Sinonasal or nasopharyngeal undifferentiated Carcinoma?: diagnostic pitfall and the role of Epstein-Barrvirus (EBV) and human papillomavirus (HPV) examination

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ABSTRACT

Undifferentiated carcinoma of the head and neck is frequently observed in nasopharynx, however it may also occur in oropharynx, salivary gland and sinonasal. Overlapping lesions in those regions create difficulty in determining the origin of the tumor. Thus, it causes diagnostic pitfall not only for pathologists, but also for clinicians. A 40 year old man, presented with nasal obstruction, epistaxis, diplopia, and headache for a year and showed nasal cavity sinistra and nasopharynx masses on CT-scan. Lymph node enlargement was not detected. First biopsy was performed and histopathologically diagnosed as nasopharyngeal undifferentiated carcinoma (NPC), extended into nasal cavity. Chemo-radiation protocol for NPC was conducted, and showing incomplete response. Second biopsy was done, and reviewed with the first biopsy result. The tumour was arranged in solid, syncytial and trabecular pattern, with vesicular nuclei, prominent nucleoli, and lack of lymphoplasmacytic infiltrat. Immunohistochemistry (IHC) analysis of p16, EBNA1 and LMP1 were negative. PCR analysis of HPV-18 was positive, while EBV detection showed negative result. General association of EBV with NPC suggests that the presence of latent EBV infection can serve as a positive marker for NPC. Therefore, in this case, the EBV negativity and strong HPV association led to diagnosis of SNUC. The distinction of sinonasal undifferentiated carcinoma (SNUC) or from NPC was important for appropriate management and therapy.
INTRODUCTION

Undifferentiated carcinoma of the head and neck mostly occurred in the nasopharynx.\(^1\) However, tumors with identical morphological features can also occur in oropharynx, salivary gland and sinonasal.\(^2\)\(^-\)\(^4\) Undifferentiated carcinoma in the sinonasal tract may occur as primary lesion (sinonasal undifferentiated carcinoma / SNUC) or extension from a destructive undifferentiated nasopharyngeal carcinoma (NPC).\(^5\)\(^,\)\(^6\) Palomba A & Cardesa A (2011 Overlapping lesions in these region and similarity in morphology produced difficulty in determining definitive diagnosis of the tumor, and often bring diagnostic pitfalls not only for pathologists, but also for clinicians. The difference of SNUC and NPC has traditionally rested on histopathology finding, however the association of EBV with NPC suggests that the presence of latent EBV infection serve as a marker to discern these tumors.\(^5\)\(^,\)\(^6\)\(^,\)\(^10\)

SNUC usually presents as large necrotic exophytic masses involving multiple sites in sinonasal tract and may extend into the nasopharynx. The tumor is invasive, with common orbital and cranial infiltration.\(^7\) This complex anatomic region may represent the site of aggressive, non-squamous cell epithelial and nonepithelial malignant neoplasms of varying histogenesis, which are grouped under the term undifferentiated malignant neoplasms. Frequently, these undifferentiated malignancies share clinical and light microscopic features, which makes differentiation of one from the other virtually impossible without the use of adjunct analyses (eg, immunohistochemistry, electron microscopy, or molecular biologic studies).

SNUC harbors high-risk association with HPV DNA (types 16 and 18) virus infection.\(^8\) Its immunohistochemical staining profile has been incompletely characterized, and little work has been done on its expression of the markers for human papillomavirus (HPV). In contrast to NPC, an association with Epstein-Barr virus infection has not been clearly demonstrated.\(^6\)\(^,\)\(^9\) SNUC have been incompletely characterized immunohistochemically, and their undifferentiated appearance often requires such ancillary studies to aid in their distinction from other high-grade neoplasms. To address these two issues, 25 cases of SNUC diagnosed between the years 1983 and 1999 were selected from our files. EBER in situ hybridization (ISH) Morphologically, SNUC are composed of nests cells with a delicate fibrous background, distinct cellular borders, coarse cromatin, prominent nucleoli, brisk mitotic, extensive necrosis, and scant lymphoplasmacytic infiltrate.\(^5\)\(^,\)\(^6\)\(^,\)\(^10\) SNUC have been incompletely characterized immunohistochemically, and their undifferentiated appearance often requires such ancillary studies to aid in their distinction from other high-grade neoplasms. To address these two issues, 25 cases of SNUC diagnosed between the years 1983 and 1999 were selected from our files. EBER in situ hybridization (ISH) There is no optimal management guidelines proved to be successful in treating this tumor.\(^11\) Combined radical resection, radiotherapy and chemotherapy have evolved to the current recommendation treatment regimens.\(^12\)

NPC presents with one or more of 4 groups of symptoms; including nasal symptoms (epistaxis), nasal obstruction and discharge; otologic symptoms (deafness and tinnitus); cranial nerve palsies (commonly 5\(^{th}\) and 6\(^{th}\) cranial nerves); and neck masses (usually appear in the upper neck).\(^13\) EBV plays a role in the development of NPC.\(^6\)\(^,\)\(^14\)\(^,\)\(^15\) Histopathologically, NPC are composed of syncytial cell aggregates with indistinct cellular borders, vesicular cromatin, inconspicuous nucleoli, variable mitotic, infrequent necrosis, and
usually with a lot of lymphoplasmacytic infiltrat. NPC has a better prognosis and more responsive to radiation therapy than SNUC. The distinction of undifferentiated carcinoma from the nasal cavity (SNUC) or the nasopharynx (NPC) is important for appropriate management and therapy.

**CASE**

A 40 year old man, present with nasal obstruction, epistaxis, diplopia, and headache for a year before admitted to Dr. Sardjito General Hospital, Yogyakarta, Indonesia. CT Scan showed right nasopharynx mass, with infiltration to surrounding tissue and infiltrated to intracranial. Biopsy from ear and nasal cavity was performed in other hospital, and histopathologically diagnosed as undifferentiated carcinoma, with possible extension from nasopharynx. Chemo-radiation protocol for NPC was conducted. The patient received cisplatin 100 mg/m² for the first day, and 5-fluorouracil 1000 mg/m² for the first until the fourth day, in 28 day cycle, for 4 cycle, continued with secondline chemotherapy regimen for 4 cycle. The patient also received 31 cycle radiotherapy. Six months following completion of treatment, the tumor showed uncomplete response, and repeat imaging was performed as shown in FIGURE 1.

![FIGURE 1. Head and neck MSCT scan after complete chemo-radiotherapy. The analysis result showed inhomogen mass in the left nasal cavity, left nasopharynx, left maxillary sinus and infiltrated into left fossa temporalis.](image)

The second biopsy was performed in Dr. Sardjito General Hospital, Yogyakarta, Indonesia, and reviewed with the first biopsy result. Macroscopic features showed fragmented tissue from nasal cavity sinistra, 0.8 cc, tan to white and rubbery in consistency, mean while sample from the nasopharynx was 0.5 cc, tan to white in colour and also rubbery. Microscopically, nasal cavity specimens showed tumour nests arranged insolid, and trabecular pattern, with vesicular nuclei and prominent nucleoli. The background showed desmoplastic stroma with scant lymphoplasmacytic infiltrat, without prominent necrosis (FIGURE 2). There was no tumor in the nasopharynx specimen. IHC analysis was performed, using p16, EBNA1 and LMP1 (Abcam and Novocastra) antibodies. The staining revealed negative result as shown in FIGURE 3.
FIGURE 2. Microscopic features of sinonasal specimen. Tumor composed of solid, syncytial and tubercular nests. The cells were pleomorphic, with indistinct borders, vesicular nuclei, and prominent nuclei. The background was desmoplastic stroma with scanty lymphoplasmatic infiltrate (HE, 40, 200x, 400x).

FIGURE 3. Immunohistochemistry result. Staining for p16, EBNA1, LMP1 showed negative result (100x)

PCR analysis of HPV and EBV were then performed. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) tissue specimens. DNA amplification for HPV was performed with AB Analitica Ampliquality HPV-TYPE Express v3.0, and continued with genotyping with reverse line blot. The test revealed HPV-18 positive as shown in FIGURE 4.
DISCUSSION

SNUC is a rare malignancy and firstly described by Frierson et al.\textsuperscript{16} It was originally defined as a high-grade malignant epithelial neoplasm of the nasal cavity and paranasal sinuses of uncertain histogenesis with or without neuroendocrine differentiation, but without evidence of squamous or glandular differentiation. Furthermore, World Health Organization classification redefined SNUC as a highly aggressive and clinicopathologically distinctive carcinoma of uncertain histogenesis that typically presents with locally extensive disease. Although the exact histogenesis are unknown, it has been believed that SNUC was originate from schneiderian epithelium or from the nasal ectoderm of the paranasal sinuses.\textsuperscript{7,16}

The etiology of SNUC is unknown. Some cases have occurred after prior radiation therapy for nasopharyngeal carcinoma.\textsuperscript{17} Association of EBV with SNUC have shown controversial result.\textsuperscript{6} SNUC have been incompletely characterized immunohistochemically, and their undifferentiated appearance often requires such ancillary studies to aid in their distinction from other high-grade neoplasms. To address these two issues, 25 cases of SNUC diagnosed between the years 1983 and 1999 were selected from our files. EBER in situ hybridization (ISH) However, a significant subset of SNUC (7 of 11) was associated with EBV, whereas all Western cases were negative for EBV. The Asian SNUC that showed EBV positivity were limited to those showing similar features to anaplastic large cell lymphoma, or mimicking undifferentiated nasopharyngeal carcinoma.\textsuperscript{6,18} The latter were then referred to primary sinonasal nasopharyngeal-type undifferentiated carcinoma.
Sinonasal carcinoma, including SNUC, harbor high-risk HPV DNA (types 16 and 18) virus infection. Several studies have confirmed its causal role in a subgroup of upper aerodigestive tract tumours. Of the non-genital cancers, tonsillar carcinomas (TCs) Recent study reported that HPV-positive cancers of the head and neck, including the tonsil, oropharynx, and oral cavity had a better overall survival than HPV-negative cancers. Although the exact mechanism is not fully understood, three possible explanations could be a) the genome of HPV positive cancer cells is less unstable and/or, b) HPV-positive cells suffer from hypoxia and can be more easily induced to apoptosis or, c) treatment improves the local immunity favouring the eradication of HPV (and regression of the tumour).

HPV status is also associated with p16 expression, and HPV-positive tumours are less likely to harbour p53 mutations. Rb inactivation by the viral oncoprotein E7 induces overexpression of p16, therefore immunohistochemical detection of p16 serves as a surrogate marker for high risk HPV in oropharyngeal cancers. The HPV viral E6 protein can cause a p53 gene mutation that inactivates p53 protein and interferes with p53 function by targeting it for ubiquitination and degradation. However, in this case, IHC staining for p16 was failed to demonstrated the correlation of HPV with the tumor. Therefore PCR analysis was performed and proved the involvement of HPV infection.

The HPV testing methods are mostly based on detecting HPV DNA in cancer tissues either with in situ hybridization (ISH) or PCR or both. With regard to the detection method, PCR-based studies reported a higher prevalence rate than ISH-based rates, especially in the oral cancer subgroup.

CONCLUSION

The universal association of EBV with NPC suggests that the presence of latent EBV infection serve as a marker to distinguish NPC from SNUC. SNUC are typically negative for EBV and positive for HPV. The distinction of SNUC or NPC is important for appropriate management and therapy.

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REFERENCES


