a retrospective Danish national survey covering the period between 1975 and 1995, the annual incidence of CUP metastatic to the cervical lymph nodes with SCC histology was constant at 0.34 cases per 100,000/year, with the proportion in relation to total head and neck cancers decreasing from 2.5% to 1.7% over the same period.[14] Whether the observed decline was due to a more comprehensive initial diagnostic workup could not be established from the retrospective data. According to the National Cancer Registry of Slovenia, 125 SCC cases were identified among 234 patients with cervical lymph node metastases from a clinically undetectable primary in the period from 1975 to 1994. These cohorts represent 1.7% and 3.12%, respectively, of 7548 new patients treated in Slovenia for head and neck malignancies during the same time span.[6,15]

The typical patient with CUP metastatic to cervical lymph nodes is male, 55 to 65 years old, with a history of chronic tobacco and/or alcohol use.[6,8,16,17] This pattern is expected to change with an increase in younger nonsmokers presenting with human papillomavirus (HPV)-related oropharyngeal cancer.[18] Discrepancies observed in some series may result from inclusion of patients with tumors of other histological types (ie, undifferentiated carcinomas, lymphomas, melanoma, and others).[14,19–21] The symptom that usually prompts initial consultation is a mass in the neck, and this accounted for 94% of the 352 patients in the series reported by Grau et al[14] with pain and weight loss only reported in 9% and 7%, respectively. In a series of 167 patients reported by Issing et al,[8] the incidences of cervical "swelling,'' pain, and dysphagia were 100%, 9%, and 3.6%, respectively. Typically, the enlarged cervical lymph nodes are located at level II, followed by level III, with bilateral involvement reported in $<10\%$.[6,8,14,16,17,22,23] The clinical N classification in the majority of cases is N2a, N2b, and N2c, with a median nodal size of 3.5 to 5 cm.[6,14,16,17,23,24] The time interval between the appearance of cervical mass and diagnosis ranges from 2 to 5 months.[6,8,25]

Diagnostic workup

The main objectives of the diagnostic evaluation of a patient with CUP are: to determine the histology of the metastatic tumor and to identify the primary tumor. These 2 objectives are interdependent because the results of the first inform the search for the primary. Furthermore, the diagnostic workup should determine the N and M classification of the disease.

Tissue diagnosis

After a detailed office examination of the head and neck and upper aerodigestive tract (UADT) with

nasopharyngoscopy, the initial tissue diagnostic procedure is a fine-needle aspiration biopsy (FNAB). This is an efficient, minimally invasive, and cost-effective diagnostic method with negligible risk of seeding tumor cells along the needle track.[26] In comparison, core needle biopsy is technically more demanding and less comfortable for the patient, usually requires local anesthesia,[27] and has potentially more serious complications such as bleeding, infection, and fistula formation.[26,27] FNAB results in a representative cellular sample in the majority of patients. The diagnosis is usually established with routine histologic staining, supplemented by immunohistochemistry,[26,28] and with an experienced histopathologist, achieves a diagnostic sensitivity of 83% to 97% and a specificity of 91% to 100% for metastatic lesions.[26,29] Although image-guidance technology has been shown to improve the efficacy of fine-needle biopsies, particularly in an N0 neck, in patients with a CUP the nodes will usually be palpable and image guidance is of lesser importance. However, on-site assessment of the aspirant (with repeated passage, if necessary) and ultrasound-guidance are useful, particularly with large, cystic nodes to direct the needle to the wall of the cystic mass.[26,28]

In cystic metastases, a false-negative rate of 42% with FNAB has been reported[29] with sensitivities ranging from 33% to 50%,[30] because it can be difficult to distinguish cystic metastases from benign branchial (lymphoepithelial) cysts. The differential diagnosis may also include an abscess and tuberculosis.[26,29–31] Excisional biopsy might be needed to confirm malignancy but only after nondiagnostic image-guided FNAB and core biopsy. In a recent report by Goldenberg et al,[32] FNAB was found to be positive for malignant cells in 80% of cystic lymph node metastases, confirming the efficacy of FNAB even with cystic metastases. Ultrasound guidance of the FNAB enables the guidance of the needle to the relevant parts of the neck mass (eg, the wall of a cystic lesion).

Open cervical lymph node biopsy is indicated only when repeated FNABs followed by a core-needle biopsy are nondiagnostic or in patients with masses clinically and histologically suspicious for lymphoma.[33] Even in the latter situation, an ultrasound-guided cutting needle biopsy is an alternative to the more invasive open biopsy, with full subclassification of the disease possible in 92% of malignant lymphoma cases.[34] In 1944, Martin and Morfit[35] described open biopsy as "ill-advised and needless surgery.'' Increased rates of local complications, neck recurrence, and systemic dissemination have also been reported by other authors after this procedure.[36,37] In contrast, no adverse effects in relation to neck control or survival were reported in more recent studies of open neck node biopsy, provided that biopsy was followed by adequate definitive treatment, either in the form of radiotherapy, comprehensive neck dissection, or both.[38–40] In view of these data, it seems that it is not imperative to perform an immediate subsequent neck dissection when an open biopsy with frozen sections reveals metastatic cancer in the excised node. It should be emphasized that an open neck node biopsy should not be performed before a thorough clinical, radiological, and endoscopic search for the primary tumor has been completed.

The light microscopic examination of routine Giemsa and Papanicolaou stained FNAB specimens (or the hematoxylin-eosin staining of tissue sections) allows for the characterization of cell morphology and tumor differentiation. In Papanicolaou-stained specimens, the orangeophylic staining of cytoplasm of malignant cells is suggestive (but not absolute proof) of SCC and its variants.[41] Additional stains and, in particular, immunohistochemistry are subsequently used for the tissue diagnosis of undifferentiated malignancies.[42] An initial screening panel using antibodies against a wide spectrum of cytokeratins, common leukocyte antigen, carcinoembryonic antigen, S100 protein, and desmin should differentiate carcinoma, lymphoma, melanoma, and sarcoma. Vimentin can be misleading, because it can be positive in spindle cell carcinomas that are cytokeratin negative.[43] Further differentiation can be achieved using antibodies against specific cellular components, (ie, enzymes, structural tissue components, hormones, and hormonal receptors; see below).

Identification of the primary tumor

The tonsillar fossa and the base of tongue are by far the most common sites harboring an occult primary tumor in patients presenting with metastatic SCC in cervical lymph nodes and an unknown primary. In a series of 236 patients,[33] almost 90% of all primaries were identified in these 2 oropharyngeal subsites, which are notoriously anatomically complex and difficult to assess. The decreased likelihood of identifying the primary tumor in other sites (eg, the nasopharynx or hypopharynx) observed in recent series compared to earlier reports[44] is most likely the result of the availability of fiberoptic endoscopy and the advancement in CT and MRI technology. A proper diagnostic evaluation identifies the primary tumor in more than 50% of these patients overall,[33,44] and in two thirds of patients with suspicious findings on physical and/or radiological examinations, but only 30% of patients without such findings.[33]

History and physical evaluation. These are the first steps in a directed search for the occult primary. A history of excessive alcohol consumption and heavy smoking may suggest a primary tumor outside the nasopharynx, while a history of multiple sexual partners and orogenital contact may suggest a primary tumor within the oropharynx. An accurate history of previous skin lesions (in particular melanoma and SCC) with careful questioning of both the patients and their physicians is important. Occasionally the dermatologist or surgeon might not have sent an excised skin lesion for histopathology.[9]

The location of cervical lymph node metastases may suggest the location of the primary tumor.[9,45] Tumors of the lip and oral cavity usually metastasize to lymph nodes in levels I to III, whereas metastases from oropharyngeal, hypopharyngeal, laryngeal sites, and the thyroid tend to appear lower in the jugular chain (levels II–IV) or centrally in level VI. Approximately 50% of masses limited to level IV and/or the supraclavicular fossa are from a primary tumor arising below the clavicle (ie, the lung, breast, gastrointestinal tract, kidney, and ovary). Cervical lymph node metastases localized to level V are commonly of nasopharyngeal or cutaneous origin. Enlarged parotid

TABLE 2. The main panels of immunohistochemical markers by tumor type (adapted from Reference 43).

Tumor type	Marker
Carcinoma	Cytokeratins
Lymphoma	CLA, ALK1, CD30, CD43
Melanoma	S-100, HMB45, Melan-A
Sarcoma	Vimetin, Actin, Desmin , MyoD1, Myogenin, S100, CD34, CD99
Squamous cell carcinoma	CK5/6, p63
Squamous cell carcinoma (oropharynx)	p16
Adenocarcinoma	
Prostate	PSA, PAP
Lung	TTF-1, $CK7^+/CK20^-$
Thyroid	TTF-1, Thyroglobulin
Breast	GCDFP-15, mammaglobin, ER
Colon	CDX2, $CK20^+/CK7^-$
Pancreas/biliary	CDX2, $CK7^+/CK20^+$ or $CK7^+/CK20^-$
Germ cell tumors	PLAP, OCT4, AFP, HCG
Hepatocellular carcinoma	Hepar 1, AFP
Renal cell carcinoma	RCC, CD10
Neuroendocrine carcinoma	Chromogranin, Synaptophysin, PGP9.5

Abbreviations: CLA, common leukocyte antigen; ALK1, anaplastic lymphoma kinase; protein; HMB45, anti-human melanosome; CK, cytokeratin; PSA, prostate-specific antigen; PAP, prostatic acid phosphatase; TTF-1, thyroid transcription factor-1; GCDFP-15, cystic disease fluid protein 15; ER, estrogen receptor; PLAP, placental alkaline phosphatase; OCT4 – Octamer-binding transcription factor 4; AFP – α-fetoprotein; HCG – Human chorionic gonadotropin; pCEA – Polyclonal carcinoembryonic antigen; PGP9.5 – Protein gene product 9.5.

nodes should direct the search for a primary tumor of cutaneous or salivary origin. Bilateral cervical lymph node metastases should focus attention on the nasopharynx, base of tongue, hypopharynx, and midline structures.[45]

Information on hoarseness (vocal cords), dysphagia, or referred pain (oropharynx or hypopharynx and supraglottis) could also suggest the primary site.[9] Sudden (overnight) enlargement of a neck mass, particularly in nonsmokers and nondrinkers, at a rate beyond that which is expected due to tumor cell proliferation should raise suspicion of an oropharyngeal malignancy, and especially if cystic, of HPV-associated disease, or lymphoma.[32] In the series of Goldenberg et al,[32] 85% of 20 patients with cystic metastases had primaries arising in palatine or lingual tonsil, and the remaining 3 were CUP cases. Cystic metastases should be considered in all patients with suspected branchiogenic cysts who smoke and drink, especially if >40 years of age.[46]

A useful tip for clinical detection of the primary tumor is to re-examine the patient looking for bleeding after the initial palpation of the tonsil and tongue base, and retraction of the tonsillar pillars.

Lymphadenopathy elsewhere in the body is indicative of a primary tumor outside the UADT, mainly lung, breast, lower gastrointestinal tract, and lymphoma.[9]

Immunohistochemistry. A variety of antibodies directed against specific components of the neoplasm can assist in establishing the histological type of the metastasis and hence, the most likely site of origin. A panel of immunostains should be interpreted together with the morphological characteristics and clinical presentation. The number of antibodies used in the panel depends on the type/size of specimen submitted for cytopathological or histopathological examination and the differential diagnosis after initial cellular and immunohistochemical assessment.[43]

The diagnostic value of an immunohistochemical panel of markers to determine the origin of the SCC metastatic to cervical lymph nodes in 101 patients (with an otherwise known primary) was studied by Park et al.[47] Using the classification and regression trees method, a combination of p16, cytokeratin 10, cytokeratin 19, and pRb correctly determined that 89.5% (34 of 38) cervical lymph node metastases originating from an oropharyngeal primary, the frequency of classification was much lower for other sites (oral cavity, 25%; hypopharynx, 30.8%; and larynx, 57.1%).[47]

For adenocarcinomas, there are relatively specific tumor markers that may help identify the site of the index cancer: prostate, prostate-specific antigen and prostatic acid phosphatase[48] (Table 2); lung, thyroid transcription factor-1 and cytokeratin 7^+/cytokeratin 20^-[49,50]; thyroid, thyroid transcription factor-1 and thyroglobulin; breast, gross cystic disease fluid protein 15, mammaglobin, and estrogen receptors (ERs)[51]; colon, CDX2, cytokeratin 20^+/cytokeratin 7^-[52]; pancreas/biliary, CDX2, cytokeratin 7^+/cytokeratin 20^+ or cytokeratin 7^+/cytokeratin 20^-[53]; ovary, ER, CA125, and mesothelin.[54]

Less common cancer types can also be characterized using an appropriate panel of immunohistochemical markers: germ cell tumors, placental alkaline phosphatase, OCT4, α-fetoprotein (AFP), human chorionic gonadotropin; hepatocellular carcinoma, Hepar 1, AFP, canalicular pCEA/CD10/CD13; renal cell carcinoma (RCC) – CD10; neuroendocrine carcinomas, chromogranin, synaptophysin, protein gene product 9.5.[43] Detection of tumor markers in needle washout fluid after the FNAB or in fluid retrieved from cystic lesions is particularly useful for characterization of thyroid lesions, where increased thyroglobulin and calcitonin levels are highly indicative of differentiated or the rarer medullary carcinoma, respectively.

Imaging. Diagnostic imaging is aimed at assessing the extent of cervical lymph node metastases, to identify the index cancer, and to determine M classification. As mentioned previously, imaging should be performed before any invasive diagnostic procedure, such as guided biopsies or tonsillectomy, in order to avoid false-positive results or other misinterpretation due to tissue trauma, and to assist with directing the tissue biopsies.

The mainstay of the imaging workup is contrast-enhanced CT scan from the skull base to clavicles. It is quick, has good spatial resolution, and is inexpensive. The extent and location of cervical disease and its relationship to neighboring structures, the presence of extracapsular extension, the status of the retropharyngeal nodes, and contralateral neck should be addressed. In the search for a primary tumor in the head and neck, a CT scan may be either complemented or supplanted by an MRI with gadolinium contrast, which has superior soft tissue resolution, particularly for evaluation of nasopharynx or oropharynx. The potential of a CT, MRI, or both to detect a primary tumor

is in the range of 9.3% to 23%,[7,13,55,56] rising to 60% when suspicious radiologic findings direct subsequent endoscopic biopsies.[33] Certain other examinations can be obtained when a primary tumor is suspected to originate from sites outside the head and neck (eg, a mammography or MRI of the breast, or an octreotide scan for tumors of neuroendocrine histology).

During the last 2 decades, F-18-fluorodeoxyglucose (FDG) PET has been increasingly used in diagnostic algorithms for CUPs and to evaluate the neck for residual disease after chemoradiotherapy. It has recently been supplemented by fusing functional information (FDG-PET) with morphological data obtained by CT (FDG-PET/CT). However, the resolution of FDG-PET limits its detection capability to tumors $\geq$5 mm, and basal uptake of FDG in normal lymphoid tissues of Waldeyer's ring, and the secretion of FDG from salivary glands may further limit the identification of small and superficial lesions.

Several studies have evaluated the ability of PET/CT to detect the primary tumor in patients with cervical lymph node metastases of unknown origin. In a meta-analysis conducted by Rusthoven et al[57] (16 studies published between 1994 and 2003, 302 patients), the detection rate of the primary tumor was 24.5% (sensitivity 88.3%; specificity 74.9%; diagnostic accuracy 78.8%). Tonsils accounted for the highest false-positive rate (39.3%) while the lowest sensitivity rate was for the base of tongue (80.5%).[57] In a more recent meta-analysis for hybrid FDG-PET/CT[58] (11 studies published between 2005 and 2007, 433 patients), the primary tumor detection rate was 37% (sensitivity and specificity 84%). The false-positive rate (15%) was highest for oropharyngeal and lung primaries.[58] Analyzing another set of 8 studies from 2000 to 2009, in which FDG-PET or FDG-PET/CT was used in 180 patients with cervical lymphadenopathy of unknown origin, Al–Ibraheem et al[59] reported a 28.3% detection rate of primary tumors with 37% false-positive scans. No analysis by tumor site or PET type was provided by the authors.[59] In the past 2 years, 4 studies have addressed this issue, and the results are in line with those presented above.[13,60–62] In 2 of these studies, the diagnostic performances of whole-body FDG-PET and integrated FDG-PET/CT were also compared and both indicated a superiority of the latter. Thus, Waltonen et al[13] reported the following figures for FDG-PET (41 scans) and FDG-PET/CT (52 scans): primary detection rate, 14.6% versus 44.2%; sensitivity, 42.9% versus 74.2%; specificity, 72.4% versus 72%; positive predictive value, 42.9% versus 76.7%; negative predictive value, 72.4% versus 69.2%; and accuracy, 55.1% versus 68.3%. Keller et al[62] reported similar figures.

However, when attempting to assess the added value of FDG-PET (with or without CT) to a conventional workup in an unknown primary setting, one must be aware of several obstacles and limitations related to studies already published. As summarized by de Bree,[63] the main issues are the inclusion criteria (physical examination only vs complete imaging and/or endoscopic workup with directed biopsies), the definition of sensitivity (if the possibility of spontaneous regression of the primary is ignored, all included patients and not only the sum of the positive cases from all the different diagnostic techniques should be used as a reference standard), and the standardization of scanners and protocols which differ across the studies, making the interpretation and comparison of results difficult. The other potential drawbacks of PET scanning are the high rate of false-positive findings, particularly if performed after a biopsy or in the oropharynx,[57,58] false-negative cases, the limited availability of the procedure, the costs, the exposure to radiation, and burden to the patient.[63] Moreover, according to the University of Florida's experience, a multivariate analysis for primary tumor detection revealed that these studies had no significant impact when weighted against other diagnostic procedures; however only 21 of the 236 patients studied had FDG-PET or FDG-PET/CT.[33] On the other hand, the probability of a subsequent primary tumor becoming apparent after a negative PET and panendoscopy is low (ie, less than 6%).[64] Also, despite the rather low primary detection rate, each patient in whom the primary tumor is correctly identified could benefit from less extensive and, in some cases, more specific treatment which should result in less morbidity for the patient.[63] In view of the above, a prospective, multicenter Dutch study (PRIMUS) was conducted, in which patients without a primary tumor detected after standard workup—including physical examination, indirect laryngoscopy, and CT/MRI—were directed to a blinded FDG-PET and panendoscopy with directed biopsies. During the same general anesthetic session, the results of the PET scan were provided to the surgeon for additional biopsies if still required. Unfortunately, the results of this important study are not yet available.[63]

Endoscopy with Biopsy. Definitive evaluation of the unknown primary is panendoscopy of the UADT (pharyngoscopy including nasopharynx, laryngoscopy, and esophagoscopy), with the patient under general anesthesia, assisted by careful palpation of accessible regions, and directed biopsies of clinically or radiologically suspicious areas. Bronchoscopy is warranted when there is an abnormality of the lung on chest imaging.[9]

The likelihood of discovering a primary tumor by endoscopy correlates with presence of suspicious findings on preliminary examinations. When routine diagnostic workup failed to identify a primary tumor, Cianchetti et al[33] found subsequent panendoscopy successful in 29.2%. This figure doubled in patients with suspicious findings present on any of the 2 examinations. A repeated panendoscopy is warranted only when the suspicious site was not biopsied adequately during the first procedure.[33,44]

Although most hidden primaries are eventually identified in the tonsillar fossa and the base of tongue, given the sensitivity of modern imaging modalities, random tonsillar biopsies and tonsillectomy are controversial.[32] Also, random sampling of normal appearing mucosa from nasopharynx and pyriform sinus may no longer be justified. As a directed procedure, an ipsilateral tonsillectomy in patients with lymphoid tonsil tissue is warranted and yields an 18% to 44.6% primary tumor detection rate,[22,33,65–68] the wide range likely reflecting differences in imaging. In the absence of a visible or palpable lesion, Waltonen et al[69] showed that tonsillectomy results in a significantly higher likelihood of detecting an occult

tonsillar tumor then a deep tonsil biopsy (29.5% versus 3.2%; $p = .0002$). The evidence supporting bilateral tonsillectomy is less convincing, although rates of detection of a primary in the contralateral tonsil of 10% (4 of 41)[70] and 23% (5 of 22)[71] have been reported.

It is possible that a formal lingual tonsillectomy may have a higher yield than random mucosal biopsies of base of tongue should a palatine tonsillectomy prove unrevealing.

Molecular Studies. Better insight into etiology and pathogenesis of head and neck tumors has led specific diagnostic tests and effective therapeutic strategies. Molecular studies have the potential to improve diagnosis further.

Recently, a causal relationship between HPV, especially type 16, and increased oropharyngeal SCC has been documented. Meta-analysis (Mehanna et al., unpublished data) showed the overall prevalence of HPV in oropharyngeal SCC was 47.7% (95% confidence interval, 42.9% to 52.5%) compared to 21.8% (95% confidence interval, 18.9% to 25.1%) for tumors at other head and neck sites. In the oropharynx, prevalence of HPV has significantly increased over the last decade in North America and Europe; thus, the gap between these 2 regions that existed before the year 2000 has now disappeared (69.8% versus 73.1%; $p = .8$). Hence, there are strong arguments for HPV testing all FNAB specimens from cervical lymph nodes in CUP.

Begum et al[72] used in situ hybridization for HPV16 testing on 77 consecutive aspirated cervical masses diagnosed as metastatic SCC. HPV16 was detected in 10 of 19 (53%) metastases from oropharyngeal and none from nonoropharyngeal sites. To exclude possible bias due to limited tumor sampling by FNAB, HPV16 status of the primary oropharyngeal carcinomas was also determined: in all but 1 case, HPV profiles were concordant. In the same study, p16 expression (a surrogate HPV marker) was also determined. The 2 markers were strongly associated, with p16 over-expression recorded in 12 of 13 HPV16-positive tumors and only 4 of 64 HPV16-negative tumors (92% versus 6%; $p < .0001$).[72] The same group also assessed HPV16 status in surgically excised cervical lymph node from 68 patients with SCC and found HPV16 was only detected in oropharyngeal tumors.[73] Similar findings have been reported by Zhang et al[74] (in FNAB samples) and by El–Mofty et al[75] (in tissue sections), who also noted HPV-positivity is strongly associated with nonkeratinizing SCC.

Recently, Desai et al[76] reported on the HPV status of SCC metastatic to cervical lymph node from 41 patients (37 tissue sections, 4 FNAB aspirates). Using a polymerase chain reaction (PCR) with multiple primers, the HPV-positivity rate was 44.4% in patients with oropharyngeal primaries (4 of 9; all 3 cases with tonsillar tumors were HPV-positive); 25% (8 of 32) for those with other tumor sites, and 60% (3 of 5) for those where the primary tumor remained unknown. No HPV-positive lesions were found in the larynx or hypopharynx.[76] The HPV16 status was reported to be strongly associated with the presence of cystic cervical lymph node metastases, which seems to be a reliable marker of oropharyngeal origin: using in situ hybridization, HPV DNA in tumor cells was confirmed in 13 of 15 cases with cystic lymph nodes (all with oropharyngeal or an unknown primary) and in none of 21 cases with solid lymph nodes (4 of 21 had an oropharyngeal primary).[32] Recently, HPV-associated nasopharyngeal cancer has been reported in white North American patients, thus indicating that the nasopharynx should remain a potential site of origin in patients with HPV-positive lymph nodes.[77,78]

Similarly, detection of Epstein–Barr virus (EBV) in an involved cervical lymph node may suggest a nasopharyngeal origin because this virus is implicated in the etiology of undifferentiated nasopharyngeal tumors. EBV assay should be considered in younger patients with poorly differentiated SCC or undifferentiated carcinoma. Using PCR, Feinmesser et al[79] detected EBV genomes in all 9 nasopharyngeal carcinomas (NPCs; 1 primary lesion and 8 nodal metastases; tissue samples and fine-needle aspirates were analyzed) but none of 20 lymph nodes originating from other cancer types, 10 negative lymph nodes, or 105 normal control samples. EBV was found also in 2 additional patients with CUP and both developed overt NPCs within 1 year.[79] Conversely, only 1% of 300 primary tumor samples exclusive of NPC were positive for EBV DNA in a North American study.[80]

Although sensitivity and specificity rates close to 90% have been reported for EBV in FNAB samples,[81–84] the sensitivity of PCR in nasopharyngeal biopsy tissue samples seems to be higher.[83] Furthermore, in situ hybridization for small EBV-encoded RNA is more sensitive and specific than PCR in detecting EBV.[85]

The demonstration of EBV DNA in the plasma/serum of patients with NPC has provided a new tool for NPC detection and monitoring. Comparing genotypes of paired samples from plasma and primary tumor, Lin et al[86] have found consistent similarity between both samples, suggesting circulating cell-free EBV DNA may originate from the primary tumor. The sensitivity and specificity of using circulating EBV DNA for detection of NPC with real-time PCR is 96% and 93%, respectively.[87]

Other methods for localizing the primary tumor

Several diagnostic methods are either under development or too complex at the moment to be introduced into daily practice. However, they may represent a clinically useful tool in the search for the primary origin in the future.

Micro RNA (miRNA) comprises approximately 22 nucleotides – noncoding RNA molecules involved in regulation of key cellular pathways associated with development, differentiation, and tumorigenesis.[88] As miRNA seem to be a molecular fingerprint, Barker et al[89] hypothesized that their expression profile, determined by quantitative real-time PCR, could be predictive of the site of metastatic disease. Analyzing matched-pairs of primary tumor and metastatic cervical lymph node from 6 patients with tonsillar, base of tongue, and nasopharyngeal tumors the miRNA expression profile was consistent between the primary site and nodal metastasis for individual patients and between different patients within an individual anatomic site (each primary site had a distinct miRNA

expression profile). These results were confirmed by a validation analysis conducted in a group of an additional 6 patients.[89]

Califano et al[2] used microsatellite analysis to study the genetic relationship between the directed biopsy samples of clinically/histologically benign mucosa and the metastatic tissue of cervical lymph nodes in 18 patients with CUP to determine whether the site of origin could be identified by molecular genetic means. A clinically detectable primary tumor developed in 4 of the 10 cases with at least 1 mucosal specimen that shared genetic alterations with a cervical metastasis. In 3 of these, the primary tumor had genetic changes identical to those in the benign mucosal biopsy specimen and in the metastatic cervical lymph node. In the fourth patient, no benign mucosa sample was collected during the diagnostic procedure from the site where the primary tumor subsequently appeared.[2]

Image cytometry measures numerous nuclear features: the foremost nuclear texture and organization on a 2-dimensional snapshot of the chromatin structure could be a surrogate marker for endpoint molecular genetic changes in a nucleus.[90] In a series of 21 patients, statistically significant differences between several nuclear morphometric and textural features were found in the cellular samples from cervical metastases obtained by FNAB when patients were grouped according to the origin of index cancer (oropharynx vs other sites).[91]

Finally, fluorescence-guided screening and diagnostic evaluation of early neoplasia has also been used in CUP. Kulapaditharom et al[92] used laser-induced fluorescence (LIF) endoscopy in parallel with a panendoscopy in 13 patients diagnosed with cervical metastasis and no visible primary tumor. A primary tumor was disclosed with LIF imaging and conventionally (after ipsilateral tonsillectomy) in 5 (36.5%) and 2 (15.4%) patients, respectively, whereas the LIF also effectively reduced the number of unnecessary biopsies.[90] Hayashi et al[93] described the use of narrow-band imaging in detection of the primary tumor site in 46 patients with CUP with metastatic SCC in cervical lymph nodes. After unsuccessful conventional diagnostic evaluation, including white-light endoscopy, 26 suspicious mucosal lesions in 25 patients were identified, of which 16 lesions were histologically confirmed as potential index SCC. All lesions were superficial neoplasia and located in hypopharynx (10 lesions) or oropharynx (6 lesions).[93] Recently, autofluorescent imaging detected a clinically innocuous palatal tumor in a patient previously treated for CUP.[94]

Assessment for distant metastases

Distant metastases at presentation are reported in 14% of patients with cervical metastases from CUP.[7] This critically impacts the treatment strategy and adversely affects patient survival. A conventional diagnostic workup to determine the M classification usually comprises a chest CT scan and abdominal and pelvic ultrasound or CT. A chest CT is useful in evaluating the mediastinum, and especially valuable in cases with a higher risk of systemic dissemination (level IV/V, N3, or bilateral nodal disease) or when there is a strong suspicion of an index lung cancer (eg, positive thyroid transcription factor-1 staining of the cervical node).[50,95,96] Nevertheless, the low sensitivity and specificity of a chest CT in detecting systemic metastases (73% and 80%, respectively) indicates a need for a more sensitive and whole-body screening technique.[97] Other examinations (eg, skeletal scintigraphy and endoscopies) are performed as clinically indicated, whereas blood tests and abdominal CTs have only a low sensitivity for the detection of extrapulmonary disease.[98,99]

More recently, whole-body FDG-PET, with or without CT integration, has been introduced for the M-staging of head and neck cancer. Senft et al[100] reported a prospective multicenter study with 145 consecutive patients with head and neck cancer with an increased risk of distant metastases, comparing chest CT and whole-body FDG-PET for screening for distant metastases. With a sensitivity of 53% (vs 37%) and a positive predictive value of 80% (vs 75%), the superiority of PET scanning was confirmed over a conventional workup with CT, whereas the combination of both modalities resulted in the highest sensitivity (ie, 63%).[100] No significant additional costs were linked with more advanced screening procedures, and with all 3 (CT, PET, PET+CT) found to be cost-effective.[101] Furthermore, assessing the interobserver variability in chest CT and whole-body FDG-PET screening for distant metastases in patients with head and neck SCC, higher kappa values for origin, susceptibility, and overall conclusions were found for PET.[102] These results indicate that in clinical practice PET can be scored by 1 observer, but a CT should probably be scored by different observers in consensus or combined with PET.[102]

FDG-PET and FDG-PET/CT were compared by Xu et al[103] who conducted a meta-analysis on 15 studies using FDG-PET scanning in an initial systemic evaluation of patients with head and neck cancer (FDG-PET, 7 studies with 797 patients; FDG-PET/CT, 8 studies with 795 patients). The authors concluded that both examinations have good diagnostic performance although FDG-PET/CT tends to have higher diagnostic accuracy than FDG-PET.[103] Finally, a 3.0 Tesla whole-body MRI was weighted against FDG-PET/CT scanning for assessment of M-staging and distant-site synchronous primary tumors in 150 patients with NPC.[104] Both methods showed similar diagnostic performances with a sensitivity of 77.8% versus 72.2% ($p > .999$), a specificity of 98.5% versus 97.7% ($p > .999$), and a diagnostic capability of 0.905 versus 0.878 ($p = .669$). A combined interpretation of the 2 modalities demonstrated no significant benefit over the sole use of either technique.[104]

CONCLUSION

Over the past several decades, substantial improvements in diagnostics have allowed more accurate determination of the extent of disease in the neck, systemic spread to distant organs, and identification of the hidden primary tumor in more than half the patients with CUP presenting as a neck mass. In cases with no primary tumor on initial investigations, the site of the index primary can be predicted with a high level of probability.

Supported by literature data, in an optimal scenario, the diagnostic workup should be conducted in 4 steps. First,

history, clinical examination with UADT assessed by multiple examiners and modern imaging using contrast-enhanced CT or MRI as a method of choice. The added value of FDG-PET or FDG-PET/CT to conventional workup has yet to be determined.

Second, tissue diagnosis is preferably obtained by a FNAB; if the FNAB is nondiagnostic, it should be repeated (image-guided). A core needle biopsy is indicated after multiple inconclusive FNABs followed by excisional biopsy of the enlarged node. Appropriate immunohistochemical studies allow tissue characterization of tumor in the vast majority of patients.

Third, panendoscopy under general anesthesia with ipsilateral tonsillectomy and directed biopsies. Optimal imaging information of potential sites of the hidden primary tumor is crucial as this guides mucosal sampling. Reversing the order of examinations increases the rate of false positive findings on CT or PET. A second panendoscopy is indicated only when a suspicious mucosal site(s) was inadequately biopsied during the previous procedure.

Fourth, molecular studies. For optimal predictive information about a potential primary site, the HPV and/or EBV immunostaining of the FNAB sample obtained from the enlarged cervical lymph node is warranted, particularly in cases where no suggestive findings are recorded on imaging and panendoscopy and/or in patients without a history of smoking and alcohol abuse.

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