

CASE REPORT

Nasopharyngeal undifferentiated carcinoma with sarcomatoid features: Pitfalls in the immunohistochemistry

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Abstract

We present a case of an undifferentiated subtype of non-keratinizing squamous cell carcinoma (NK-SCC) with sarcomatoid features in the nasopharynx in a 69-year-old man who was difficult to diagnose due to spindle-shaped malignant cells. He was admitted because of a right nasal obstruction and right headache, and imaging revealed a heterogeneously enhanced irregularly shaped mass at the nasopharynx. Histopathologically, the tumour was partially organised, and the tumour cells were epithelioid or spindle-shaped. Initially, we erroneously diagnosed the tumour as an angiosarcoma owing to its false-negative immunoreaction for cytokeratins and a mistaken interpretation for CD31. After in situ hybridization for Epstein-Barr virus was positive, a consultation and additional immunostaining (including re-staining for cytokeratin with varying dilutions) were performed, and the diagnosis was revised to NK-SCC with sarcomatoid features. We believe that sarcomatoid features may be observed in nasopharyngeal carcinoma and in this case, immunostaining using various epithelial markers is necessary and careful attention should be paid to the interpretation of immunostaining.

Keywords: Nasopharyngeal carcinoma, sarcomatoid, cytokeratins, Epstein-Barr virus, immunostaining

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a carcinoma arising in the nasopharyngeal mucosa that shows light microscopic or ultrastructural evidence of squamous differentiation. According to the World Health Organization (WHO) pathologic classification, NPCs are largely divided into non-keratinizing (NK), keratinizing, and basaloid squamous cell carcinoma (SCC). NK-NPC is further subclassified into undifferentiated and differentiated subtypes, but this subclassification is morphologically somewhat subjective and has no clinical or prognostic value.¹ In the practical diagnosis, pathologists have observed that malignant cells of undifferentiated subtype of NK-NPC occasionally can be observed as fusiform in fascicular arrangements focally or extensively. Recently, Wang *et al.* proposed a new prognostic histopathologic classification of NPC into epithelial carcinoma (EC), sarcomatoid carcinoma (SC), mixed sarcomatoid-epithelial carcinoma (MSEC), and SCC and they suggested that their classification offered more information for predicting NPC prognosis than the WHO

classification.²

Here, we present a 69-year-old man with an undifferentiated subtype of NK-NPC with sarcomatoid features who experienced difficulty in his practical diagnosis owing to spindle-shaped malignant tumour cells and inevitable errors in immunostaining implementation and interpretation. Furthermore, we believe that the current WHO classification of NPC needs to be reconsidered to avoid diagnostic errors due to spindle-shaped malignant cells that may be common in NPC.

CASE REPORT

A 69-year-old man was admitted to the otolaryngology department from the emergency room because of otalgia and ear fullness of right ear and right headache. The patient was undergoing treatment for middle ear infection in his right ear after he had cold 16 months ago. He underwent radical total gastrectomy for gastric cancer 9 years ago and histologically poorly differentiated adenocarcinoma (pT2apN0). Computed tomography (CT) and magnetic

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resonance imaging (MRI) of the head and neck revealed a heterogeneously enhanced irregularly shaped mass at the nasopharynx, which obliterated both the fossa of Rossemuller and extended to the right nasal cavity and right ethmoid sinus (Fig. 1A, B). Several enlarged lymph nodes with internal low-density necrosis were also observed in the right retropharyngeal and level IIB areas (Fig. 1C, D). Because of the suspicion of NPC and lymph node metastasis, we had frozen sections for rapid diagnosis and treatment.

Based on the frozen sections, poorly differentiated malignant tumour was suspected, and in the frozen permanent sections, the tumour was partially organised and the tumour cells were epithelioid (Fig. 2A) or spindle-shaped (Fig. 2B), and highly pleomorphic, and showed frequent mitoses (Fig. 2C). It was necessary to differentiate primary malignant tumours, such as undifferentiated NK-NPC, sarcoma, malignant melanoma, and other malignant tumours, and the possibility of metastatic gastric cancer was considered to be low, but it

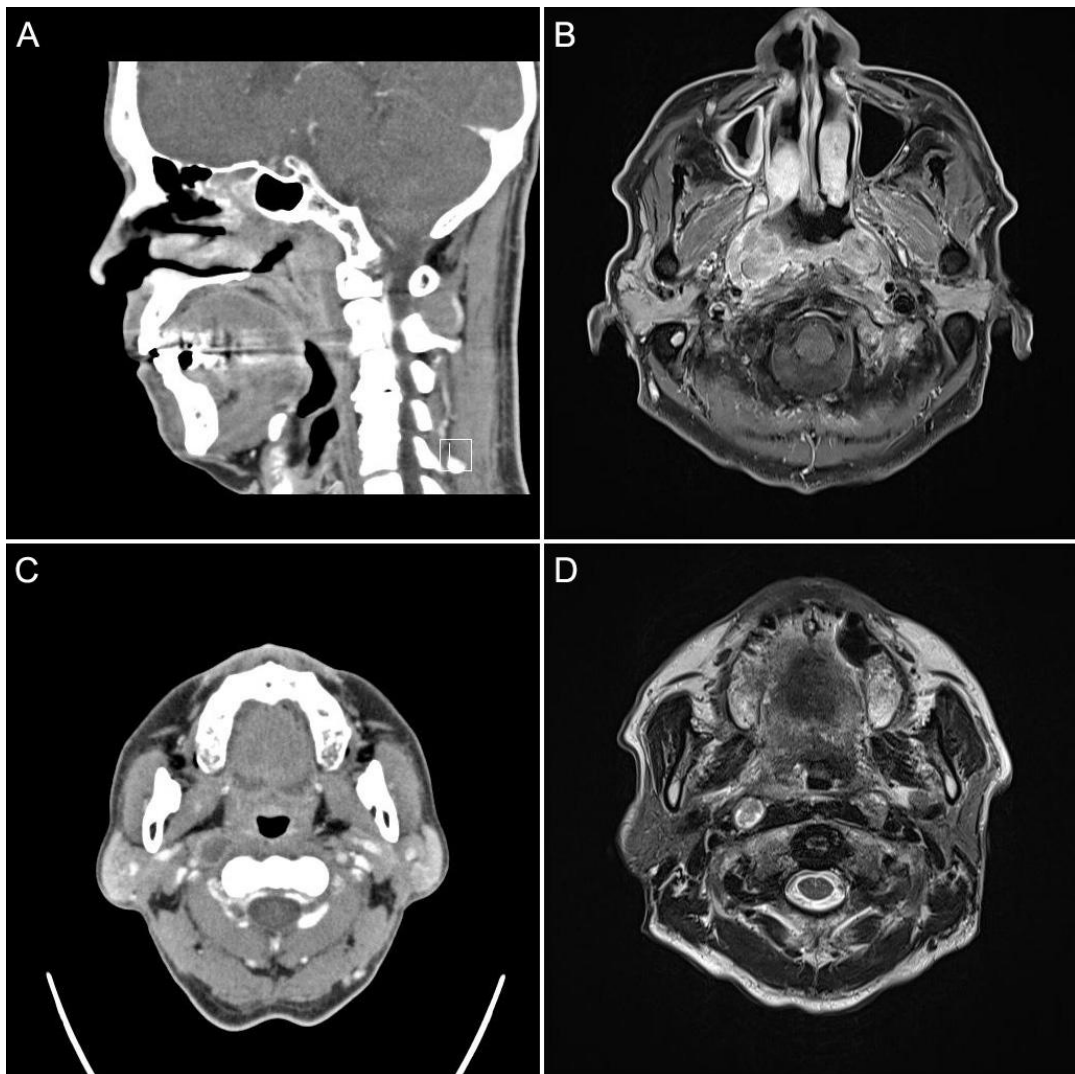


FIG. 1: Computed tomography (CT) (A) and magnetic resonance imaging (MRI) (B) of the head and neck showed that the heterogeneously enhancing irregular shape mass was observed at the nasopharynx which obliterated both fossa of Rossemuller and extended to right nasal cavity and right ethmoid sinus. (C) and (D) Several enlarged lymph nodes with internal low-density necrosis in right retropharyngeal area are also identified (A, CT sagittal image; B, MRI axial T1-weighted image; C, CT axial image; D, MRI axial T2-weighted image).

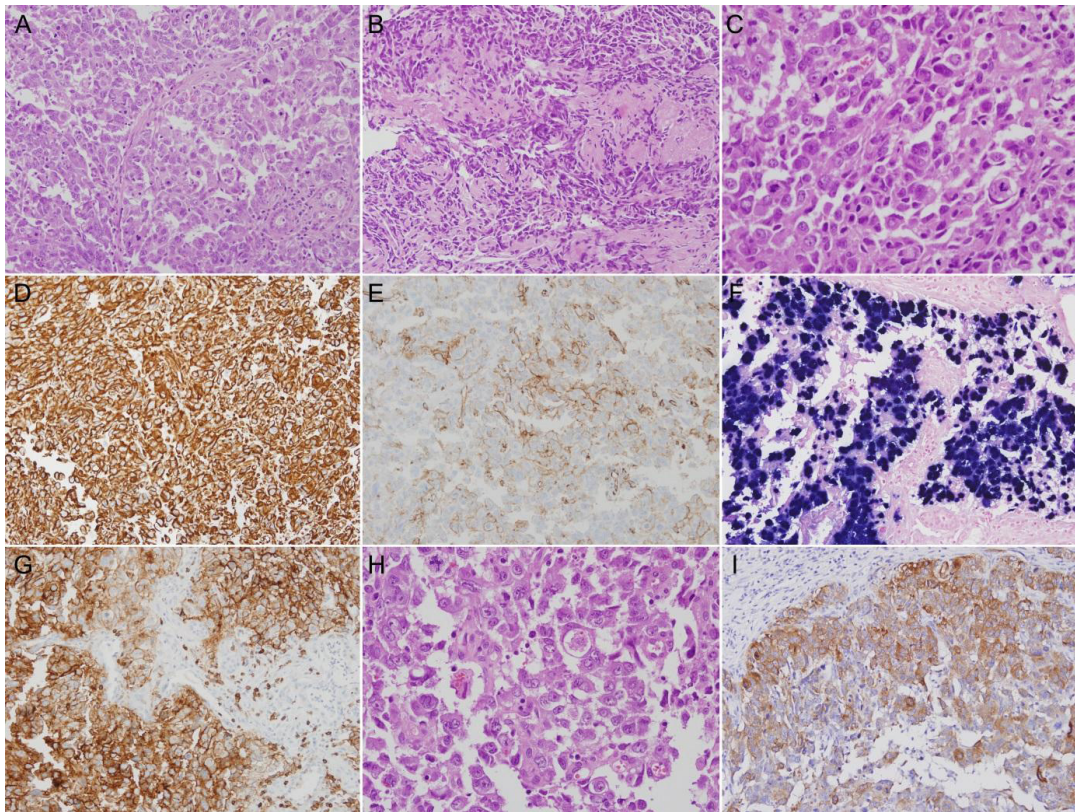


FIG. 2: The tumour cells were epithelioid (A) or spindle (B) and highly pleomorphic and showed frequent mitoses (C). Tumour cells were diffuse strong positive for vimentin (D) and focally weak for CD31 (E). (F) There was positive reaction in in situ hybridization for Epstein-Barr virus. (G) The tumour cells were strongly positive for CD5. (H) The cannibalism in some tumour cells was noted. (I) Immunostaining for pan-CK was performed on the organs that were commissioned by external consultants and the tumour cells were partially positive.

was thought that it was necessary to exclude the possibility. To distinguish among those tumours, immunohistochemical staining of the following antibodies was performed (Table 1); pan-cytokeratin (pan-CK, 1:200, Novocastra, Newcastle, UK), CK7 (1:50, Dako, CA, USA), CK20 (pre-dilution, Novocastra), vimentin (1:50, Novocastra), S100 (1:200, Novocastra), α -smooth muscle actin (α -SMA, 1:100, Novocastra), desmin (1:50, Dako), myoglobin (1:10, Novocastra), CD31 (1:50, Abcam, Cambridge, UK), CD34 (pre-dilution, Novocastra), D2-40 (pre-dilution, Dako), HMB-45 (1:40, Dako), Melan-A (1:50, Dako), chromogranin (1:50, Novocastra), and synaptophysin (1:100, Novocastra). As a result, there was diffuse strong positivity for vimentin (Fig. 2D), focally weak positivity for CD31 (Fig. 2E), and negativity for all other antibodies. Based on the results of the immunohistochemical staining, we suspected that metastatic gastric cancer, primary NK-NPC, and melanoma could

be excluded and reported the possibility of angiosarcoma.

In situ hybridization (ISH) analysis for Epstein-Barr virus (EBV) was performed externally with frozen tissue sections, and the result was positive after the patient was diagnosed with angiosarcoma (Fig. 2F). Because angiosarcoma was not known to be positive for EBV, additional tests for other EBV-positive tumours were warranted. To more precisely exclude the possibility of NK-NPC, immunostaining for CK of other molecular weights (high molecular weight-CK (HM-CK (1:150, Dako), CK5/6 (1:50, Dako), CK19 (1:100, Dako)) and p63 (pre-dilution, Dako) was performed. We also performed immunostaining with the following antibodies to exclude lymphoma and Kaposi sarcoma: LCA (1:20, Novocastra), CD3 (1:100, Novocastra), CD5 (1:40, Novocastra), CD20 (1:20, Dako), CD30 (1:20, Dako), ALK (pre-dilution, Dako), and

TABLE 1: Results of immunohistochemistry

Results of immunohistochemistry	Primary antibodies
Diffuse strong positive	CD5, Vimentin
Partially positive	Pan-CK (1:100), CD31
Negative	Pan-CK (1:200), CK5/6, CK7, CK19, CK20, HM-CK, p63 (-SMA, CD34, D2-40, Desmin, HMB-45, Melan-A, Myoglobin, S100 Chromogranin, Synaptophysin ALK, CD3, CD20, CD30, CD56, Granzyme B, LCA, TIA-1 HHV-8

HHV-8 (external referral). As a result, except CD5, all of the antibodies showed negative responses, and the tumour cells were strongly positive for CD5 (Fig. 2G). Moreover, all of the other NK/T cell markers (CD56 (1:50, Dako), TIA-1 (1:40, Beckman Coulter, France), and Granzyme B (1:25, Dako)) that were tested for NK/T cell lymphoma were negative.

Despite the many immunohistochemical staining tests that were performed, we decided that it was difficult to make a pathological diagnosis, and we sought outside consultation with a specialised pathologist. We were informed that the possibility of NK-NPC was more likely to be based on the positive reaction in EBV ISH, organised patterns in some areas, and cannibalism in some tumour cells (Fig. 2H). Furthermore, some carcinomas may be positive for CD5 antibodies. Immunohistochemical staining for pan-CK was performed for organs that were commissioned by external consultants, and the tumour cells were partially positive (Fig. 2I). In our department, the dilution ratio of pan-CK was doubled, and the specimens were again immunostained. The tumour cells were partially positive, which was in contrast to previous staining results, and the patient was eventually diagnosed with undifferentiated NK-NPC with sarcomatoid features. The clinical stage was T2N2M0, and the patient received three cycles of chemotherapy (cisplatin 110 mg) and 45 days of computer-controlled radiation therapy (volumetric-modulated arc therapy, 1 arc, total radiation dose of 6,996 cGy; 212 cGy/fraction). The sizes of the tumour and enlarged neck lymph node were observed to be significantly reduced in MRI images that were taken 1 month after the end of the treatment (from 34.9 mm to 6.7 mm, -80.8% partial response), but on a subsequent positron emission tomography-CT, multiple bone metastases were newly found in the left

humeral head, axial skeleton, and both femurs, and symptomatic nerve blocks were performed several times owing to severe bone pain. The patient died 5 months after his visit.

This study was approved by the Institutional Review Board of Eulji University Hospital (EMC 2018-05-008), and informed consent was waived.

DISCUSSION

Pathologists generally make a diagnosis within the existing diagnostic categories, and they experience embarrassment or difficulties when the observing findings fall outside the scope of diagnosis or cannot be included in a diagnosis. To easily solve this problem, immunostaining is performed, but sometimes immunostaining can result in confusion. When we misinterpret the findings of hematoxylin-eosin (HE) staining or emphasise only specific findings, immunohistochemical staining can be additionally performed on the side that matched the diagnosis and it can also result in a serious diagnostic error that reads in a direction that is appropriate for it (e.g., positive for false positives). Some sarcomas that develop in the head and neck may also express CK with markers that are associated with each sarcoma. Synovial sarcoma, especially the biphasic type, shows patchy to focal reactivity with epithelial markers such as epithelial membrane antigen, CK, and BerEP4.³ CK expression is observed in approximately 30% of epithelioid hemangioendothelioma, which may cause these cancers to be misdiagnosed as carcinomas or myoepithelial tumours.^{4,5} Furthermore, several cases have reported that other sarcomas, such as biphenotypic sinonasal sarcomas, rhabdomyosarcomas, and malignant peripheral nerve sheath tumours, may express epithelial markers, including CK.^{6,7} On the other hand, the diagnosis of laryngeal and hypopharyngeal

spindle cell SCC (SCSCC) requires the identification of epithelial differentiation, either by routine morphology or immunohistochemical analysis for CK. However, a significant subset of SCSCC may be negative for epithelial markers⁸ and may exhibit a true or false-positive response to immunostaining, which is suggestive of various sarcomatous phenotypes.⁹ In this case, we diagnosed the patient with angiosarcoma because of immunohistochemical staining (positive for vimentin, weakly positive for CD31, and negative for CK, CK7, and CK20), regardless of the HE findings.

We made a mistake and realised that the diagnosis should be reconsidered after identifying positive EBV ISH results. The CD5 antibody showed a strong positive response, which made another mistake of the extensive immunostaining for lymphocytic tumours, because the literature review shows that CD5 can be observed in epithelial cancers, especially carcinoma showing thymus-like elements.¹⁰ We have learned from this case that serious errors can arise when the immunostaining that is performed to differentiate a poorly differentiated malignant tumours into carcinomas, sarcomas, and lymphomas is not properly assessed (e.g. false positives or cross-reactions). In addition, if it is difficult to determine the diagnostic immunostaining patterns, we think that the HE staining slides should be reevaluated, recognising the limitations of immunohistochemistry, and compared with the expression of other reference antibodies or other test results. Considering the possibility that the immunostaining markers that are used to find the tumour origin may not appear as expected, we emphasise again that it is necessary to consider the possibility of false negatives or false positives, depending on the antibody concentration or antigen retrieval.

Pathologists often see cases in which the tumour cells of NPC show varying morphologic features, with cells that are small and round, large and round, spindle-shaped, with or without vesicular nuclei, or mixed round and spindle-shaped, and fail to correctly categorise in any of the current WHO categories. In most other organs, the term 'sarcomatoid carcinoma' or the corresponding histologic diagnosis is used but this is not the case in NPC. In this classification, if sarcomatoid features, such as those that were observed in this case, are prominent, the diagnosis is made according to the existing classification and may be ambiguous. Unnecessary immunostaining may also be

performed with a diagnostic approach closer to a sarcoma than a carcinoma. In the actual diagnosis, the immunostaining that was used to differentiate carcinoma and sarcoma may result in an overlap or a false cross-reaction, resulting in trial and error. In addition, the classification itself may hinder the specific histological diagnosis at a specific site or stop subsequent investigations. The most powerful prognostic factor of NPC is the stage of the patient at presentation, and the issue of histopathological type (NK, keratinizing, and basaloid SCC) in relation to prognosis is complex. Furthermore, the subclassification of NPC into undifferentiated and differentiated subtypes is somewhat morphologically subjective and is of insufficient clinical or prognostic value.^{1,11,12} Recently, Wang *et al.* developed a new histopathologic classification based on morphologic traits and cell differentiation of tumours that were analysed among 2,716 patients with NPC (EC, SC, MSEC, and SCC).² According to their results, the difference in the 5-year overall survival (OS) rate was 8.9% between the most common subtypes (EC and MSEC), which together comprised 82.2% of all patients and on the other hand, the difference of only 0.6% OS rate was detected between the two most common subtypes of WHO classification, which together comprised 98.2% of all patients. Wang *et al.* stated that their classification offered more information for the prediction of NPC prognosis compared with the WHO classification and thus might be a valuable tool in guiding treatment decisions for patients with NPC subtypes that are associated with poor prognosis. Through our case report, we believe that sarcomatoid features may be observed in nasopharyngeal carcinoma and in this case, immunostaining using various epithelial markers is necessary and careful attention should be paid to the interpretation of immunostaining.

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