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Patterns of EBV-positive cervical lymph node involvement in head and neck cancer and implications for the management of nasopharyngeal carcinoma T0 classification

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ABSTRACT

Objectives: Epstein-Barr virus (EBV)-positive cervical lymph node (CLN) metastasis of unknown primary origin is classified as nasopharyngeal carcinoma (NPC) T0 by the American Joint Committee on Cancer staging manual (8th edition). We aimed to investigate the possible primary sites and patterns of EBV-positive CLN metastases and to provide implications for the management of NPC T0 classification.

Materials and methods: We retrospectively reviewed 269 patients with newly diagnosed EBV-positive CLN metastatic disease who underwent EBV detection via EBV-encoded RNA in situ hybridization. Fifteen patients with unknown primary tumors underwent follow-up after initial treatment.

Results: In patients with EBV-positive CLNs, the most common primary sites after the nasopharynx (51.7%) were the salivary gland (24.5%), lung (7.8%), oropharynx (3.3%), nasal cavity/maxillary (3.3%), oral cavity (2.2%), orbit (1.1%), and liver (0.4%). No primary site was found in 15 patients (5.6%). For salivary gland malignancies, level II and I were the most frequently involved regions. Tumors arising from the lung or liver metastasized to the lower neck (level IV, V, and VI) rather than the upper neck. After initial treatment, 2/15 patients with EBV-positive CLNs of unknown primary exhibited primary NPC and oropharyngeal tumor, respectively. Further, even without prophylactic irradiation to the nasopharynx, only one of 13 unknown primary patients developed NPC.

Conclusions: The origins of EBV-positive CLNs may not be restricted to the nasopharynx alone, and are likely to involve the head and neck or non-head and neck regions. NPC T0 classification should be cautiously assigned to such tumors.

Introduction

An accurate TNM staging system is crucial for evaluating the prognosis and determining appropriate treatment of malignant tumors [1]. To provide the best possible TNM staging system, the International Union against Cancer (UICC) and the American Joint Committee on Cancer (AJCC) periodically modify the same in response to newly acquired clinical data and rapidly evolving knowledge of the prognostic factors of cancer [1].

It is well documented that the natural history and response to treatment of cervical lymph node (CLN) metastases from Epstein-Barr virus (EBV)-related nasopharyngeal carcinoma (NPC) are different from those of virus-unrelated head and neck cancers in terms of impact on prognosis, and hence, they warrant distinct N classification schemes [1]. This difference also has led to the addition of T0 for EBV-positive unknown primary tumors with CLN involvement in the most recent 8th edition of the AJCC staging manual for NPC [1]. This change could likely eliminate the uncertainty of optimal management in EBV-positive

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unknown primary tumors with CLN involvement.

However, this assignment is based on evidence level III, which means the supporting evidence is somewhat problematic. To our knowledge, EBV is not only related to NPC. For instance, lymphoepithelioma-like carcinoma (LELC), a rare disease arising from mucosal sites outside the nasopharynx could also be EBV-related [2–6]. It is morphologically similar to undifferentiated NPC with high frequency of concurrent CLN metastases but could occur in multiple sites, including the salivary gland, oropharynx, sinonasal tract and non-head and neck regions [2–6]. LELC may be confused with NPC when presenting initially with CLN metastasis. Therefore, it requires great caution to correctly identify the origin of EBV-positive metastatic cervical lymphadenopathy. In addition, once assigned an NPC T0 category, patients typically receive radiotherapy that encompasses the nasopharynx, the entire retropharyngeal lymph node (LN) and bilateral CLN region [7–10]. This might lead to unnecessary irradiation of certain areas and, in turn, reduced quality of life.

To date, little has been reported on whether EBV-positive metastatic CLNs from unknown primary could successively present NPC. Thus, the present study sought to illustrate the possible primary sites and metastatic patterns of EBV-positive CLN involvement and to further provide implications for the management of NPC T0 classification.

Materials and methods

Patients

We performed in situ hybridization of EBV-encoded RNA (EBER) on all available biopsies from 325 patients with newly diagnosed LELC in head and neck and EBER-positive (EBER +) CLN metastatic disease who presented at our institution between December 2001 and January 2016. Among 313 EBER + patients, 269 (85.9%) with EBER + CLN metastases were enrolled. The study was reviewed and approved by the Clinical Research Ethics Committee of Sun Yat-sen University Cancer Center. The key raw data have been uploaded onto the Research Data Deposit public platform (RDD), with the approval number of RDDA2019000977.

In situ hybridization of EBER

The EBV Probe In Situ Hybridization Kit (DIG-AP, A300K.9901, PanPath Company, Amsterdam, Netherlands) was used to detect EBER. Briefly, paraffin sections were deparaffinized and rehydrated using xylene and a series of graded ethanol solutions. Then, the sections were pretreated with 0.4% pepsin for 10 min, followed by hybridization with digoxigenin-conjugated EBV probes at 37 °C for 3 h. The immune reaction signal was detected using peroxidase-conjugated anti-digoxigenin antibody and 3,3′-diaminobenzidine (DAB); sections were finally counterstained with hematoxylin solution. The positive signals were stained brownish-yellow and localized within the nuclei (Fig. 1). EBV-positive NPC slides served as positive controls and blank slides as negative controls.

Determination of primary sites and metastatic CLNs

All patients underwent a preliminary workup comprising a complete history, clinical examination, hematology and biochemistry profiles, computed tomography (CT), magnetic resonance imaging (MRI) of the head and neck, chest radiography, abdominal sonography, bone scanning, and/or positron emission tomography (PET)-CT. Additionally, given the close relationship between EBV and NPC, all patients were examined by nasopharyngoscopy and biopsy to identify potential primary sites in the nasopharynx. All patients were restaged based on the AJCC staging manual (8th edition) [1].

Two radiologists specializing in head and neck cancers separately evaluated all scans; disagreements were resolved by consensus. The CLN levels were determined according to the Radiation Therapy Oncology Group guidelines [11]. When CLNs were located at the border of two regions that crossed in different axial planes, the nodal status was recorded in both regions. When CLNs overlapped within the same axial plane, the assignment was made according to the location of the main body of the nodes.

Treatment

Patients were treated depending on their diagnosis, general health, and the clinicians' discretion. For non-nasopharyngeal LELC, treatment strategies included surgery, radiotherapy, and/or chemotherapy. Either unilateral or bilateral surgery was performed. Irradiation covered the unilateral neck, bilateral neck, or putatively involved mucosa. Delineation of the target volumes was performed according to our institutional treatment protocol and the International Commission on Radiation Units and Measurements reports, 50 and 62. Regarding chemotherapy, patients with adverse features such as T3/T4 or N2/N3 classification, extracapsular spread, and perineural invasion received platinum-based concurrent chemoradiotherapy (CCRT), neoadjuvant and/or adjuvant chemotherapy.

Follow-up

Patients were examined at least every three months for the first two years, then every six months for three years thereafter or until death. In this study, we focused on the survival data of the 15 patients with unknown primary tumors at diagnosis. End points (time to the first defining event) assessed included overall survival (OS), local-regional relapse-free survival (LRRFS), and distant metastasis-free survival (DMFS) that were calculated from the first day of therapy to the date of death or the last follow-up visit, local regional failure, and distant failure, respectively.

Results

Patient characteristics and primary sites of EBV-positive CLN metastases

The clinical features of the patients diagnosed with EBER + CLN metastatic disease are shown in Table 1. The median age was 44 years (range: 10–77 years; 178 men and 91 women; male-to-female ratio:1.96). Fig. 1 depicts examples of hematoxylin-eosin staining and in situ hybridization of acquired specimens. Regardless of the primary site, malignant cells shared identical undifferentiated and syncytial appearance with marked lymphoplasmacytic infiltrate and were positive for EBER in the nuclei by in situ hybridization.

The incidence of EBER + CLNs metastases was 85.9% (269/313). Bilateral CLN involvement was present in 34.6% (93/269) patients. The majority of patients had CLN metastases from the nasopharynx (51.7%, 139/269). Concerning the non-nasopharyngeal cases, the salivary gland (24.5%, 66/269) accounted for the most common primary site, followed in order by the lung (7.8%, 21/269), oropharynx (3.3%, 9/269), nasal cavity/maxillary sinus (3.3%, 9/269), oral cavity (2.2%, 6/269), orbit (1.1%, 3/269), and liver (0.4%, 1/269). No primary site was found in 15 patients (5.6%, 15/269).

Patterns of EBV-positive CLN metastases by primary sites

Incidence and distribution of the 130 cases with EBER + CLN metastases from different origins outside the nasopharynx are shown in Table 2. Among the 174 EBER + non-nasopharyngeal cases, 130 (74.7%) had EBER + CLN metastases. Tumors of head and neck origins including the parotid gland, submandibular gland, nasal cavity/maxillary sinus, oral cavity, orbit, and sublingual gland spread most frequently to level II (71.4%, 60/84) and level I (40.5%, 34/84), whereas tumors from the oropharynx never spread to level I (0/9). Furthermore,

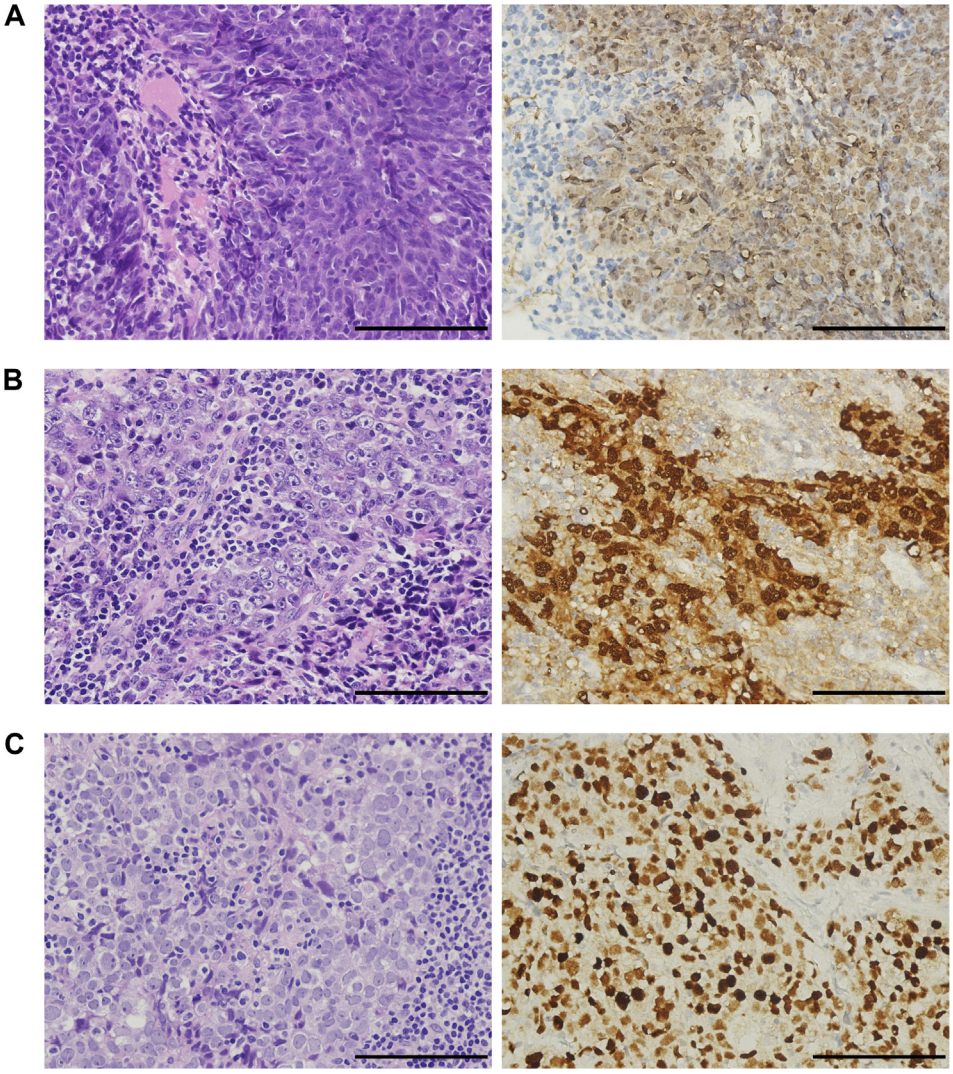

Fig. 1. Representative hematoxylin-eosin staining (left panels) and in situ hybridization (right panels) of Epstein-Barr-virus-encoded RNA-positive specimens (magnification, 400×; scale bars, 100 μm). (A) Primary site of nasopharyngeal carcinoma (NPC), (B) Primary site of parotid gland lymphoepithelioma-like carcinoma (LELC), (C) Metastatic cervical lymph node from a parotid gland LELC.

Table 1
Characteristics of the 269 cases with EBV-positive CLN metastases.

Characteristic	No. of patients (%)
Total	269 (100)
Age, y	
Median	44
Range	10–77
Gender	
Male	178 (66.2)
Female	91 (33.8)
Primary site	
Nasopharynx	139 (51.7)
Parotid gland	45 (16.7)
Lung	21 (7.8)
Submandibular gland	20 (7.4)
Unknown primary	15 (5.6)
Oropharynx	9 (3.3)
Nasal cavity/maxillary sinus	9 (3.3)
Oral cavity	6 (2.2)
Orbit	3 (1.1)
Sublingual gland	1 (0.4)
Liver	1 (0.4)

Abbreviations: EBV, Epstein-Barr virus; CLN, cervical lymph node.

those of non-head and neck origins (lung and liver) metastasized only to the lower neck (level IV, V, and VI) rather than the upper neck, and the majority of level IV metastases originate from the lung (58.3%, 21/36).

For tumors of parotid gland origin, level II (77.8%, 35/45) and level I (35.6%, 16/45) were the most frequently involved regions. Among 11 patients who had level III involvement and 4 with level IV involvement, all had ipsilateral level II metastases. Level IX and X metastases were only observed in parotid gland tumors, without the involvement of other LNs than level II LN. Among 11 patients who had level Ib involvement, 18.2% (2/11) did not have level II metastases (Table 3).

With regard to submandibular gland malignancies, all patients had metastases in level I (55.0%, 11/20) or level II (65.0%, 13/20) regions, which were the most frequently involved. Among 4 patients who had level III involvement and 3 who had level IV involvement, all showed ipsilateral level II metastases. Of the 9 patients who had level Ib involvement, 66.7% (6/9) did not have level II metastases (Table 4).

Characteristics and follow-up of the 15 patients with EBV-positive CLN metastases of unknown primary origin

The median age of the 15 patients with EBER+ CLN metastatic disease of unknown primary was 44 years (range: 17–66 years; 11 men and 4 women; male-to-female ratio: 2.75). All 15 patients had unilateral

Table 2
Incidence and distribution of EBV-positive CLN metastases from outside the nasopharynx.

Primary site	EBER + CLNs cases/EBER + cases	Level involved										
		Ia	Ib	II	III	IV	V	VI	VII	VIII	IX	X
Parotid gland	45/69	5	11	35	11	4	7	–	2	13	1	2
Lung	21/21	–	–	–	–	21	1	2	–	–	–	–
Submandibular gland	20/30	2	9	13	4	3	1	–	2	–	–	–
Unknown primary	15/15	1	2	10	6	6	2	–	3	1	–	–
Oropharynx	9/9	–	–	9	4	–	–	–	2	–	–	–
Nasal cavity/maxillary sinus	9/14	–	3	6	1	1	1	–	3	2	–	–
Oral cavity	6/7	–	1	3	–	–	–	–	–	–	–	–
Orbit	3/7	–	2	2	1	–	–	–	–	–	–	–
Sublingual gland	1/1	1	–	1	–	–	–	–	1	–	–	–
Liver	1/1	–	–	–	–	1	–	–	–	–	–	–
Total (%)	130/174	9	28	79	27	36	12	2	13	16	1	2

Abbreviations: EBV, Epstein-Barr virus; CLN, cervical lymph node; EBER+, EBV-encoded RNA-positive.

CLN metastases. Overall, no patient was in stage I; 40.0% (6/15) were in stage II; 6.7% (1/15), stage III; and 53.3% (8/15), stage IV. The median duration of follow-up was 49.1 months (range: 14.4–110.6 months). Table 5 presents the clinical and follow-up data. Of the 15 reviewed patients, 6 received ipsilateral neck management (4 ipsilateral neck dissection, one ipsilateral neck CCRT, and one ipsilateral neck radiotherapy), and 5 received bilateral neck management (3 bilateral neck CCRT and 2 bilateral neck radiotherapy). Prophylactic irradiation of potential primary sites was prescribed to only 3 patients: case 2 in the oropharynx and hypopharynx, and cases 8 and 12 in the nasopharynx. Five patients received induction chemotherapy and four received CCRT. Four patients with more advanced tumors underwent palliative chemotherapy.

By the end of the follow-up, the primary sites were identified in 2/15 patients in total. One (case 3) among 13 patients without prophylactic irradiation to the nasopharynx exhibited NPC 47.0 months after initial treatment, who later underwent salvage radiotherapy to the nasopharynx and was alive until the last follow-up. The other patient (case 2) with suspected hypopharynx involvement detected by PET-CT underwent radical irradiation (70 Gy) to the hypopharynx and prophylactic dose to the oropharynx, who presented a primary tumor in the oropharynx 36.0 months after the initial management; however, he was lost to follow-up after identification of the primary site. Altogether, 13 patients survived, and 2 patients died of bone metastasis without the apparent presence of a primary tumor. The OS and DMFS rates were the same (86.7%).

Discussion

The 8th edition AJCC manual system adds the T0 classification for NPC on the basis of the previous edition, referring to EBV-positive unknown primary tumors with CLN involvement (level of evidence: III) [1,12]. Similarly, the system defines human papilloma virus (HPV)-positive CLN metastases of unknown primary as T0 using the criteria for oropharyngeal carcinoma [1]. The rationale behind these assignments may be the same: the vast majority of EBV/HPV-positive tumors originate from nasopharynx or oropharynx, respectively [13,14]. However, these recommendations warrant further investigation and confirmation. In our study dealing with EBV-positive CLN metastases, we showed that a large fraction of EBV-positive metastatic cervical lymphadenopathies originate from outside of the nasopharynx and that the diagnosis of NPC T0 may be questionable.

Primary sites of EBV-positive CLN metastases

EBV, a human cancer-associated virus, is closely associated with a spectrum of diseases including NPC, LELC, lymphoma, and melanoma [3,5,13]. While lymphoma and melanoma can be readily distinguished from NPC by immunohistochemistry [5,15], differentiating LELC from NPC is a clinical challenge. This can be illustrated by several aspects. First, LELC, a variant of undifferentiated carcinoma outside the nasopharynx accompanied by a significant lymphoplasmacytic infiltrate, is morphologically similar to undifferentiated NPC [2–6]. Second, LELC and NPC have strong associations with EBV in overlapping endemic regions (e.g., southern China and Southeast Asia) [16–18], although laryngeal and tracheal LELCs are typically EBV-negative [19]. Third, both NPC and LELC in the head and neck harbor high rate of CLN metastasis. Previous studies showed that 70–80% of NPC patients have CLN metastases [20], compared with 40–77% of non-nasopharyngeal LELC patients presenting with CLN metastases [3,21]. In accordance with previous data, our study reported that 74.7% of EBV-positive non-nasopharyngeal tumors had CLN metastases originating from various origins, including the salivary gland, lung, oropharynx, oral cavity, orbit, nasal cavity/maxillary, and liver. Thus, the presence of EBV-positive metastatic CLNs of unknown primary may not lead to the diagnosis of an NPC.

Patterns of EBV-positive CLN metastases

Malignancies originating from the head and neck are liable to spread to CLNs. However, comprehensive literature reviews have indicated that in the previously untreated neck, the LN drainage of different sites in the head and neck follows a sufficiently predictable pattern, which serves as the rationale behind selective neck treatment

Table 3
Relation between level III, IV, Ib and level II metastases from the parotid gland.

Level II LNs	No. of level III LNs (%)			No. of level IV LNs (%)			No. of level Ib LNs (%)		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	11 (24.4)	24 (53.3)	35 (77.8)	4 (8.9)	31 (68.9)	35 (77.8)	9 (20.0)	26 (57.8)	35 (77.8)
Negative	0	10 (22.2)	10 (22.2)	0	10 (22.2)	10 (22.2)	2 (4.9)	8 (17.8)	10 (22.2)
Total	11 (24.4)	34 (75.6)	45	4 (8.9)	41 (91.1)	45	11 (24.4)	34 (75.6)	45

Abbreviation: LNs, lymph nodes.

Table 4
Relation between level III, IV, Ib and level II metastases from the submandibular gland.

Level II LNs	No. of level III LNs (%)			No. of level IV LNs (%)			No. of level Ib LNs (%)		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
Positive	4 (20.0)	9 (45.0)	13 (65.0)	3 (15.0)	10 (50.0)	13 (65.0)	3 (15.0)	10 (50.0)	13 (65.0)
Negative	0	7 (35.0)	7 (35.0)	0	7 (35.0)	7 (35.0)	6 (30.0)	1 (5.0)	7 (35.0)
Total	4 (20.0)	16 (80.0)	20	3 (15.0)	17 (85.0)	20	9 (45.0)	11 (55.0)	20

Abbreviation: LNs, lymph nodes.

Table 5
Characteristics and follow-up of the 15 patients with EBV-positive CLN metastases of unknown primary tumors.

Case	Age, y/ sex	Site of CLN metastasis	Stage	Neck management	Chemotherapy strategy	Additional irradiated site (Total doses, Gy)	Primary site identified during follow-up	Follow-up duration	Relapse or metastasis
1	32/M	II	T0N1M0	Unilateral surgery	–	–	–	110.6 mo	No
2	30/M	II, VII	T0N1M0	Bilateral radiotherapy	–	Oropharynx (60) + hypopharynx (70)	Oropharynx, 36.0 mo after radiotherapy	36.0 mo	No
3	61/M	II	T0N1M0	Unilateral surgery	–	–	Nasopharynx, 47.0 mo after surgery	86.1 mo	No
4	66/M	III	T0N1M1[a]	–	Chemotherapy	–	–	91.8 mo	No
5	33/F	II, III, IV	T0N3M0	–	Chemotherapy	–	–	54.1 mo	No
6	50/F	II, III, IV, VIII	T0N3M0	–	Chemotherapy	–	–	14.4 mo	Died, bone
7	44/M	IV	T0N3M0	Bilateral CCRT	IC + CCRT	–	–	15.9 mo	Died, bone
8	37/M	II, V, VII	T0N1M0	Bilateral CCRT	IC + CCRT	Nasopharynx (70)	–	47.4 mo	No
9	47/M	I, II, III	T0N3M0	Unilateral CCRT	IC + CCRT	–	–	41.2 mo	No
10	45/M	II, III, IV, V	T0N3M0	Bilateral CCRT	IC + CCRT	–	–	49.1 mo	No
11	48/F	VII	T0N1M0	Bilateral radiotherapy	–	–	–	48.1 mo	No
12	38/M	II, III	T0N1M0	Unilateral radiotherapy	IC	Nasopharynx (70)	–	30.0 mo	No
13	46/F	II	T0N2M0	Unilateral surgery	–	–	–	66.4 mo	No
14	23/M	I, IV	T0N3M0	Unilateral surgery	–	–	–	95.4 mo	No
15	17/M	IV	T0N3M1[a]	–	Chemotherapy	–	–	60.1 mo	No

Abbreviations: CLN, cervical lymph node; M, male; F, female; CCRT, concurrent chemoradiotherapy; IC, induction chemotherapy.
[a] The patient was classified as stage M1 because of metastasis to abdominal lymph nodes.

[22,23]. Our previous studies have proven that, in the case of NPC, metastatic CLNs spread in order from the upper neck to the lower neck, and involvement of level Ib is a rare incidence, which always occurs in combination with ipsilateral level II metastases [24]. For malignancies of the parotid and submandibular glands, the present study showed that level II and I LNs were the most frequently involved, which is consistent with previous reports [25,26]. Furthermore, metastases in level II were commonly accompanied by those in level III and IV, and level Ib could be involved without level II metastases, suggesting that both level II and Ib LNs may be the first-echelon nodes in these tumors. Oropharyngeal tumors never spread to level I, which is similar to that of NPCs.

Tumors arising outside the head and neck can also metastasize to the neck. Anatomically, LNs in the left supraclavicular fossa receive afferent lymphatics from the thorax, abdomen, and pelvis [27,28]. It is well documented that CLN metastases of lung cancer occur mostly in supraclavicular region [28]. We also observed that in tumors of non-head and neck primary origin (lung and liver), EBV-positive CLNs were inclined to metastasize to the lower neck (level IV, V, and VI) rather than the upper neck.

The location and level of the metastatic LNs may provide clues in localizing tumors of obscure origin. As previously reported, metastases in the upper and middle neck tend to originate from tumors of head and neck origin, while the lower neck involvement often indicates tumors from the infraclavicular origin [29,30], which is in accordance with our results. Above all, when determining the primary sites or treatment for patients with CLN metastases, clinicians could take advantage of the spread patterns and the site-specific distribution of metastatic CLNs.

Implications for the management of NPC T0 classification

Advances in imaging and pathological technologies have contributed to an increasing number of identified primary tumors initially presenting with metastatic CLNs. Our results found that the primary sites of patients with EBV-positive CLN metastases remained unknown in few patients (5.6%, 15/269) through comprehensive diagnostic workup.

To our knowledge, studies concerning EBV-positive CLNs of unknown primary are rare. Feinmesser et al. reported two patients with unknown primary CLN metastases to be EBV-positive, both of whom manifested NPC within one year from diagnosis [31]. In another series, EBV was detected in two of three cases with unknown primary CLN metastases and one of them (50%) demonstrated NPC after initial diagnosis [32]. However, these small sample sized studies lacked the ability to determine whether EBV-positive CLNs of unknown primary are of nasopharyngeal origin or to investigate the treatment for EBV-positive CLNs of unknown primary.

In terms of occult primary CLN metastases, the optimal management remains uncertain. Proposed treatment modalities include surgery and/or radiotherapy, depending on initial nodal disease burden [33–36]. Given that EBV-positive metastatic CLNs may originate from nasopharynx or outside the nasopharynx, the experience in treating NPC or LELC should be of use when treating EBV-positive CLNs of unknown primary. Similar to NPC, LELC is described to be radiosensitive. It confers a relatively favorable prognosis among undifferentiated carcinomas presumably because of its prominent lymphoplasmacytic infiltrate with strong immune response [2,3]. Thus, radiotherapy alone may serve as a radical treatment for locoregional LELC. Irradiation usually encompasses the most common primary sites including the nasopharynx, hypopharynx, and oropharynx. However, our study showed that even without prophylactic irradiation to the nasopharynx, only one (7.7%) of 13 patients with EBV-positive unknown primary tumors developed NPC after initial treatment. We also found a patient

presenting a primary tumor outside the nasopharynx (oropharynx) after initial treatment.

Collectively, the results indicated that the diagnosis of NPC T0 may be questionable and with close follow-up, the prophylactic irradiation of the nasopharynx is probably not a must for EBV-positive CLN metastatic disease of unknown primary.

Owing to the rarity of EBER+ CLN metastases of unknown origin, the sample size was not large enough to conclude a more generalized result, and follow-up evaluation should be continued to acquire long-term data. Nevertheless, we illustrated that a large fraction of EBV-positive metastatic cervical lymphadenopathies originate from outside of the nasopharynx and that we should exercise caution when classifying EBV-positive unknown primary tumors with CLN involvement as NPC T0.

Conclusions

EBV-positive CLNs are a heterogeneous disease, whose origins may not only be in the nasopharynx but also elsewhere in the head and neck or non-head and neck regions. Given that in the absence of prophylactic irradiation to the nasopharynx few patients with EBV-positive unknown primary CLN metastases could successively develop NPC after initial treatment, NPC T0 classification should be cautiously assigned to EBV-positive unknown primary tumors with CLN involvement.

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Conflict of interest statement

None declared.

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