

Histopathological and immunohistochemical study of laterocervical lymph node metastases of unknown primary origin

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Abstract

In this study, we examined histopathologically and immunohistochemically 24 cases of laterocervical lymph node metastases with unknown primary origin. For immunohistochemical study, we used a large panel of antibodies represented by CK7, CK19, CK20, CKA1/EA3, CK34betaE12, TTF1, HBME-1, CEA, MUC5AC and EBV. In the cases studied tumors accompanied by seemingly primitive adenopathies were located in the thyroid, lung, esophagus, stomach, rhinopharynx, hypopharynx, oropharynx and larynx.

Keywords: laterocervical lymph node metastases, unknown primary carcinomas, histopathology, immunohistochemistry.

Introduction

Lymph node metastases represent an important prognostic factor in malignant tumors. Most tumors are being classified using the TNM system, and their treatment and prognosis are changed when accompanied by lymph node metastases.

Carcinomas of unknown primary (CUP) are histologically defined as the presence of a metastasis without detection of the primary tumor [1]. CUP is a very heterogeneous condition, in which the type of the tumor, its extension and the treatment vary widely. Since this condition is not included in the *International Classification of Diseases* (ICD), it is very difficult to compare different data from the literature. However, approximately 3–15% of all cancers are designated as CUP [2, 3].

The diagnosis, treatment and monitoring of patients with laterocervical metastases of unknown primary involves a wide range of oncologic entities. Therefore, patients with CUP must undergo an appropriate sequencing of the primitive tumor [4]. This includes investigation of known patterns of tumor spreading in an effort to locate the primary tumor, usually squamous cell carcinoma type, and assessing the possibility of spontaneous regression of primary tumor or metastatic potential.

Investigation of laterocervical lymph node metastases of unknown primary involves corroborating all clinical, laboratory and pathological data. In case the morphological appearance of the lymph node metastasis is less

differentiated, complex immunohistochemical and cytogenetic techniques must be applied.

Materials and Methods

Our study comprised 24 cases with a clinical tumor adenopathy, with unknown primary tumor site.

Lymph node biopsy fragments were fixed in 10% formalin, processed through the usual technique for paraffin inclusion and stained with the Hematoxylin and Eosin technique.

Four µm-thick serial sections were cut and subjected to immunohistochemical processing using LSAB2/HRP visualizing system (Dako, code K0690).

Signal detection was performed using 3,3'-diaminobenzidine (DAB). We used a broad panel of antibodies orienting us towards the location of the primary tumor, which included: CK7, CK19, CK20, CKA1/EA3, CK34betaE12, TTF1, HBME-1, CEA, MUC5AC and EBV (Table 1).

Table 1 – Antibodies used for the immunohistochemical study

Antibody	Clone	Dilution	Antigen retrieval	Incubation time
CK7	OV-TL 12/30	1:200	Tris-EDTA, pH 9	one hour
CK19	RCK108	1:50	Proteinase K	one hour
CK20	Ks20.8	1:50	Tris-EDTA, pH 9	one hour
CKA1/EA3	AE1/EA3	1:50	Sodium citrate, pH 6	one hour
CK34betaE12	34betaE12	1:50	Sodium citrate, pH 6	one hour

Antibody	Clone	Dilution	Antigen retrieval	Incubation time
TTF1	8G7G3/1	1:100	Sodium citrate, pH 6	one hour
HBME1	HBME1	1:50	Sodium citrate, pH 6	one hour
CEA	II-7	1:100	Sodium citrate, pH 6	one hour
Epstein-Barr virus, LMP	CS.1-4	1:100	Sodium citrate, pH 6	one hour
Muc5AC	CLH-2	1:100	Sodium citrate, pH 6	one hour

For validation, we used external negative controls by omitting the primary antibody.

Results

The study, which covered a period of five years (2004–2008), included 252 cases of which 228 cases (90.48%) with secondary adenopathy and 24 cases (9.52%) with apparently primitive adenopathy, which are adenopathies of unknown primary origin.

Distribution by age groups shows an increased incidence of apparently primitive adenopathies between 51–60 years (33.33%) and 41–50 years (20.83%); the 20–30-year-old and 61–70-year-old groups had an equal number of cases (16.66%); the fewest cases were present in age groups 31–40 years (8.33%) and 71–80 years (4.16%). Gender distribution of apparently primitive adenopathies showed an increased incidence of in males (70.83%, 17 cases) compared with females (29.16%, seven cases).

In terms of topography of apparently primitive adenopathies (24 cases), we found a predominance of

the jugular-carotid ones (16 cases, 66.66%): 12 on the right (50%), three on the left (12.5%) and one bilateral localization (4.16%). Other locations were supraclavicular (three cases, 12.5%): one on the right (4.16%), two on the left (8.33%), spinal (two cases, 8.33%): one on the right (4.16%) and one on the left (4.16%), and three cases with right submandibular location (12.5%).

For the 24 lymph node metastases with apparently primitive origin, following clinical, laboratory, histopathological and immunohistochemical investigation, we identified the following locations of the primary tumor (Table 2).

Table 2 – Location of the primary tumor accompanied by apparently primary adenopathies

Location of primary tumor	No. of cases	%
Thyroid	2	8.33
Lungs	2	8.33
Digestive tract	2	8.33
Rhinopharynx	9	37.5
Oropharynx	6	25
Hypopharynx (piriform sinus)	2	8.33
Larynx	1	4.16
Total	24	100

Lymph node metastases with a primitive thyroid tumor starting point were present in 8.33% of the cases analyzed, which corresponded to papillary thyroid carcinomas confirmed histologically (Figure 1a) and by IHC positivity for CK19 (Figure 1b) and HBME1 (Figure 1c).

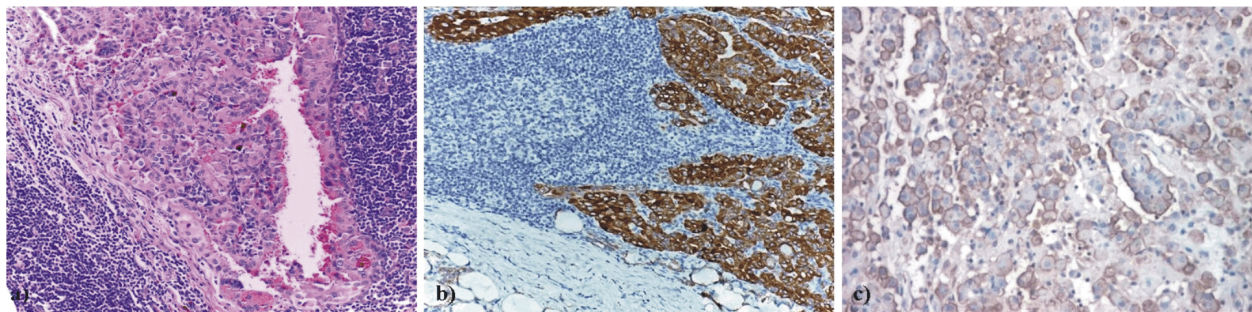


Figure 1 – Metastasis of a papillary thyroid carcinoma: (a) HE stain, ×100; (b) CK19 immunostaining, ×100; (c) HBME1 immunostaining, ×100.

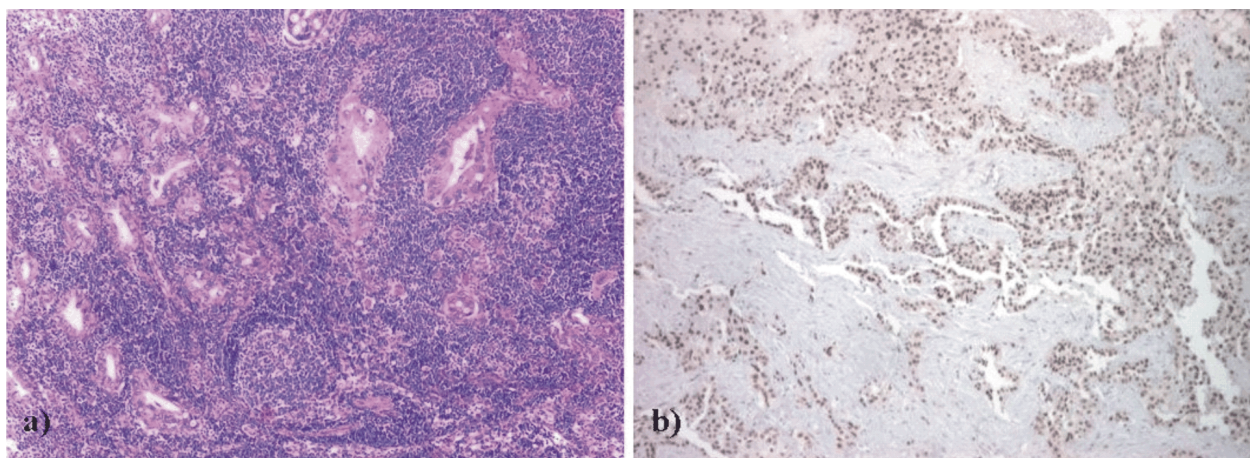


Figure 2 – Metastasis of a pulmonary adenocarcinoma: (a) HE stain, ×100; (b) TTF1 immunostaining, ×40.

Lymph node metastases with a starting point in a primary lung tumor were encountered in 8.33% of the cases. The two cases with lymph node metastases from primitive lung tumors corresponded to large cell lung carcinoma with squamous differentiation, and adenocarcinoma (Figure 2a). In our study, we used an algorithm consisting of TTF1 positivity in adenocarcinomas and TTF1 negativity in squamous carcinomas, CK7 positivity in adenocarcinomas and CK7 negativity in squamous carcinomas, CKAE1/AE3 negativity in adenocarcinomas and CKAE1/AE3 positivity in squamous carcinomas and diffuse positivity for CK34betaE12 in squamous carcinomas.

Lymph node metastases having as starting point a primary digestive tumor were found in other 8.33% of

the cases. Of the two digestive metastases, one had a left supraclavicular location while the other had a jugular-carotid location; in terms of location of the primary tumor, one had a gastric starting point, and the other originated from an esophageal cancer (Figure 3a). The metastasis with a gastric starting point corresponded to a poorly differentiated adenocarcinoma and the esophageal cancer metastasis was represented by a well-differentiated squamous cell carcinoma. From the immunohistochemical point of view, the esophageal carcinoma was positive for CKAE1/AE3, while the gastric adenocarcinoma was positive for CEA (Figure 3b), CK7 (Figure 3c) and MUC5AC (Figure 3d), and negative for CKAE1/AE3 and CK20.

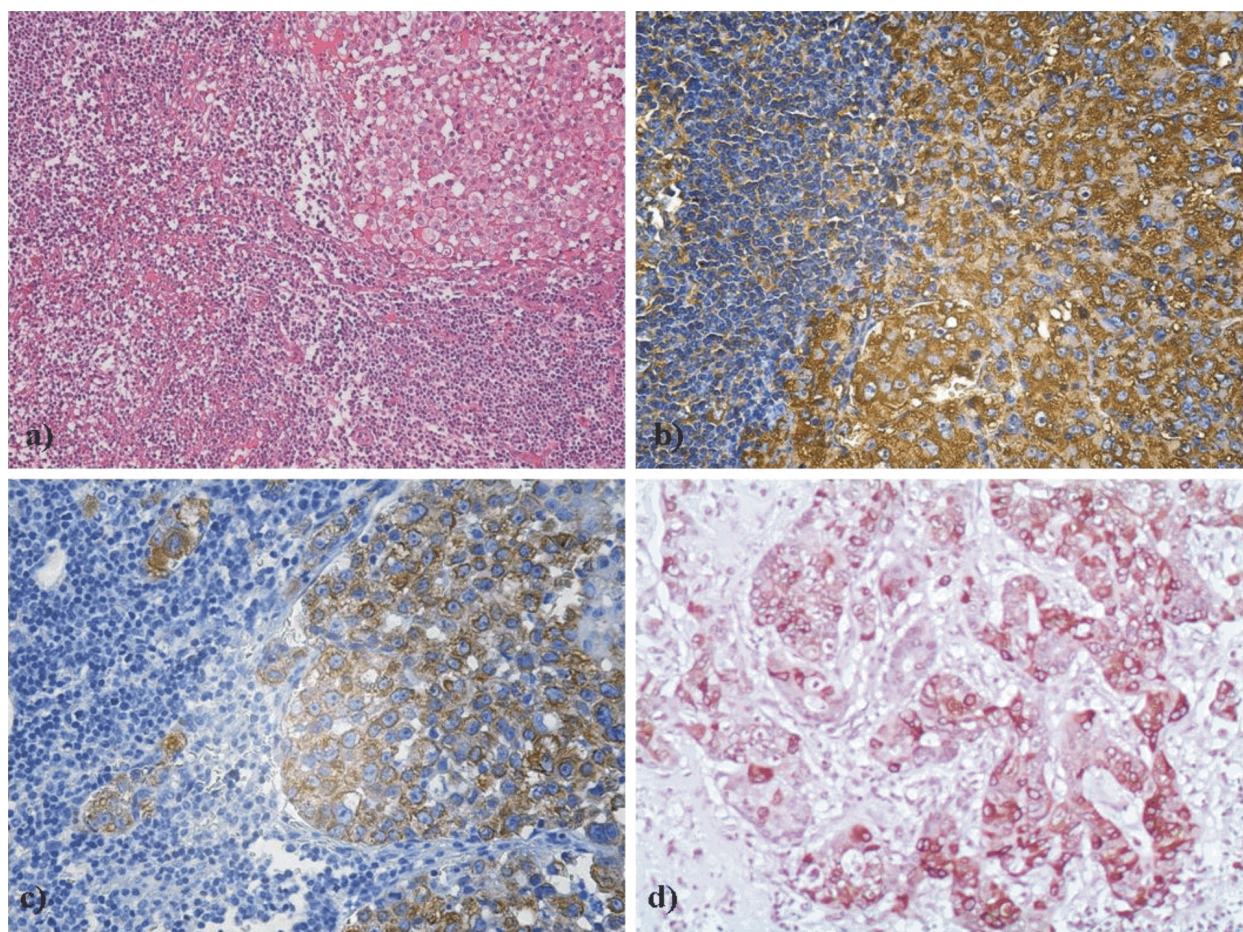


Figure 3 – Lymph node metastasis of a poorly differentiated gastric adenocarcinoma: (a) HE stain, $\times 100$; (b) CEA immunostaining, $\times 100$; (c) CK7 immunostaining, $\times 100$; (d) MUC5AC immunostaining, $\times 100$.

Lymph node metastases with an ENT primary tumor as a starting point (rhinopharynx, oropharynx, hypopharynx and larynx) were diagnosed in 74.99% of the cases. In our study we found 17 such cases: nine cases in the rhinopharynx (seven poorly differentiated squamous cell carcinomas and two undifferentiated carcinomas), six cases in the oropharynx (five poorly differentiated squamous cell carcinomas and one undifferentiated carcinoma), one case in the hypopharynx (poorly differentiated squamous cell carcinoma) and one in the larynx (moderately differentiated squamous cell carcinoma) (Figure 4a). All tumors were positive for CKAE1/AE3 (Figure 4b).

Lymphoplasmocytic infiltrate can often be present in lymph node metastases of undifferentiated carcinomas, suggesting a lymphoepithelial carcinoma (Figure 5a).

In three cases, the undifferentiated carcinomas with strong lymphatic component were difficult to delineate from the adjacent lymphoid tissues. In these cases, we performed an immunohistochemical staining using a cocktail of cytokeratins (AE1/AE3) (Figure 5b) which marks the epithelial cells of the carcinoma, and for EBV detection (Figure 5c).

The EBV immunostaining showed positivity in tumor cells, but all cases showed rare lymphocyte nuclear positivity for the same marker.

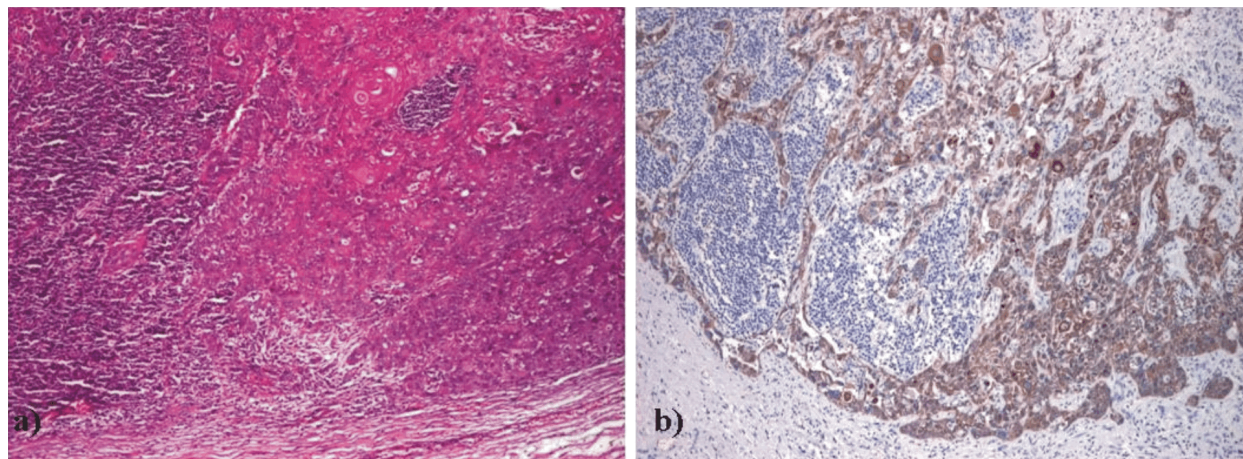


Figure 4 – Lymph node metastasis of a poorly differentiated squamous cell carcinoma: (a) HE stain, ×100; (b) CKAE1/AE3 immunostaining, ×100.

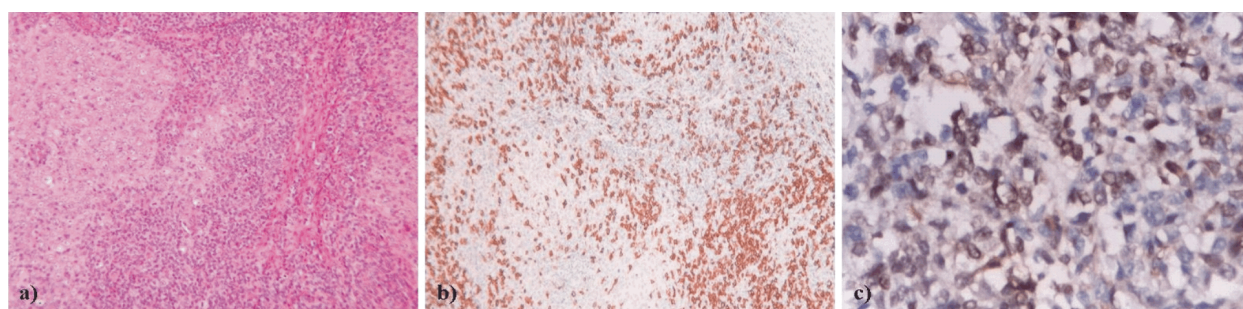


Figure 5 – Lymph node metastasis of an undifferentiated lymphoepithelial carcinoma: (a) HE stain, ×100; (b) CKAE1/AE3 immunostaining, ×40; (c) EBV immunostaining, ×200.

Discussion

The literature reported variable incidences of CUP. The most common histopathological entities are squamous carcinomas, followed by undifferentiated carcinomas, adenocarcinomas and malignant melanomas [5]. Up to 10% of all laterocervical lymph node metastases have unknown origin, but 70–80% of primary tumors are located in the head and neck, since cervical lymph nodes ensure the lymphatic drainage of these areas [6].

Our study comprised 24 cases of CUP, representing 9.52% of metastatic adenopathies, for a period of five years. They had an increased incidence in the age group 51–60 years (33.33%), affecting predominantly males (70.83%). In terms of the topography of lesions, we found a predominance of the jugular-carotid ones (16 cases, 66.66%). A similar study on 71 patients with CUP report a male/female ratio of 1.7/1, with most of the patients aged between 51 and 70 years [7]. The authors report the identification of the location of the primary tumor in only 11 patients.

In patients with CUP there is no consensus regarding the necessary diagnostic steps. The studies showed that an effective algorithm requires diagnostic lymph node biopsy, panendoscopies with systematic biopsies of suspicious areas and endoscopic biopsies of endoscopically discrete regions, including the base of the tongue and nasopharynx, as well as bilateral tonsillectomy [8].

Depending on the histopathological appearance of the lesion, different panels of antibodies are used. The

immunohistochemical study of CUP is performed in several steps according to the following algorithms:

- in a laterocervical metastasis of unknown origin there is a question of excluding a primitive lymphoma-type tumor, then differentiating a metastasis from carcinoma, sarcoma or melanoma;
- for carcinoma metastasis it is important to assess the type of carcinoma using immunohistochemical markers.

Immunohistochemistry is not 100% specific or sensitive, but may be useful in determining the tumor origin [2]. There are more than 20 known types of cytokeratins (CK), useful in identifying the origin of the disease. CK7 is present in patients with lung cancer, endometrial, ovarian or breast cancer, while CK20 is present in urothelial, Merkel and gastrointestinal tract cells [9]. Several staining patterns are highly suggestive of tumor origin. For example, CK7-/CK20+ suggests an immunoprofile characteristic for colorectal cancer, while CK7+/CK20 is associated with lung, breast, upper gastrointestinal tract, pancreas and biliary tract cancer [9]. TTF-1 is positive in about 68% of lung adenocarcinomas and 25% of squamous carcinomas of the lung, and is also a marker for thyroid cancer [10].

Thyroid laterocervical metastases are sometimes the first step in detecting thyroid cancer. In terms of histopathology the most common type is papillary carcinoma as confirmed by our study, the origin of tumors being established after histopathological and immunohistochemical examination. The panel of antibodies used by us consisted of CK19 and HBME1 as specific

markers for the diagnosis of metastatic thyroid papillary carcinoma.

The literature shows a 26.5% incidence of laterocervical lymph node metastasis of papillary thyroid carcinoma [11]. In the immunohistochemical studies of thyroid cancers, El Demellawy D *et al.* (2008) used an antibody panel composed of CK19, CD56, E-cadherin and p63 [12]. CK19 was positive in 85% of papillary carcinoma cases, CD56 was negative in papillary carcinomas but positive in other types of carcinoma and adenoma, p63 showed a focal positivity in 70% of cases, and the expression of E-cadherin was inconclusive. Many authors emphasize the importance of CK19 and HBME1 in the diagnosis of metastatic papillary carcinoma and the detection of lymph node metastases [13–15]. CK19 is the most common marker used to diagnose papillary thyroid carcinomas, but we must keep in mind that it is also positive in other types of cancer (pancreatic or biliary carcinoma), and it is therefore necessary to use a panel of antibodies.

Lymph node metastases with a starting point in a primary lung tumor were present in 8.33% of the cases investigated. The histopathological appearance suggested in one case an adenocarcinoma and in the other a poorly differentiated squamous cell carcinoma. In our study, we used an algorithm consisting of TTF1 that was positive in adenocarcinomas and negative in squamous carcinomas, CK7 positive in adenocarcinomas and negative in squamous carcinomas, CKAE1/AE3 negative in adenocarcinomas and positive in squamous carcinomas, and diffuse positivity for CK34betaE12 in squamous carcinomas.

Immunohistochemistry may be useful to differentiate a metastasis from lung adenocarcinoma from one arising from a squamous carcinoma of the same location. In their study, Rekhtman N *et al.* (2011) [16] used a panel of antibodies consisting of TTF1, CK5/6, p63 and CK34betaE12 to differentiate the two types of cancer. The squamous cell carcinoma immunoprofile was TTF1- and diffuse positivity for p63, CK5/6 and CK34betaE12. When it comes to the “squamous markers”, the adenocarcinoma showed a heterogeneous immunoreactivity (p63 – 32%, CK5/CK6 – 18%, CK34betaE12 – 82%, TTF1 – 89%).

Laterocervical lymph node metastases arising from digestive carcinomas represented 8.33% of the cases analyzed. One of the cases corresponded to a primitive esophageal squamous cell carcinoma and the other to a primitive gastric adenocarcinoma.

One study showed a correlation between the location of the primitive esophageal tumor and laterocervical metastases, the latter being more frequent in tumors with upper and middle location [17].

Lee MJ *et al.* (2003) initiated a classification model for gastric cancers based on the immunohistochemical profile expression [18]. Mucins and cytokeratins showed different expression patterns that allow the classification of primary sites of digestive cancers. The first category includes CK7+/MUC5AC+ tumors (tumors of the exocrine pancreas and extrahepatic bile ducts) and CK7+/MUC5AC- tumors (tumors of the ampulla of Vater and gallbladder), the stomach showing variable

CK7+/MUC5AC± expression. The second category includes CK7- tumors, which can be located in the colon or anus (CK7-/CK20+/MUC5AC-/MUC1+), appendix (CK7-/CK20+/MUC5AC+) or liver (CK7-/CK20-/CK13+/CK19+).

In our study, we used a panel of antibodies consisting of CK7, CK20, CKAE1/AE3, CK34betaE12 and MUC5AC. Metastases of esophageal carcinoma were of squamous-cell type, and gastric adenocarcinoma was CK7+/CK20-/MUC5AC+.

Metastases of squamous cell carcinoma in the lower neck region may come from a primitive tumor located in the head and neck, esophagus, lung and urogenital tract. The main tumors that metastasize in this region are those of the nasopharynx, probably secondary to an Epstein–Barr virus (EBV) infection [19]. Cervical node metastases are often the first symptom, the lymph node biopsy playing an important diagnostic role, just like in the cases we studied. In the case of undifferentiated lymphoepithelial carcinomas, tumors arise in the majority of cases in the nasopharynx, but also in the tonsillar region, most cases being associated with the Epstein–Barr virus. Undifferentiated lymphoepithelial carcinomas typically lead to early cervical lymph node metastases, which can also represent the initial clinical signs, while the primary tumor may remain initially occult [20, 21]. In ambiguous cases the immunohistochemical staining for CK is recommended which unmistakably marks tumor epithelial cells of the lymphoepithelial carcinoma for which malignant lymphomas are negative [22]. It is noteworthy that EBV is a virus with tropism for lymphoid cells [23, 24]. In the cases investigated, the EBV immunostaining showed EBV positivity in tumor cells, but all cases showed rare lymphocytic nuclear positivity for the same marker. The presence of EBV expression in lymphocytes suggests the possibility of a secondary location of the virus after initiation of carcinogenesis in epithelial cells.

Conclusions

Sequential analysis of clinical, histopathological and immunohistochemical data can be of great use in the diagnosis of laterocervical lymph node metastases of unknown primary origin. The initial histopathological interpretation provides valuable information for establishing the location of the tumor, data that should be completed with those obtained by using immunohistochemical tests. A panel of antibodies including CK7, CK19, CK20, CKAE1/AE3, CK34betaE12, TTF1, HBME1, CEA, MUC5AC and EBV can identify the location of the primary tumor in most cases of laterocervical lymph node metastases with unknown primary origin.

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