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Case Report

Integrated Diagnostic Model That Incorporates Epstein-Barr Virus DNA, Imaging, and Nasal Endoscopy to Stratify Primary Tumor and Lymph Nodes in a Patient with N1 Nasopharyngeal Carcinoma: Multidisciplinary Management

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Keywords

Epstein-Barr virus DNA · Nasopharyngeal cancer · Undifferentiated carcinomas of the nasopharyngeal type · EBV-DNA · Intensity-modulated radiation therapy

Abstract

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy, with a high metastatic potential. Epstein-Barr virus (EBV) infection plays a fundamental role, even if it is not well understood.

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The diagnosis of the disease in its early stage is infrequent. Imaging studies, positron emission tomography scans in addition to clinical examination, endoscopic examination, and biopsy provide information on the extent of the disease. The application of neoadjuvant chemotherapy followed by concomitant chemoradiation can improve the control of NPC. In March 2016, a 54-year-old male with NPC cT1 cN2 cM0, stage III (8th edition of American Joint Committee on Cancer (AJCC) staging system) underwent to a two-step treatment: induction chemotherapy by TPF regimen (docetaxel, cisplatin, 5-fluorouracil), followed by concomitant chemoradiotherapy (weekly cisplatin). The quantity of free plasma EBV-DNA can be related to the disease stage, and the detection of EBV-DNA during follow-up can be predictive of distant metastases. Especially, either plasma or serum EBV-DNA titer is estimated to reflect tumor volume. Biologically, such EBV-DNA reflects reproduced or released DNA from dead or dying tumor cells. On the other hand, EBV-specific DNA released as exosome may reflect the biological feature of the alive NPC tumor cell. The follow-up is ongoing after 21 months from a complete response.

Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy with a high potential for metastatic behavior. Epstein-Barr virus (EBV) infection plays a fundamental role in NPC development, but its oncogenic role is not well understood. EBV is a member of the human γ-herpesvirus, which infects >90% of the world's population. EBV has been implicated to be strongly associated with the development of several malignancies including Burkitt's lymphoma and NPC. Distant metastasis is still the dominant treatment failure of NPC, although the current chemotherapy and radiotherapy (RT) are effective. The highest incidence of NPC occurs in South China, with an annual incidence of 15–50 cases per 100,000 population. RT is the mainstay treatment modality for all patients with locoregional NPC. NPC differs from other head and neck cancers in its epidemiology, natural history, and response to treatment. EBV-positive NPC is highly invasive for local and distant metastasis but sensitive to both chemotherapy and RT. On the other hand, EBV-negative NPC shows a clinical behavior comparable to that of head and neck cancer of different site.

Case Report

In March 2016, A 54-year-old male presented with a 4-week history of fatigue, sore throat, fever, and swollen lymph nodes. The physical examination revealed on left laterocervical region a node 3 cm in diameter. The serum level of EBV IgG was 539 UA/mL, EBV-VCAIgG: 133 UA/mL, and EBV-VCA IgM: 10 UA/mL. We suspected EBV-related viral infection. One month later, he had an ultrasound evaluation for a progressive node enlargement and an FNA was performed for suspicious echogenicity. A diagnostic workup with positron emission tomography/computed tomography (PET/CT) scan, head and neck MRI, and nasopharyngoscopy was started to assess the presence of malignant cells. It showed a tumor lesion with an irregular border, located along the nasopharyngeal region. Biopsy of the tumor was performed, and histological findings revealed EBV-associated NPC of nonkeratinizing subtype; the immunophenotype CK AE1–3: cytoplasm positive; p40: nuclear positive; KI67: positive 90%; EBV-encoded RNA (EBER)/EBV: nuclear widespread positive. A determination EBV-DNA was done and the result was positive. The MRI, performed with and without paramagnetic contrast (Gd-

DPTA) showed a lesion with irregular border, located along the nasopharyngeal region, having a transverse diameter of about 13 mm and extending 15 mm, hyperintense signaling on T2-weighted and homogenous on T1-W. Enlarged cervical lymph node, was present, at level IIa, left side, with an axial diameter of 27 mm, and right cervical lymph node with an axial diameter of 14 mm (Fig. 1). PET/CT was performed to exclude distant metastasis confirming a locally advanced nasopharyngeal lesion. The clinical stage was cT1 cN2 cM0, stage III (according to the 8th edition of American Joint Committee on Cancer (AJCC) staging system). The patient underwent two-step treatment: induction chemotherapy by TPF regimen (docetaxel, cisplatin, 5-fluorouracil) for two cycles, followed by concomitant chemoradiotherapy (CRT; weekly cisplatin 40 mg/m^2; RT by image-guided Volumetric Modulated Arc Therapy [VMAT-IGRT]: 70 Gy/35 fractions on primary tumor and residual nodes, 63 Gy on initially PET-positive nodes, 54 Gy bilaterally on node levels from Ib to V and retropharyngeal nodes. After RT and concomitant chemotherapy, the nasopharyngoscopy showed a decreasing trend of the lymph nodes from 17 mm (left) and 11 mm (right) to 8 × 6 mm (left) and 5 mm (right), and nasopharyngeal tumor volume from 14 × 11 to 4 × 6 mm. The first-year follow-up includes the serum EBV-DNA level determination every 4 months, and head/neck MRI every 6 months. The patient has shown a complete response during 18 months of follow-up.

Discussion

NPC tumors present with varying degrees of differentiation and have been classified by the World Health Organization (WHO) into three categories. Squamous cell carcinoma, WHO-I tumors, are highly differentiated with characteristic epithelial growth patterns and keratin filaments. Nonkeratinizing WHO-II carcinomas retain their epithelial cell shape and growth patterns. Undifferentiated carcinomas, WHO-III, do not produce keratin and lack a distributive growth pattern [1]. Recently, based on an etiological viewpoint, an alternative, simpler classification has been proposed that divides NPC into two histological types, namely squamous cell carcinomas and undifferentiated carcinomas of the nasopharyngeal type. This classification has been correlated with EBV serology tests. Using EBER in situ hybridization, EBER signal was present in virtually all tumor cells. Premalignant lesions of the nasopharyngeal epithelium have also been shown to harbor EBV, suggesting the EBV infection occurs in the early phases of carcinogenesis. Specific EBV latent genes are consistently expressed within the NPC tumors and also in early, dysplastic lesions. Blood test for EBV-DNA has now become a screening test for the high-risk patients, aiming to diagnose the disease in its early stages. Malignant cells have a high turnover rate, and on cell lysis there is an increased EBV-DNA. These circulating free EBV-DNA are released into the blood and can be detected by polymerase chain reaction. The increased number of copies of EBV-DNA found in the blood during the initial phase of RT can suggest that the viral DNA is released into the circulation by dying cells [2]. The quantity of free plasma EBV-DNA can be related to the disease stage and the detection of EBV-DNA during follow-up can be predictive of distant metastases [3–5]. MRI provides better resolution than CT in terms of assessing parapharyngeal spaces, marrow infiltration of the skull base, intracranial disease, and deep cervical nodes. ^{18}F-fluorodeoxyglucose (18F-FDG)-PET is sensitive and accurate in the detection of nodal metastasis but lacks the soft tissue resolution of MRI for the assessment of the primary tumor. In terms of distant metastasis staging, several studies have concluded that 18F-FDG-PET is substantially more sensitive and accurate than the conventional workup consisting of chest radiography, abdominal ultrasound, and skeletal

scintigraphy [6]. One of the unique clinical features of NPC is the propensity for distant metastasis. The tumor, nodes, and metastases (TNM) staging is the most important prognostic factor for NPCs. Among the changes in the eight edition of the TNM staging system (2016), the medial pterygoid muscle and/or lateral pterygoid muscle involvement was changed from T4 to T2, prevertebral muscle involvement was added as T2, the supraclavicular fossa was replaced with the lower neck, and this was merged with a maximum nodal diameter >6 cm as N3, and T4, and N3 were merged as stage IVA criteria. These changes not only will lead to a better evaluation of risk but also will optimize the balance in clinical practicability and global applicability. A study showed that the tumor's WHO histological type and the RT dosage and coverage were also significant independent prognostic factors [7]. The histological type, WHO-I, which is frequently seen among the Caucasian population, was found to be associated with adverse prognosis. Indeed, most other known prognostic factors are directly or indirectly related to the extent or bulk of the tumor. A large variation of tumor volume is present in T stages, and primary tumor volume represents an independent prognostic factor of local control. Its validity has been confirmed in patients with T3 and T4 tumors, and there is an estimated 1% increase in the risk of local failure for every 1 cm^3 increase in primary tumor volume. Although the incorporation of tumor volume into TNM classification is attractive, there are still important issues to be addressed. Measurement of tumor volume can be affected by imaging modalities, measuring protocols, interobserver and intraobserver variability. The amount of circulating EBV-DNA in an NPC patient is estimated to reflect the tumor load and is positively correlated with disease stage. Further, it has been shown to have prognostic importance. Especially, either plasma or serum EBV-DNA titer is estimated to reflect tumor volume. Biologically, such EBV-DNA reflects reproduced or released DNA from dead or dying tumor cells. On the other hand, EBV-specific DNA released as exosome may reflect the biological feature of the alive NPC tumor cell [8]. Most of the NPC cases have wild-type p53, which is highly radiosensitive. Thus, RT plays a central role in the treatment of all stages of NPC patients without distant metastases. A good locoregional control should be the prime objective of treatment, as locoregional relapses represent a significant risk factor for the development of distant metastases. Conventional 2-dimensional (2D) RT successfully controlled between 75 and 90% of patients with T1 and T2 tumors and 50–75% of T3 and T4 tumors. Nodal control is achieved in 90% for N0 and N1 cases, but the control rate drops to 70% for N2 and N3 cases. Because interruptions and prolonged treatment adversely affect outcome in RT for NPC, every effort should be made to maintain the treatment schedule. The major limitations of conventional 2D RT for NPC can now be overcome with three-dimensional (3D) conformal RT and intensity-modulated radiation therapy (IMRT) [9]. IMRT is an advanced form of 3D conformal RT, in which the tumor is irradiated with a high dose, while normal tissues are irradiated with a low dose. Such ability of IMRT to deliver a more conformal radiation dose to the target area and spare surrounding structures seems to decrease the toxicity of CRT. Some researchers reported excellent local control as more than 90% of NPC patients achieved local control with IMRT, even in cases of advanced T3–4 diseases. It has also been shown that preservation of salivary function and quality of life improves in IMRT survivors. A recent multicenter study also showed that in a multi-institutional setting, it was possible to achieve 90% local control rate excellence with IMRT as reported in single institutions [10, 11]. Thus, IMRT has gradually been considered as the new standard RT for NPC. Radiosensitivity correlates well with chemosensitivity; thus, NPC is also chemosensitive (Fig. 2, 3). Many clinical studies investigated the advantages of chemotherapy for NPC. Compared with RT alone, CRT significantly improved progression-free survival and overall survival. The intergroup then conducted other randomized trials, using a similar design, in endemic regions in Asia, to validate the intergroup results.

Three randomized trials were subsequently reported from Hong Kong, Singapore, and China, respectively. The advantage of neoadjuvant chemotherapy (NAC) has not been established in combination with RT alone. Furthermore, the role of NAC in combination with concurrent CRT (CCRT) is yet to be confirmed. In a phase 3, multicenter, randomized controlled trial, the addition of NAC with docetaxel, cisplatin, and 5-fluorouracil (TPF) to CCRT significantly improved failure-free survival in advanced NPC. TPF may play an important role in improving the treatment results of NPC. Moreover, for patients with intracranial invasion, re-planning the delineation for tumor volume after NAC improved the local disease control and reduced IMRT-associated adverse events. This meta-analysis compared the overall survival, locoregional failure, and distant metastasis-free survival between NAC + CCRT and CCRT. The addition of AC to CRT achieved the highest survival benefit and consistent improvement for all end points. The addition of NAC to CRT achieved the highest effect on distant control. A sufficient amount of an anticancer agent is required to control the distant metastasis. However, at present, it is unclear whether the addition of NAC to CCRT improves survival rates compared with CCRT. Among the molecular markers, the most studied is plasma EBV-DNA, which is universally associated with the nonkeratinizing subtype of NPC. EBV-DNA is not only a good prognosticator, but it is also useful for assessing treatment response and detecting disease relapse. The levels of posttreatment plasma EBV-DNA in patients with NPC appear to strongly predict progression-free survival and overall survival. Additionally, this is accurate, and accurately reflects the posttreatment residual tumor load. The current research focuses on the effect on the selected patients that probably referred to maximal benefit from AC. High circulating plasma EBV-DNA loads of 500 copies per mL were also suggested. Similarly, the EBV-DNA tested at 6 weeks after primary treatment can predict the probability of subsequent relapse of NPC. This study led to the adoption of posttreatment plasma EBV-DNA load as a prognostic marker. Further studies are needed to investigate the utility of posttreatment plasma EBV-DNA in individualizing AC [12]. The target delineation and prescribed doses of RT and chemotherapeutic regimens were the same as those described previously. The patient is followed up every 3 months during the first 2 years, every 6 months during the next 2 years, and annually thereafter. Routine follow-up included complete head and neck examination, nasopharyngoscopy, hematology and biochemical profiles, chest radiography, and abdominal sonography. Bone scan and CT of the chest or abdomen and even PET/CT were performed every 6 months. The role of PET in the detection of distant metastasis NPC and other malignancies has been established. It has been reported that PET is more sensitive than CT and MR at detecting residual and recurrent tumors in the nasopharynx. Relative roles of EBV-DNA, endoscopy, and fluorine-18-deoxyglucose in staging and follow-up is very important. NPC is notorious for its metastatic features among head-and-neck malignancies and is closely associated with EBV infection. The metastasis at multiple sites such as neck lymph nodes and distant organs including bone, liver and intracranial invasion, is a common event. The relationship between NPC, EBV, and metastasis remains poorly defined [13].

Conclusion

The introduction of IMRT was a pioneering breakthrough that significantly improved local control of NPC. Currently, the locoregional control rate of NPC treated with IMRT is greater than 90%; distant metastasis is now the main failure pattern. Moreover, several studies have found that over 50% of patients with NPC presented with N1 disease at initial diagnosis. However, patients with N1 NPC are relatively underresearched, and the metastasis risk of this

group is not well-stratified. A recent study by Lu et al, showed that the gross tumor volume of the lymph nodes (GTVnd) was a significant factor affecting distant metastasis in NPC patients. Additionally, previous studies have demonstrated that pretreatment serum Epstein–Barr virus (EBV) DNA copy number is also a reliable predictor for metastasis of NPC [14]. However, no prognostic model for the prognostic prediction has been investigated to date in patients with NasoPharyngeal Cancer N1 [15]. Recent study demonstrates that EBV has evolved sophisticated strategies by driving epithelial cells to obtain malignant features, particularly in NPC metastasis, providing novel biomarkers for the therapy and prognosis of EBV-associated NPC. Our diagnostic model that incorporates endoscopic study, GTVnd, and EBV-DNA copy number may be useful for predicting distant metastasis in patients with undifferentiated carcinomas of the nasopharyngeal type.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors declare that they have no conflict of interest.

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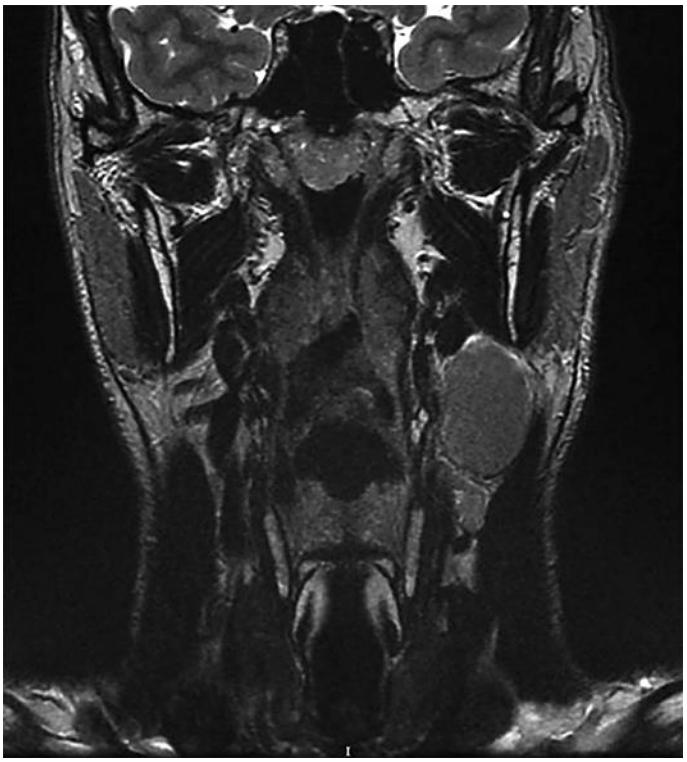

Fig. 1. T2 FSE, coronal view, MRI of the neck, with and without paramagnetic contrast (Gd-DPTA), shows a lesion with an irregular border, located along the nasopharyngeal region, having a transverse diameter of about 13 mm and extending 15 mm with hyperintense signaling on T2-weighted imaging.

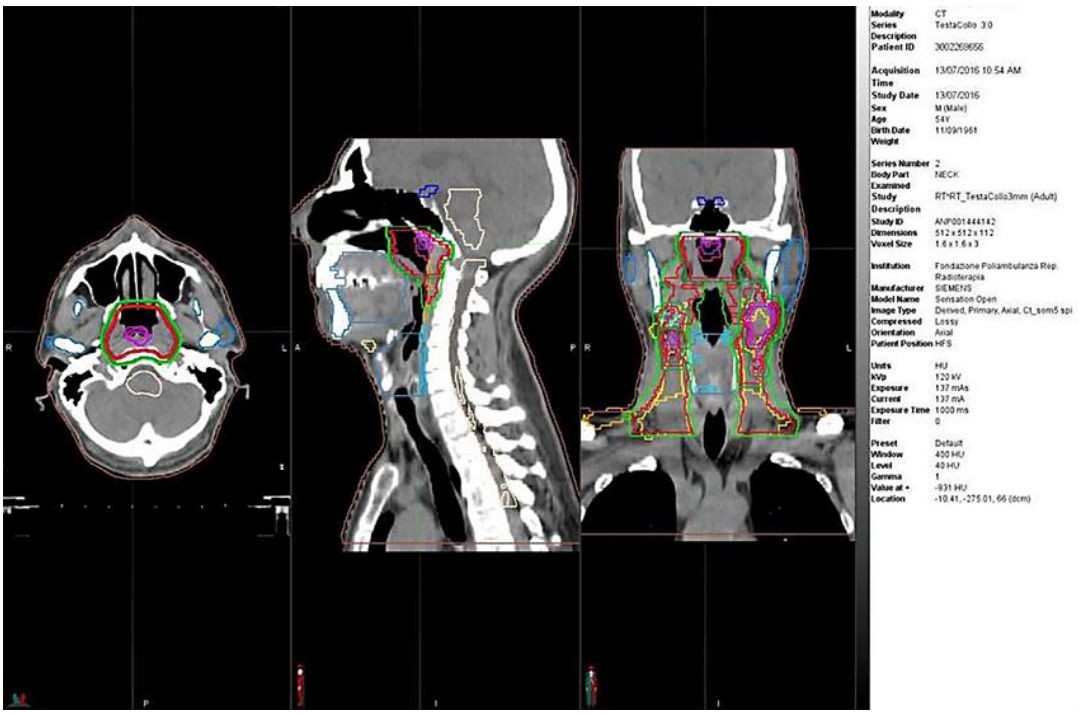

Fig. 2. Radiotherapy dose distribution.

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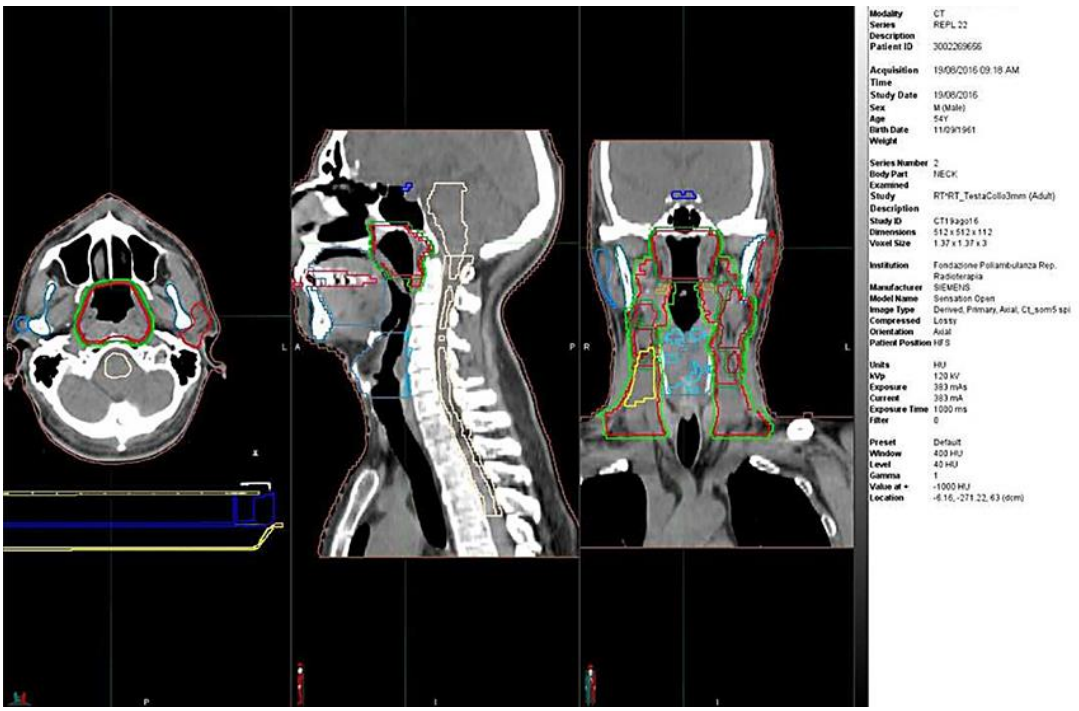

Fig. 3. After radiotherapy and concomitant chemotherapy, there was a decreasing trend of the lymph nodes from 17 mm (left) and 11 mm (right) to 8 × 6 mm (left) and 5 mm (right), and nasopharyngeal tumor volume from 14 × 11 to 4 × 6 mm.