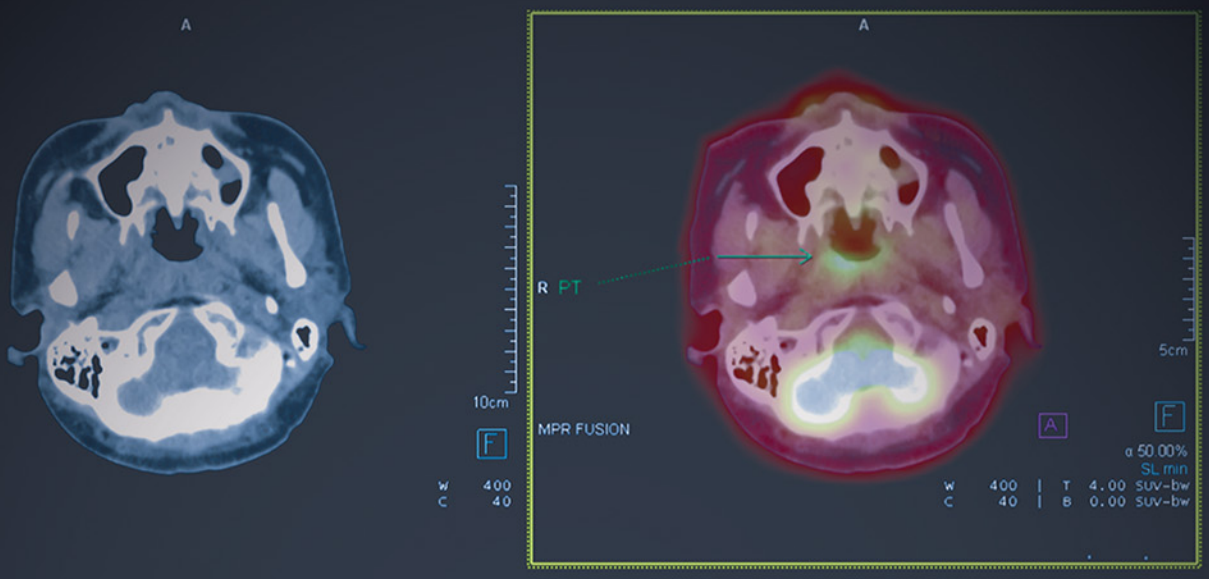

An Evidence-Based Approach to the Management of Nasopharyngeal Cancer

From Basic Science to Clinical Presentation and Treatment

Edited by
Baharudin Abdullah
Anusha Balasubramanian
Norhafiza Mat Lazim

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Dedication

Baharudin Abdullah
To my loving wife, Dr. Eka Sumianti, who has always been patient with me and my two beautiful girls, Clarissa Andini and Carneisha Aleeya. I will always love and cherish you all until the end of time.

Anusha Balasubramanian
To my beloved parents, husband, godparents, teachers, patients, colleagues and friends; thank you for guiding me through the path of life, I remain eternally grateful and indebted to you. God bless.

Norhafiza Mat Lazim
I would like to dedicate this book to my caring husband Associate Professor Dr. Zul Izhar Mohd Ismail, who has been very supportive through the journey of completing the chapters for this book. My sincere gratitude also goes to my three beloved children, Arieff Iskandar, Adry Zahrin, and Alyssa Yasmin, who have been my endless motivation for me. I am deeply grateful and blessed to have continuous support and encouragement from my other immediate family members, friends and close colleagues at work.

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He is a Senior Consultant at the Department of Otorhinolaryngology – Head & Neck Surgery at the Universiti Sains Malaysia. He graduated with an MBBS in Medicine and Surgery from the Universiti Malaya (1994) and a Master of Medicine (MMED) in Otorhinolaryngology – Head & Neck Surgery from the Universiti Sains Malaysia (2002). He was an Honorary Visiting Scholar at the Chinese University of Hong Kong, Hong Kong (2006) and an Asian Surgical Association Clinical Fellow (Head and Neck Surgery) at the University of Hong Kong, Hong Kong (2006). He was also a Fellow in Rhinology and Endoscopic Sinus Surgery at the University of Graz, Austria (2014) and Senior Fellow at the Head and Neck Optical Diagnostics and Intervention Society. He is on the editorial board of several international medical journals, and has published and presented many scientific studies and papers on allergy, rhinology, and head and neck surgery. He has contributed significantly to the successes of symposiums and workshops at the Universiti Sains Malaysia (USM), and other symposiums, congresses, and workshops both at the national and international level. He is currently the President Elect of the Malaysian Society of Allergy and Immunology (MSAI).

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Anusha Balasubramanian, an otorhinolaryngologist, graduated with a double gold medal in her postgraduate exams, and subsequently went on to complete her fellowship in Head and Neck Surgery and Oncology with Honours under the IFHNOS programme. She is currently a clinical fellow at The Royal Marsden Hospital London and simultaneously pursuing a degree in Medical Law in London. Having completed fellowship attachments at MSKCC New York and AIMS Kerala India, she realizes the gap in knowledge in the

common public as well as the medical community with regard to nasopharyngeal carcinoma (NPC), a common presentation among the Asian population. Her passion lies in exploring the latest developments in NPC, as well as bridging the gap between patients, treating physicians, and researchers. As an editor of this project, she hopes to gather knowledge from the basic sciences to advanced molecular genetics and deliver it in a clear and comprehensive manner for students, researchers, treating physicians, and the general public with an interest in the subject.

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Acknowledgment

Writing a book is indeed an arduous task but the long journey has finally come to an end. While the task is very demanding and requires a lot of hard work, it is deeply gratifying and satisfying. We are delighted to present this book to academia from various disciplines, both clinical and nonclinical, either directly or indirectly involved in the management of nasopharyngeal carcinoma.

The aim of this book is to improve head and neck cancer care, particularly nasopharyngeal carcinoma, regionally and globally. It is crucial to cover the wide range of issues surrounding nasopharyngeal carcinoma and thus we have made an effort to include the basic sciences including anatomy and pathology together with the clinical sciences of oncology care, surgical procedures, and advanced future technology. We believe these are all crucial components in our endeavor to provide the best and optimal patient care in the coming years.

We would like to express our sincere thanks to our contributing authors as well as the publisher and editorial team. This book would certainly not be possible if not for our past and present patients, and we thank them for giving us the opportunity to care for them.

Lastly, we hope our readers will enjoy this book and find it useful for their work.

Baharudin Abdullah
Anusha Balasubramanian
Norhafiza Mat Lazim

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1 Introduction

William Ignace Wei[1] *and Raymond K. Tsang*[2]
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Nasopharyngeal carcinoma (NPC) is a unique cancer in every aspect, including its epidemiology, pathogenesis, and the evolution of its treatment. It is also a cancer for which modern medicine can be proud of the progress made in combatting it. From the first description of NPC in the late 19th century, to introduction of the first curative therapy via radiation in the first half of the 20th century, to modern diagnosis and treatment in the 21st century, we have come a long way in understanding this cancer and are now able to offer curative treatment for the majority of the patients. Yet this cancer is still not fully understood. The precise cause of differences in distribution by race is still an enigma and we have not elucidated the exact role of the Epstein–Barr virus (EBV) in carcinogenesis. Much more research, both basic and clinical, is still required in order to eradicate this disease. Reviewing the history of how modern medicine learned to understand this cancer is a synopsis of how modern medicine came to understand and treat many cancers in the last one and a half centuries.

Historical aspect

As NPC is relatively rare in Europe, there is no record of the disease until the 19th century. Early reports of suspected cases of NPC might be confused with other cancers like paranasal sinus cancers, which probably involved the nasopharynx and adjacent structures in their late stage. Muir in 1960 summarized the reports of the suspected cases of NPC in the non-English literature (Muir, 1983). Bosworth was the first to write about the disease in English in his textbook *Diseases of the Nose and Throat* and devoted a chapter to describe the disease (Bosworth, 1892). Bosworth considered the disease incurable and advised for palliative treatment only. The famous endoscopist Chevalier Jackson wrote about the disease in 1901 and also agreed that radical curative surgeries did not offer a cure and only increased the suffering of the patient (Jackson, 1901).

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While the disease was relatively rare in the West, Western medical practitioners working in the Far East recognized the common occurrence of the disease and wrote about the presentation of the cancer. While practicing medicine in Guangzhou, Todd wrote about cancers in the posterior nares metastasizing to the cervical glands (Todd, 1921). K.H. Digby, Professor of Surgery in the University of Hong Kong, wrote in detail regarding the different presentations of the disease, including the various cranial neuropathies, for the benefit of the medical students and young doctors (Digby, Thomas, & Tse, 1930). A sample of his drawings can be seen in Fig. 1.1.

Despite the rarity of NPC in the West, it received attention from the media and medical community when the famous baseball player Babe Ruth suffered from the disease and received an early form of radiotherapy and chemotherapy in 1946 and 1947 (Bikhazi, Kramer, Spiegel, & Singer, 1998). Babe Ruth's disease was initially controlled with teropterin, a folic acid analog, which resulted in regression of the cancer. The cancer ultimately became nonresponsive to teropterin and Babe Ruth succumbed to the disease. Teropterin later was developed into amethopterin, now known as methotrexate. Babe Ruth's treatment was one of the earliest uses of chemotherapeutic agents in a solid organ cancer.

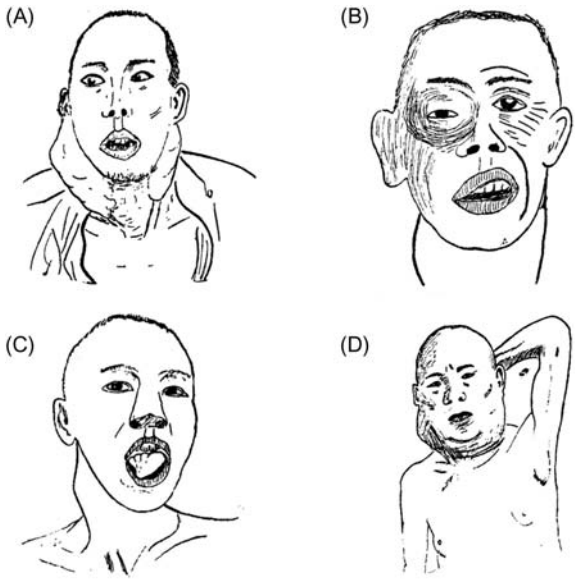

Figure 1.1 Selected drawings from Digby's monograph on presentation of nasopharyneal carcinoma (NPC). (A) Proptosis of right eye with ophthalmopeglia and bilateral massively enlarged neck lymph nodes. (B) Right proptosis with right facial nerve palsy. (C) Right hypoglossal nerve palsy with atropy and fibrillation of right tongue. (D) Enlarged neck lymph nodes and left axillary lymph node. Source: *From Digby, K., Thomas, G. H., & Tse, H. S. (1930). Notes on carcinoma of the nasopharynx. The Caduceus, 9, 45–68.*

Geographical distribution and familial clustering

NPC is a peculiar cancer that has a ten-fold difference in incidence in endemic areas versus nonendemic areas. As mentioned above, Western physicians working in South China in the early 20th century noted the remarkably high incidence of NPC among the local population in South China, especially in Guangdong province. By 1950–60, the disparity in the incidence of the disease worldwide was well recognized. Increased incidence of the disease was noted in the overseas Chinese communities both in South East Asia and the West. Hayes Martin from Memorial Sloan Kettering Hospital in New York City described his case series of 358 cases of NPC and noted a disproportionally high percentage (10%, 37 patients) of Chinese individuals suffering from the disease (Martin & Quan, 1951). The stark difference in the incidence among the local population and the immigrant Chinese population had already led to a speculation on the genetic basis of the disease. Fig. 1.2 shows the difference in incidence of the cancer among different countries in 2012.

The disease has the highest incidence in Southern China, whereas in Northern China the incidence is lower and comparable to that of the West. Even within Guangdong province, the area with highest incidence in China, there was a difference in the incidence among population groups speaking different dialects. The population that spoke Cantonese had a higher incidence compared with the population that spoke Hakka. Further, among the Cantonese speaking

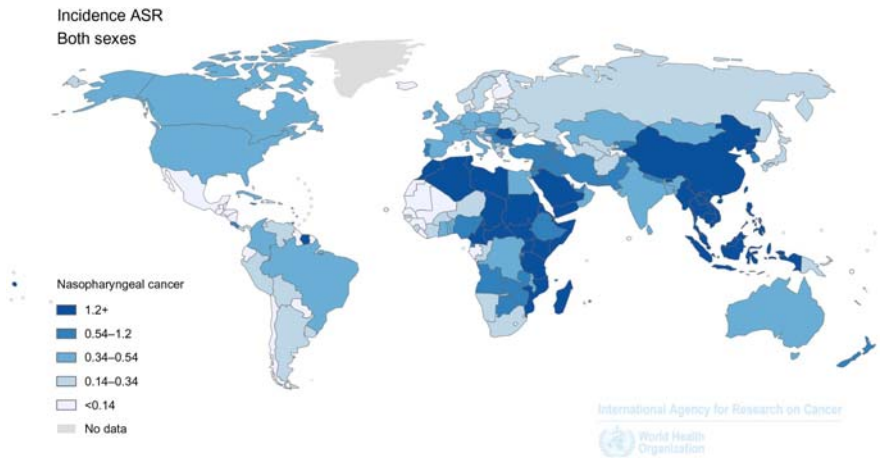

Figure 1.2 Worldwide incidence of nasopharyngeal carcinoma, age-adjusted, both sexes, in 2012. Source: *Adapted from Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, ... Bray, F. (2013). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon: International Agency for Research on Cancer. <http://gco.iarc.fr/today/home> Accessed 16.04.18.*

population, fisherman that lived in boats had a two-fold increased risk compared to the population living on land (Li, Yu, & Henderson, 1985). Here both genetic and environmental factors cause the disparity in the incidence. Compared to the land living counterparts, Cantonese fishermen consumed large amounts of preserved salted fish, which has been shown to increase the risk of developing NPC.

In areas of the West with high incidence of NPC like New York City and San Francisco, invariably there are high concentrations of immigrants from China, mostly from arriving from Guangdong province since the late- 19th century. Other areas in South East Asia like Singapore, Malaysia, Thailand, and Indonesia also have significant numbers of Chinese immigrants and relatively high incidence of the disease.

Apart from the high incidence in Southern Chinese communities, indigenous populations from Malaysia and Indonesia also have moderate incidence of NPC, though not as high as Southern Chinese. Another population with moderate incidence are people of Middle East and North African origin. Inuit people residing in the Artic circle of North America and Greenland also have moderate incidence of the disease (Torre et al., 2015). On the other hand, Northern Chinese, Koreans, and Japanese populations have a low incidence of the disease similar to the incidence in Caucasians (Forman et al., 2014).

Apart from the wide difference in incidence among different population, the phenomenon of familial clustering of NPC has long been observed in both high incidence and low incidence populations (Albeck et al., 1993; Gajwani, Devereaux, & Beg, 1980; Jia et al., 2004). The cause of familial clustering can be inheritance of a susceptibility gene or common exposure to the environmental carcinogen in the family. A complex segregation analysis done in Southern China on more than 1900 Cantonese patients showed that the inheritance was multifactorial and no single gene was responsible for susceptibility to the cancer (Jia et al., 2005).

With the presence of familial clustering, family members of NPC patients have increased risk of developing the disease. The risk was estimated to be increased 4–10 fold in individuals with a first degree relative suffering from the disease (Chang & Adami, 2006). Therefore it is logical to offer screening to family members of NPC patients. A study in Hong Kong showed that compared with symptomatic patients, NPC patients detected by the familial screening program were both younger and suffering from earlier stages of the disease (Ng et al., 2009).

Association with Epstein–Barr virus

NPC is one of the first cancers found to be associated with a viral infection, the EBV. It is the first head and neck cancer found to have a virus as a

causative agent. As NPC is a relatively rare disease in the West, the implication of a virus associated cancer was less studied until the discovery of another virus associated cancer four decades later, the human papilloma virus associated with oropharyngeal cancer. Old et al first identified the presence of antibodies against an antigen in Burkitt's lymphoma cell line in sera from patients suffering from NPC in 1966 (Old et al., 1966). The antigen was later identified as the EBV, a virus belonging to the family of herpes viruses. Later, it was confirmed that patients suffering from NPC had elevated EBV antibodies, especially anti-EBV IgA antibodies (de Schryver et al., 1969; Henle et al., 1970). The anti-EBV IgA antibodies have since been used as a tumor marker for NPC for more than four decades.

By the turn of the 21st century, improvement in molecular biology technologies allowed detection of the DNA of EBV in both NPC cells and the plasma of NPC patients. Lo et al. first developed the use of plasma EBV DNA titers as a screening tool for NPC (Lo et al., 1999) and a recent large-scale population study has shown that plasma EBV DNA titer is a sensitive and specific tool for screening high risk population for NPC. The test is able to detect asymptomatic individuals and diagnose patients in earlier stages of disease (Chan et al., 2017). Moreover, the use of the plasma EBV DNA titer is not just limited to screening. The plasma EBV DNA titer can be used as an assessment of tumor load (Mäkitie et al., 2004), a prognostic marker (Lo et al., 2000), for monitoring therapeutic response (Ngan et al., 2001), and for detection of recurrence (Lo et al., 1999). NPC is the first head and neck cancer to have such a unique and versatile biomarker for clinical use. These applications will be further discussed in subsequent chapters of this book.

Improvement in treatment outcomes in the last five decades

NPC is one of the few head and neck cancers that have shown dramatic reductions in death rates in the last half century. In 1983, the age adjusted mortality from NPC was 9.8 per 100,000 individuals in Hong Kong, but by 2015 the age adjusted mortality rate had reduce to 2.8 per 100,000 (Fig. 1.3). This nearly fourfold reduction of morality, which could not be attributed to the 30% reduction in incidence of the disease in the last three decades. More importantly, the main contributing factor in the dramatic increase in cure rate is the improvement in modern radiotherapy techniques, primarily the introduction of intensity modulated radiotherapy (IMRT), and application of adjuvant treatment modalities like chemotherapy.

The nasopharynx is situated in the center of the skull, adjacent to critical structures like the optic nerves and brain stem (Fig. 1.4). The dose limits of these

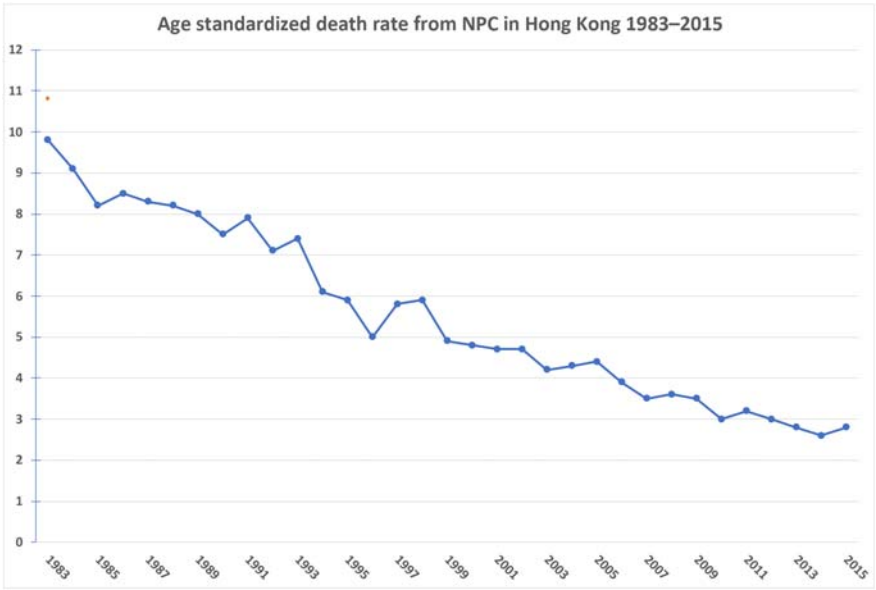

Figure 1.3 Age standardized death rate from nasopharyngeal carcinoma in Hong Kong, both sexes, 1983–2015. Source: *Data from Hong Kong Cancer Registry, Hospital Authority of Hong Kong.*

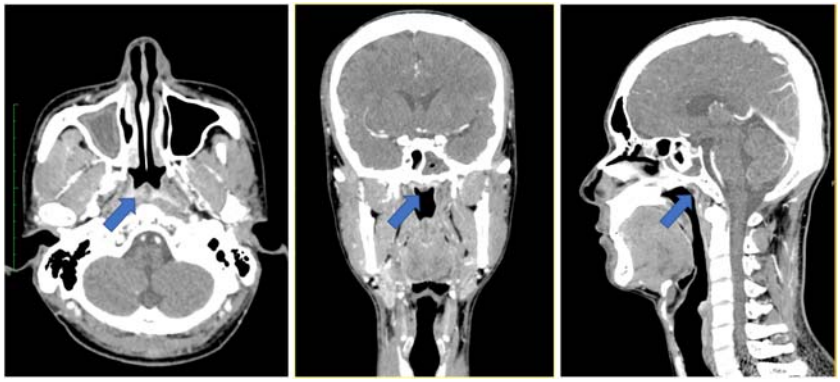

Figure 1.4 From left to right, axial, coronal, and sagittal CT scan of the head with arrow pointing to a small tumor in the central nasopharynx. Note the nasopharynx is in the center of the skull, far away from the surface of the head in all directions.

critical structures are less than 60 Gy, while over 66 Gy of radiation would be required to successfully control the tumor. With older two dimensional radiation techniques, many times it would be very difficult, if not impossible, to cover the tumor with adequate radiation dosage without imparting significant toxicities to the adjacent critical structures. With the introduction of new radiation machines like IMRT and tomotherapy machines around the turn of the

21st century, radiation oncologists could deliver adequate radiation dosage to cover the whole tumor while sparing the adjacent critical structures from high levels of radiation. This ability dramatically improved control of tumor spreading into the skull base, parapharyngeal space, and muscles of mastication. This improvement in local control is reflected in the new eighth edition of the Union for International Cancer Control (UICC)/American Joint Committee on Cancer (AJCC) cancer staging manual where invasion of tumor to the prevertebral muscles, medial pterygoid, and lateral pterygoid muscles are now staged as T2 instead of T3 as in the previous eighth edition staging system (Lydiatt et al., 2017).

By the end of the 20th century, it was recognized that addition of chemotherapy to radiotherapy would improve local control and possibly reduce distant failure in NPC. The landmark trial of Intergroup 0099 reported by Al-Sarraf et al. in 1998 demonstrated the benefit of concurrent chemotherapy in addition to radiotherapy in improving the progression free survival and overall survival in advanced stage NPC (Al-Sarraf et al., 1998). Since then, multiple trials have confirmed the benefit of concurrent chemoradiotherapy and now concurrent chemoradiotherapy is the standard of care in stage II or above NPC.

While surgery has never played a role in the primary treatment of NPC, since the late 1980s salvage surgery has proven to be an effective and less toxic alternative in the management of small, local, recurrent disease that failed radiotherapy. While there are no large randomized control trials to compare the efficacy of salvage surgery versus second radiation in managing locally recurrent NPC, multiple case series with various surgical techniques have shown that surgery is comparable to second radiation in salvaging the local recurrence (Wei, Chan, Ng, & Ho, 2011; Yu et al., 2005). Second radiation also incurs a significant morbidity due to the large dose of radiation the surrounding normal tissue must receive. Therefore in centers where there is surgical expertise, salvage surgery is routinely performed for locally recurrent NPC.

The techniques of salvage surgery have also improved over the last three decades. While open approaches to the nasopharynx were standard in the 1980s and 1990s, by the first decade of the 21st century improvement in endoscopes and endoscopic instruments allowed selected tumors to be resected with a minimally invasive approach. Endoscopic nasopharyngectomy and its derivative, robotic-assisted nasopharyngectomy (Tsang et al., 2015) has been described in the literature, mainly to salvage smaller local recurrences (Fig. 1.5). Early results showed that these minimally invasive approaches have similar local control when compared with open nasopharyngectomy and second radiation (Ho et al., 2012; You et al., 2015).

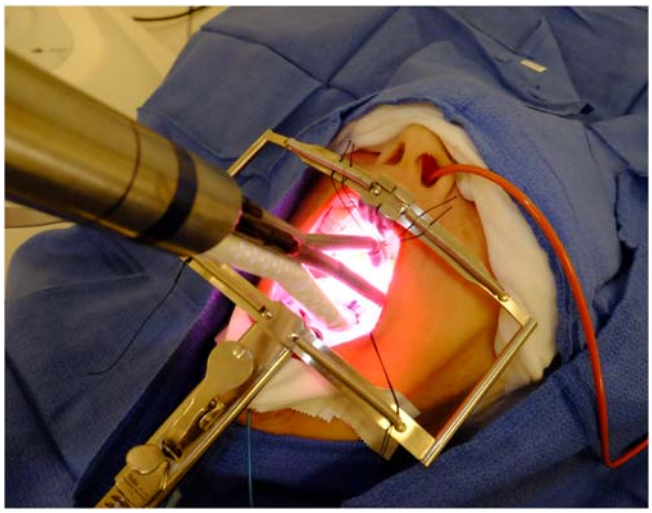

Figure 1.5 Photo showing deployment of the new da Vinci SP surgical robot for transoral robotic nasopharyngectomy.

Conclusion

Nasopharyngeal carcinoma is truly a unique cancer in every aspect. It is a model for the study of virus-induced cancer. In the last five decades, ongoing research has steadily improved our understanding of the cancer and our ability to cure it. There are still significant gaps in our understanding of the carcinogenesis and behavior of the cancer. This book aims to present our current understanding in the pathophysiology and management of the cancer and highlight the deficits in our knowledge. Closing these gaps of knowledge will allow us to understand and conquer this cancer and other cancers in the future.

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2

Risk factors and etiopathogenesis of nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy that arises from the nasopharynx, the region posterior to the nasal cavity. The most common site of origin is at the Fossa of Rosenmuller. NPC is prevalent in certain geographic areas such as China, Southeast Asia, India, the Arctic, the Middle East, and South Africa. This distribution is due to several existing risk factors that place certain populations at risk for NPC. Currently, the worldwide incidence of NPC is on the rise and will continue to increase in the coming decades as the at risk population also continues to grow. Presently, a trend is developing towards diagnosis of younger patients with NPC. This trend can be attributed to several important risk factors, has clinical implications, and significantly impacts management strategy. Epidemiologic studies indicate that specific racial and ethnic minorities have higher disease rates compared to the Caucasian population at large. This finding can be attributed to genetic, behavioral, and environmental factors.

The presentation of patients with NPC can be subtle, as it can mimic the symptoms of other common nasal pathologies and diseases such as rhinosinusitis. Patients normally exhibit symptoms when their disease progresses and thus are most often diagnosed with late stage disease. Presence of an alarming symptom such as epistaxis should raise a high index of suspicion of the presence of malignancy or other sinister pathology. NPC patients commonly present with neck swelling, hearing loss, tinnitus, blurring of vision, headache, and cranial nerve palsies. Neck swelling is the most common symptom. Some literature also reports ear or nasal symptoms as the most common presentation (Adham et al., 2012).

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Regardless of geographical location, there are two distinct peaks of incidence, early adolescence (18–24 years old) and elderly (65–70 years old). Males are affected twice as often as females. Individuals of Chinese or Cantonese ancestry have a higher risk of developing NPC. In clinical practice, the conventional wisdom is for a Chinese male patient presenting with tinnitus and hearing loss to be thoroughly investigated for NPC until proven otherwise. The nasopharyngeal area should be inspected and urgently biopsied for tissue diagnosis in addition to thorough investigation of medical history and clinical examination.

The identifiable risk factors for NPC have been extensively investigated and discussed in the current literature. These factors include genetic background, viral infection, dietary habits, cigarette smoking, alcohol consumption, as well as the exposure to other environmental carcinogens. These risk factors may act alone or in combination in initiating and promoting carcinogenic events. The etiology of NPC is multifactorial and involves complex interactions between genetic, environmental, and dietary factors. Consumption of salted fish and smoked foods as well as infection with the Epstein–Barr virus (EBV) are the most prominent established risk factors of NPC. Genetic factors and positive family history are also strongly associated with the development of NPC. Hypotheses regarding the development of NPC vary. However, the most likely mechanism is related to dysplasia of the epithelia followed by the action of several cofactors (i.e., the risk factors discussed above) that lead to field cancerization.

Chang and Adami highlighted that the well-established risk factors for NPC are elevated antibody titers against the EBV, consumption of salt-preserved fish, a positive family history of NPC, and certain human leukocyte antigen class I genotypes (Chang & Adami, 2006). Other factors such as consumption of preserved leafy vegetables, smoking and a history of chronic respiratory tract infection are associated with elevated NPC risk. Consumption of fresh fruits and vegetables and other human leukocyte antigen genotypes may be associated with decreased risk of developing NPC.

The survival of patients with NPC is mainly dependent on the clinical stage at diagnosis as well as the treatment instituted. In areas where NPC is prevalent, the majority of patients are diagnosed at late stage. Individuals diagnosed with late stage disease have a 50% survival rate in contrast to 90% for those diagnosed with early stage disease. Thus early diagnosis is crucial for optimal prognosis. Imperatively, diagnosis can be facilitated by the identification of existing risk factors in individuals at high risk. Dissemination of knowledge of the symptoms of NPC and improvement of health awareness among the public will also help detect NPC early in the disease process.

Risks factors of nasopharyngeal carcinoma

Family history

It is widely accepted that positive family history of NPC is associated with increased risk of NPC. This holds true especially for first-degree relatives who have 4–10 fold increased risk of acquiring NPC compared to those without positive family history (Chang et al., 2017). This familial segregation can be strongly observed in endemic areas of NPC such as Guangdong province in China as well as in low incidence areas. Of note is that this genetic factor may act in combination with environmental factors in promoting NPC occurrence. As stated in the study by Chang et al., this familial clustering can be a result of shared genetic susceptibility, shared environmental risk factors, or both. They further stated that a complex segregation analysis of familial NPC in Southern China revealed that multiple genetic and environmental factors, rather than a single major susceptibility gene, seemed most likely to explain the observed pattern of inheritance (Chang et al., 2017).

The fact that multiple genetic and environmental factors, rather than a single susceptibility gene, may be responsible for the specific pattern of inheritance of NPC is truly captivating. The majority of the field agrees that family history is the strongest predictor of development of NPC. This is also true for other head and neck malignancies, which commonly occur in individuals with family members who have been diagnosed with a similar malignancy. In other words, an individual from a family diagnosed with NPC has a higher probability of developing NPC. The importance of family history in relation to patient survival is also currently being investigated. Family history as a primary determinant of patient overall survival times has been controversial and remains a focus of current research.

The relationship between family history and patient survival is currently being studied. Ouyang et al. studied 1773 NPC patients including 207 (11.7%) with a first-degree relative with NPC This study demonstrated that having a positive first-degree family history of NPC was associated with a significant reduction in risk of death, locoregional relapse, and distant metastases (Ouyang et al., 2013). Importantly, this effect remains even after adjusting for known prognostic factors. The authors attributed this finding to the diagnosis of early stage disease in patients with positive family history leading to monitoring of this subset of patients. Further, screening family members with positive family history will aid in early disease diagnosis and early therapeutic intervention leading to better prognosis and survival.

Ren et al. documented that the risk of NPC was positively associated with history of head and neck cancers (HNCA) among parents. Another study by Liu et al. revealed that an individual with a first-degree family history of NPC was found to be at more than four-fold increased risk of NPC compared with

individuals who do not have such history. The excess risk was higher for maternal compared to paternal history and was slightly stronger for a sibling with NPC compared with a parental history (Liu et al., 2017).

Abdul Amir et al. in his study of 122 patients found out that there were 48 HNCA (i.e., a history of HNCA within the first three degree relatives, namely parents, brothers/sisters, and cousins). It was shown that 6 (14.3%) of NPC patients had positive family history. On the other hand, out of 14 hypopharyngeal carcinoma (HPC) and 66 laryngeal carcinoma (CL) patients that were studied, it was revealed that 9 (64.3%) of HPC and 33 (50%) of CL patients had positive family history (Abdul Amir, Hafidh, Abdul Muhaimen, Abu Bakar, & Abbas, 2008). The authors concluded that positive family history of NPC is not a predictive risk factor for NPC development and implies the presence of other risk factors that may be more predictive than familial history of the disease.

Ethnic and racial distribution

Among the HNCAs, NPC is known to be strongly related to ethnicity. The striking pattern of ethnic and racial distribution of NPC may be associated with other environmental and geographical factors. Inspection of the geographical distribution of NPC worldwide reveals significant areas with high incidences located where the majority of the population is Chinese, Cantonese, or African in origin, and in the Arctic where consumption of salted fish and preserved vegetables is high.

Generally, literature on NPC originates from South East Asia, China, Hong Kong, and Nordic countries. These countries have been widely described as the areas where NPC is prevalent. In Southeast Asia, NPC risk varies with the degree of racial and social admixture with Southern Chinese. The incidence is low among Singaporean Indians but much higher in Thai, Macaonese, and the Malays, which have a history of intermarriage with Chinese ancestors. In the Chinese province of Guangdong where the overall NPC incidence rate is >20 per 100,000 person years among males, rates in Cantonese speakers are double those in other dialect groups such as the Hakka, Hokkien, and Chiu Chau. In the Malaysian state of Selangor, the rates in Chinese residents have historically been highest among Cantonese, intermediate among Khek, and lowest among Hokkien and Teochiu. In the United States rates are highest among Chinese Americans, followed by Filipino Americans, Japanese Americans, Blacks, Hispanics, and finally whites (Chang, Adami, 2006). Notably, the geographical distribution of NPC is correlated with the histological types of NPC. The keratinizing and nonkeratinizing types are prevalent in regions with lower incidence of NPC such as the North America and Europe. In contrast, the undifferentiated type is predominant in countries with high incidence of NPC namely China, Indonesia, Malaysia, Singapore, the Arctic, North Africa, and South India.

The implications of the migration of populations to other geographical regions for incidence of NPC is also important to highlight. The majority of studies show that there is a persistent risk of developing NPC in individuals who migrate from high incidence areas to low incidence areas. Interestingly, the risk of NPC among southern Chinese living in Singapore, Malaysia, and Japan is comparable with natives in southern China. NPC incidence is also higher in North African migrants to Israel and their offspring than in natives Israelis. Even though the incidence of NPC among Chinese in the United States remains 10–20 times higher than among US whites and blacks, it is approximately half as high as that observed in southern China. This trend is also observed among Chinese who migrate to the United Kingdom and Australia (Chang & Adami, 2006). Importantly, the risk seems to decrease with longer duration of residence and with succeeding generations in the West. In addition, risk of NPC increases among white males born in China or the Philippines compared to those born in the United States as well as among males of French origin born in North Africa compared with those born in southern France.

Genetic factors

Human leukocyte antigen

Human leukocyte antigen (HLA) is one of the most widely studied regions in the human genome and HLA molecules are widely expressed on cell surfaces. Generally, HLA present specific antigens to T lymphocytes and thus modulates the immune response to inflammatory as well as malignant diseases. HLA is a strong genetic factor associated with NPC and studies have documented that younger NPC patients have different pathological mechanisms that lead to NPC compared to older NPC patients due to differences in HLA. HLA comprises a family of Class I and Class II genes within the major histocompatibility complex, which is located on the short arm of chromosome 6 (Lye et al., 2015; Nor Hashim et al., 2012). They present antigenic peptides to specific T-cells to initiate a cell mediated immune response to EBV infections. HLA Class II genes encoded by *DR, DQ*, and *DP* genes are expressed in immune cells and are important in regulation of immune responses to foreign antigen and discrimination of self from nonself antigen. The HLA Class II is highly polymorphic due to differences in the B chain and allelic differences in the *DQa* and *DPa* chain (Chang & Adami, 2006).

Importantly, HLA is also associated with survival rate after the diagnosis of NPC. Several haplotypes of HLA are currently being investigated in order to come up with better methods of diagnosis and treatment for NPC. Chattopadhyay et al. stated that several important genetic factors are implicated in the pathogenesis of NPC. These include polymorphisms in HLA, cytochrome P450, P53, and some signaling pathways that promote NPC (Roy Chattopadhyay, Das, Chatterjee, & Choudri, 2017). Chang et al. (2006) documented that some HLA alleles have been consistently associated with NPC risk. In southern Chinese

and other Asian populations, HLA-AR-B46 and B17 were generally associated with a 2–3 fold increased risk of NPC. On the contrary, 30%–50% lower risk of NPC was found in association with HLA-A11 in both Chinese and Whites, B13 in Chinese, and A2 in Whites (Chang & Adami, 2006; Chang et al., 2017).

The HLA-DRb1 chain gene is localized in the MHC class II region that encodes immunomodulatory factors involved in recognition of extracellular proteins and autoantigens. Some studies documented that genetic alteration of HLA-DRB1 could modulate NPC susceptibility, whereas in other studies the findings were inconclusive. Yao et al. revealed in their study that the HLA-DRB1 confers both promoting and protective effects toward NPC. The HLA DRB1 03, 08, 09, and 10 allele polymorphisms contributed to the susceptibility of NPC, whereas the HLA-DRBII 12 allele polymorphism may be an important protective factor for NPC, especially in Asian populations (Yao, Yang, Shen, Zhang, & Li, 2017).

Lye et al. stated that the human xeroderma pigmentosum group D (XPD) gene is an important component in the transcription factor IIH complex and is responsible for encoding an ATP-dependent 5′–3′ DNA helicase protein (10). Their study revealed that the genotype frequencies of the XPD K751Q polymorphism in cases were 85.7%, homozygous Lys/Lys 13.8% which are higher than those in the control groups. Their study revealed that the genotype frequencies of the XPD polymorphism were homozygous Lys/Lys, which are higher than those in control groups.

Tumor oncogens and tumor suppressor genes

Oncogenes and tumor suppressor genes (TSGs) are known factors involved in the carcinogenesis process that leads to the development of many cancers. Several oncogenes such as *raf-kinase*, *myc*, *abl*, *erb*, *ras*, and *c-Sis* are linked to breast cancer, lung cancer, gastric cancer, and colon cancers. TSGs mainly regulate the cell cycles that maintain cells in their normal shape and character thus avoiding biologic events that lead to carcinogenesis. Human p53 is the most dominant suppressor gene, and in the majority of cancers mutation of P53 leads to cancer formation. In the pathogenesis of NPC, it is the overexpression of p53 that has been implicated in the cancer.

Epigenetic alterations especially cytosine-guanine dinucleotides (CpG) methylation have been shown to be as important as genetic abnormalities during tumor initiation and progression. In contrast, genetic alterations in tumors such as homozygous deletion or loss of heterozygosity pinpoint potential loci for critical TSGs. Several critical TSGs within specific regions inactivated by genetic or epigenetic mechanisms have been identified for NPC and other tumors like *FHIT* (3p14.2), *PTPRG* (3p21–p14), *ADAMTS9* (3p14.1), *RASSF1A* (3p21.3), and *MLH1* (3p21.3) (Shu et al., 2013). A recent model of the cancer gene island phenomenon was proposed revealing that hemizygous deletions preferentially occur in small gene islands harboring high density TSGs to maximize the

proliferative advantage of tumor cells. Loss of TSGs is common in NPC and other tumors like lung, esophageal, and breast cancers (Shu et al., 2013).

Chromosome 3p shows a high frequency of allelic loss in NPC and has a vital role in NPC suppression. *NotI* chromosome-3 specific microarray profiling is useful for screening of inactivated genes by deletion and/or hypermethylation. Involvement of fibulin in NPC was identified by its frequent deletion and methylation in NPC cell lines, which was detected by chromosome 3 genomic *NotI* microarray analysis in a study by Law et al. (2012). This study suggested that the significant downregulation and frequent promoter hypermethylation of fibulin further provides evidence for its involvement in NPC. The acquisition of sustained angiogenesis is essential for both tumor growth and metastases. Fibulin (FBLN2S) contributes to in vivo tumor suppression through cell proliferation inhibition and angiogenesis suppression. Transient transfection of FBLN2S suppressed the colony formation ability of the NPC cell lines. This fibulin protein expression is clearly able to inhibit in vitro cell proliferation, angiogenesis, migration, as well as invasion in NPC.

The Epstein–Barr virus

Since last decade, viruses have been known to be associated with multiple significant cancers, especially HNCA. This has significant sequalae either in the diagnosis of the type of cancer or on the management of these virus-related cancers. The commonly known oncogenic viruses include human papilloma virus (HPV), the EBV, human T-cell lymphotropic virus, and human immunodeficiency virus (HIV). HPV is known to have a strong association with cervical cancer and at this juncture it also has significant usage in the diagnosis as well as prognosis of oral cavity and oropharyngeal carcinoma.

EBV belongs to the herpesvirus group and causes a lifelong asymptomatic infection in 95% of the individuals around the globe. This virus also has been designated as a class I oncovirus by the World Health Organization (WHO). It remains in memory B cells with low copy numbers of episomal virus in the latent phase in healthy individuals. It has strong clinical implications in the management of NPC. The tumorigenic activity of EBV was first discovered in a childhood cancer common in South African children, the Burkitt's lymphoma. The virus is able to infect and destroy B cells as well as epithelial cells. EBV can be reactivated at particular times and it can switch from the latent to lytic phase with the production of viral particles. This is turn triggers the elevated immune response, which causes high antibody surge against specific antigens such as early antigen (EA) and viral capsid antigen (VCA). The infection of B cells is a highly efficient process in contrast to epithelial cell infection. B cell infection involves an intricate interaction between the surface proteins on the B cells. This virus has been linked to multiple malignancies namely Burkitt's lymphoma and Hodgkin's lymphoma.

In NPC, EBV typically exists in type II latency phase, particularly in the undifferentiated or poorly differentiated types. Type II latency is characterized by the expression of a subset of latent genes including the EBV-determined nuclear antigen 1 (EBNA1), latent membrane proteins (LPP1, LMP2, and LMP2B) and several EBV noncoding RNAs (Shen, Zhang, Sun, Wu, & Qian, 2015). The detection of EBV in NPC and the prominent role of EBV in promoting tumor development support EBV as a potential therapeutic target for NPC. Furthermore, with the addition of knowledge regarding EBV oncogenicity and interactions between EBV and host immune responses, immunological approaches such as adoptive T cell immunotherapy may be explored.

Tsang et al. report that infection of EBV is ubiquitous and tumorigenesis only occurs in a small fraction of the infected population. This suggests that the tumorigenic transformation of infected EBV human cells involves a complex virus–host interaction and other additional cofactors (Tsang & Tsao, 2015). The oncogenic latent membrane proteins are frequently expressed in EBV seropositive patients and might have role in transformation of the infected cells to become malignant (Dawson, Port, & Young, 2012). Numerous studies have shown that NPC patients and those at high risk of developing the disease show a distinctive anti-EBV antibody profile. The majority will have elevated immunoglobulin A (IgA) antibodies with specific EBV antigens. The most widely used serological biomarkers includes VCA-IgA, EA-IgA, and EBNA1-IgA.

The pathogenesis of EBV is linked to the suppressed immune microenvironment of a host as well as inflammatory changes that contribute to the progression of malignant change. Abdul Amir et al. conducted a study on 100 patients with NPC and documented that the NPC patients had higher EBV IgA and IgG antibodies compared to the other HNCA group and control group (Abdul Amir et al., 2008). Zheng et al. also found that high sensitivity and specificity were observed in the present study using both the EBV DNA load per ng DNA (96% sensitivity and 97% specificity) and EBV DNA load per β-globin DNA (95% sensitivity and 96% specificity) (Zheng, Lu, Li, & Jia, 2015). Fung et al. documented that the histological subtype of tumor is another factor that affects the detection rate of plasma EBV DNA in NPC patients (Fung, Lam, & Chan, 2016). In endemic areas, most NPC cases are nonkeratinizing and undifferentiated (WHO type III) and these tumors typically harbor the EBV genome.

The dietary factors

Generally speaking, food that contains high fiber such as leafy vegetables, fruits, and nuts are associated with a reduced risk of cancer. The protective effect of a healthy diet is well known and is due to multiple components of vegetables which have antioxidant, antiinflammatory, and antimutagenic activity. These components include beta carotene, retinoids, folate, phytoestrogen, tocopherol, and flavonoids, which possess numerous positive effects and are

involved in biological process that may modify cancer risks. These constituents of vegetables may have actions such as inhibition of cell growth, DNA methylation, and oxidative stress mechanisms.

On the contrary, consumption of salted fish and preserved vegetables has been known to be associated with risk of developing NPC. In most of the countries where the majority of patients are Chinese, such as China, Singapore, Taiwan, and Korea, salted fish and preserved vegetables are widely consumed as a regular daily diet and are readily available at the supermarket. The salted vegetable preparation called suan cai is a traditional Chinese pickled cabbage usually prepared by slow fermentation followed by pickling with salt and brine. This is similar to sauerkraut common in Central and Eastern Europe, which has also been shown to increase risk of NPC (Chang & Adami, 2006).

Different levels of nitrosamines, which are carcinogenic, are attributed to the different types and processes of fermentation used. Salted fish such as Cantonese style salted fish has been categorized as a group 1 (mild) carcinogen by the International Agency for Research on Cancer. Other salt fish are categorized as group IV (severe) carcinogens. These carcinogens may interfere with cell cycle checkpoints and cause specific abnormalities that promote carcinogenesis. Interestingly, other types of preserved foods have also been shown to increase risk of NPC. Adham et al. documented that the major etiologic factors for NPC include genetic susceptibility and early age exposure to carcinogen (e.g., preserved foods other than salted fish such as beef liver and salted eggs) are significantly associated with increased risks of NPC. In addition, other factors such as the presence of nitrosodiethyl amines in dried and smoked meat and the use of nasal herbal medicine are well-known risk factors for NPC (Adham et al., 2012).

Polesel et al. conducted a study among White patients and found that the consumption of vegetables, especially yellow and red colored vegetables, is associated with reduced risk of NPC (Polesel et al., 2013). This risk reduction is higher if the individual is also a nonsmoker. Bidoli et al. also found that the consumption of fiber, both the soluble and insoluble types such as cellulose and lignin, are associated with the reduced risk of NPC (Bidoli et al., 2013). Turati et al., conducted a hospital-based case-control study in the low NPC risk country of Italy that included 198 histologically confirmed NPC cases and 594 matched controls. In this study dietary habits were collected by means of a validated food-frequency questionnaire including 83 foods, food groups, or beverages. Adherence to the traditional Mediterranean diet was assessed through a Mediterranean Diet Score based on nine dietary components, namely high intake of vegetables, fruits, nuts, cereals, legumes, and fish; low intake of dairy products and meat; high monounsaturated to saturated fatty acid ratio; and moderate alcohol intake (Turati et al., 2017). This study concluded that a Mediterranean diet has a favorable effect on NPC risk.

Gallicchio et al. documented that the consumption of preserved leafy vegetables is associated with high risk of developing NPC, as these preserved vegetables contain nitrosamines and nitrates that are mutagenic (Gallicchio et al., 2006). N-nitrosamine induced the formation of DNA adducts that are responsible for carcinogenesis. Different concentrations of nitrosamines are found in preserved food at different geographical areas. The consumption of preserved leafy vegetables is not only related to NPC but also to gastric, colon, and esophageal cancers.

Jia et al. conducted studies in China where NPC is prevalent and stated that the consumption of salted fish, salted shrimp paste, moldy bean curds, preserved meat, and various preserved vegetables is associated with high risk of NPC (Jia et al., 2010). They further documented that the intake of herbal tea and slow cooked herbal soup are associated with reduced risk of NPC. Several properties that might be related to the herbal tea are its effects on apoptosis, immunomodulation, inhibition of angiogenesis, and reversal of multidrug resistance. Importantly, coix seed has been shown to have an inhibitory effect on EBV and has antitumor properties.

Salted fish

Consumption of salted fish has been linked to the pathogenesis of NPC, especially in Chinese populations and other Southeast Asian countries. The salted fish contains bacteria and pathogens that convert nitrates and nitrites into carcinogenic amines. Certain salted fish also contains mutagens, genotoxins, and EBV reactivating substance (Roy Chattopadhyay et al., 2017).

Traditional preserved foods such as the salted fish, salted meat, and preserved vegetables appear to be associated with increased risk of NPC. A study by Yong et al. among Singaporean patients stated that patients who consumed salted meat at least once a month were found to have double risk of developing NPC compared with those who never or rarely consumed salted meat (OR = 2.10, 95% CI 1.18−3.50) (Yong et al., 2017). They also suggested that in addition to salted meat, salted vegetables consumed at least once a week were also found to be significantly associated with an increased risk of developing NPC (OR = 3.70, 95% CI 1.58−8.64) compared with those who rarely consumed. Importantly, they highlighted that the trend of increasing risk of NPC was significantly associated with increasing frequency of salted fish, salted meat, and salted vegetable consumption (P-trend = .033, .003, and $<$.001, respectively).

Another study by Lau et al., showed markedly decreasing trends in salted fish intake and tobacco consumption, but an increasing trend in vegetable consumption in Hong Kong over the past several decades. All of these environmental risk factors were individually highly correlated with age standardized mortality rate and age-standardized incidence rate (Lau et al., 2013). The decrease in salted fish consumption may represent a change in dietary culture with Westernization of dietary

patterns among Hong Kong citizens from a traditional Cantonese diet and from increased awareness of the carcinogenicity of salted fish. Yong highlighted that people who had a least one salted vegetable in a week had four times increased risk of developing NPC compared to those who do not consume salted vegetables (Yong et al., 2017). In Singapore at least 20% of the population consumes salted vegetables in their daily diet. This comes in the form of pickled cabbage and green mustard also called suan cai.

Environmental carcinogens

Recently a change has been observed in the geographic pattern and distribution of NPC. There has been a decreasing incidence of NPC in high risk areas, which is probably due to lifestyle changes and better health awareness, namely less consumption of the preserved food and salted fish, a reduction in cigarette smoking, and increased adoption of western dietary habits. The early screening of EBV antibodies also contributes to these new observed changes. Xie et al. documented that occupational exposure to cotton dust, chemical fumes, and welding fumes are significantly associated with increased risks of NPC. The duration of exposure to cotton dust is also significant, with exposure of more than 15 years resulting in high risk of developing NPC (Xie, Yu, Tse, Au, & Lau, 2017).

Burning incense is a new factor capturing scientific interest. This practice is prevalent in the Southeast Asian and Cantonese populations where Buddhism and Taoism are the main religions. It has been associated with harmful effects due to the gases and volatile organic compounds produced, which can be mutagenic (e.g., formaldehyde and carbonyls) (He et al., 2018).

Tobacco smoking

Cigarette smoking has been linked to multiple cancers. Importantly, it causes lung cancers that are responsible for the highest mortality rates among men afflicted with cancer across the globe. Numerous studies have also shown associations between smoking and pathogenesis of NPC, whereas other literature does not support smoking as a risk factor for NPC. Yong stated that studies conducted in Taiwan, China, Thailand, Wuhan, and Shanghai in China, and the United States found that smoking was a risk factor for NPC, although other studies in Singapore, Malaysia, Serbia, and China found less clear association between smoking and risk of NPC (Roy Chattopadhyay et al., 2017). In low endemic areas of NPC such as in Europe and the Western world, tobacco smoking appears to be a risk factor for NPC. Multiple studies documented that smoking is a risk factor of the squamous cell type of carcinoma in low risk geographical areas.

Cigarette smoking produces many reactive agents such as polyaromatic hydrocarbons, aromatic amines, and *N*-nitrosamines, which result in formation of

DNA adducts that subsequently cause DNA damage. This DNA damage accumulates and subsequently causes cancers. The effects of smoking can be direct damage by these carcinogenic materials in tobacco smoke or alternatively the smoke may carry EBV, which eventually is deposited in the nasopharynx mucosa and adjacent airways.

Several studies in the United States documented an increased risk of NPC in heavy smokers (6 times risks) compared to those who never smoked, and the association is much stronger for differentiated NPC rather than undifferentiated NPC (Polesel et al., 2011). A study by Lin et al. provides new prospective evidence that heavy and chronic smokers have significant risk of NPC mortality, which is consistent with most case control studies and the four cohort studies from Taiwan, Singapore, and the United States (Lin et al., 2015). Lin et al. further documented that the adjusted hazard ration from their study was 3.26 (1.14−9.36) in heavy smokers who smoked more than 15 cigarettes per day. The authors also noted significant trends that suggest dose-response relationships for daily smoking amount and cumulative tobacco use among all cohort studies above. This study used lower smoking levels to define heavy and chronic smokers (more than 10 pack-years, more than 10 years smoking, or 15 cigarettes per day), but their results still showed a strong and significant association between smoking and NPC mortality.

A study by Xue et al., incorporated a total of 10,274 cases of NPC and 415,266 subjects to evaluate the effect of tobacco smoking on NPC risk with reasonable quality control and revealed that ever smokers had a significantly higher risk of NPC than never smokers, and this association was relatively stable across almost all of the subgroup analyses, with a robust dose-dependent pattern (Xue, Qin, Ruan, Shugart, & Jia, 2013). In contrast, other studies demonstrated a significantly higher risk only for smokers with very high levels of smoking (more than 30 pack years or cigarettes per day). The study also found that both former smokers and low-dose smokers had an increased risk of NPC and the risk rose by 1%−2% with each pack-year of smoking ($P < .001$), which seems to indicate that this meta-analysis made a more accurate assessment of the association than any other single study.

A recent study has revealed a new role of cigarette smoking in the carcinogenesis of NPC by induction of EBV reactivation. The importance of active versus passive smoking and risk of NPC has garnered scientific interest and more studies have been conducted to observe the different effects of active and passive smoking. Several studies found significant positive associations of active tobacco smoking (among men) and passive smoking (among men and women) with risk of NPC, with greater excess risk in concert with longer smoking duration and exposure to passive smoking in childhood or from a spouse.

Xu et al. showed that cigarette smoking is associated with EBV-IgA seropositivity (Xu et al., 2012). This is a vital finding that supports the interaction between

the risk factors that are responsible for NPC. A 20 year follow up study in Taiwan documented that a long-term cigarette smoker has higher anti-EBV seropositivity compared to nonsmokers or short term smokers. Studies have documented that smoking is associated with the antigens expressed in the lytic phase, not in the latent phase, and smoking has a dose response relationship with seropositivity for EBNA1-IgA and *Zta-IgA*. The study by Lin et al. confirmed that smoking reactivates EBV because they found similar dose-response relationships between smoking and EBNA1-IgA and *Zta-IgA* seropositivity (Lau et al., 2013; Lin et al., 2015). Smoking was not associated with seropositivity for LMP1-IgA, which is an antibody against an antigen specifically expressed during EBV latent infection. This finding suggests that smoking is associated with antibodies against infection stage-specific antigens.

In addition, smokers have 5- to 6-fold increased risk of simultaneous seropositivity for EBNA1-IgA, *Zta-IgA*, and VCA-IgA compared with nonsmokers who were instead seronegative for all three antibodies. This study further documented that cigarette smoking might contribute to NPC risk in an indirect way by synergistically elevating anti-EBV IgA levels, in addition to contributing directly to NPC carcinogenesis by the introduction of multiple carcinogenic materials. However, the exact materials that result in higher EBV-IgA antibody levels and the detailed mechanisms by which cigarette smoking modulates EBV reactivation remain to be further elucidated (Lin et al., 2015; Xie et al., 2017; Xu et al., 2012).

Alcohol consumption

Alcohol consumption has been suggested to be protective against certain diseases at specific intake levels. However, it is widely appreciated that alcohol has more harmful effects than benefits. Chronic alcohol intake has been associated with liver disease and development of liver cancers. It also predisposes to esophageal and gastric cancers. There is no strong evidence to implicate alcohol consumption in the pathogenesis of NPC; however, it is found that individuals who smoke and drink alcohol have higher risk to develop NPC compared to those who just smoke cigarettes. Ethanol itself is responsible for the effects of alcoholic beverage consumption on cancer.

There are numerous mechanisms that have been proposed that could contribute to ethanol associated cancer development. Acetaldehyde, an oxidative product of alcohol, is known to be toxic, carcinogenic, and mutagenic. Many studies have consistently shown that acetaldehyde interferes at many sites with DNA synthesis and repair and can result in tumor development. In addition, chronic alcohol consumption has been shown to induce cytochrome P450 enzyme (CYP2E1) activity in mucosal cells, which can lead to stimulation of free radical formation and consequent cell injury. Furthermore, chronic and heavy alcohol consumption may result in various deficiencies of vitamins and trace elements including folate, iron, zinc, and vitamin A, which are involved in gene

regulation and cell differentiation. These nutritional deficiencies may further enhance ethanol associated carcinogenesis.

The increased risk of NPC in individuals who both drink and smoke cigarettes could be explained by the fact that there is a potential interaction between alcohol and cigarette consumption in escalating carcinogenesis. A study by Chen et al. suggested that an increased NPC risk with increased alcohol intake is biologically plausible and consistent with well-established associations between increasing alcohol intake and increased risk for other upper aerodigestive tract disorders (Chen et al., 2009). In their meta-analysis study, they found that alcohol intake was associated with increased risk in both United States and Chinese populations, but the association was stronger and statistically significant in the United State studies. The authors speculate that alcohol intake is much lower in the Chinese population compared to the Western population, which translates to the fact that the Chinese populations may drink alcohol at a lower level that is not detrimental for NPC development.

The relationship between alcohol consumption and risk of NPC is also influenced by the histological type of the NPC. The undifferentiated and nonkeratinizing carcinomas are the major forms of NPC identified in high-risk regions such as China and parts of Asia, whereas the keratinizing squamous cell carcinoma is identified predominantly in low risk areas like North America. Some studies documented that heavy alcohol intake may be a risk factor for differentiated squamous cell cancer but not undifferentiated and nonkeratinizing squamous cell carcinomas. In the United States, a large proportion of NPC cases are differentiated carcinomas for which the associations between alcohol drinking and NPC would be expected to be the strongest.

Interaction between the risk factors and pathogenesis of nasopharyngeal carcinoma

At present, the mechanism of the development of NPC is under scrutiny by many scientists as younger people become afflicted with the disease and display fast disease progression. Many studies have identified the important risk factors associated with NPC and focused on the multiple interactions that exist between these factors that are responsible for the development of NPC. Interestingly, the interaction between several risk factors that contribute to the formation of NPC has been consistently shown to be responsible for the pathogenesis of NPC. This involves a complex interaction between EBV, smoking, genetic, and environmental factors.

The tumorigenic potential of viral infections is associated with their carriage of genes associated with cell transformation and their ability to induce chronic inflammation. The HLA plays a central role in viral antigen presentation, which

is key to determine the outcomes of the host immune response to lifelong viral infections. HLA genes play roles in the development of NPC because they have a functional impact on the innate and adaptive immune response against the viral etiologic agent, EBV. NPC cells expressing specific EBV proteins that are processed and the antigen presented in association with HLA class 1 alleles may be recognized by EBV specific CD8 cytotoxic T cells. Some evidence supports the hypothesis that EBV may down regulate the expression of HLA alleles resulting in immune escapes of NPC cancer cells by decreasing the recognition of EBV expressing cancer cells (Lung et al., 2014).

Association between stages of nasopharyngeal carcinoma at diagnosis with risk factors of nasopharyngeal carcinoma

Jen et al. found out that several factors are associated with diagnosis of NPC at late stage. For instance smoking more than 30 cigarettes per day is strongly associated with diagnosis at late stage compared to smoking less than ten cigarettes per day. Similarly, eating salted fish on a weekly basis is also associated with diagnosis of NPC at late stage. The numbers and the concentration of inhaled carcinogens cause more cell growth disruption and mutations that lead to more carcinogenic events (Jen et al., 2010).

Additionally, factors such as patient knowledge of the early symptoms of NPC and physical examination can increase the likelihood of getting the NPC diagnosis early. This is crucial because early intervention can be implemented if the early diagnosis is made. This translates to high chance of cure and good survival rates and prognosis for this subset of patients. Ren et al. studied a few important factors that related to the early diagnosis of NPC and highlighted numerous factors such as exposure to environmental carcinogens (e.g., EBV and cigarette smoking) that can facilitate early diagnosis of NPC (Ren et al., 2010).

Implications for the management of nasopharyngeal carcinoma

NPC is prevalent in the Southeast Asia region, China, Hong Kong, South Africa, India, the Middle East, and the Artic. Certain geographic areas are known to have certain predisposing factors. By studying these risk factors in depth, a potential screening and intervention program can be developed.

Family members at risk can be screened as early as possible and educated on NPC and its management. Genetic testing can also be carried out to facilitate early identification of those who are at risk. The development of a simple kit to

detect the EBV in saliva or serum could be an effective method to diagnose NPC early. Advancement in technology and instrumentation will determine the focus of NPC management to ensure the best survival rates for these patients and better quality of life.

Epstein–Barr virus as a screening biomarker

Numerous studies have suggested that the EBV is an established and appropriate candidate to be a screening marker for individuals at high risk. The majority agrees that the antibody to EBV antigen is highly sensitive and specific for NPC and the test is an effective screening tool, especially in endemic areas. Saliva collection instead of blood is a noninvasive, easy, and convenient method for screening a large cohort of high risk patients (Lourembam et al., 2016; Ng et al., 2005).

A study by Coghill et al. investigated use of the EBV antibody to nuclear antigen EBNA1-IgA in families with positive family history in Taiwan. They stated that in a high risk multiplex with multiples family with an underlying NPC incidence on the order of 100×105, it was estimated that only 164 high risks individuals would need to have blood drawn to be screened at baseline for each case of NPC successfully detected over the following 5 years. In the Taiwanese population at risk of NPC, approximately 1250 individuals need to be screened for NPC which are equal to NPC detected by EBNA1 IgA markers (Coghill et al., 2014).

How to identify and reduce risk

Three are multiple risk factors for the development of NPC and the interactions between these factors are complex. Some risk factors can be recognized early and mitigated, whereas other risk factors are well beyond control and difficult to manage. However, most of these risk factors are associated with changes in lifestyle such as the dietary habits, alcohol consumption, and cigarette use. Educating the community, especially those who consume salted fish and preserved vegetables regularly, to minimize their intake by introducing another healthy diet regime will help in reducing NPC. A health campaign on EBV and its dangers as well as on alcohol and smoking, especially to younger and middle age groups, can be effective in reducing the risk of NPC.

Above all local health experts and professionals can collaborate with government and nongovernment agencies in conducting workshops and campaigns to educate remote populations on the disease and it risk factors as well as the symptoms that the patient might be experiencing and what are correct next step to be taken. Hospitals and local clinics have close contact with individuals at high risk of NPC and should participate in health promotion campaigns and related activities.

Conclusion

The incidence of NPC is on the rise, especially in endemic areas where the population has increased over the years. Several risk factors are well known to associate with the carcinogenesis of NPC. Despite different geographical regions, the risk factors appears to be somewhat similar and the afflicted population is confined to individuals of Chinese or Cantonese origin. The well-established risk factors of NPC include salted fish, EBV, preserved leafy vegetables, and select HLA alleles. Other factors like alcohol, tobacco, chemical dust, and burning incense have been showed to correlate with NPC development in certain regions of the globe, while no association with these factors and NPC was found in other regions. The risk factors may act alone or in combination to lead to NPC. The complex interactions between these cofactors are being researched so that this disease can be combatted efficiently to save lives.

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Genetics of nasopharyngeal carcinoma and molecular signaling pathways

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Genomic and epigenetic landscapes of nasopharyngeal carcinoma

Introduction

Nasopharyngeal carcinoma (NPC) is a unique type of head and neck cancer that is mainly seen in Southern China, Southeast Asia, and North Africa regions, but is rarely diagnosed in Caucasians (Chua, Wee, Hui, & Chan, 2016; Petersson, 2015). Evidence, especially from the unique geographic and ethnic distributions of NPC, strongly suggests a link between its development and genetic/epigenetic factors (Dai, Zheng, Cheung, & Lung, 2016) with Epstein–Barr virus (EBV) infection (Chua et al., 2016). Furthermore, it has been suggested that EBV genome diversity may also contribute to increased incidence of EBV-associated NPC in certain geographical regions (Tu et al., 2017). There is an increased relative risk for developing NPC in individuals with a first-degree family history of NPC as compared to those without (Liu et al., 2017). In addition to the already established genetic changes, epigenetic deregulation is also implicated in the initiation and progression of NPC (Shyamasundar, Dheen, & Bay, 2016).

Interestingly, the presence of mutation in NPC may not be related to the stage of the disease, age or gender of the patients (Fountzilas et al., 2018). Some of

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the critical molecular events involved in the development of NPC include the overexpression of cyclin D1 and activation of nuclear kappa B signaling pathway as well as dysregulation of p16 and Ras association domain family 1A (RASSF1) (Lo, Chung, & To, 2012; Lo & Huang, 2002).

In general, the rate of somatic mutation in NPC is moderately low compared to other cancer types. Mutation in *TP53* is the most frequently reported alteration associated with NPC. Strikingly, mutation in most of the well-established oncogenes (such as *EGFR*, *PIK3CA*, *KRAS*, *HRAS*, *NRAS*, and *BRAF*) is seldom observed in NPC patients. On the other hand, epigenetic regulators such as *ARID1A*, *BAP1*, *KMT2B*, *KMT2C*, *KMT2D*, *TSHz3*, *HDAc4*, *PAXIP1*, and many others are commonly mutated in NPC patients. In addition, copy number variations in chromosomes 1q, 2q, 3q, 3p, 4q, 6q, 7q, 8p, 8q, 9p, 9q, 11q, 12p, 12q, 13q, 14q, 16q, and 17q have also been frequently observed in NPC.

Histopathologically, NPC can be broadly classified into three major categories: nasopharyngeal squamous cell carcinoma (which may be keratinizing or nonkeratinizing); nasopharyngeal undifferentiated carcinoma (NPUC; poorly or undifferentiated carcinoma); and nasopharyngeal adenocarcinoma (Ali et al., 2017). There is also an interindividual difference in the genomic landscape of the three subtypes of NPC that supports the hypothesis that the etiopathogenesis of different NPC subtypes may be associated with different genetic alterations. The diversity seen is crucial in the discovery of novel potential treatment strategies for NPC (Ali et al., 2017). The relationship between various factors associated with the pathogenesis of NPC is illustrated in Fig. 3.1.

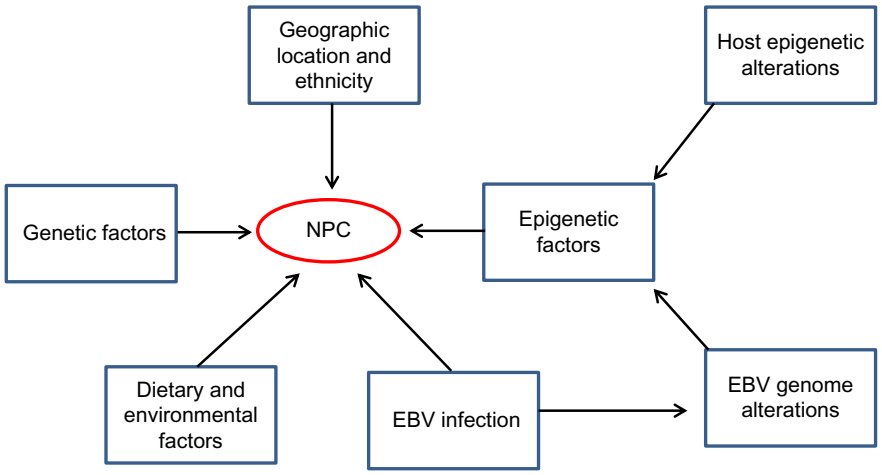

Figure 3.1
Various factors contributing to the development of nasopharyngeal carcinoma (NPC).

Genetic susceptibility to nasopharyngeal carcinoma

Genetic variations can lead to differences in gene function, which in turn can lead to increased or decreased susceptibility to diseases like cancer. A lot has been revealed about the involvement of genetic factors in the etiology of NPC together with the interplay of EBV infection and environmental factors. It has been observed that NPC is prevalent in Southern China, Northern African regions, and Alaska (Bei, Zuo, Liu, Guo, & Zeng, 2016; McDermott, Dutt, & Watkinson, 2001), suggesting a significant contribution by geographic location. As far back as almost half a century ago, there was strong evidence that migration significantly increased the risk of NPC among Chinese migrants in California when the incidence was compared with that of the local Caucasians (Buell, 1974). This is further supported by the high prevalence of the NPC among Chinese in Malaysia and Singapore (Curado et al., 2007), thus suggesting genetic predisposition in the pathogenesis of NPC.

Epidemiological association studies conducted between the year 2000 and 2011 (Hildesheim & Wang, 2012) as well as case–control studies (Bei, Jia, & Zeng, 2012) have also revealed a relationship between genetic polymorphisms and risk of developing NPC. Although most of these studies have not been replicated elsewhere, a number of genes including human leukocyte antigen genes such as *DRB1*, *DQA1*, *DQB1*, and *DPB1*; DNA repair gene *RAD51L1*; cell cycle control genes *MDM2* and *TP53* as well as cell adhesion/migration gene *MMP2* were consistently reported to be associated with NPC occurrence (Hildesheim & Wang, 2012). Furthermore, genetic polymorphism involving cytokines genes such as *IL1A* (rs3783553), *IL1B* (−511T), *IL2* (−330G), *IL8* (−251A), *IL10* (−1082G), *IL12* (rs3212227), *IL16* (rs11556218), and *IL18* (−137C) is also associated with NPC development (Bei et al., 2012). However, most of the cytokines genes require further studies to replicate their contributions in NPC pathogenesis. Other immune-related genes observed to be associated with NPC development include toll-like receptor (*TLR*)3 (829A > C), *TLR4* (11350G > C), *TLR10* (haplotype GCGTGGC for rs10856837, rs11466651, rs11466652, rs11466653, rs11096956, rs11096955, and rs11466655) [74], DC-SIGN (−139A > G and −939G > A), CTLA-4 (+49A > G), MKK4 (−1304T > G), and IGk (rs232230, C allele) (Bei et al., 2012).

It is well known that consumption of salted fish and other food preservatives as well as smoking and tobacco consumption are implicated in NPC etiology (Roy Chattopadhyay, Das, Chatterjee, & Choudhuri, 2017). Carcinogens such as *N*-nitrosamines, aromatic amines, and polycyclic aromatic hydrocarbons that are commonly found in these dietary risk factors are predominantly metabolized by the cytochrome P450 (CYP) super family (Wahlang, Falkner, Cave, & Prough, 2015). Gene polymorphisms in some of these important enzymes influence the metabolism of carcinogens that may in turn alter the risk of NPC development (Bei et al., 2012). One of the most implicated members of the CYP super family involved in increasing susceptibility to NPC is CPY2E1, which is consistently

demonstrated to exhibit association with NPC risk (Bei et al., 2012). DNA damage due to EBV infection or other carcinogenic factors is also believed to contribute to NPC tumorigenesis (Huang et al., 2011; Wang et al., 2017). On the other hand, although linkage studies have been reported on susceptibility loci on 3p21.31-21.2 (Xiong et al., 2004), 4p15, 1-q12 (Feng et al., 2002), and 5p13 (Hu et al., 2008), the findings were inconsistent (Bei et al., 2016). A number of reasons including poor statistical power, variation in NPC types, and heterogeneity of population were among other factors suggested to contribute to the lack of concordance (Bei et al., 2012). Thus more linkage studies are warranted to delineate the existence of susceptibility loci for NPC development.

Epigenetic alterations and nasopharyngeal carcinoma

Epigenetics, as a relatively recent field, has improved the understanding of the contribution of genetics in the pathogenesis of NPC. In general, unlike genetic changes that are typically irreversible, epigenetic modifications are believed to be reversible (Yoo & Jones, 2006) and recent studies suggest that epigenetic mechanisms such as DNA methylation, histone modifications, and microRNAs (miRNAs; miRs), which are frequently deregulated in both host cells and EBV genome may be implicated in the initiation and progression of NPC (Shyamasundar et al., 2016).

DNA methylation and nasopharyngeal carcinoma

DNA methylation entails the addition of a methyl group to the 5-carbon of cytosine in CpG islands (Cheng & Blumenthal, 2008). The EBV genome is often found in all NPC cells of patients with EBV associated NPC, indicating a strong link between EBV latent infection and NPC development (Lo & Huang, 2002). DNA methylation can result in the silencing of both EBV genome and host genome. EBV latent infection is believed to be crucial in NPC development and it has been observed that the latent EBV genes are silenced by DNA methylation (Li & Minarovits, 2003). The EBV-encoded latent membrane protein 1 (LMP1), which is believed to enhance the activity of DNA methyltransferase 1 (a DNA methylating enzyme) in EBV infected NPC cells (Tsai et al., 2006), is significantly expressed in the majority of NPC patients, suggesting a role in NPC development (Lao, Nguyen, Nguyen, & Le, 2017). The LMP1 is also believed to promote NPC metastatic potentials via a number of intracellular signaling mechanisms including neutralization of integrin-α5 and *N*-cadherin molecules (Wasil & Shair, 2015). Silencing of E-cadherin gene (that has been consistently reported in NPCs) due to hypermethylation promotes tumor invasion and metastasis (Semb & Christofori, 1998) and in fact, loss of membranous E-cadherin expression is reported to be associated with NPC metastasis and poor patient survival (Zheng et al., 1999). Furthermore, abnormal methylation of tumor suppressors *RASSF1*, *CDKN2A*, *ADAMTS9*, *PTPRG*, *ZMYND10*, *FBLN2*, *CRYAB*, *CADM1*, *THY1*, *MMP19*, *DUSP6*, *MIPOL1*, and *LTBP2* were seen in NPC.

Earlier reports implicated the presence of hypermethylation of a single gene *EDNRB* that was detected in 90.5% of NPC tumors. Similarly, it was observed that 74% of primary NPC tumors expressed hypermethylation of *BLU* promoter region, in contrast to nonneoplastic nasopharyngeal tissues that exhibited low methylation. Several other gene methylations were reported in NPC that include *RARbeta2* (80%), *DAP-kinase* (76%), *p16* (46%), *p15* (17%), *p14* (20%), *MGMT* (20%), *CCNA1* (48%), *RARRES1* (51%), and *HRASLS* (17%) (Jiang, Cai, & Chen, 2015). Similarly, several investigations have established a high frequency of inactivation of *UCHL1*, *WIF-1*, *RASSFIA*, *FEZF2*, *LOX*, *Kank1*, and *RRAD* through promoter methylation in NPC (Jiang, Cai, et al., 2015).

Moreover, DNA methylation involving the tumor suppressor genes (TSGs) is also crucial in molecular signaling pathways regulating the NPC progression. For instance, hypermethylation of *ADAMTS8* is associated with progression of NPC by antagonizing EGFR–MEK–ERK signaling pathway (Choi et al., 2014). In addition, significant epigenetic disruption of Wnt, mitogen-activated protein kinase (MAPK), transforming growth factor beta (TGF-β), and Hedgehog signaling pathways were also observed in NPC (Li et al., 2015). A summary of hypermethylated genes associated with NPC is shown in Table 3.1

Table 3.1 A list of hypermethylated genes and their potential clinical application in nasopharyngeal carcinoma (NPC).

Marker gene	Geographic location	Ethnic group	Potential clinical application	References
RASSF1A	East Asia and North Africa	Chinese and Moroccans	Diagnostic	Chang et al. (2003), Nawaz, Moumad, et al. (2015), Tian et al. (2013), Tong et al. (2002), and Wong, Tang, et al. (2003)
P16	North Africa	Moroccans and Tunisians	Diagnostic	Ayadi et al. (2008), Challouf et al. (2012), and Nawaz, Moumad, et al. (2015)
ITGA9	North Africa	Moroccans	Diagnostic	Nawaz, Hu, et al. (2015)
PCDH20	East Asia	Chinese	Diagnostic	Chen et al. (2015)
SLIT2	East Asia	Chinese	Diagnostic	Li et al. (2018)
TIPE3	East Asia	Chinese	Prognostic	Ren et al. (2018)
MSH3	East Asia	Chinese	Prognostic	Ni et al. (2017)
ECRG4	East Asia	Chinese	Prognostic	You et al. (2015)
WWOX	East Asia	Chinese	Diagnostic	Yang et al. (2014)
CYB5R2	East Asia	Chinese	Diagnostic	Xiao et al. (2014)
SHISA3	East Asia	Chinese	Therapeutic	Zhang, Li, et al. (2019)
NFAT1	East Asia	Chinese	Therapeutic	Zhang, Zheng, et al. (2019)

(*Continued*)

Table 3.1 A list of hypermethylated genes and their potential clinical application in nasopharyngeal carcinoma (NPC). ***Continued***

Marker gene	Geographic location	Ethnic group	Potential clinical application	References
miRNA-148a	East Asia	Taiwanese	Diagnostic and therapeutic	Li et al. (2014)
PTEN	East Asia	Chinese	Diagnostic	Li et al. (2014)
LOX	East Asia	Chinese	Diagnostic	Sung et al. (2014)
SOX11	East Asia	Chinese	Diagnostic and prognostic	Zhang, Li, and Gao (2013)
UCHL1	East Asia	Chinese	Diagnostic	Tian et al. (2013)
TTC40	North Africa	Tunisians	Diagnostic	Ayadi et al. (2014)
CDK10	East Asia	Chinese	Diagnostic	You et al. (2013)
RAB37	East Asia	Chinese	Prognostic	Li et al. (2018)
CLDN11	East Asia	Taiwanese	Therapeutic	Li et al. (2018)
DLEC1	East Asia	Chinese	Diagnostic	Tian et al. (2013)
FEZF2	East Asia	Chinese	Diagnostic	Shu et al. (2013)
RRAD	East Asia	Chinese	Diagnostic	Mo et al. (2012)
CACNA2D3	East Asia	Chinese	Diagnostic and therapeutic	Wong et al. (2013)
E-cadherin	East Asia	Chinese	Therapeutic	Ran, Wu, and You (2011)
MORF4L1	East Asia	Chinese	Therapeutic	Sang et al. (2019)
PMS2	East Asia	Chinese	Therapeutic	Ni et al. (2016)
DAPK	East Asia and Europe	Chinese and Dutch	Diagnostic and prognostic	Chang et al. (2003) and Ooft et al. (2018)
CDH1	East Asia	Chinese	Diagnostic	Wong et al. (2004)
HIN-1	East Asia	Chinese	Diagnostic	Wong, Kwong, et al. (2003)
DACT2	East Asia	Chinese	Diagnostic and therapeutic	Zhang et al. (2018)
ZNF154	East Asia	Chinese	prognostic	Hu et al. (2017)
MGMT	East Asia	Chinese	Diagnostic	Wong, Tang, et al. (2003)
MLH1	East Asia	Chinese	Diagnostic	Wong, Tang, et al. (2003)
p15	East Asia	Chinese	Diagnostic	Wong, Tang, et al. (2003)
CADM1	Southeast Asia	Indonesians	Diagnostic	Hutajulu et al. (2011)
DLC1	Southeast Asia	Indonesians	Diagnostic	Hutajulu et al. (2011)
PCDH8	East Asia	Chinese	Diagnostic	He et al. (2012)

Histone modifications

Histones are proteins found in the nucleus that are packaged together with DNA to form the nucleosomes (Peterson & Laniel, 2004). Histone modification refers to posttranslational alterations in the N-terminus of histones that affect gene expression due to the resulting modification in chromatin packaging in the nucleus without alterations in DNA sequence (Chrun, Modolo, & Daniel, 2017; Kouzarides, 2007; Lachner, O'Sullivan, & Jenuwein, 2003).

Evidence from both in vitro and clinical studies suggest that trimethylated histone H3 lysine 27 (H3K27me3), which is associated with tumor aggressiveness and chemoresistance, is upregulated in both NPC cell lines and tissues compared to normal cells and nonneoplastic tissues (Cai et al., 2011). In addition, similar to other human cancer types, Enhancer of Zeste homolog 2 (EZH2), which is a mediator of H3K27 methylation (Cao et al., 2002) and a crucial factor in silencing of E-cadherin in cancer (Cao et al., 2008), was found to be upregulated in NPC patients (Tong et al., 2012). Additionally, enhanced phosphorylation of histone H3 serine 10 (p-H3Ser10) whose activation during cell division is crucial for cancer development (Kim et al., 2008) is reported to play a key role in NPC pathogenesis (Li et al., 2013).

Histone modifications involving the EBV genome have also been reported (Shyamasundar et al., 2016). Of note, is the contribution of specific histone modifications involving histone H3 lysine 27 trimethylation (H3K27me3), H3K9me2/me3, and H4K20me3, which have been reported to regulate EBV latent and lytic cycles in EBV infected Raji cells (Murata et al., 2012).

Long noncoding RNAs and nasopharyngeal carcinoma

Recent technological advances have indeed triggered renewed interest in the discovery of potential genetic biomarkers of NPC. One of such markers that have received a lot of attention is a class of nonprotein coding RNA transcripts known as long noncoding RNAs (lncRNAs). LncRNAs are typically defined as transcripts with greater than 200 nucleotides in length without protein-coding sequence (Quinn & Chang, 2016). Once thought to be mRNA-like transcripts, the lncRNAs have recently been reported to be very distinct from mRNAs. It is suggested that the quantity of lncRNAs may be equal to that of protein-coding genes in the human genome (Prensner & Chinnaiyan, 2011). X inactivate-specific transcript (XIST) is a lncRNA located on Xq13 that has been implicated in several types of cancer. XIST exerts oncogenic effect on NPC development by upregulating the expression of miR-34a-5p targeted gene E2F3. Another recently discovered lncRNA known as lncRNA-ROR (located on 18q21) was reported to be upregulated in NPC tissue samples and is associated with proliferation, metastasis, and chemoresistance in NPC by way of suppressing the p53 signaling pathway. HOX antisense intergenic RNA (Hotair), a lncRNA located on 12q13.13 is also

associated with progression of NPC by promoting angiogenesis through activation of vascular endothelial growth factor A, glucose regulated protein 78 (GRP78), and angiopoietin2. Other lncRNAs implicated in the development and/or progression of NPC include actin filament-associated protein 1 antisense RNA1 (AFAP1-AS1, located on 4q16.1), while HNF1A-AS (located on 12q24.31), lncRNA-LET (located on 15q24.1), H19 (located on 11p15.5), NEAT1 (located on 11q13), MALAT1 (located on 11q13.1), LOC401317, and LINC00312 (located on 3p26) (He et al., 2017; Zhao et al., 2018) make up the rest.

miRNAs and nasopharyngeal carcinoma

MiRNAs are small noncoding RNA molecules of approximately 22 nucleotides in length that regulate posttranscriptional gene expression. Deregulated miRNAs have been implicated in the initiation, progression, and response to treatment of cancers including NPC (Spence, Bruce, Yip, & Liu, 2016). In addition to the human genome miRNAs, several EBV genome miRNAs including the highly expressed (in the epithelium of NPC) *EBV-mir-BARTs* and a host of others like *miR-29c*, *miR-9*, *miR-26a*, *miR-34c*, *let-7* family, *miR-98*, *miR-200* family, *miR18a/b*, *miR-141*, *miR-155*, *miR-214*, *miR-375*, and *miR-451* have also been implicated in the pathogenesis of NPC or its response to treatment (Spence et al., 2016; Wang et al., 2017). For instance, the low expression of miR-29c is associated with chemo- and radio-resistances is believed to occur via repression of expression of two important antiapoptotic factors-mcl-1 and bcl-2 (Zhang et al., 2013). A number of miRNAs including miR-30a, miR-93, miR-149, miR-155, and miR378 that are known to be metastasis promoters and others like miR-9, miR-29c, and miR-200a that are reported to be metastasis inhibitors are selectively dysregulated in NPC (Tan, Tang, & Tang, 2015).

In general, the role of miRNAs in the pathogenesis of NPC and its response to treatment is diverse, ranging from acting as TSGs to being oncogenic. The miRNAs that act as TSGs are mostly underexpressed; a phenomenon that may lead to deregulation of cellular processes and apoptosis, as well as invasion, metastasis, angiogenesis, and epithelial–mesenchymal transition (EMT) among other mechanisms (Spence et al., 2016). On the other hand, the miRNAs that act as oncogenic are usually overexpressed and may operate by causing a downregulation of other miRNAs or gene expression leading to proliferation, migration, and invasion of NPC cells (Spence et al., 2016).

Although it has been widely reported that EBV infection is closely associated with the pathogenesis of NPC, the exact mechanism through which it causes the disease is yet to be fully established (Wang et al., 2017). The detection of *EBV-mir-BARTs* at significant levels in NPC has therefore triggered a renewed interest in investigating the roles of EBV-encoded miRNAs in the pathogenesis

of NPC. Some of the major *EBV-mir-BARTs* implicated in the development of NPC include *EBV-mir-BART2*, *EBV-mir-BART3**, *EBV-mir-BART5*, *EBV-mir-BART6-5p*, *EBV-mir-BART7*, *EBV-mir-BART9*, *EBV-mir-BART10*, and *BART1-5p*, *16*, *17-5p* (Wang et al., 2017). mir-BARTs can promote NPC cell growth and development by targeting a number of proliferative and apoptosis-related factors such as p53, Bcl-2 interacting mediator of cell death (Bim), deleted in cancer 1, caspase 3, and a host of other factors (Huang, Tsao, & Tsang, 2018). In addition to this role, the mir-BARTs are also implicated in NPC cell invasion and metastasis (Huang et al., 2018). A list of both human genome and EBV genome miRNAs is given in Table 3.2.

Other Epstein–Barr virus genome products and nasopharyngeal carcinoma

In addition to the EBV-encoded miRNAs, a number of EBV genome products including the viral proteins and other RNAs such as nonadenylated EBV-encoded small RNAs (EBERs 1 and 2) may be implicated in the pathogenesis of NPC (Huang et al., 2018; Raab-Traub, 2002). However, the mechanism of how other EBV genome products promote host cell proliferation in NPC patients requires further investigation so as to serve as future potential therapeutic targets in developing new armamentarium against NPC.

Molecular signaling pathways in nasopharyngeal carcinoma

Molecular signaling pathways that are crucial for cell survival, growth and metastasis have been reported to be altered in NPC where various biomarkers that promote abnormal proliferation of the NPC cells are implicated in these signaling pathways (Liu, Chen, Huang, & Huang, 2015). Epigenetic inactivation of negative wingless-type (Wnt)/β-catenin signaling regulators is associated with abnormal activation of the said pathway in NPC development (Li, Shu, Wang, Cao, & Tao, 2011; Li et al., 2015). The altered Wnt/β-catenin that promotes NPC development is linked to epigenetic silencing of WIF-1 (Li et al., 2011). PRDM5, which has been shown to inhibit aberrant Wnt/β-catenin, was reported to be downregulated in NPC cell lines suggesting a loss of its tumor suppressor activity that potentially promotes NPC development (Shu et al., 2011).

Epidermal growth factor receptor (EGFR) is one of the crucial regulators of cell growth that has been shown to be highly expressed in NPC (Leong, Loh, Putti, Goh, & Tan, 2004; Pan et al., 2013). The overexpression of EGFR is associated with poor overall survival and disease-free survival in NPC (Ma et al., 2014; Ooft et al., 2015; Sun et al., 2014). The overexpression of vascular endothelial growth factor in tumors is associated with lymph node metastasis and a poorer prognosis in NPC patients (Chang et al., 2011; Krishna, James, & Balaram, 2006; Li et al., 2008; Wakisaka et al., 1999).

Table 3.2 A summary of human and Epstein–Barr virus (EBV) genomes miRNAs, their targeted signaling pathways, and potential clinical applications in nasopharyngeal carcinoma (NPC).

miRNAs	Targeted signaling pathway(s)	Potential clinical application(s)	References
Human			
miR-9	CXCR4/SDF-1/p38 mitogen-activated protein kinase (MAPK) pathway	Diagnostic and therapeutic	Lu et al. (2014)
miR-16	FMNL1 and metastatic tumor antigen 1 (MTA1)	Prognostic and therapeutic	Chen et al. (2018)
miR-17-5p	p21	Diagnostic and therapeutic	Chen, Yang, Zhang, and Wang (2016)
miR-18a	STK4	Diagnostic	Li, Ju, Ling, Jiang, and Peng (2017)
miR-26a	Enhancer of Zeste homolog 2 (EZH2)	Therapeutic	Yu et al. (2013)
miR-27a-3p	Mapk10	Prognostic and therapeutic	Li and Luo (2017)
miR-29c	TIAM1 and ITGB1	Prognostic and therapeutic	Huang et al. (2019) and Liu, Tang, et al. (2013)
miR-30a	E-cadherin	Prognostic and therapeutic	Wang et al. (2014)
MiR-30e-5p	USP22	Therapeutic	Ma, Zhang, Li, and Liu (2018)
miR-34a	Transforming growth factor beta (TGF-β) pathway and SMAD4	Therapeutic	Huang et al. (2018)
miR-34c	MET	Therapeutic	Li et al. (2015)
miR-92b	Smad3	Prognostic, therapeutic	Zhao, Zhao, Feng, Xu, and Qin (2017)
miR-93	TGFβR2, Smad-dependent TGF-β, and PI3K/Akt	Prognostic, therapeutic	Lyu et al. (2014)
miR-98	MTDH	Prognostic	Tan et al. (2017)
miR-99a	HOXA1	Therapeutic	Wang, Tang, Liao, Liu, and Ai (2017)
miR-122	TRIM29 and PI3K/Akt	Diagnostic, therapeutic	Yang, Li, and Guo (2018)
miR-122-5p	SATB1	Therapeutic	Liu et al. (2019)
miR-124	MALAT1/miR-124/Capn4	Therapeutic	Shi, Wang, and Yin (2017)
miR-130a-3p	BACH2 and EMT	Prognostic	Chen et al. (2017)
miR-141	UBAP1, PTEN, and BRD3/Rb/E2F	Diagnostic and therapeutic	Liu et al. (2018) and Zhang et al. (2010)
miR-143	ERK-5, KRAS, caspase 3, and Bcl-2	Diagnostic	Chen et al. (2016)

Table 3.2 A summary of human and Epstein–Barr virus (EBV) genomes miRNAs, their targeted signaling pathways, and potential clinical applications in nasopharyngeal carcinoma (NPC). ***Continued***

miRNAs	Targeted signaling pathway(s)	Potential clinical application(s)	References
miR-144	PTEN and PI3K/Ak	Prognostic	Zhang et al. (2013)
miR-148b	MTA2	Therapeutic	Wu et al. (2017)
miR-149	E-Cadherin	Prognostic	Luo et al. (2011)
miR-153	TGF-β_2/Smad2	Therapeutic	Guo, Zhang, Hu, and Bian (2019)
miR-155	JMJD1A and BACH1	Therapeutic	Du et al. (2011)
miR-184	Notch2	Prognostic	Zhu, Ma, Zhuang, and Jin (2018)
miR-185	TGF-β1/mTOR and HOXC6	Therapeutic	Cheng, Chen, Wang, and Yu (2018)
miR-185-3p	WNT2B	Therapeutic	Liu, Li, Ren, et al. (2017)
miR-194	MAP3K3	Therapeutic	Yin, Shi, and Mao (2019)
miR-200a	ZEB2 and E-cadherin	Therapeutic	Korpal, Lee, Hu, and Kang (2008)
miR-200c	PTEN	Diagnostic, prognostic, and therapeutic	Cao and Sun (2019) and Chen et al. (2017)
miR-203a-3p	LASP1	Therapeutic	Jiang et al. (2017)
miR-212	SOX4	Therapeutic	Jiang et al. (2017)
miR-214	LTF, Akt	Therapeutic	Deng et al. (2013)
miR-216b	KRAS/Akt and ERK	Therapeutic	Deng et al. (2011)
miR-324-3p	WNT2B	Therapeutic	Liu, Li, Yang, et al. (2017)
miR-328	CD44, E-cadherin, *N*-cadherin, and Snail	Prognostic and therapeutic	Lin, Chiang, and Chen (2018)
miR-342	ZEB1	Therapeutic	Zhu, Li, Zhang, and Liu (2018)
miR-342-3p	FOXQ1	Therapeutic	Cui and Zhao (2019)
miR-346	BRMS1	Therapeutic	Yan, Li, Li, Zhang, and Xu (2016)
miR-371-5p	BCL2	Therapeutic	Deng, Su, Xie, and Tang (2018)
miR-372	PBK/p53	Therapeutic	Wang et al. (2019)
miR-374a	CCND1 and PI3K/Akt	Therapeutic	Zhen et al. (2017)
miR-379	TPD52	Therapeutic	Zhao and Chu (2018)
miR-425	HDGF	Therapeutic	Zhu et al. (2018)

(*Continued*)

Table 3.2 A summary of human and Epstein–Barr virus (EBV) genomes miRNAs, their targeted signaling pathways, and potential clinical applications in nasopharyngeal carcinoma (NPC). ***Continued***

miRNAs	Targeted signaling pathway(s)	Potential clinical application(s)	References
miR-451	MIF	Therapeutic	Liu, Jiang, et al. (2013)
miR-597	14-3-3σ	Therapeutic	Xie et al. (2018)
miR-629	PDCD4	Therapeutic	Zheng et al. (2019)
miR-663b	TUSC2 and SMAD7	Therapeutic	Liang et al. (2017) and Wang, Jia, and Yuan (2018)
miR-944	MACC1 and E-cadherin	Prognostic and therapeutic	Wen et al. (2017)
miR-1181	Wnt/β-catenin	Prognostic and therapeutic	Hua and Fan (2019)
miR-4288	ELF3	Preventive	Ke, Xie, Zheng, and Chen (2019)
EBV			
EBV-miR-BART1	PI3K-Akt, FAK-p130 (Cas), and Shc-MAPK/ERK1/2	Therapeutic	Cai, Ye, et al. (2015)
miR-BART4	PTEN	Therapeutic	Wu, Han, Sheng, Zhang, and Wang (2018)
miR-BART6-3p	LOC553103	Diagnostic and therapeutic	He et al. (2016)
miR-BART7-3p	PTEN, PI3K/Akt, and c-Myc c-Jun	Therapeutic	Cai, Li, et al. (2015)
miR-BART8-3p	NF-κB and Erk1/2	Therapeutic	Lin et al. (2018)
miR-BART9	E-Cadherin	Prognostic	Hsu et al. (2014)
miR-BART10-3p	BTRC, β-catenin, and Snail	Diagnostic, prognostic, and therapeutic	Yan et al. (2015)
miR-BART13	NKIRAS2/NF-κB	Therapeutic	Xu et al. (2019)

PTEN, Phosphatase and tensin homolog; *KRAS*, Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; *BTRC*, beta-transducin repeat containing; *NF-κB*, nuclear factor kappa-B.

It is known that EBV infection induce modulation of cell signaling pathways in nasopharyngeal cells that contribute to proliferation of the infected cells and subsequent NPC development (Lo et al., 2006). The EBV latent membrane proteins (LMPs) particularly LMP1 have been reported to facilitate NPC development by alteration of a number of signaling pathways that include the overexpression of cluster of differentiation 40 and intercellular adhesion molecule-1 (ICAM-1), activation of NF-kB pathway, activation of c-Myc, and activation of mechanistic target of rapamycin (mTORC1)/NF-kB pathways (Huang et al., 2018).

Alteration of cell signaling pathways can also promote NPC metastasis and/or invasion as observed from the ability of LMP1 to: (1) promote migration of epithelial cells via modulation of integrin-α5 and *N*-cadherin (Wasil & Shair, 2015); (2) induce miR-10b that facilitates NPC metastasis (Li et al., 2010); (3) downregulate the tumor suppressor activity of miR-204, subsequently promoting the invasion of nasopharyngeal cells (Ma, Deng, Wu, Zhang, & Huang, 2014); and (4) induce cell motility by promoting transcription of tumor necrosis factor α-induced protein 2 (Chen et al., 2014).

The overexpression of EBV-encoded LMP2A has also been consistently documented in NPC cells (Busson et al., 1992; Heussinger et al., 2004) and is associated with increased invasiveness and metastasis in NPC (Kong et al., 2010; Pegtel et al., 2005). LMP2A may also enhance NPC cells invasion through ERK/Fra-1 pathway by overexpression of matrix metalloproteinase 9 (Lan et al., 2012). LMP2A is also reported to promote EMT in NPC via metastatic tumor antigen 1 (MTA1) and mTOR pathways (Lin et al., 2014). A recent finding revealed that LMPA2 promoted NPC metastasis by interfering with cofilin (an actin depolymerizing factor) and proteasomal degradation that in turn facilitates cellular motility (Gainullin et al., 2017).

Liang et al. who recently investigated the effects of LMPA2 on EGFR/Ca^{2+}/caplain/ITGβ4 signaling pathways in stimulating the migration of NPC cells found that it causes increased motility of NPC cells (Liang et al., 2017). In a related study, Lee et al. investigated the contribution of LMP2A on NPC cells migration through the oncogenic sphingosine-1-phosphate (S1P) signaling pathway and observed that its expression is associated with the upregulation of sphingosine kinase 1 enzyme that is key to S1P synthesis (Lee et al., 2017). S1P is believed to promote cancers including NPC as well as cell migration via activation of protein kinase B (AKT) (Lee et al., 2017; Patmanathan, Wang, Yap, Herr, & Paterson, 2017).

Role of genomic profiling

Although targeted therapy for common cancers such as breast, colorectal, and lung cancers appears to be promising (Abubakar & Gan, 2016), to date no molecular targeted therapy has been approved for NPC (Ali et al., 2017). A successful discovery of novel therapeutic targets to improve NPC treatment therefore requires a wide range of genomic profiling. Genomic profiling refers to a laboratory technique that is utilized to investigate all the genes in an individual or in a specific cell type and to explain the way the involved genes interact with one another and the environment. Although no well-established classification of NPCs into clinically relevant treatment groups has been developed (Spence et al., 2016), genomic profiling can potentially be used to classify tumors into different subtypes in order to provide an accurate diagnosis of each subtype. In recent times, several genomic profiling studies have

consistently indicated that genetic alterations in nuclear factor kappa-B (NF-κB) and ErbB-phosphatidylinositol 3-kinase (PI3K)-protein kinase B (AKT) signaling pathway may predict response to molecular targeted therapy (Ma, Hui, & Chan, 2017).

Genetic and epigenetic screening

Epstein–Barr virus DNA testing

It is well established that EBV infection is associated with NPC development. Additionally, it has also been shown that EBV DNA analysis can be used for early detection and monitoring of response of NPC to therapy that can potentially be utilized in the clinical setting and may play a key role in the choice of treatment options for NPC. It has been suggested that a strong association exist between NPC and the presence of high levels of EBV DNA in the plasma of patients with NPC (Leung et al., 2006; Lo, Chan, Chan, et al., 1999; Mutirangura et al., 1998). In fact, it is believed that the plasma EBV DNA load is inversely correlated with NPC prognosis (Leung et al., 2006).

The current standard treatment for NPC in many centers (although still controversial) is an initial chemoradiation step, followed by an adjuvant chemotherapy (Lee et al., 2011; Sze, Blanchard, Ng, Pignon, & Lee, 2015). However, the benefit of adjuvant chemotherapy remains inconclusive (Chen et al., 2012; Ribassin-Majed et al., 2017). Considering these controversies, it is believed that testing the presence (or absence) of EBV DNA in the plasma of NPC patients following chemoradiation may be useful in selecting patients likely to benefit from the additional adjuvant chemotherapy (Kim et al., 2017). At the time of writing, a clinical trial to test the hypothesis that posttreatment EBV DNA testing is useful in choosing patients who may benefit from adjuvant chemotherapy regimens is in progress (Kim et al., 2017). The EBV DNA testing may also be useful in the screening of asymptomatic individuals in endemic NPC regions for early detection of the malignancy (Chan et al., 2017; Kim et al., 2017) and in prognostication and monitoring of NPC recurrence (Leung et al., 2014; Lo, Chan, Chan, et al., 1999).

One of the major challenges of using EBV DNA as a reliable marker of NPC screening and diagnosis is the fact that it is also detected in non-NPC apparently healthy individuals (Kanakry & Ambinder, 2015; Lo, Chan, Lo, et al., 1999). Interestingly, a recent work by Lo et al. demonstrated that a difference exists in the molecular characteristics of EBV DNA in the plasma of NPC patients as compared to healthy individuals (Lam et al., 2018). In this large-scale study, they observed that using sequencing-based DNA assay, NPC patients had significantly longer fragment lengths and higher amounts of EBV DNA compared to non-NPC individuals.

DNA methylation screening

It is an interesting fact that the DNA methylation pattern often varies between normal and malignant tissues. With recent evidence demonstrating that NPC is associated with several genetic mutations and epigenetic alterations, it is plausible that the discovery of genes related to DNA methylation could pave ways for further delineation of molecular pathogenesis of NPC development that will subsequently aid in the development of biomarkers for early detection of NPC; serving as therapeutic targets in molecular targeted therapy. Especially important is the fact that DNA methylation occurs early, which makes it an excellent potential biomarker for early detection of NPC (Delpu, Cordelier, Cho, & Torrisani, 2013). For instance, the development and progression of NPC has been linked to aberrant DNA methylation at the promoter regions of NPC-related genes (Razak et al., 2010; Tao & Chan, 2007).

Evidence have also emerged that several TSGs and miRNAs are constantly silenced by DNA methylation in NPC cells (Bruce, Yip, Bratman, Ito, & Liu, 2015; Jiang, Cai, et al., 2015; Li et al., 2011). Interestingly, demethylation of these genes restores their function and results in suppression of cellular proliferation, promotion of apoptosis of NPC cells, and inhibition of their migration and invasion. It was recently revealed that following restoration of miR148a, miR31, miR34c, and miR24 expressions, NPC cell growth and migration are remarkably inhibited by targeting several different downstream genes (Jiang, Cai, et al., 2015).

Although epigenetic biomarkers appear promising as potential biomarkers of early diagnosis of NPC, a single biomarker may not be sensitive enough to accurately detect early NPC, hence necessitating the application a multiplex methylation-specific polymerase chain reaction technique that can simultaneously detect multiple relevant genes to improve the sensitivity of the early diagnostic strategies (Nawaz, Moumad, et al., 2015). In line with this, it can be hypothesized that in the very near future, DNA methylation biomarkers could be employed in early diagnosis of NPC.

Prediction of prognosis of NPC using DNA methylation biomarkers is also promising (Jiang, Cai, et al., 2015). For instance, methylation of 14-3-3 sigma (a downstream target of p53) WIF-1, DAP-kinase, RASSF1A, and RARβ2 was found to be correlated with some important prognostic factors for NPC that include lymph node status, distant metastases, and clinical stage (Fendri et al., 2010; Ye, Huang, Ni, Yang, & Chen, 2017; Yi et al., 2009). DNA methylation may also be associated with demographic factors like age at diagnosis of NPC as well as cell histological type. Hypermethylation of RASSF1A and RARβ may be used to predict the age at diagnosis of NPC and its histological types (Fendri et al., 2009). The evidence of WIF-1, UCHL1, RASSF1A, CCNA1, TP73, and SFRP1 as prognostic markers in NPC are further provided in a recent investigation (Jiang, Liu, et al., 2015).

miRNAs screening

With increasing evidence supporting the selective presence of circulating EBV-miRNAs in NPC patients, it is possible that these miRNAs could serve as biomarkers of NPC diagnosis and prognosis in the future, particularly in cases where isolation of EBV DNA is not applicable (Wang et al., 2017). However, this requires further evaluation using large-scale validation studies to establish the mechanism by which EBV-miRNAs are released and how they influence NPC development and eventually be used in clinical settings as biomarkers for both NPC diagnosis and prognosis (Spence et al., 2016).

Cost and efficacy

The use of molecular technologies in the diagnosis and prognostication of various types of cancer is on the rise; however, there is limited data on the cost of these services especially in molecular targeted therapy of NPC and other cancer types. Interestingly, over the last decade, there has been a fall in the cost of one of the most relevant techniques (next-generation sequencing) used in targeted treatment of cancer (Pages et al., 2017).

With the recent report on the implementation of molecular profiling in clinical practice that is targeted toward individualized treatments (Biankin, Piantadosi, & Hollingsworth, 2015), one of the major challenges of precision medicine in cancer therapy is the progressive discovery of an increasing number of molecular subtypes that equally require more targeted therapy to ensure high efficacy (Hollingsworth & Biankin, 2015). Interestingly, a recent report indicates that molecular diagnostic techniques in oncology constitute only about 6% of the overall cost of molecular guided therapy per individual patient, but the bulk of the cost comes from drug treatments and hospital admissions (Pages et al., 2017).

Clinical implications

Currently, major treatment modalities for NPC include surgery, chemotherapy, radiotherapy, or their combinations. Nevertheless, despite the fact that early-stage NPC is highly sensitive to radiotherapy, a significant number of patients have locally advanced disease that almost always necessitates combination of chemotherapy and radiotherapy. Although many early-stage NPC considerably responds to treatment at the initial stage, the majority of patients with advanced-stage NPC who experience severe toxicity from the chemo-radiotherapy eventually develop resistance to the combination therapy that contribute to reduction in the median survival time of the advanced cases (only 5–11 months) (Ali et al., 2017). In fact, poor understanding of the molecular events involved in the etio-pathogensis of NPC has limited the progress of discovery of novel targeted therapy (currently used in the treatment of breast, lung, and colorectal cancers and is believed to be associated with lesser toxicity and little or no resistance to combination therapy).

The prognostication of NPC (in the past) has also been largely based on its clinical TNM staging that has not been able to sufficiently predict the prognosis of the disease in patients presenting at similar clinical stage but ending up with a variety of clinical outcomes (Liu et al., 2015).

To date, the documented clinical trials on targeted therapy of NPC exist with only a few reported clinical trials targeting the vascular endothelial growth-factor receptor and epidermal growth-factor receptor (EGFR) showing some promising results (Zhang, Chen, Liu, Tang, & Mai, 2013). In addition, frequent mutations in *IDH2* (a gene that encodes isocitrate dehydrogenase 2 enzyme that converts isocitrate to α-ketoglutarate) in a number of malignancies including leukemias, gliomas, chondrosarcomas, and intrahepatic cholangiomas have been reported (Mondesir, Willekens, Touat, & de Botton, 2016; Ragon & DiNardo, 2017). Ali and colleagues recently demonstrated significant *IDH2* mutations in NPUC subtype (Ali et al., 2017). An example is enasidenib (AG-221), which is a first-in-class oral, potent, reversible, selective inhibitor of IDH2 mutant enzyme that is under clinical trial and has shown promising results in patients with advanced hematologic malignancies (with *IDH2* mutation), solid tumors, glioma, intrahepatic cholangiocarcinoma, angio-immunoblastic T-cell lymphoma, and chondrosarcoma with *IDH2* mutations (Mondesir et al., 2016). The case with enasidenib suggests that in the future, it may be possible to incorporate detection of *IDH2* mutation in molecular targeted therapy of NPC especially NPUC subtype (Ali et al., 2017). However, the outcomes of anti-EGFR therapy have so far been inconclusive with some discouraging findings attributed to the absence of *EGFR* mutations or failure in amplification as commonly observed in other types of cancers (Petersson, 2015).

With the discovery of a variety of actionable therapeutic targets in NPC through whole exome sequencing (WES) and whole genome sequencing (WGS) studies, a number of drugs are currently undergoing clinical trials. There is a growing number of studies on the genomic landscape of NPC using the WES and WGS as well as other genomic analysis (Ali et al., 2017; Li et al., 2017; Lin et al., 2014; Zhang et al., 2017; Zheng et al., 2016). There are consistent genetic alterations affecting the ErbB-PI3K-AKT signaling pathway and the numerous negative regulators of the NF-kB pathway could serve as potential therapeutic targets.

Owing to the reversible nature of epigenetic changes, it is possible to target DNA methylation in targeted therapy of NPC. Preliminary investigations with NPC cell lines have suggested some beneficial effects of decitabine (5′-aza-2′-deoxycytidine), which has been shown to decrease cell viability and promote apoptosis in NPC (Li et al., 2011; Luo et al., 2015; Zhang et al., 2013; Zhao et al., 2017) as well as enhancing chemosensitivity to cisplastin in NPC cells possibly through demethylation of ECRG4 (You et al., 2015). Similarly, it is also proposed that 5-azacytidine may enhance NPC radiosensitivity (Jiang et al., 2014). However, to date, clinical trials on NPC targeting epigenetic alterations are still lacking and are needed.

The recent understanding of crucial role played by histone modifications involving H3K27me3 and p-H3Ser10 in NPC pathogenesis also makes exploration of these alterations a worthwhile recommendation for discovery of novel therapeutic targets in the management of NPC.

Conclusions and future directions

NPC is a multifactorial disease that embroils host genetic factors, environmental factors, and EBV infection. In recent years, understanding the molecular mechanisms involved in cancer development has revolutionized its diagnostic and treatment approaches. To date, molecular targeted therapy has shown promising results in many cancers. Recent studies have shed more light on the contribution of molecular events including epigenetic alterations, cell signaling mechanisms and miRNAs dysregulation in the development of NPC. This has led to the discovery of biomarkers that can be potentially targeted therapeutically and can also be used for early detection and prognostication of NPC. Although epigenetic alterations such as DNA methylation constitute an array of potential therapeutic targets in the management of NPC, additional high-quality studies including clinical trials may help to establish the benefits of these biomarkers for early detection and improved prognosis of NPC. Furthermore, there is a paucity of data on molecular targeted therapy of NPC; thus more studies are needed to explore the potentials of the genetic and molecular biomarkers as therapeutic targets in the management of NPC. Another hurdle is the integration of genomic profiling data into clinical practice that could assist in further exploration of the molecular biomarkers and their clinical relevance.

List of abbreviations

ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9
AFAP1-AS1	Actin filament-associated protein 1 antisense RNA1
Ang2	Angiopoietin2
ARID1A	AT-rich interactive domain 1A
BAP1	BRCA1 associated protein 1
BART	BamHI fragment A rightward transcript
BRAF	v-raf murine sarcoma viral oncogene homolog B1
CADM1	Cell adhesion molecule 1
CCNA1	Cyclin A1
CD	Cluster of differentiation
CDKN2A	Cyclin-dependent kinase inhibitor 2A
CRYAB	Crystallin alpha B
bcl-2	B-cell lymphoma 2
DAP-kinase	Death associated protein kinase
DICE1	Deleted in cancer 1
DUSP6	Dual specificity phosphatase 6

EBERs	Nonadenylated EBV-encoded small RNAs
EBV	Epstein–Barr virus
ECRG4	Oesophageal carcinoma-related gene 4
EDNRB	Endothelin receptor type B
EGFR	Epidermal growth factor receptor
EMT	Epithelial–mesenchymal transition
FBLN2	Fibulin-2
FEZF2	Fez family zinc finger protein 2
GRP78	Glucose regulated protein 78
HDAc4	Histone deacetylase 4
HNF1A	Hepatocyte nuclear factor 1A
Hotair	HOX antisense intergenic RNA
HRAS	Harvey rat sarcoma viral oncogene homolog
HRASLS	HRAS like suppressor
ICAM-1	Intercellular adhesion molecule-1
IDH2	Isocitrate dehydrogenase 2
Kank1	KN motif and ankyrin repeat domain-containing protein 1
KMT2B	Histone-lysine *N*-methyltransferase 2B
KMT2C	Lysine (K)-specific methyltransferase 2C
KMT2D	Histone-lysine *N*-methyltransferase 2D
KRAS	Ki-ras2 Kirsten rat sarcoma viral oncogene homolog
lncRNAs	Long noncoding RNAs
LMP	Latent membrane proteins
LTBP2	Latent-transforming growth factor beta-binding protein 2
LOX	Lysyl oxidase
MALAT1	Metastasis associated lung adenocarcinoma transcript 1
MAPK	Mitogen-activated protein kinase
MGMT	*O*-6-Methylguanine-DNA methyltransferase
mcl-1	Myeloid cell leukemia 1
miRNAs	MicroRNAs
mTORC1	Mechanistic target of rapamcycin
MIPOL1	Mirror-image polydactyly 1
MMP	Matrix metallopeptidase
MTA1	Metastatic tumor antigen 1
NEAT1	Nuclear Enriched Abundant Transcript 1
NF-kB	Nuclear kappaB
NGS	Next-generation sequencing
NPAC	Nasopharyngeal adenocarcinoma
NPC	Nasopharyngeal carcinoma
NPSCC	Nasopharyngeal squamous cell carcinoma
NPUC	Nasopharyngeal undifferentiated carcinoma
NRAS	Neuroblastoma-ras-viral-oncogene-homolog
PAXIP1	PAX-interacting protein 1

PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PRDM5	PR domain zinc finger protein 5
PTPRG	Receptor-type tyrosine-protein phosphatase gamma
RARβ2	Retinoic acid receptor beta2
RARRES1	Retinoic acid receptor responder 1
RASSF1A	Ras association domain family protein 1A
RRAD	Ras-related associated with diabetes
S1P	Sphingosine-1-phosphate
SFRP1	Secreted frizzled-related protein 1
SPHK1	Sphingosine kinase 1
TGF-β	Transforming growth factor beta
THY1	Thy-1 cell surface antigen
TNFAIP2	Tumor necrosis factor α-induced protein 2
TSG	Tumor suppressor gene
TSHz3	Teashirt Zinc Finger Homeobox 3
UCHL1	Ubiquitin carboxyl-terminal hydrolase isozyme L1
VEGFA	Vascular endothelial growth factor A
WES	Whole exome sequencing
WGS	Whole genome sequencing
WIF-1	Wnt inhibitory factor 1
XIST	X inactivate-specific transcript
ZMYND10	Zinc finger MYND domain-containing protein 10

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4

Surgical anatomy of the nasopharynx

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Introduction

The pharynx is a conical fibromuscular tube that forms the upper part of the air and food passages. It is approximately 14 cm long and extends from the base of the skull to the lower border of the cricoid cartilage where it becomes continuous with the esophagus. The pharyngeal wall consists of four layers. They are the mucous membranes, pharyngobasilar fascia, muscular coat, and buccopharyngeal fascia. Anatomically, the pharynx is divided into the nasopharynx, oropharynx, and laryngopharynx.

Surgical anatomy of nasopharynx

The nasopharynx lies posterior to the nasal cavities in between the floor of the sphenoid sinus and the soft palate. It has a roof, a floor, an anterior wall, a posterior wall, and lateral walls. The roof is supported by the body of the sphenoid and the basilar part of the occipital bone. A collection of lymphoid tissue, called the pharyngeal tonsil, is present in the submucosa of this region. The floor is formed by the sloping upper surface of the soft palate. The pharyngeal isthmus is the opening in the floor between the free edges of the soft palate and the posterior pharyngeal wall. The posterior wall forms a continuous sloping surface with the roof. It is supported by the anterior arch of the atlas. The anterior wall is formed by the posterior nasal apertures, separated by the posterior edge of the nasal septum (Snell, 1992). Located on each side of the lateral wall is the pharyngeal orifice of the Eustachian tube. It is bounded posterosuperiorly by a mucosal elevation called the torus tubarius. It is an elevation formed by the cartilage of the Eustachian tube. Located superior and posterior of the tubal

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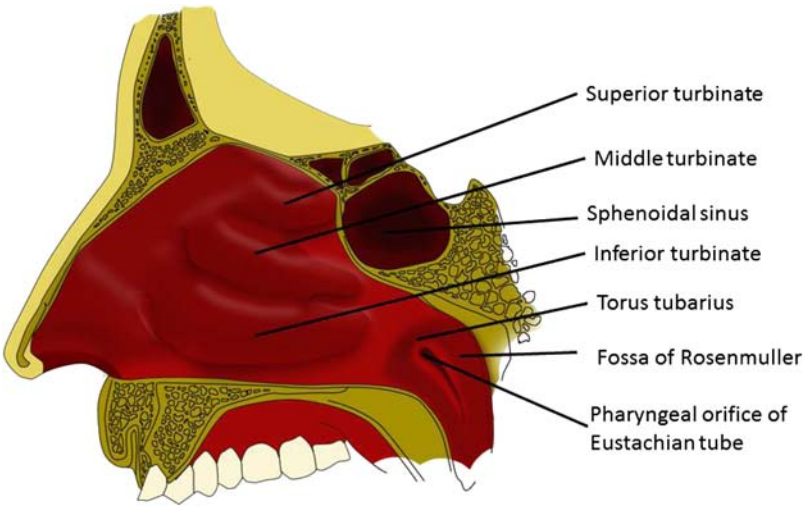

Figure 4.1 Schematic diagram illustrating the lateral wall of the right nasal cavity and nasopharynx.

elevation is a recess called the fossa of Rosenmuller. There is a mucosal ridge extending from the inferior end of the torus tubarius to the lateral pharyngeal wall. This mucosal ridge is called the salpingopharyngeal fold. It is a mucosal fold formed by the salpingopharyngeus muscle (Dhingra, 2008) (Fig. 4.1).

The fossa of Rosenmuller is a bilateral projection of the nasopharynx just inferior to the skull base. It is also called the lateral pharyngeal recess or simply the pharyngeal recess. The fossa is covered by nasopharyngeal mucosa and is the most common site of origin of nasopharyngeal carcinoma. It is lined by pseudostratified ciliated columnar epithelium with goblet cells. It is located posterior to the torus tubarius, a prominence caused by the medial cartilaginous end of the Eustachian tube (Gray, 1918). The torus tubarius is larger on the superior and posterior lips of the Eustachian tube, effectively hiding the fossa (Fig. 4.2). The fossa extends through a defect between the fibers of the superior constrictor muscle and the base of the skull. The fibers of the superior constrictor muscle project from multiple parts of the lower oropharynx to the skull base, but the fibers only reach the skull base at the midline. The lateral defect, called the sinus Morgagni, is covered by a fibrous band known as the nasopharyngeal aponeurosis. Therefore the boundaries of the fossa of Rosenmuller are defined anteriorly by the Eustachian tube and the levator veli palatini muscle, posteriorly by the posterior wall of the nasopharynx and the retropharygngeal space, laterally by the parapharyngeal space and the tensor veli palatini muscle, and inferiorly by the upper edge of the superior constrictor muscle. The fossa's superior boundary is formed by the skull base with its various openings and prominences, including the foramen spinosum, the carotid canal, foramen lacerum, and foramen ovale. From its opening in the lateral nasopharynx, the fossa projects laterally posterior to the pharyngeal orifice of the Eustachian tube. At the apex of the fossa of

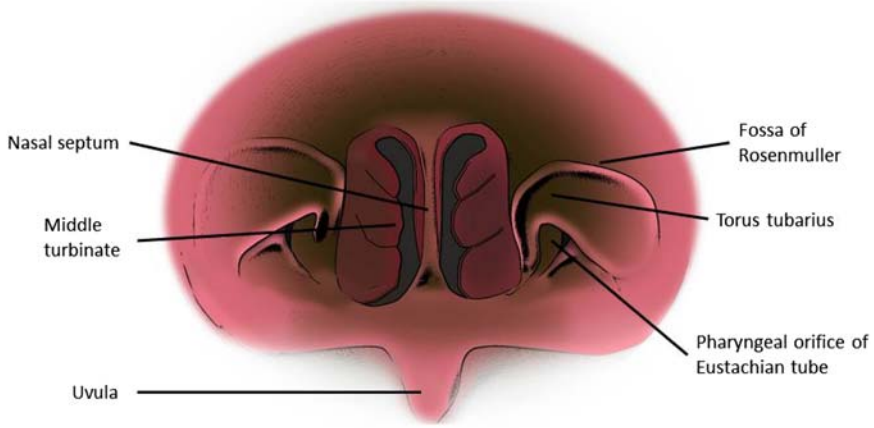

Figure 4.2
Anterior view of the nasopharynx as seen with a laryngoscope, depicting the fossa of Rosenmuller.

Rosenmuller, only a thin layer of fibroconnective tissue separates the mucosa from the cervical internal carotid artery (Amene et al., 2013).

The arterial supply of the nasopharynx is derived from branches of the ascending pharyngeal, the ascending palatine, the facial, the maxillary, and the lingual arteries. The veins drain into the pharyngeal venous plexus, which in turn drains into the internal jugular vein.

The nerve supply of the nasopharynx is mainly from the pharyngeal plexus. This plexus is formed from the branches of the glossopharyngeal, vagus, and sympathetic nerves. The sensory nerve supply of the mucous membrane of the nasal part of the pharynx is mainly from the maxillary nerve. The motor nerve supply is derived from the cranial part of the accessory nerve, which, via the branch of the vagus to the pharyngeal plexus, supplies all the muscles of the pharynx except the stylopharyngeus, which is supplied by the glossopharyngeal nerve (Snell, 1992).

The lymphatic drainage of the nasopharynx, including those of the adenoids and pharyngeal end of the Eustachian tubes, drain into upper deep cervical nodes either directly or indirectly through the retropharyngeal and parapharyngeal lymph nodes. They also drain into the spinal accessory chain of nodes in the posterior triangle of the neck. Lymphatics of the nasopharynx may also cross the midline to drain into contralateral lymph nodes.

Important surgical adjacent structures

The infratemporal fossa (ITF) is an anatomical space located under the floor of the middle cranial fossa, posterior to the maxilla, medial to the ramus of the mandible, and lateral to the nasopharynx. The greater wing of the sphenoid

bone and the subtemporal surface of the temporal bone form the roof of the ITF. The lateral pterygoid plate along with the Eustachian tube forms its medial wall. The temporalis muscle attaching to the mandibular ramus and the temporomandibular joint form its lateral border (Fig. 4.3). The infratemporal fossa communicates with the pterygopalatine fossa (PPF) via the pterygomaxillary fissure, which is continuous with the inferior orbital fissure medially (Hosseini et al., 2012). It contains the parapharyngeal and masticator spaces. The styloid diaphragm, formed by the stylopharyngeal aponeurosis, separates the parapharyngeal space into pre- and poststyloid compartments. The prestyloid compartment is a narrow and fat-containing space between the medial pterygoid and the tensor veli palatini muscles. The poststyloid compartment contains the internal carotid artery, internal jugular vein, and lower cranial nerves (9th to 12th). The masticator space contains the masseter muscle, medial and lateral pterygoid muscles, the tendon of the temporalis muscle, the internal maxillary artery, the mandibular branch of the trigerminal nerve, the tensor and levator veli palatini muscles, the styloid diaphgram, and the Eustachian tube (Falcon et al., 2011; Fernandes, Lobo, Castro, Oliveira, & Som, 2013) (Fig. 4.4).

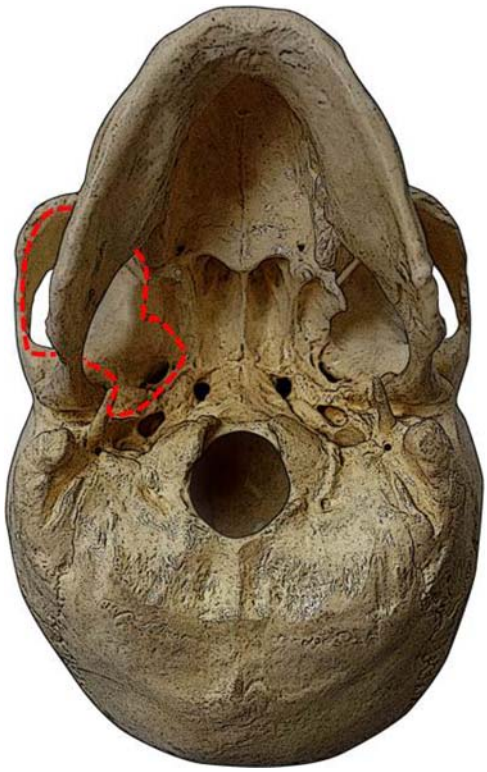

Figure 4.3
Inferior view of the base of skull. The boundary of infratemporal fossa is delineated by the *dotted line*.

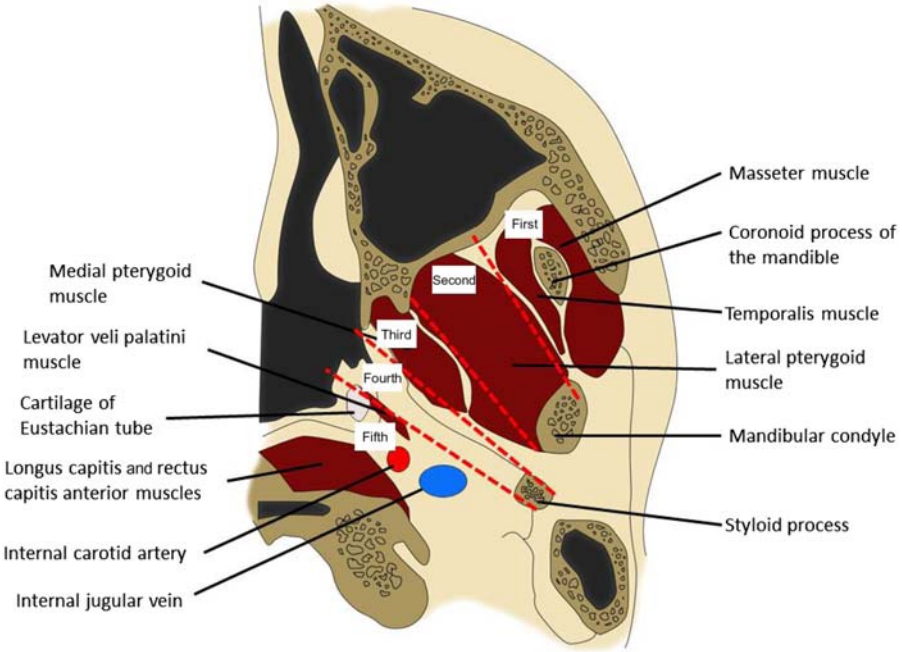

Figure 4.4
A cross-sectional view of the anatomical structures of the infratemporal fossa and its plane of dissection.

The ITF is defined in five planes of dissection, as we dissect from superficial to deep planes. The first plane of dissection lies between the lateral pterygoid muscle medially and the deep part of the temporalis muscle and mandibular ramus laterally. The second plane of dissection consists of the lateral pterygoid muscle. The third plane of dissection is located posterior to the lateral pterygoid plate, medial to the middle meningeal artery and mandibular nerve, and extends laterally to the temporomandibular joint. The fourth plane of dissection lies between the medial pterygoid and the tensor veli palatine muscles. The fifth plane of dissection lies in the poststyloid parapharyngeal space and contains the internal carotid artery (Hosseini et al., 2012).

The PPF is a pyramidal space limited by the pterygoid plates posteriorly, the palatine bone anteromedially, and the maxilla anterolaterally. The anterior compartment of the PPF contains the third portion of the internal maxillary artery and its terminal branches, whereas the posterior compartment contains the maxillary division of the trigeminal nerve and the sphenopalatine ganglion and its branches. The pterygomaxillary fissure forms the boundary as well as communication between the PPF and the infratemporal fossa. The fissure is also continuous with the infraorbital fissure. This portion of the infraorbital nerve delineates the border between the ITF and the PPF (Falcon et al., 2011) (Fig. 4.4).

Conclusion

Surgical anatomy of the nasopharynx and adjacent structures is essential for understanding the diseases of this region and especially for planning of surgical interventions at the nasopharynx.

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5

Pathology classification of nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) is an uncommon cancer of the back of the head and throat, which has a distinctive ethnic and geographic distribution. It is endemic to southern China and Asia and is etiologically associated with the Epstein–Barr virus (EBV) (Ma, Hui, & Chan, 2017). Since NPC is located in a silent and painless area, diagnosis typically occurs at the advanced stages. Often, the biopsy taken from the fossa of Rosenmuller (FOR) is inadequate for histopathological assessment and diagnosis.

The evolved history of nasopharyngeal carcinoma pathology classifications

Classification schemes of certain tumors are meant to be used to provide a foundation for tumor diagnosis with a reproducible approach to terminology. Classification schemes avoid confusion among a patient's primary care physician, surgeon, oncologist, radiation therapist, or geneticist, which could result in unnecessary delay of treatment. The classification should aid them in the treatment plan, give an indication of prognosis, allow for an evaluation of the treatment results, and facilitate the effectiveness of patient management.

Historically, there have been consistent debates on the histopathological classifications of NPC. It became the focus of interest to many pathologists from all

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parts of the world, who created varying terminologies and classifications. China is one of the countries with higher incidences of NPC. However, despite continuous advance of Chinese and International NPC histopathological classification research, it was found to be difficult to unify and standardized these two classifications.

The biggest obstacle in classifying NPC is that it lacks a universally accepted system for a comprehensive, but prognostically significant, histopathologic classification. There is no unification between global and Chinese classifications. The terminologies and the incidences according to a different type of classifications between global and Chinese classifications was not tally with each other and show obvious regional discrepancies.

Michaux was the first pathologist to describe NPC, calling it the "skull base cancer" in 1845. It was a very general terminology, which Trotter then renamed "endothelioma" in 1911. Reverchon and Coutard (1921) brought up "lymphoepithelial carcinoma" 10 years later, which described the morphology of NPC (Wei et al., 2011). From 1935 to 1940 the Quick and Cutler (1927) "transitional cell carcinoma," and believed it to have the same histogenesis as the lymphoepithelioma but without the lymphocytic component. Both Ewing (1929) and Cappell (1934, 1938), however, emphasized the close relationship between lymphoepithelioma and transitional cell carcinoma and others began to doubt that they were separate pathological entities (Linsell, 1963). Ewing (1929) described other morphological features of NPC and divided histopathologically into five types: squamous cell carcinoma (SCC), transitional cell carcinoma, lymphoepithelial carcinoma, malignant adenoma and cystic adenoid basal cell carcinoma. Later in 1938, Cappell further divided the lymphoepithelial carcinoma into Schimincke and Regauds subtypes (Wei et al., 2011). Within a few years, many pathologists had changed the nomenclature to suit their understanding and created even more terminologies.

During the 1950s a number of research papers on NPC pathological classifications were published by Gui et al. (1956) titled *Analysis on the Clinic and Pathology of 138 Cases of Nasopharyngeal Malignant Tumour*. The essays correlated clinical data with histomorphological features of NPC cases. In 1959, a teaching and research group of pathology anatomy at Zhong Shan Medical College divided the NPC pathology into four types: SCC, transitional cell carcinoma, lymphoepithelial carcinoma, and adenocarcinoma. Three of them were similar to Ewing's.

Many more morphological features of NPC were identified in the nasopharyngeal region, and pathologists everywhere raised up new terminologies and further subdivided them into smaller categories. In 1962, Liang et al. first presented NPC International Histopathological Classification and divided NPC based on morphology into three major categories: undifferentiated carcinoma (pleomorphic carcinoma); poorly differentiated carcinoma (large round cell

carcinoma, spindle cell carcinoma, SCC grade III); and well-differentiated carcinoma (SCC grade I and II, basal cell carcinoma, columnar cell carcinoma/adenocarcinoma) (Liang et al., 1962). From 1961 to 1976, there were four successful meetings/conferences held in China, in which many issues related to NPC were discussed.

Simultaneously, International groups actively reviewed previous classifications, and in 1967, Yeh presented at Union for International Cancer Control (UICC) NPC Symposium and fewer new terminologies were brought up (Li, Pan, & Chen, 1983). The NPC histopathology was divided into seven major types: (Classic) epidermoid carcinoma, clear cell carcinoma, spindle cell carcinoma, transitional cell carcinoma, lymphoepithelial carcinoma, pleomorphic carcinoma, and mixed-cell carcinoma.

WHO classifications

Previous classifications

April 4–6, 1977, UICC and WHO together held their first NPC symposium in Kyoto, Japan and published a monograph on NPC pathology. This was the first edition of WHO Classification (1978) that simplified NPC into three major types: Type I (SCC); Type 2 (nonkeratinizing SCC); and Type 3 (undifferentiated carcinoma) (WHO Regional Office for the Western Pacific, 1982). However, many reported figures on the frequencies of various subtypes indicated that the boundaries between the types were not always clear [such as less well-differentiated forms of keratinizing squamous cell carcinoma (KSCC) vs nonkeratinizing carcinoma (NKC), and NKC vs undifferentiated carcinoma]. This posed a problem in diagnosis, especially from a limited biopsy sample of nasopharynx. The intra- and interobserver reproducibility of the classification was suboptimal (Chan et al., 2005; Shanmugaratnam et al., 1979). In the same year, 1977, the Chinese group had proposed a new classifications in which there were more deviations and did not show the unification link with the WHO classification. Chen and Zong (1978) presented at Fujian Conference and divided NPC histopathological classification into two major types, carcinoma in situ (CIS) and invasive carcinoma—which were further subdivided into four subtypes: microinvasive carcinoma (invasive scope not beyond a light microscope visual field 400x amplifications); well-differentiated carcinoma (SCC and adenocarcinoma), poorly differentiated carcinoma (SCC and adenocarcinoma); and undifferentiated carcinoma and other rare cancers (adenoid cystic, basal cell carcinoma, mucoepidermoid carcinoma). Even though the nasopharynx or nasopharyngeal primary cancer is the cancer occurring in the mucosa lining epithelium (squamous, columnar and transitional epithelium) and the minor salivary glands of nasopharynx (Hong & Guo, 2003; Zong, Liu, Zhong, Chen, & Wu, 2001), the WHO classification only considered it as NPC when it only showed evidence of squamous differentiation under the light microscope, contrary to the Chinese classification. In 1979, the Nanning

film reading meeting on NPC held in Changsa refined the terminology of large round cell carcinoma (based on Liang et al classification in 1962) to "vesicular nucleus cell carcinoma." The 5th Meeting of China NPC Prevention Collaborative Group categorized the term vesicular nucleus cell carcinoma under poorly differentiated carcinoma. Three years later, another NPC seminar sponsored by WHO was held in Guangzhou, where it was suggested to simplify NPC classification to only two major: Type 1 SCC, and Type 2 (combination of WHO's 1978 Types 2 and 3). However, the China experts did not accept the suggestion. They said it was too simple and too deviated from what they used in practice. They did accept the definition: nasopharyngeal cancer with squamous differentiation.

In 1991, experts at Chinese University of Hong Kong co-edited a monograph titled *Second Edition of WHO Histopathological Classifications on NPC*, which divided NPC into two major types: KSCC (well and moderately differentiated; poorly differentiated) and nonkeratinizing SCC (differentiated and undifferentiated). It was a reclassification, and refined the first edition WHO Classification of 1978.

In the 1991 WHO classification, the SCC subtype (KSCC) was retained, while the last two subtypes in the previous classification were combined under a single category of "nonkeratinizing carcinoma," which was further subdivided as being "differentiated" or "undifferentiated." The lymphoepithelioma-like carcinoma was considered a morphologic variant of undifferentiated carcinoma, and transitional cell-like morphology was considered as differentiated NKC. The use of numerical designation of WHO types 1, 2, and 3 was eliminated. However, another groups of Chinese experts who didn't accept the WHO classifications produced a book titled the *Norms of China Common Cancer Clinical Diagnosis and Treatment*, which adopted the previous Chinese classifications of 1962, 1978, and 1979 into two major types. Vesicular nucleus cell carcinoma was listed as an independent category under invasive carcinoma, which they claimed was unique and had a good prognosis for radiation therapy. This opinion was not in agreement with international and WHO pathologists, who believed that the vesicular nucleus cell carcinoma should be placed under undifferentiated carcinoma, which ended up being supported by data in China thereafter (Zhang & Xu, 2005; Zong et al., 2001; Wei, Xu, Liu, Zhang, & Liang, 2011).

Zong et al., (2001) proposed a new NPC pathological classification in the year 2000 to cater to both Chinese and International/WHO classifications, as follows: (1) precancerous lesions; (2) CIS and microinvasive carcinoma; (3) KSCC or SCC (well, moderately, and poorly differentiated; pleomorphic or anaplastic SCC, papillary SCC, adenoid SCC, basal cell-like SCC and clear cell SCC); and (4) NKC (also known as lymphatic epithelial carcinoma). These four classes further subdivided as: (a) differentiated (include spindle cell NKC), (b) undifferentiated carcinoma/large-cell carcinoma/vesicular nucleus cell carcinoma, and

mixed types, (c) nasopharyngeal adenocarcinoma—which Zong et al. then split into (i) traditional adenocarcinoma with focal squamous metaplasia, adenosquamous carcinoma, papillary adenocarcinoma, intestinal type carcinoma, signet ring adenocarcinoma (well, moderate, and poorly differentiated), and (ii) salivary glands adenocarcinoma (most commonly, adenoid cystic carcinoma, and mucoepidermoid carcinoma). Zong et al. provided a very comprehensive classification combining both Chinese and International/WHO Classifications (Wei et al., 2011).

In the year 2005, the third edition of WHO Classification of head and neck tumors highlighted another type of NPC as a separate entity known as basaloid squamous cell carcinoma (BSCC), it was divided into three major types: KSCC, nonkeratinizing SCC, and BSCC. According to this edition, subclassification into the undifferentiated and differentiated subtypes of nonkeratinizing SCC is optional, since their distinction is of no clinical or prognostic significance. For example, the patient exhibits features of one or the other subtype when biopsies are taken from different areas of the same tumor, whether simultaneously or at different times. When both subtypes are seen in a specimen, the tumor may be classified according to the prominent subtype, or—as NKC—with features of both subtypes. BSCC was first described to have distinct features and be highly malignant (Wain, Kier, Vollmer, & Bossen, 1986). Until 2003, few cases of BSCC as a primary tumor in the nasopharynx were reported. Muller and Beleites (2000) reported one, which was found to behave aggressively. BSCC was then introduced in the 2005 classification as one of the major types. It is a rare morphologic variant of SCC, which is more common in the head and neck. However, it appears to show a lower clinical aggressiveness, compared with BSCC occurring in other head and neck sites.

Four out of six cases reported at that time were positive for EBV (Wan, Chan, Lau, & Yip, 1995). Recently in 2016, Wang et al. found that even this current system is insufficiently informative, as clinical outcomes vary substantially among patients with the same clinical stage and histopathologic subtype. They proposed another NPC histopathologic classification that could potentially be used to predict prognosis and treatment response. They proposed NPC to be divided into four subtypes based on morphologic features: epithelial carcinoma (EC), sarcomatoid carcinoma, mixed sarcomatoid-epithelial carcinoma (MSEC), and SCC. Based on the new classification, they found that MSEC had a poorer prognosis with a low survival rate, whereas EC had a better prognosis with a good response to chemoradiation therapy.

Is carcinoma in situ universally accepted as a separate entity?

One of the pitfalls and challenges in the diagnosis of NPC is that most of the samples are small biopsies. It is quite difficult to access and completely remove

a tumor from the FOR region. Thus, it remains a challenge for the pathologist to make a diagnosis with limited diagnostic material. In one extreme, it may be non-representative of the tumour which may lead to a false-negative result. While in another extreme, the sampling may be positive but it does not provide the actual main morphological features due to diverse morphological areas in a same tumour mass.

In Chen and Zong (1978), 1991 and 2001 Chinese NPC classifications "nasopharyngeal carcinoma in situ" and "microinvasive carcinoma" were proposed as variants of NPC. However, it was rare and only detected in about 3%–8% of biopsies. In the third edition WHO classification in 2005, the precursor lesion was mentioned in the NPC (chapter 2) but was not classified as a separate entity. It was defined histologically as a pure nasopharyngeal CIS, which is characterized by atypical epithelial change confined to the surface, or crypt epithelium, and lacking an invasive component. The epithelium is usually slightly thick, and consists of cells with varying loss of polarity, nuclear enlargement, nuclear crowding and distinct nucleoli. However, it is not easy to determine from a small biopsy sample whether the invasive carcinoma has originated from the overlying in situ carcinoma or has merely invaded the surface epithelium.

A few groups of researchers have also supported this concept that the small amount of biopsy material did not facilitate an accurate, consistent and meaningful classification of NPC. Furthermore, they also concluded that due to its anatomical location of the nasopharynx, it is not an easily accessible site to assess NPC in situ as compared to cervical pathology (Weiland, 1978; Zong & Li, 1986; Zong et al., 1993). Thus the identification of CIS histopathologically in biopsy sample may not be the best solution to predict early NPC but detection of EBV by in situ hybridization (ISH) could be.

Nonkeratinizing NPC is associated with EBV in almost all of the cases (Pathmanathan et al., 1995; Plaza et al., 2002), irrespective of the ethnic background of the patient, and most of the CIS described morphological features of mostly undifferentiated carcinoma. ISH for EBV encoded early RNA (EBER) appears to be the simplest and most reliable way to demonstrate EBV, which is abundant in cells latently infected by EBV. ISH for EBER also can aid in the diagnosis of NPC if there are difficulties in distinguishing between carcinoma and reactive epithelial atypia (Tables 5.1 and 5.2).

Latest WHO classification

"Nasopharygeal carcinoma" still remains the diagnostic term of choice for all SCCs arising from the nasopharynx, which by definition should exhibit light microscopic or ultrastructural evidence of squamous differentiation. The WHO then subdivided these SCC to nonkeratinizing (further subcategorized as

Table 5.1 Chinese nasopharyngeal carcinoma (NPC) classifications.

Year	Description	Changes
Liang et al. (1962)	NPC histopathology is divided into three major categories. a. Undifferentiated i. Pleomorphic cell carcinoma b. Poorly differentiated i. Large round cell carcinoma ii. Spindle cell carcinoma iii. Squamous cell carcinoma (SCC) grade III (poorly diff SCC) c. Well differentiated i. SCC grade I and II ii. Basal cell carcinoma iii. Columnar cell carcinoma (adenocarcinoma)	Large round cell carcinoma was renamed as vesicular nucleus cell carcinoma in 1978 and 1979
1978	NPC pathology is divided into two major types: a. Carcinoma in situ b. Invasive carcinoma i. Microinvasive carcinoma (invasive scope not beyond a light microscope visual field with 400 times amplification) ii. Well-differentiated carcinoma (SCC and adenocarcinoma) iii. Poorly differentiated carcinoma (SCC and adenocarcinoma) iv. Undifferentiated carcinoma and other rare cancers such as cystic adenoid, basal cell carcinoma, and mucoepidermoid carcinoma	
5th China NPC Collaborative Group Meeting 1979	NPC histopathology is divided into two major types: a. Carcinoma in situ b. Invasive carcinoma i. Well-differentiated cancer (including adenocarcinoma and SCC) ii. Poorly differentiated cancer (including adenocarcinoma, SCC, and vesicular nucleus cell carcinoma) iii. Undifferentiated carcinoma and other rare cancers such as column tumor-type adenocarcinoma, mucoepidermoid carcinoma, malignant mixed tumor, basal cell carcinoma, etc.	Vesicular nucleus cell carcinoma was added under poorly differentiated cancer
The Norms of China Common Cancer Clinical Diagnosis and Treatment 1991	NPC is divided into two major types: a. Carcinoma in situ b. Invasive carcinoma i. Microinvasive carcinoma, ii. SCC (mild, moderate, and poorly differentiated) iii. Adenocarcinoma (mild, moderate and poorly differentiated) iv. Vesicular nucleus cell carcinoma	Vesicular nucleus cell carcinoma listed as independent category.

(*Continued*)

Table 5.1 Chinese nasopharyngeal carcinoma (NPC) classifications. ***Continued***

Year	Description	Changes
	v. Undifferentiated carcinoma	
Zong et al. (2001)	NPC pathological classification divided as follows: 1. Precancerous lesions 2. Carcinoma in situ and microinvasive carcinoma 3. Keratinizing squamous cell carcinoma (KSCC) or squamous cell carcinoma (well, moderately, and poorly differentiated) a. Pleomorphic or anaplastic SCC b. Papillary SCC c. Adenoid SCC d. Basal cell-like SCC e. Clear cell SCC 4. Nonkeratinizing carcinoma (NKC), some of which are also known as lymphatic epithelial carcinoma a. Differentiated type (including spindle cell NKC) b. Undifferentiated type (also known as nasopharyngeal undifferentiated carcinoma and once called large round cell carcinoma and vesicular nucleus cell carcinoma) and mixed type three subtypes c. Nasopharyngeal adenocarcinoma, which can be divided as: i. Traditional (adenocarcinoma with focal squamous metaplasia, adenosquamous carcinoma, papillary adenocarcinoma, intestinal type adenocarcinoma, signet ring adenocarcinoma—well, moderately, and poorly differentiated) ii. Salivary gland adenoca (adenoid cystic carcinoma, mucoepidermoid carcinoma, and other rare salivary glands tumor types)	Large round cell carcinoma/vesicular nucleus cell carcinoma was lump into undifferentiated carcinoma category

undifferentiated or differentiated) and keratinizing types, as well as BSCC. This classification system is in keeping with the previous (third) edition.

The histopathological classification of NPC in the recently published fourth edition (2017) retained similar classification as the third edition (2005) (Table 5.3). The diagnostic morphological features remain constant. The current edition highlights the interplay of roles between infection, genetic and environmental factors. It also elaborates on the contribution of the above factors toward the tumor's geographical distribution, development, biological behavior, and histopathological type.

Table 5.2 International/WHO nasopharyngeal carcinoma (NPC) pathological classifications.

Year	Description	Changes
Ewing (1929)	NPC can be histopathologically divided into five types: a. Squamous cell carcinoma (SCC) b. Transitional cell carcinoma c. Lymphoepithelial carcinoma d. Malignant adenoma e. Cystic adenoid basal cell carcinoma	
UICC (Union for International Cancer Control) NPC Symposium (1967)	NPC can be histopathology divided into seven major types: a. Typical (or classic) epidermoid carcinoma b. Clear cell carcinoma c. Spindle cell carcinoma d. Transitional cell carcinoma e. Lymphoepithelial carcinoma f. Pleomorphic carcinoma g. Mixed-cell carcinoma subtypes	
First Edition WHO NPC histopathological classification (1978)	NPC can be histopathology divided into three major types: a. Type 1 (SCC) b. Type 2 [nonkeratinizing carcinoma (NKC)] c. Type 3 (Undifferentiated carcinoma)	WHO defined NPC is nasopharyngeal cancer with evidence of squamous differentiation by light microscopy (adenocarcinoma and salivary gland tumors were not included)
Second Edition WHO NPC histopathological classification (1991)	NPC histopathological classification can be divided into two major types: a. Keratinizing squamous cell carcinoma (KSCC) (two subtypes) i. Well and moderately differentiated ii. Poorly differentiated b. NKC (two subtypes) i. Differentiated ii. Undifferentiated	The use of numerical designation of WHO types 1, 2, and 3 was eliminated
Third Edition WHO NPC histopathological classification, (2005)	NPC histopathological classification can be divided into three types: a. KSCC b. NKC i. Undifferentiated ii. Differentiated c. Basaloid squamous cell carcinoma (BSCC)	BSCC was introduced as independent category

Table 5.3 Classification of nasopharyngeal carcinoma (NPC) WHO fourth edition (2017).

Nasopharyngeal carcinoma	
Nonkeratinizing squamous cell carcinoma	8072/3
Keratinizing squamous cell carcinoma	8071/3
Basaloid squamous cell carcinoma	8083/3

Histomorphological features of nasopharyngeal carcinoma

The diagnostic term "nasopharyngeal carcinoma" is used to describe SCC that occurs primarily at nasopharyngeal mucosa. Although this tumor is of squamous cell in origin, the etiology and its pathogenesis, epidemiology (i.e., geographical distribution and ethnicity), and biological behavior, are distinctive from SCC that occurs in other head and neck areas. Any tumor with glandular differentiation, and salivary gland-type tumors, are excluded from this classification.

Classification of the NPC based on its histomorphological features is further subdivided into: (1) nonkeratinizing SCC, and its undifferentiated and differentiated subtypes, (2) KSCC, and (3) BSCC.

Nonkeratinizing squamous cell carcinoma

This type is further subdivided into differentiated and undifferentiated variants. However, there is no recognized clinical or prognostic significance of this subdivision (Petterson et al., 2015). It does exhibit differences in etiological factors, geographical distribution and morphological features. The nonkeratinizing SCC (notably the undifferentiated variants) is the most common subtype occurring in endemic areas (i.e., South China and Southeast Asia) and exhibits high association with EBV infection (Adham et al., 2012). High nitrosamine content within the diet (Yuan et al., 2000) and genetic susceptibility (Tse et al., 2011) are also identified as contributing factors in those regions. In nonendemic areas, high-risk human papilloma virus (HPV) infection has been described as the etiological factor in a subset of NPC cases (Robinson et al., 2013). The differentiated variant occurs more often in low-incidence areas. It is associated with tobacco and alcohol as the carcinogenic agents (Long, Fu, Li, & Nie, 2017).

As the name implies, the features of squamous differentiation in this subtype are ambiguous. It can be represented by only primitive differentiation (i.e., greater amount of lightly eosinophilic cytoplasm and slightly distinct cell border) or not at all evident by light microscopic examination. Ultrastructurally, at least some of the malignant cells show evidence of squamous differentiation characterized by presence of tonofilaments and desmosomes.

The malignant cells of undifferentiated variants show heterogenous arrangement even within the same tumor mass. They can range from solid to trabeculae (Figs. 5.1 and 5.2) to individually arranged. They are mixed intimately with variable proportions of lymphoplasma cells, and devoid of desmoplastic stromal reaction. The cells are large in size, and contain round to oval vesicular nuclei, large central nucleoli, and scanty eosinophilic to amphophilic cytoplasm. The indistinct cell border leads to a syncytial appearance (Figs. 5.3 and 5.4). In some cases, the lymphoplasma cells are so intense causing the breakage of malignant cells clusters. This leads to formation of tiny clusters or individual

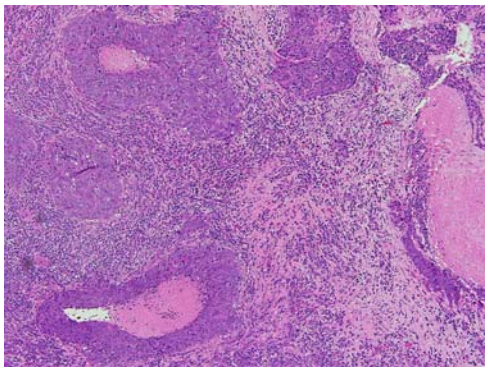

Figure 5.1
Nasopharyngeal nonkeratinizing carcinoma (NKC), undifferentiated subtype. The tumor is arranged in irregular islands with comedo necrosis. The stroma is rich in lymphoplasma cells (H&E 100 ×).

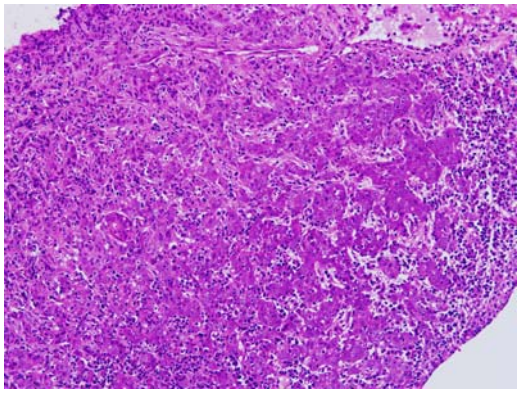

Figure 5.2
Nasopharyngeal nonkeratinizing carcinoma (NKC), undifferentiated subtype. The tumor shows trabeculae arrangement with lymphoplasma cells seen in between (H&E 200 ×).

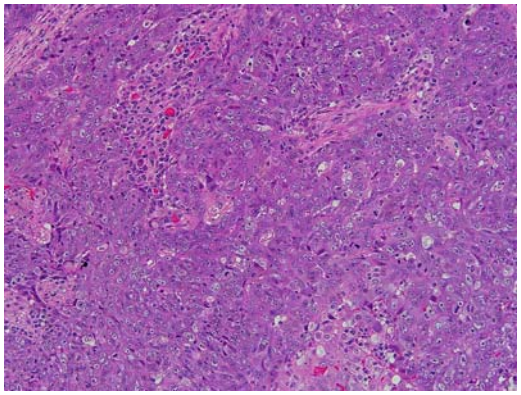

Figure 5.3
Nasopharyngeal nonkeratinizing carcinoma (NKC), undifferentiated subtype. The tumor cell border is indistinct leading to syncytial appearance (H&E 200 ×).

arrangements of neoplastic cells embedded within lymphoplasma cell-rich background. Thus the term "lymphoepithelial carcinoma" is used to describe this type of tumor. Besides lymphoplasma cells, other inflammatory cells may also present in abundance; eosinophils, neutrophils and even epithelioid

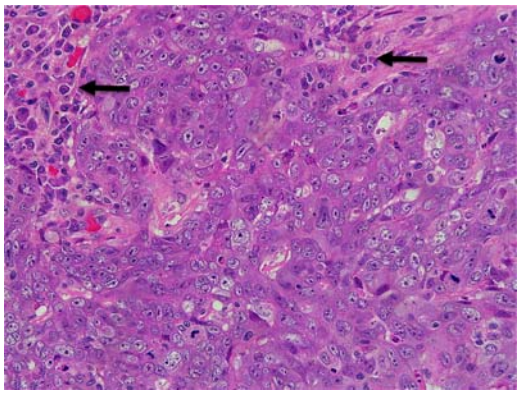

Figure 5.4
Nasopharyngeal nonkeratinizing carcinoma (NKC), undifferentiated subtype. The tumor cells display overlapping of cells. The nuclei are vesicular with prominent centrally located nucleoli. Lymphoplasma cells are seen in between the clusters (H&E 400 ×).

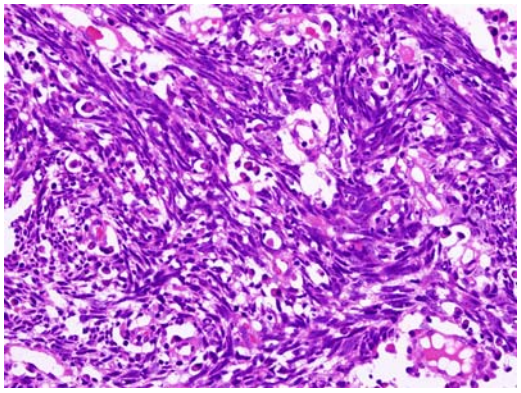

Figure 5.5
Nasopharyngeal nonkeratinizing carcinoma (NKC), undifferentiated subtype. The tumor cells appear spindly and are arranged in fascicles (H&E 400 ×).

granuloma. About 12% of NPC cases show presence of intracellular and extracellular amyloid globules. This element can be used as a supportive feature of diagnosing NPC in a biopsy with suspicious islands of epithelial cells as it is absent in normal nasopharyngeal mucosa (Chan, 2017). Uncommon morphological features include, spindly malignant cells arranged in fascicles (Fig. 5.5) and papillary frond formation.

The differentiated variant exhibits stratification, or pavement arrangement. Sometimes it can be in a plexiform growth pattern, resembling transitional cell carcinoma of the bladder (Shanmugaratnam, Sobin, & Barnes, 1991). Similar to the undifferentiated type, the malignant cells are embedded within lymphoplasma cells and devoid of desmoplastic reaction. As compared to the undifferentiated type, the sizes of the malignant cells are smaller with fairly well-defined cell borders. Sometimes vague intercellular bridges and occasional

keratinized cells can be observed. The cells display chromatin-rich nuclei and less prominent nucleoli.

Keratinizing squamous cell carcinoma

KSCC is usually seen in low-incidence regions. Tobacco and alcohol play a major role as etiological agents (Dietz et al., 2004). EBV infection is commonly absent in this type, particularly in nonendemic areas (Marks, Phillips, & Menck, 1998), as well as radiation-induced tumors (Chen & Hsu, 2000). This subtype can arise de novo or secondary to radiotherapy for nonkeratinizing SCC (Chen & Hsu, 2000). Clinically, it is more locally advanced and exhibits less lymph node or distant metastasis (Reddy, Raslan, Gooneratne, Kathuria, & Marks, 1995). Its responds less to radiotherapy and has a worse prognosis, as compared to the nonkeratinizing subtype.

Morphologically, this tumor displays similar features to SCC in other head and neck areas (Fig. 5.6). It shows obvious squamous differentiation that can be appreciated microscopically, intercellular bridges, keratin pearls, and/or keratinization (Figs. 5.7 and 5.8). The stroma often shows marked desmoplastic reaction.

Basaloid squamous cell carcinoma

The BSCC variant is rare. EBV positive cases with this variant are observed in high-incidence ethnic groups (Chan, 2017). Clinically, it is less aggressive and exhibits a better prognosis than basaloid neoplasm occurring in other areas of the head and neck (Wan et al., 1995). Morphologically, it composed of basaloid cells and SCC. The basaloid cells are arranged in closely packed lobules, some of which exhibit peripheral palisading. The cells are small in size with

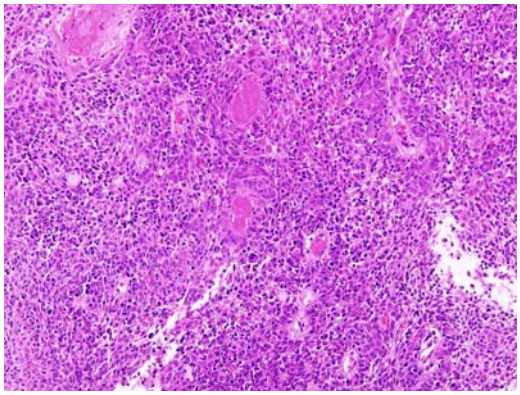

Figure 5.6
Nasopharyngeal keratinizing carcinoma, moderately differentiated. The tumor cells invade in an irregular island pattern with intermingling lymphoplasma cells. The tumor cells show distinct cell borders as compared to nonkeratinizing variant (H&E 200 ×).

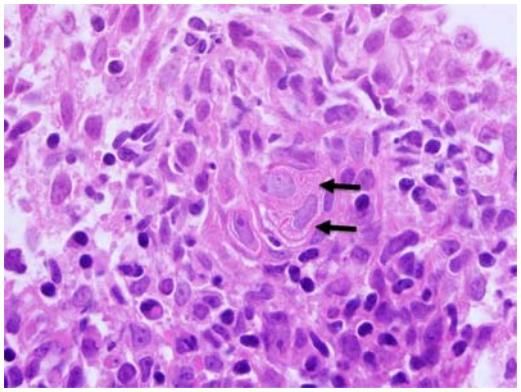

Figure 5.7
Nasopharyngeal keratinizing carcinoma. The squamous differentiation is evidenced by the presence of intercellular bridges (*arrow*) on light microscopy (H&E 1000 ×).

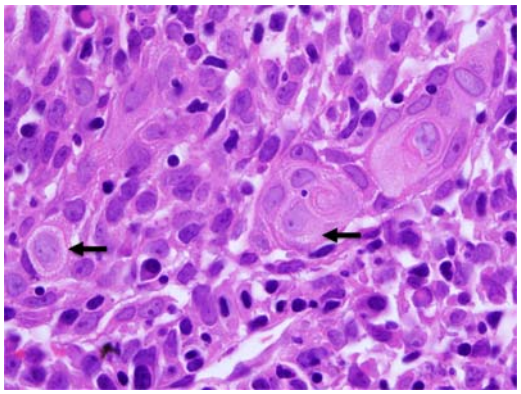

Figure 5.8
Nasopharyngeal keratinizing carcinoma. The cells display squamous appearance; polygonal in shape with abundant eosinophilic cytoplasm. Squamous differentiation evidenced by intercellular bridges (*arrow*) are also observed (H&E 1000 ×).

hyperchromatic nuclei, inconspicuous nucleoli and scant cytoplasm. Comede-type of necrosis frequently is observed. The peculiar features of this tumor are the presence of small cystic spaces containing periodic acid Schiff - and Alcian blue-positive material, and stromal hyalinization. The tumor is always accompanied by either in situ or invasive SCC. Metastases appear in the form of basaloid carcinoma, squamous carcinoma, or both.

Ancillary studies

Immunohistochemistry

Normally, NPC tissue samples are stained with p63, pancytokeratin and cytokeratin. NPC tissues all have robust staining, in addition to detectable sporadic stains that detect low molecular weight cytokeratin and epithelial membrane

antigen, besides the absence of CK7 and CK20. However, these stains are not specific for NPC. During latent viral infection, EBV expresses latent genes, including EBNA1 and LMP1, which are important for NPC progression. EBNA1 and LMP1 are considered to be the most important latent viral proteins due to their ability to utilize host cellular proteins that are involved in various functions, such as cell signaling and apoptosis, to enhance NPC pathogenesis. Detection of EBNA1 and LMP1 is thus a potential tool for NPC diagnosis. A study utilizing formalin-fixed, paraffin-embedded tissues from 40 NPC patients and 20 healthy subjects revealed that EBNA1 and LMP1 are potential markers for NPC. The study showed that EBNA1 (92.5%) and LMP1 (90%) expression was observed in NPC tissues, and was absent in normal tissue samples. Further analysis of LMP1 in different histological types of NPC revealed that in NKC, 49% of all samples tested showed LMP1 positivity, and in KSCC, 66.7% of samples tested showed LMP1 positivity.

Nasopharyngeal carcinoma genetic predisposition

EBV is known as a common virus, and it has been found that the majority of the population it infects experience no symptoms. Analysis of EBV in the blood also indicates association between EBV infection and NPC carcinogenesis. The knowledge regarding the capability of the virus to inflict changes that lead to NPC carcinogenesis remains incomplete. However, initiation and progression of NPC was suggested to result from a combination of events associated with EBV latent infection, the presence of genetic predisposition and susceptibility markers, environmental conditions, and lifestyle changes adopted by the host.

It is well established that those of southern Chinese descent are more susceptible to NPC in comparison to people that resides in western countries. Incidentally, the descendants of the migrated southern Chinese population remain susceptible, generations after their assimilation into the United States. Other factors such as smoking, the uptake of nitrosamine-rich diet and prolonged subjection to wood dusts are also vital in NPC carcinogenesis. Advancement in genotyping technology has also revealed genetic susceptibility markers present within these populations that increases their susceptibility toward acquiring EBV associated NPC.

Multiple genome-wide association studies (GWAS) have consistently showed that the polymorphism within the major histocompatibility complex, in particular the human leukocyte antigen (HLA), to be a genetic susceptibility marker for NPC, which strongly suggests the importance of the immune response in EBV associated NPC. Populations that are determined to be high risk have been found to have certain HLA polymorphism that reduced the ability of their HLA class I to recognize and mount an effective immune response to control EBV infection within infected individuals, thus increasing the chance of EBV infection

progressing to NPC. A study conducted among 277 patients with NPC revealed other immunological genetic polymorphisms that occur within *GABBR1* and *MICA* that reside within the HLA region to be strongly associated with NPC. Other GWAS also showed that in addition to HLA gene, there are other immunological loci polymorphisms that determine susceptibility toward NPC, which include *TNFRSF19* and *MDS1-EVI1* polymorphisms. Apart from genes associated with immunology, polymorphisms within functional genes from other cellular pathways have also been found to be closely associated with NPC. These genes include *MDM2*, which is involved in regulation of the cell cycle, and the apoptotic genes *TP53* and *MMP2*, which are important in cell proliferation and migration.

Molecular diagnosis

EBER analysis using ISH is considered the gold standard in EBV detection, and displays 100% EBER detection in cancer cells. This diagnostic method detects EBV small RNAs that are expressed during the viral latent phase, 48 hours postcommencement of the lytic infection. During this latent phase, the virus expresses two EBV specialized noncoding RNAs, termed as EBER-1 and EBER-2 alongside other BamH1-A rightward frame transcripts. EBER is used due to the abundance of expression during EBV latency state and can be readily detected using fluorescence ISH. Utilization of probes conjugated with chromogen allows for the detection and visualization of EBER in tissues from NPC patients, and pathologists can differentiate between cancer cells and reactive epithelial atypia. Detection of signals corresponding to EBER in tissues characterized as metastatic NKC can also be a strong indicator that the tissue originates from the nasopharynx. In KSCC, EBER signal can also be detected; however, the signal is predominantly within cells that are undergoing reduced differentiation. This diagnostic method was also shown to be more sensitive when compared to LMP1 immunohistochemistry. In addition, EBER ISH was also shown to be more reliable than polymerase chain reaction (PCR), due to the specific nature of the test, which detects EBER in host cells affected by EBV latent infection.

Initially EBV DNA was detected by PCR in the serum of 31% of NPC patients, but was absent in normal healthy individuals tested, thus considered to be highly specific. However, this method was later determined to be unsuitable for clinical usage due to the low sensitivity of the procedure. Real-time PCR analysis was later introduced as a tool for the detection of EBV DNA due to higher sensitivity and the capability to quantify EBV DNA isolated from patients. The study by Lo et al. (1999) also became among the first to reveal the association between levels of EBV DNA and advance stages of NPC (Lo et al., 1999). More recent studies have also shown the capability of real-time PCR to be used as a tool for NPC diagnosis. Real-time PCR was shown to have high sensitivity and specificity in the detection of EBV DNA in plasma and serum. The method was

shown to have 91.4% specificity and 93.2% specificity in plasma, 84.4% sensitivity, and 76.0% specificity in serum, with pooled sensitivity and specificity of 89.1% when used to detect EBV DNA in plasma or serum. Although useful, this method is prone to result in variability when carried out in different diagnostic centers, and requires the use of similar materials and methodology by the participating diagnostic centers.

Nasopharyngeal carcinoma diagnostic problems

Difficulties in making accurate diagnosis based on nasopharyngeal biopsy can be attributed to factors affecting biopsy materials, the morphological heterogenousity of the tumor itself, and mimickers (Chan, 2017; El-Naggar, Chan, Grandis, Takata, & Slootweg, 2017).

Crush artifacts

Crush artifacts are common in nasopharyngeal biopsy. It is difficult to identify the neoplastic cells in the crush area. Assessment of the crush area should be avoided and make with caution. Better-preserved areas with better-preserved cells are the best areas for evaluation. Immunostain for cytokeratin could be used to highlight the malignant epithelial cells, which usually appear as invasive clusters.

Morphological variations

As previously mentioned, amounts of lymphoplasma cells vary. Marked infiltration of the tumour area by lymphoplasma cells can lead to confusion with lymphoma. Morphologically, lymphoma cells show poorly outlined cell borders whereas the malignant epithelial cells show distinct cell borders. Presence of malignant cells clustering and amyloid globules can aid in making a diagnosis. The cytokeratin immunostain can be used to identify the malignant epithelial cells.

Epithelioid granuloma formation can mask the clusters of malignant cells, and can also be seen in NPC cases. It can represent part of an immune reaction toward tumor or to infection. However, granuloma is uncommon in this area. Thus biopsy with granulomatous inflammation should be thoroughly examined for possibility of NPC and to exclude other causes.

Mimickers

Clustering of germinal center cells can mimic carcinoma, particularly where the mantle zone is not well defined. Morphologically, it is composed of large cells with vesicular nuclei and prominent nucleoli that resemble NKC cells. The presence of other lymphoid cell components, such as centrocytes and tingible body

macrophages, would hint at germinal center cell components. Immunostain to confirm for lymphoid origin and exclude epithelial origin is very helpful. Islands of benign crypt cells in tangential cutting can also contribute to the confusion of NPC diagnosis. Morphologically, the crypt epithelial cells are smaller in size, not overlapping with prominent nucleoli. The absence of invasive growth and continuity with the surface epithelium should be assessed.

Reactive lymphoid hyperplasia with increased components of immunoblast may lead to mimicry of NPC. The immunoblasts are also large in size. However, they do not form clusters and display well-defined amphophilic cytoplasm, contrary to carcinoma cells, which are arranged in clusters and display eosiphilic cytoplasm. Confirmation of lymphoid origin by using immunostain should be helpful.

Large-cell lymphoma can mimic NKC and vice versa. Morphological cellular features that favor NPC include cells clustering, ill-defined cell borders and prominent nucleoli. The difference is large-cell lymphoma cells display amphophilic cytoplasm and marked nuclear folding, and the malignant cells are singly arranged.

Conclusions

Evolution of NPC classification is vast, whether by Chinese or International (WHO) classifications. Unfortunately, the two views are not connected, which hinders smooth international information exchange and analysis of the disease. Another issue of NPC classification is lack of its significance regarding clinical aspects; prognosis, disease progression, and treatment. The classification is mainly based on the tumor's morphological features, without proper relation to its clinical effects. Thus more efforts are needed to standardize the classification, and clinicopathological correlation studies should be carried out extensively to aid in that development.

The latest WHO classification, published as the fourth edition, maintained the NPC classification of the third edition. It emphasized the relationship between the etiological factors (such as EBV, HPV, and genetic predisposition) and the tumor, demonstrated via an array of techniques. This helps to bring essential insight toward the underlying pathogenesis of NPC. It may lead to recognition of new biomarkers that are beneficial for early detection, clinical perspective, diagnostic and therapeutic approach, and prognosis.

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6

Clinical presentation of nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) is a unique disease with a clinical presentation, epidemiology, and histopathology differing from other squamous cell carcinomas of the head and neck. It is a nonlymphomatous squamous cell carcinoma that occurs in the epithelial lining of the nasopharynx. This neoplasm shows varying degrees of differentiation and is most frequently seen in the pharyngeal recess (Rosenmüller's fossa), posteromedial to the medial crura of the Eustachian tube (ET) opening in the nasopharynx (Sham, Poon, Wei, & Choy, 1990; Sham, Wei, et al., 1990).

Symptoms of NPC can be obscure due to its anatomic location, which begins in the upper part of the pharynx behind the nose and ends at the proximal part of the trachea and esophagus. These include nasal, aural, and neurologic symptoms and thus often presents a challenge in diagnosis. Cervical lymphadenopathy is most commonly noted during physical examination of patients with NPC. The majority of newly diagnosed NPC patients have locoregionally advanced disease, and cervical nodes are usually involved (Razak et al., 2010) (Fig. 6.1).

It is infrequent to find together the triad of clinical manifestation of nasal obstruction with epistaxis, ear involvement, and neck mass. Serous otitis should raise suspicion in an adult that has no prior history of this type of affection. In addition, patients with NPC often present symptoms or complications because of local infiltration into the surrounding areas. Early symptoms are usually because of epistaxis, discharge, and nasal obstruction, but are nonspecific and difficult to correlate to NPC. Selected patients may also present with deafness and tinnitus due to the ET dysfunction. Sixty percent of cases can present with unilateral hearing loss. The type of hearing loss relates more to the transmission subtype.

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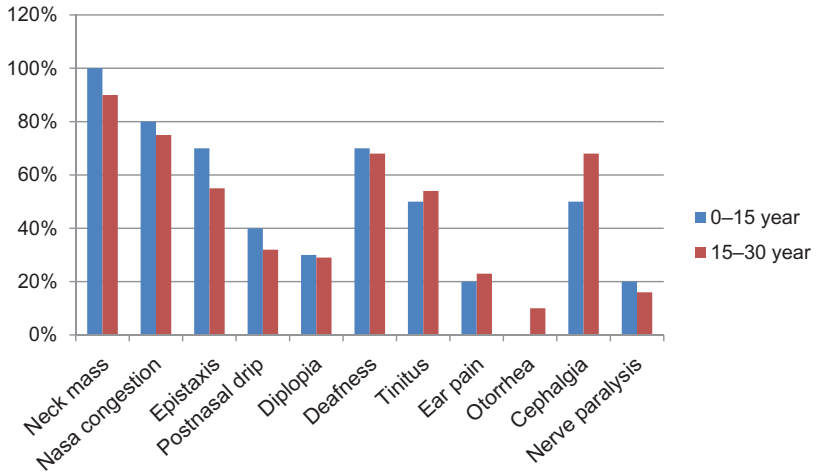

Figure 6.1
The first symptoms appearing in nasopharyngeal carcinoma (NPC) patients (Adham et al., 2014).

Figure 6.2
Patient with normal nasopharynx.

Early diagnosis of NPC can be a difficult task because the postnasal space is relatively inaccessible to examination, compounded by the fact that the presentation of NPC is variable; it usually runs silently or with nonspecific symptoms. Patients may present with headache, cranial nerve (CN) involvement, nasal obstruction, or a neck mass due to nodal metastases. A low index of suspicion and the technical challenges of postnasal space examination may also preclude earlier diagnosis, resulting in presentation with locally advanced disease that adversely influences outcome (Al Rajhi et al., 2009; Leong, Fong, & Low, 1999)

NPC can be diagnosed early by rapidly initiating an endoscopic examination, imaging, and biopsy in the suspected areas. Tumor growth occurs primarily in the pharyngeal recess (Rosenmuller's fossa) in the lateral wall of the nasopharynx, and secondarily in the superior posterior wall (Figs. 6.3–6.5). Patients may remain asymptomatic for a long time given the often clinically occult site of

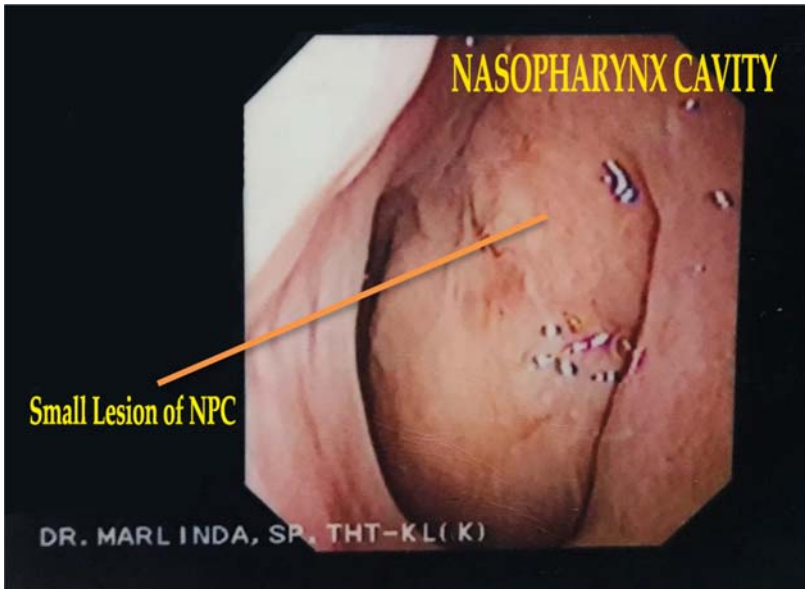

Figure 6.3
Patient with small lesion of nasopharyngeal carcinoma (NPC).

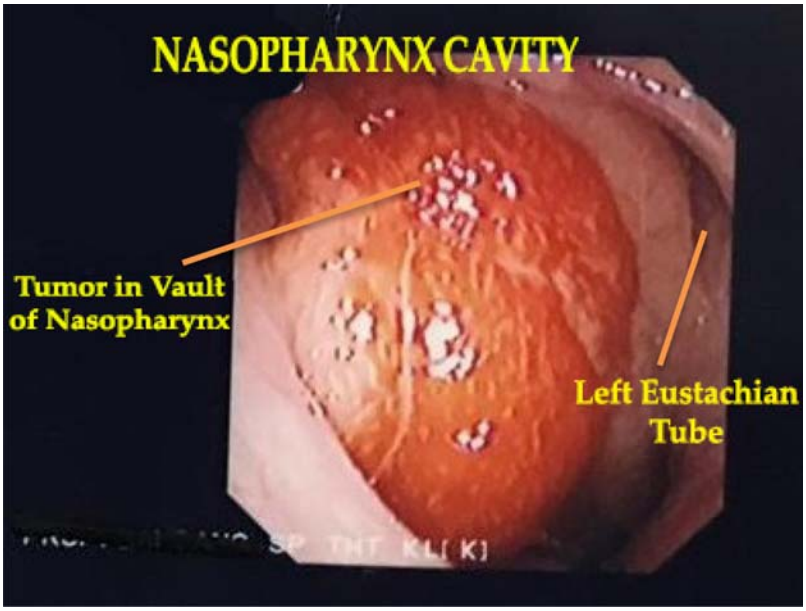

Figure 6.4
Patient with nasopharyngeal carcinoma (NPC).

presentation and consultation with physicians with inexperience in managing NPC. It is therefore not surprising that the diagnosis of NPC is delayed (Indudharam, Valuyeetham, Kannan, & Sidek, 1997). Skinner et al. noted that diagnosis of most patients is delayed by about 6 months because the

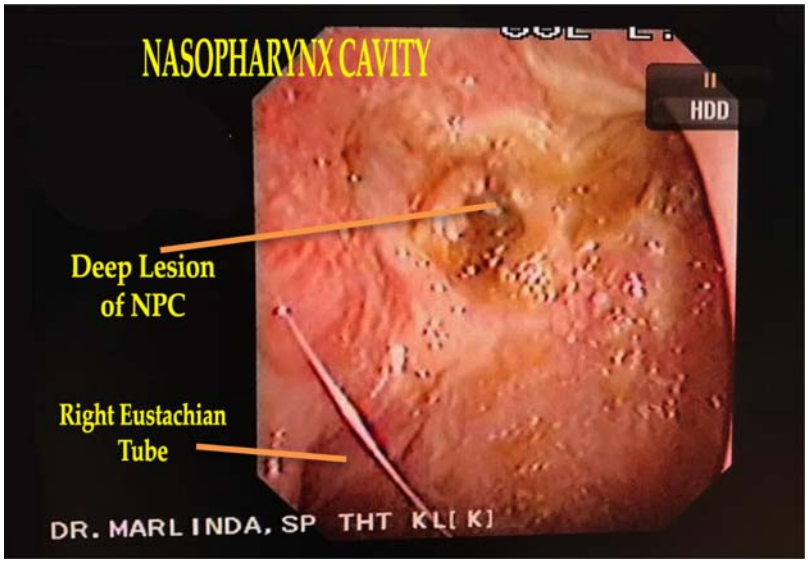

Figure 6.5
Patient with deep lesion of recurrent nasopharyngeal carcinoma (NPC).

symptoms may be nonspecific in the early stages (Skinner, Van Hasselt, & Tsao, 1991). The largest study conducted to date in Hong Kong revealed that the mean symptom-to-diagnosis duration was 8 months and that earlier presentation correlated with improved 10 years survival (Lee et al., 1997). Only10% of patients are diagnosed early at stage I (Van Hasselt & Woo, 2008).

Not many patients with NPC can be detected at an early stage due to nontypical symptoms. Hamida et al. reported that only 0.7% of 428 patients who came to one of the hospitals in Jakarta with stage I NPC and more than 50% of patients had come with advanced stage of NPC. Patients with early stages usually present with symptoms such as epistaxis, double vision, and auditory complaints while complaints such as neck mass and double vision are only found in patients with more advanced stages. Complaints of a neck mass were obtained in patients in stages II–IV and complaints of double vision were only obtained in patients with stage IV. To improve detection of early stage NPC it is necessary to conduct training programs for general practitioners in order to recognize the signs, symptoms, and risk factors of early-stage NPC (Wildeman et al., 2012).

Wei and Sham (2005) distinguished the clinical signs and symptoms of NPC patients into four groups: (1) Symptoms resulting from the tumor mass in the nasopharyngeal (NP) (nasal obstruction, epistaxis, and nasal discharge); (2) symptoms and signs related to dysfunction of the ET effusion with otitis media (OME), hearing loss; (3) symptoms resulting from extension toward the skull base involvement (erosion) with impairment of the 5th and 6th CN

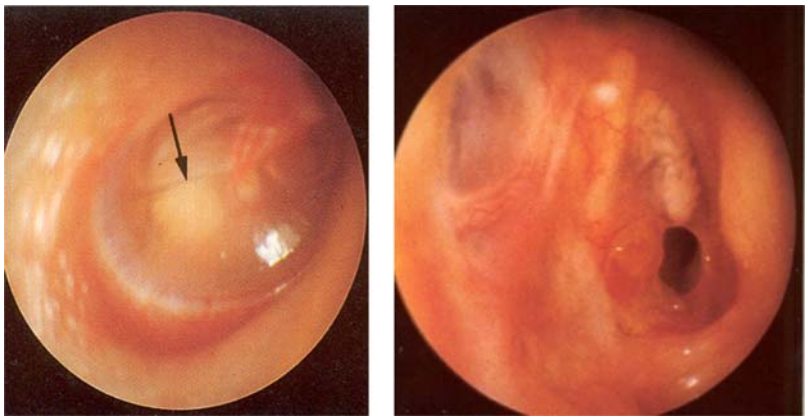

Figure 6.6
Symptoms and signs of nasopharyngeal carcinoma (NPC) related to dysfunction of Eustachian tube (ET), otitis media (OME) effusion (*left*), and OME with perforation (*right*).

(headache, diplopia, facial pain; and numbness/paresthesia); and (4) palpable neck masses (Wei & Sham, 2005). The most common clinical findings in NPC are a fast-growing neck mass (frequently localized in the posterior neck triangle and upper jugular chain) (76%); nasal symptoms including nasal obstruction, discharge, and epistaxis (73%); aural symptoms including OME, hearing loss, and tinnitus (62%); and symptoms associated with CN involvement (20%) (Sham, Poon, et al., 1990; Sham, Wei, et al., 1990; Wei & Sham, 2005) In a large retrospective study from Hong Kong comprising almost 5000 patients, the initial symptoms at presentation were, with decreasing frequency, neck mass (76%), aural dysfunction (62%), headache (35%), diplopia (11%), weight loss (7%), and trismus (3%) (Lee et al., 1997).

At the first otolaryngology-head and neck surgery (Oto-HNS) visit, at Kaiser Northern California 54 (53%) patients had a nasopharyngeal lesion diagnosed (Fig. 6.6). For the remaining patients, other diagnoses were made, most commonly middle ear effusion and neck masses. Nasopharyngoscopy was performed at the first Oto-HNS visit for 84 (83%) patients. Among initial endoscopies, 69 (68%) detected a nasopharyngeal lesion (Wang, Austin, Chen, Sonne, & Gurushanthalah, 2017).

A study by Adham et al. (2014) in Jakarta showed patients with NPC at early stages come with unspecific symptoms from two different age groups. Of the two groups, the neck mass is a symptom present in almost all age groups. It is present in all of the patients in the 0-15 year group and 90% patients in 16–30 year group come with neck mass. Nevertheless, nasopharynx and otologic symptoms are the most frequent in NPC patients.

Distant metastatic disease at presentation is rather uncommon and has been reported to be present in approximately 5% patients from southern China (Teo et al., 1996). NPC is predominantly a disease of adults with a peak occurrence

in the fourth to sixth decade but it can also affect the pediatric population. Age distribution of 1121 NPC patients in the 1995–2005 period reported from Dr. Cipto Mangunkusumo Hospital Jakarta, Indonesia. Although NPC incidence in children of <10 years old is low, a considerable number of NPC cases were observed in the age group 10–19 (juvenile) around 9.9% and 20–29 (young adult) years old. Overall the 1–30 year age group represented 21% of all cases. The NPC peak incidence in this cohort lays in the age group of 30–50 years old (Adham et al., 2012).

The distribution of age-specific incidence rates of NPC in several low-risk countries (India, Japan, North America, Europe, Australia) shows the formation of bimodal distribution in different age of groups. The first peak appears at the age of 15–19 years (late adolescence/early adulthood) and the second peak appears in the age of 65–79 years. However, this phenomenon of bimodal distribution is not found in countries with a high risk of NPC (Philippines, Singapore, and Thailand) (Bray et al., 2008).

Four groups of clinical signs and symptoms of nasopharyngeal carcinoma

Symptoms resulting from the tumor mass in the nasopharyngeal area

Symptoms are usually refered in the neck or the ear (i.e., far away from the nasal cavity and to a less extent related to the nose). This may be explained by the relatively wide postnasal space (4.3–3 cm) that makes lymphatic metastases and pressure effect on ET. It is the most common presenting feature and one that may put a different diagnostic possibility that can lead to delay of the diagnosis. This brings to light the importance of suspicion and early examination and assessment of the postnasal space to detect the tumors early. A mass in the NP area will result in symptoms of nasal obstruction and blood-stained nasal discharge and/or epistaxis. Imad et al. found 14 patients (28%) complained of off-on blood stained nasal discharge, and 13 patients (26%) had associated unilateral nasal obstruction (Imad, Mohammad, & Hidayatullah, 2005).

Nasal symptoms such as obstruction and epistaxis or sinusitis-like were was third most common upon presentation. These conditions often were initially treated with nasal steroids, antihistamines, or antibiotics by a primary care physician before referral to an Oto-HNS. The much longer mean time intervals reflect the minority of patients whose care was substantially delayed by themselves or by their physician (Wang et al., 2017).

Symptoms and signs related to dysfunction of the Eustachian tube

Adham et al. reported that most of their patients (60.6%) had unilateral ear problems as the earliest sign of NPC in Indonesia (Adham et al., 2012). Ear symptoms occur because the nasopharyngeal tumor creates a negative pressure or obstructs the torus tubarius and leads to ET dysfunction and further infiltration of the tensor veli palatine muscle, which can manifest as a middle ear effusion acute OME and conductive hearing loss (Agrawal, Baisakhiya, Vagh, & Joharapurkar, 2009).

The occurrence of OME in NPC patient also increases after irradiation due to irradiation-induced inflammation. There are many techniques used for treating OME in postirradiated NPC patients. Kuo et al. (2012) reported a high rate of dry eardrum perforation using laser myringotomy. In contrast, another study reported a low rate of dry eardrum perforation using ventilation tube insertion and traditional medicine (auripuncturem, myringotomy plus gromment insertion). The duration of relief from otologic symptoms using laser myringotomy is longer than simple tympanic aspiration (Liang et al., 2011; Xu et al., 2008).

Symptoms resulting from extension toward skull base involvement (erosion) with impairment of the fifth and sixth cranial nerves (headache, diplopia, facial pain, and numbness/paresthesia)

Horner syndrome is a combination of signs and symptoms of partial ptosis, miosis, and fascial anhidrosis caused by sympathetic nerve supply disruption (Khan & Bollu, 2019). Horner syndrome rarely presents as the first manifestation of NPC. The etiology of Horner syndrome is likely sympathetic chain and/or superior cervical ganglia involvement. Anhidrosis occurs in cases in which the lesions are located before the separation of the vasomotor and sudomotor fibers at the beginning of the internal carotid artery (Ellul, Cutajar, Borg Xuereb, & Said, 2018). Skull base invasion is the common presentation in up to one third of cases and can be the initial presentation (Yang et al., 2004).

The NPC can spread intracranially via the foramen lacerum or foramen ovale or through both the foramen ovale and foramen lacerum, and can also spread by direct erosion and will result multiple cranial deficits (Ampil, Heldman, Ibrahim, & Balfour, 2000; Taguchi et al., 1997).

Cranial nerve involvement is manifested by isolated nerve palsies or anterior or posterior syndromes. Anterior syndromes are clinical manifestations of the lesions affecting the middle cerebral fossa nerves. Some example are:

1. The superior orbital fissure syndrome: involves CN III, IV, VI, ophthalmic branch of the trigeminal nerve, and superior ophthalmic vein. Clinical

manifestations are total ophtalmoplegia, pain/paresthesias/sensory loss of the trimeginal nerve, area 1 (V1), proptosis/chemosis/lid edema: pupil is mydriatic and unreactive (Binder, Sonne, & Aschbeln, 2010).

2. Orbital apex syndrome involves CN II, III, IV, VI. Patients suffering with this syndrome have complete ophtalmoplegia, ptosis, decreased corneal sensation, and visual loss (Cho et al., 2009).
3. Jaccoud syndrome is characterized by progressive ophtalmoplegia, trigeminal neuralgia, and neurosensorial loss (Binder et al., 2010).
4. Gradenigo syndrome affects CN V and VI with facial pain/numbness, retroorbital pain, abducens nerve palsy (impairment of lateral movements of the eyeball), and otorrhea (Binder et al., 2010).
5. Reader syndrome involves CN V, VI, and symphatetic cervical nerve and includes impairment of lateral movements of the eyeball and pain/paresthesias/sensory loss in trigeminal nerve area and manifestation of Claude–Bernard–Horner syndrome-ptosis, miosis without anhidrosis, and ipsilateral head/facial/retroorbital pain (Binder et al., 2010).
6. Posterior syndrome involves the skull base or in those cases with bulky upper cervical nodes (CN XII) or the jugular foramen (CN IX, X, and CN XI) (Fig. 6.7).

Palpable neck masses

Lymphatic metastases of NPC occur to the ipsilateral neck in 85%–90% of cases and to the bilateral neck in 50% of cases. It is rare to metatisize to contralateral cervical node alone (Lu, Cooper, & Lee, 2010). This is the most common presenting symptom and 43% of patients present with unilateral or bilateral cervical mass on physical examination (Skinner et al., 1991) (Fig. 6.8).

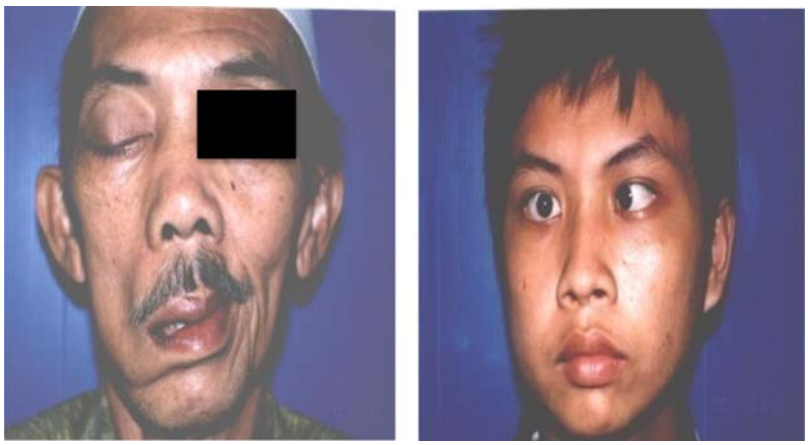

Figure 6.7
Ptosis (*left*) and opthalmoplegia (*right*) as the result of cranial nerve (CN) involvement in nasopharyngeal carcinoma (NPC).

Figure 6.8 Unilateral and bilateral neck masses in nasopharyngeal carcinoma (NPC) patients.

Table 6.1 Clinical presentation.

Structures involved	Symptoms
Metastasis	Painless cervical lymph node enlargement (*most common presentation*)
	Bone pain, organ dysfunction, and rarely, a paraneoplastic syndrome of osteoarthropathy
Nasal	Large or exophytic lesions may cause nasal obstruction or epistaxis
Aural	Unilateral hearing loss from blockage of the Eustachian tube (ET) and a middle ear effusion
	Tinnitus
	Stuffiness
Neurological [cranial nerves (CN) involvement]	Xerophthalmia involvement of the greater superficial petrosal nerve at the foramen lacerum
	Facial pain, trigeminal nerve involvement
	Diplopia-isolated abducens nerve injury
	Ophthalmoplegia involvement of cranial nerves (CN) III, IV, and VI (in the cavernous sinus or the superior orbital fissure)
	Horner's syndrome, injury to the cervical sympathetic chain
	Deficits of the lower CN (IX, X, XI, and XII), more extensive skull base involvement

Source: Data from IJCP, Vol 2, No 4, Autumn 2009 (Agrawal et al., 2009).

Mostly the upper cervical nodes enlarge before the middle and lower cervical nodes (Sham, Poon, et al., 1990; Sham, Wei, et al., 1990). In particular, the lateral and medial retropharyngeal lymph nodes (Lu et al., 2010). The upper cervical nodes are usually more bulky than the lower cervical nodes, which is indicative of an orderly spread in the craniocaudal direction. In most cases, the cervical node enlargement is unilateral, but it is not uncommon to see bilateral cervical adenopathy.

Hsieh et al. found that neck mass was the second most significant clinical finding in patients with newly diagnosed NPC and reported a sensitivity rate of 66% (Hsieh, Wang, Lin, Weng, & Lee, 2012) (Table 6.1).

Tumor invasion in the different local anatomic sites of the nasal cavity ranged from 8.8% to 97.6%. The nasopharynx can be classified according to the cumulative incidence rates of NPC invasion in high risk ($\geq$35%), medium risk ($\geq$10%–35%), and low risk ($<$10%). Posterior skull base and adjacent nasopharynx sites are considered high risk sites. The most common route to skull base invasion is through the foramen lacerum (Brown & Fee, 1998; Cao et al., 2018).

High-risk areas involved are Foramen lacerum (97.6%), basis of sphenoid bone (97.1%), sphenoidal sinus (92.2%), prestyloid compartment (89.8%), prevertebral muscle (88.8%), petrous apex (87.3%), clivus (84.9%), foramen ovale (82.4%), medial pterygoid muscle (60.0%), medial pterygoid plate (57.6%), foramen rotundum (51.7%), pterygopalatine fossa (46.8%) poststyloid compartment (46.8%), and hypoglossal canal (39.0%) (Brown & Fee, 1998).

Medium-risk invasion areas include lateral pterygoid plate (31.7%), jugular foramen (24.4%), infratemporal fossa 41 (20.0%), lateral pterygoid muscle (19.5%), orbital apex (16.6%), oropharynx (15.6%), maxillary sinus (15.1%), and nasal cavity (13.2%). Low-risk invasion is limited to the orbital wall (9.3%) and ethmoid sinus (8.8%) (Brown & Fee, 1998).

In 25%–35% of cases the skull gets destroyed when invasion of the tumor originates in the posterior-superior wall and 12% of patients present with intracranial tumor spread. Trigeminal nerve involvement may be seen first, with hypoesthesia of the involved region. In later stages, the cavernous sinus and eye movement may be impaired with associated diplopia (CN VI and III) (Adham et al., 2012). Patients may remain asymptomatic for an extended time period, since NPC commonly arises in the fossa of Rosenmuller. The tumor presentation may be an indistinct nodule with or without ulceration, an irregular infiltrating mass, or a smooth submucosal area (Licitra et al., 2003).

Impaired sensation of the lower teeth (inferior alveolar nerve), tongue numbness (lingual nerve), paresthesia of the auricle and the surrounding skin (auriculotemporal nerve), problems with mastication (pterygoid space and muscles), and swallowing (parapharyngeal space) can be seen when the tumor originates in the postero-lateral wall. Up to 30% of patients that have involvement of the oropharyngeal area have associated retropharyngeal nodes. (Adham et al., 2012). Compared to other head and neck cancers NPC tends to have more propensity for cervical lymph node metastasis. Around 75% of patients present with cervical lymph node metastasis, and over 50% of the cases are bilateral (Brown & Fee, 1998; Licitra et al., 2003) (Fig. 6.9).

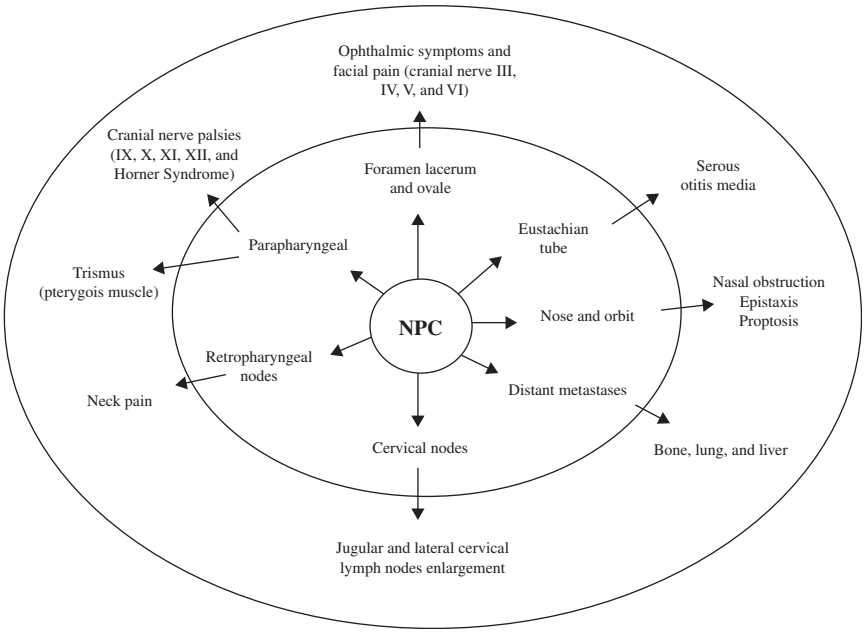

Figure 6.9 Nasopharyngeal carcinoma (NPC) sign and symptoms (Dhingra & Dhingra, 2014).

Sign and symptoms related to extension of disease out of the nasopharynx

Distant dissemination of NPC is uncommon, it can be seen in about 5%–11% of patients at presentation in the clinic. The incident is associated with metastasis through the nodes, especially the supraclavicular region. Bone is the most frequent site of distant metastasis in about 75% of the cases (60% of WHO type 1 and 85%–90% of WHO type 2), followed by liver (30%), lung (18%), and extra-regional nodes (axillary, mediastinal, pelvic, and inguinal) are more uncommon. Paraneoplastic syndromes, fever of unknown origin, and dermatomyositis can also occur. Patients can also have pneumonia, neck mass, chills, fever, headache, bone pain, and weight loss. The incidence of metastatic disease is correlated with the nodal stage (Adham et al., 2012; Kamran, Riaz, & Lee, 2015; Lu et al., 2010; Wei & Mok, 2007; Wei et al., 2001).

Paraneoplastic syndrome is a group of symptoms that occur due to immune cross-reactivity between tumors and normal tissue. Dermatomyositis might appear as the initial manifestation of paraneoplastic syndrome in NPC patients but in rare circumstances. Dermatomyositis is characterized as hyperkeratotic, follicular, and erythematous papules. In most cases, the first lesions appear on

the face and eyelids and eventually, the neck, shoulder, and upper extremities. The skin manifestation is always followed by muscular weakness. In one study, only 1% of 1154 NPC cases in Hong Kong were associated with dermatomyositis and three of the ten patients with it had paraneoplastic syndrome (Lu et al., 2010).

A common site of tumor extension of NPC is in the oropharyngeal area in 33% of the cases, and is associated with retropharyngeal nodes. The carotid sheath, as well as CN IX and XII, and the poststyloid space can be seen in advanced disease. The parapharyngeal spaces, retropharyngeal space, and poststyloid space are the first nodes to be involved. NPC has been observed more commonly with the upper and middle spinal nodes (Kamran et al., 2015; Wei & Kwong, 2010; Wei & Mok, 2007).

NPC usually originates in the fossa of Rosenmuller. Primarily at the transformation zone of fossa Rosenmuller between the ciliated columnar respiratory and the stratified squamous epithelium where metaplastic squamous cells are detected (Poh, Chua, & Wee, 2016). It can extend to the sinus of Morgagni, laterally affecting the ET, the parapharyngeal space, and the area of the foramen ovale; giving rise to Trotter syndrome, which is an ipsilateral trigeminal (mandibular) neuralgia (Lee et al., 2004).

It is important to mention that Asians have better survival rates than blacks or whites, even though they have more tendencies to have advanced neck and distant metastases (31% for Asians and 24.5% for non-Asians). Recurrent tumor in cervical lymph nodes after therapy was investigated by Wei et al. in a series of neck dissection specimens and it was found that level II was the most common site of recurrence (53%). They also found that 84% of the patients had persistent or extensive disease in the neck or extra-nodal involvement (Cannon, Zanation, Lai, & Weissler, 2006; Yoshizaki et al., 2012).

There are also complications to radiation and reradiation in recurrent disease. Common signs and symptoms include varying degrees of xerostomia, neuroendocrine imbalances, temporal lobe necrosis, and osteoradionecrosis. Otologic problems like sensorineural hearing loss and serous OME have also commonly been reported. In one study, leukopenia and thrombocytopenia with nausea and vomiting was experienced with chemotherapy and less so with biological treatment (Wei, Yuen, & Ho, 2000) (Fig. 6.10).

Despite aggressive radiotherapy, the 5 year survival rate for locoregionally advanced disease at presentation is 30%–45% (Cooper, Lee, Torrey, & Hochster, 2000). Factors associated with a poor prognosis are skull base involvement, extent of the primary tumor, and CN involvement (Kalogera-Fountzila et al., 2006; Ong et al., 2011).

A number of patients presented with recurrent disease especially at the neck a few years following chemoradiation. The neck recurrence mostly involves neck

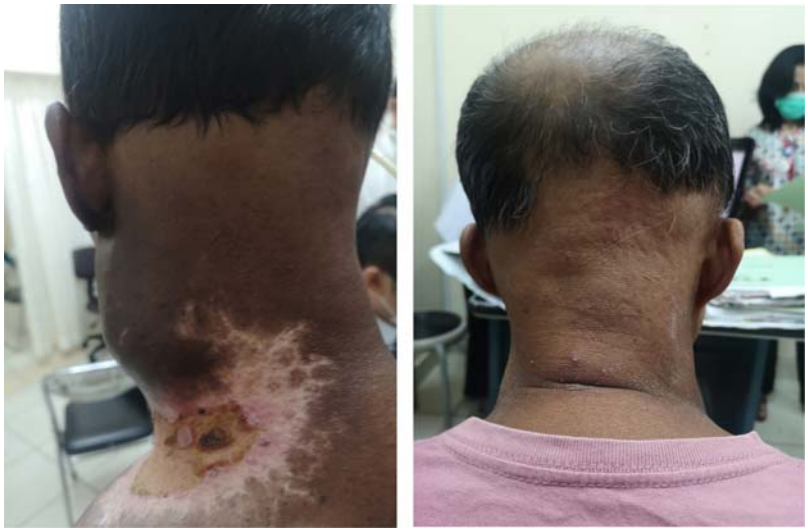

Figure 6.10
Complications of radiotherapy include fibrotic neck (*left*) and hair loss (*right*).

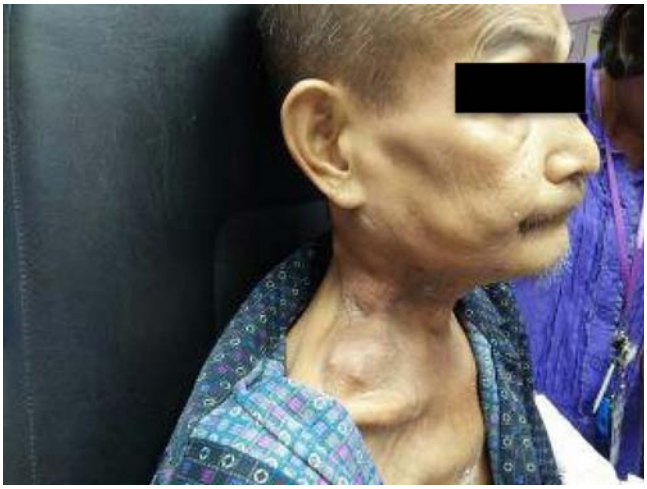

Figure 6.11
Recurrence of a nasopharyngeal carcinoma (NPC) patient after postchemoradiation with multiple neck levels affected.

levels II, III, IV, and V. This is attributed to several reasons such as poor compliance with posttreatment follow-up schedule due to social, family, and economic factors. Selected patients presented with local recurrence with superior extension to the skull base and intracranially (Fig. 6.11).

Some patients are subject to palliative chemotherapy due to extensive disease that is not amenable to surgery. A limited neck recurrence or multiple level neck recurrence without distant metastases will be an ideal case for salvage neck dissection.

Diagnoses

The definitive diagnosis for NPC is biopsy from the primary tumor. Endoscope is usually used to support the biopsy and is performed under local anesthesia to obtain an adequate sample. Patients who clinically present NPC but have negative biopsy results are recommended to repeat the biopsy using general anesthesia to obtain clearer visualization of fossa Rosenmuller (the only region of the nasopharynx). Multiple deep biopsies should be obtained from the bilateral fossae of Rosenmuller (Lu et al., 2010).

A study of Wei-ming Cai et al. (1983) showed that NPC patients who were given radiotherapy less than 14 days after the biopsy had a higher 5-year survival rate (61%) than patients who underwent radiotherapy more than 15 days after biopsy (47.5%). The study also noted that repeated biopsies did not affect treatment outcomes. Partial incision at the lymph node also provides a lower 5-year survival rate (22%) compared to biopsy with complete excision (50%).

Conclusion

NPC is one of the most easily misdiagnosed tumors because it presents initially with nonspecific symptoms and signs. Careful evaluation of the nasopharynx is therefore necessary to exclude NPC in the presence of unilateral, unexplained OME in an adult patient. Nasopharyngeal endoscopy is the initial procedure of choice for the detection of NPC, with a definitive diagnosis of NPC confirmed by endoscopic biopsy of the primary tumor. Doctors can contribute to a delayed NPC diagnosis when they ignore and misjudge the nonspecific early stage symptoms that mimic other diseases. Educating hospital staff and medical practitioners in primary health care is a critical first step for diagnosing early stage NPC, as this can improve awareness of various nonspecific symptoms and signs of NPC, especially among high-risk populations.

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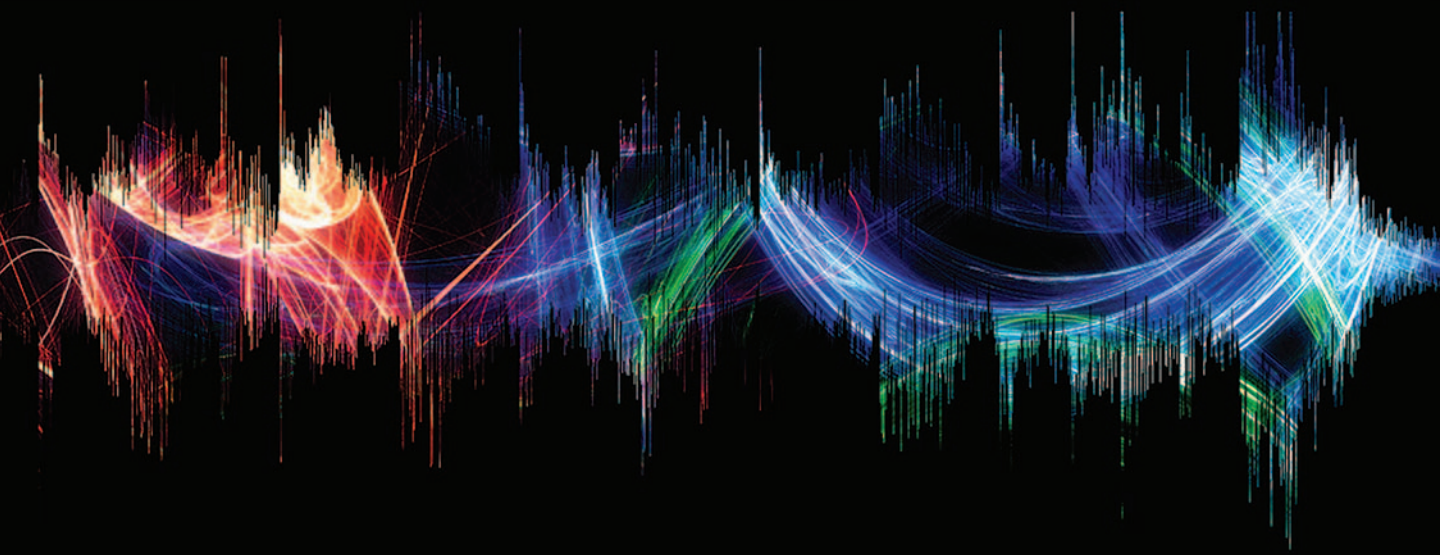

7

Assessment and staging of nasopharyngeal carcinoma

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Assessment

History

Clinical presentation

Early symptoms of nasopharyngeal carcinoma (NPC) can often be deceptive and confusing. A comprehensive history has to be taken to fully evaluate any patient with suspicion of NPC. Patients may exhibit subtle or mild clinical features. Without a proper screening program, it is not possible for NPC to be discovered in early stages before it has spread to lymph nodes or other regions. The first presentation of NPC is usually the enlargement of regional lymph nodes, presenting as neck nodes or masses. In the presence of ear and nose symptoms, such as hearing problems, serous otitis media, tinnitus, nasal obstruction, anosmia, bleeding, difficulty swallowing, dysphonia, and even eye symptoms including diplopia and pain, clinicians must be aware of the possibility of NPC and examine the patient's nasopharynx. Initial diagnosis is difficult because early signs and symptoms of NPC are not specific to the disease.

An early retrospective study of 4768 NPC patients investigated the clinical presentations commonly seen in NPC patients. This report found that neck mass (37.3%) and nasal symptoms (35.4%) including nasal discharge, bleeding, and obstruction are the most common first presentations. The location of a neck mass often indicates the primary disease. An asymptomatic posterior triangle neck mass was highly suggestive of NPC, and it was the most common presenting symptoms, occurring in 76% of NPC patients (Lee et al., 1997).

The enlargement of the tumor in the nasopharynx leads to nasal symptoms including bleeding, nasal obstruction, and nasal discharge. Nosebleed in the form of blood-stained discharge is one of the most common symptoms. Approximately 42.3% of patients revealed nosebleed at the initial stage of the

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disease but this must be differentiated from frank epistaxis, which is uncommon. Other nasal and laryngeal symptoms are also common findings, such as stuffiness, hemoptysis, running nose, dysphagia, and altered olfactory function (Epstein & Jones, 1993).

The tumor may extend into the ear, causing ear symptoms. Aural symptoms, including tinnitus and impairment of hearing, are the third most common first presentation of NPC (Lee et al., 1997). Patients may describe their auditory symptoms as plugging, drainage, ringing, deafness, or dizziness. Decreased hearing is the most common symptom among patients with auditory complaints (61.8%), which is approximately 39.9% of all NPC patients (Epstein & Jones, 1993). The resulting conductive hearing loss is usually due to tumor infiltration of the tubal musculature leading to the malfunction of the Eustachian tube opening mechanism. The tumor may further cause middle ear effusion leading to decreased hearing (van Hasselt & Leung, 1999). Sensorineural hearing loss is a relatively rare symptom of NPC; however, it is a common adverse effect in NPC patients treated with chemoradiotherapy. Tinnitus is also a common auditory complaint and can be particularly troublesome and difficult to treat.

Otitis media is a warning sign for the development of NPC. In particular, adult-onset serious otitis media is rarely caused by diseases other than NPC (van Hasselt & Leung, 1999). An 11-fold higher risk of developing NPC for adult patients with otitis media was reported by a Taiwan study, which recommended that follow-up examination should be undertaken for at least 5 years in patients with otitis media (Huang et al., 2012).

NPC may directly erode the base of the skull and invade cranial nerves (CN), causing neurological complaints including headaches or CN symptoms. These symptoms are indicative of advanced local disease. Cranial nerve invasion is negatively correlated with the prognosis of NPC. Headache is the most common neurological symptom, occurring in almost 20% of patients (van Hasselt & Leung, 1999). The trigeminal nerve (CN V) and the abducens nerve (CN VI) are the most commonly involved CN. When CN V is involved, hemifacial pain or numbness may occur and the pain may be referred to the upper jaw and teeth. Diplopia is a sign of CN VI involvement caused by paralysis of the lateral rectus muscle (Acquarelli & Peter, 1963).

Pain is a common presenting symptom of NPC. 96% of NPC patients who complained about pain described the location of their pain as headache, earache, jaw, midfacial, or neck pain (Epstein & Jones, 1993).

Social history

Globally, there were approximately 86,700 new cases of NPC and 50,800 deaths in 2012. Although NPC is relatively rare in most other parts of the world,

it is endemic in Southeast Asia. NPC is about 2 to 3 times more prevalent in males than in females. The disease is the sixth most common cancer among males in the region (Torre et al., 2015). When examining male patients from the endemic regions presenting symptoms mentioned above, clinicians must be aware of the possibility of NPC. Patients with family history may have higher incidence of NPC, with an incidence rate of 3.64% in NPC patients with family history (Wang, Shen, Lu, & Hu, 2017).

Early studies have investigated the dietary risk factors of NPC in the high risk Cantonese population. Salted fish consumption has been reported to be significantly associated with an increased risk of NPC. A large-scale study reported 2−3 fold higher risk of NPC for the highest childhood intake frequency stratum of canton-style salted fish, preserved vegetables, and preserved/cured meat (Jia et al., 2010).

Environmental and occupational risk factors of NPC have also been studied. Exposure to domestic wood cooking fires and occupational solvents are also associated with higher risk of NPC (Guo et al., 2009).

Physical examination

A full head and neck examination is essential in all NPC cases. Special attention should be paid to the head and neck area, including the nose, mouth, throat, facial muscles, and lymph nodes in the neck during physical examination. Neck mass is the most common first presentation of NPC. Lymph node enlargement can be observed and clinicians should possess knowledge of the neck lymph node groups in order to differentiate NPC from other neck masses. The neck should be examined extensively and carefully in a systemic manner to check for any lumps or bumps. An example of a routine neck examination is to start from the preauricular region, moving to the parotid and then submandibular submental region, followed by a circle around the anterior, supraclavicular, jugulodiagastric, and posterior triangle, and finishing at the postauricular area of the neck. Localization of the mass can suggest specific etiologies. Masses in the upper cervical and posterior triangle should elicit a high index of suspicion for nasopharyngeal malignancy (Ahuja & Ying, 2003). In one series of 4768 patients with NPC, an asymptomatic posterior triangle neck mass was the most common presenting symptom, occurring in 76% of patients (Lee et al., 1997; Wei & Sham, 2005). For specialist clinicians, the neck is divided into different levels from I to VI for easy communication and planning of oncological management.

Lymph node levels of the neck

The neck is divided into six levels (I to VI) and sublevels (A or B) that are used for describing the location of lymph nodes in the neck (Fig. 7.1).

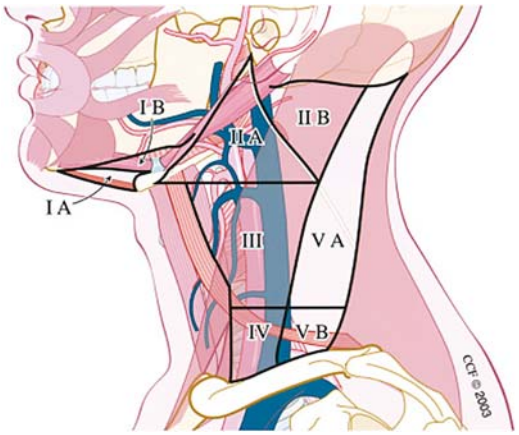

Figure 7.1 Lymph node levels of the neck. Source: *Adapted from Townsend, B., Evers, M., Townsend, C. M., Beauchamp, R. D., Evers, B. M., & Mattox, K. L. (2017). The biological basis of modern surgical practice. In:* Sabiston textbook of surgery *[electronic Resource].*

Level I	Submental and Submandibular Group (IA) Submental lymph nodes (IB) Submandibular lymph nodes
Level II	Upper Jugular Group Located from the hyoid bone to the base of the skull (IIA) anterior to the spinal accessory nerve (IIB) posterior to the spinal accessory nerve
Level III	Middle Jugular Group Located between the cricoid cartilage and the hyoid bone
Level IV	Lower Jugular Group Located from the clavicle to the cricoid cartilage
Level V	Posterior Triangle Group Located posterior to the sternocleidomastoid muscle, including spinal accessory nodes (VA) above the cricoid cartilage, supraclavicular, and transverse cervical nodes; (VB) below the cricoid cartilage.
Level VI	Central Neck Group Located between the carotid arteries from the innominate artery to the hyoid bone Including the pretracheal, prelaryngeal, and paratracheal lymph nodes

85% of NPC cases presented with lymphadenopathy. Most patients with metastatic cervical nodes present at the time of diagnosis are diagnosed with an advanced stage NPC. Lymph node metastases of NPC follow an orderly pattern. Retropharyngeal and level II lymph nodes are the most commonly involved regions with probabilities of 69% and 70%, respectively. Levels III, IV, and V nodal involvement are less common, with probabilities of 45%, 11%, and 27%, respectively. The supraclavicular, levels IA/IB and VI nodes, and parotid nodes are rarely involved, with involvement rates lower than 3%

(Ho, Tham, Earnest, Lee, & Lu, 2012). Retropharyngeal lymph node (RLN) involvement was further associated with higher rates of distant metastasis (DM) leading to poorer prognosis (Tham, Hee, Yap, Tuan, & Wee, 2009). RLNs involved by tumor may subsequently be infected to become an abscess. Retropharyngeal abscess was reported to be a rare presentation of NPC (Pak, Chan, & van Hasselt, 1999).

Nasal symptoms are a common presentation of NPC patients. Traditional indirect nasopharyngeal examination with a mirror could be limited by variations in anatomy. Direct nasopharyngeal examination is more commonly used in the outpatient clinic setting at the present time. The nasopharynx can be well examined with rigid nasoendoscopes and flexible fibroptic endoscopes for primary tumor and malignancy. The endoscopic examination of the nasopharynx will be discussed further in the next section.

Auditory complaints are also present in NPC patients. Conductive hearing loss is common in NPC patients with auditory complaints. Decreased hearing can be examined by Weber test by placing a tuning fork (512 Hz) on the vertex of the forehead. The perceived sound will be louder in the infected ear. In the rare event that patients reveal sensorineural hearing loss, the tuning fork sound may lateralize to the opposite, noninfected ear.

Otitis media is an early warning sign of NPC. The most important factor in the pathogenesis of middle ear infections is dysfunction of the Eustachian tube. Otoscopic examination is performed to examine the tympanic membrane. The tympanic membrane is normally translucent (Fig. 7.2A). While infected, it

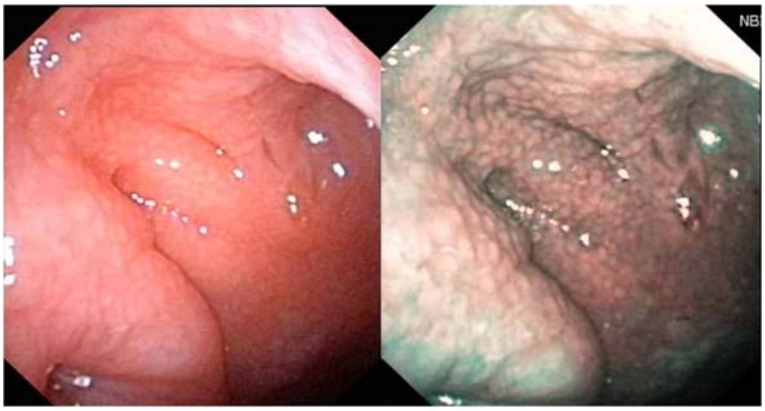

Figure 7.2 Normal nasopharynx under white light with smooth, symmetrical, regular, polygonal follicles (*left*). Normal nasopharynx under narrow-band imaging (NBI) with normal, pale, regular, polygonal follicles outlined by thin, darker reticular borders (*right*). Source: *Adapted from Vlantis, A. C., Woo, J. K. S., Tong, M. C. F., King, A. D., Goggins, W., & van Hasselt, C. A. (2016). Narrow band imaging endoscopy of the nasopharynx is not more useful than white light endoscopy for suspected nasopharyngeal carcinoma.* Head & Neck, *273(10), 3363–3369. Available from https://doi.org/10.1007/s00405-016-3940-6. Used with permission. Reprinted with permission from Springer Nature: European Archives of Oto-Rhino-Laryngology.*

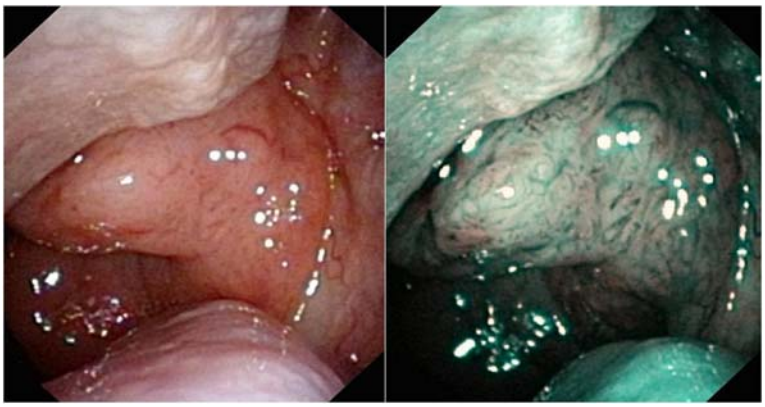

Figure 7.3 A submucosal mass in nasopharynx under white light with enlarged vessels in the overlying mucosa (*left*). A submucosal mass in nasopharynx under narrow-band imaging (NBI) with enlarged vessels—a multitude of abnormal mucosal vessels in the mucosa (*right*). Source*: Adapted from Vlantis, A. C., Woo, J. K. S., Tong, M. C. F., King, A. D., Goggins, W., & van Hasselt, C. A. (2016). Narrow band imaging endoscopy of the nasopharynx is not more useful than white light endoscopy for suspected nasopharyngeal carcinoma.* Head & Neck, *273(10), 3363–3369. Available from https://doi.org/10.1007/s00405-016-3940-6. Used with permission. Reprinted with permission from Springer Nature: European Archives of Oto-Rhino-Laryngology.*

typically demonstrates tympanic membrane redness, opacification, and bulging (Fig. 7.2B). In otitis media with effusion, the tympanic membrane appears cloudy, yellowish, or opaque. Multiple air-fluid levels are present behind the slightly retracted, translucent and non-erythematous tympanic membrane (Fig. 7.3). In addition, there may be purulence in the ear canal if there is an associated tympanic membrane rupture.

Nasoendoscopy should be performed to rule out nasopharyngeal pathology in patients with recurrent unilateral serous otitis media. There are limited data regarding the yield of nasoendoscopy in the routine workup of isolated otitis media with effusion, but it should considered that individuals from China, Southeast Asia, and Northern Africa are at increased risk for NPC.

Neurological examination

Several CN lie in close proximity to the nasopharyngeal vault. Nasopharyngeal tumors may invade CN and cause neurological disturbances. The incidence of CN palsy as a result of an adjacent extension of the primary tumor in NPC ranges from 8.0% to 12.4% (Cheng et al., 2006). The time between onset of general symptoms of NPC and neurological complaints ranges from 1 month to 7 years. The time window is less than 3 years in the majority of NPC patients (Thomas & Waltz, 1965).

In two thirds of patients the first presenting neurological complaint is either diplopia or sensory disturbance in the face, suggesting the involvement of the abducens

nerve (CN VI) and the trigeminal nerve (CN V). CN V and VI are the most commonly involved CN due to the close proximity of these nerves to the foramen lacerum (Acquarelli & Peter, 1963; van Hasselt & Leung, 1999). The abducens nerve (CN VI) was involved in 68% of patients, the trigeminal nerve (CN V) in 47%, and the glossopharyngeal-vagus nerve (CN IX) in 38% (Thomas & Waltz, 1965).

NPC can invade upward and backward through the skull base to the cavernous sinus and middle cranial fossa and invade cranial nerves CN II to VI. The oculomotor nerve (CN III) and the facial nerve (CN VII) are never involved alone, which is useful for differential diagnosis. NPC may also involve the carotid space, where it may compress or invade the hypoglossal nerve (CN XII) as it exits through the hypoglossal canal, CN IX to XI as they emerge from the jugular foramen, and the cervical sympathetic nerves (Chang et al., 2005).

Neurological examination reveals CN damage in almost all cases. It is recommended that CN invasion be assessed initially by neurological evaluation rather than by computed tomography (CT) and magnetic resonance imaging (MRI). The CN can be examined by simple hearing tests and the sensation and movement of face. Hemifacial pain or numbness occur when CN V is involved. Patients may also reveal unilateral facial pain or headache and the pain may be referred to the upper jaw and teeth when CN V is involved. Diplopia caused by paralysis of the lateral rectus muscle is a sign of CN VI involvement (Acquarelli & Peter, 1963). Electrophysiological studies and imaging studies for specific management of these CN problems may be undertaken when appropriate.

Endoscopic examination

Endoscopic examination with biopsy

The definitive diagnosis of NPC is based on endoscopic examination of the nasopharynx and the pathology of the biopsy of the primary tumor (Chan & Felip, 2009). It has been reported that the sensitivity, specificity, and accuracy, respectively, were 95%, 100%, and 98% for endoscopic biopsy of the suspected tumor (King et al., 2011).

Several early studies reported that the presence of tumor was unrelated to the macroscopic endoscopic findings. Relying just on the visual appearance through the endoscope have low sensitivity in predicting persistent disease, thus biopsy of multiple sites is indicated (Sham et al., 1989; Wei, Sham, Choy, Zong, & Ng, 1991). Repeat biopsies are indicated for those with endoscopic findings of localized tumor or positive histologic findings in the first session of biopsies in order to improve detection of persistent disease (Kwong et al., 2001). Repeated biopsies are correlated positively with the diagnosis of malignancy, implying that a single negative biopsy may not be specific to exclude malignancy indicating the importance of close surveillance of these patients (Arslan, Tuzuner, Koycu, Dursun, & Hucumenoglu, 2018).

Examination of the nasopharynx can be performed with indirect and direct nasopharyngoscopy. Traditionally, the nasopharyngeal examination was performed with indirect nasopharyngoscopy using a headlight and a postnasal mirror. The view from those procedures is adequate for most of the nasopharyngeal examination; however, there are limitations. The view can be blocked by anatomical structure such as a narrow nasopharynx, bulky tongue, or redundant soft palate. Mirror examination can also induce gag reflex causing difficulties in examination.

Currently, direct nasopharyngoscopy is a better option for a more reliable and higher resolution view of the nasopharynx. Hopkins rod telescopes are rigid nasal endoscopes with lenses at various angles such as 0-, 30-, and 90-degrees. Rigid telescopes can be inserted transnasally to evaluate the roof and recesses of the nasopharynx. The 0-degree rigid telescopes are usually used for biopsy from suspicious nasopharynx tissue. The 30-degree rigid telescopes can be used for a general examination of the whole nasopharynx and the nasal cavity. The 90-degree rigid telescope is inserted transorally to evaluate the whole nasopharynx in a wide-angle view and allow detection of minor assymetries in the anatomy. It may provide a better image quality and high-visual visualisation than indirect nasopharyngoscopy with a mirror.

The flexible fiberscope is also an ideal tool for nasopharyngeal examination. The narrow diameter and flexibility of the fiberscope tip allows for a thorough examination of every detail of nasopharynx. It gives an undistorted view and provides the flexibility required to inspect the area in detail. The excellent optics, narrow diameter, and flexible tip of the fiberscope enable accurate assessment of the entire nasopharynx and visualization of small lesions (Woo, 1999).

Both rigid and flexible nasoendoscopes can provide high resolution visualization of the nasopharynx, which may be particularly useful in cases of NPC with small primary lesions. Additionally, nasoendoscopy systems can provide high resolution imaging and video documentation (Brian & Robert, 1994). The mucosa of the normal nasopharynx should be pink and intact and the opening of the Eustachian tube visible. Mostly, NPC tumors initiate in the fossa of Rosenmüller and spread intracranially or locally. NPC originated at the submucosal level may spread outside the anatomic limits of the nasopharynx (Adham et al., 2012). The blockage of the opening of the Eustachian tube by the tumor may be observed with endoscopic examination in patients who reveal ear symptoms, including ear pain, plugging, ringing, and tinnitus.

Biopsy is done along with the endoscopic examination. The narrow diameter of nasoendoscopes and biopsy forceps allows both instruments to pass through the nasal passage at the same time, thus the biopsy can be performed under direct endoscopic visualization. The procedure may cause discomfort and local anesthesia is usually given during the procedure. Topical application of an

anesthetic transnasally ensures patient comfort and compliance with the examination. Generally, patients can tolerate this method of examination. The biopsy procedure is relatively painless, as the tumor is friable and insensitive. Biopsy under local anesthesia is safe, easy to perform, well tolerated by the patient, and can be undertaken in the outpatient clinic. This diagnostic approach achieves rapid diagnosis in patients with NPC (Van Hasselt, Mmed, & Gareth John, 1994).

High definition-endoscopic imaging and narrow-band imaging endoscopy

The authors recommend the employment of high definition (HD)-images and narrow-band imaging (NBI), in which light of a narrowed bandwidth is used to illuminate the mucosa. The wavelength used is shorter than that of standard white light. The central wavelengths of each band are 415 and 540 nm (Gono et al., 2003). This shorter wavelength light penetrates the mucosa and highlights the superficial vasculature, leading to a high contrast between blood vessels and surrounding nonvascular mucosal tissue. NBI has been successfully applied to detect malignancy in various tissues such as the colon, esophagus, duodenal ampulla, and lung (East, Tan, Bergman, Saunders, & Tekkis, 2008).

Normal nasopharynx appears smooth, symmetric, pink, and intact under white light endoscopy. Whereas in NBI endoscopy, normal nasopharyngeal mucosa in adults has minimal lymphoid tissue and regular follicular patterns. Normal, pale, regular, polygonal follicles outlined by thin, darker reticular borders can be seen on NBI (Fig. 7.2). The ratio of the pale follicle to dark border (pale-to-dark ratio) is roughly 90%. While in patients with NPC, the pale-to-dark ratio is reversed on NBI, with dark centers surrounded by pale borders and a pale-to-dark ratio of roughly 50% (Vlantis et al., 2010).

The diagnostic value of NBI for detection of NPC has been investigated. NBI was compared with conventional white light and it was reported that NBI identified distinctively adenoidal tissue from NPC (Thong, Loke, Karumathil Sivasankarannair, & Mok, 2013). Nasopharyngeal lymphoid hyperplasia (adenoid) is a vegetative mass in the posterior wall of the nasopharynx. It was identified that in NBI endoscopy, observation of a light crest (LC) on the epithelial surface in the nasopharyngeal mucosa is a highly accurate predictor of the presence of histologic lymphoid hyperplasia and may be treated as a distinctive endoscopic finding (Wang, Lin, Weng, & Lee, 2011).

Abnormal vasculature under NBI endoscopy is indicative of abnormal mucosa. In NBI endoscopy, it was found that dilated vessels were not commonly seen in normal mucosa of normal nasopharynx. Dilated and/or enlarged vessels indicated abnormal mucosa (Fig. 7.3). The presence of an earthworm-like appearance, i.e. irregular diameter and course of the microvessels, or known as vascular tufts, was associated with malignancy (Figs. 7.4 and 7.5). However, it was reported that

there was no significant difference in detection rates of NPC between NBI endoscopy of vasculature alone and white light imaging. The sensitivity and specificity of white light and NBI endoscopy for NPC was 93% and 22%, versus 92% and 98%, respectively (Vlantis et al., 2016).

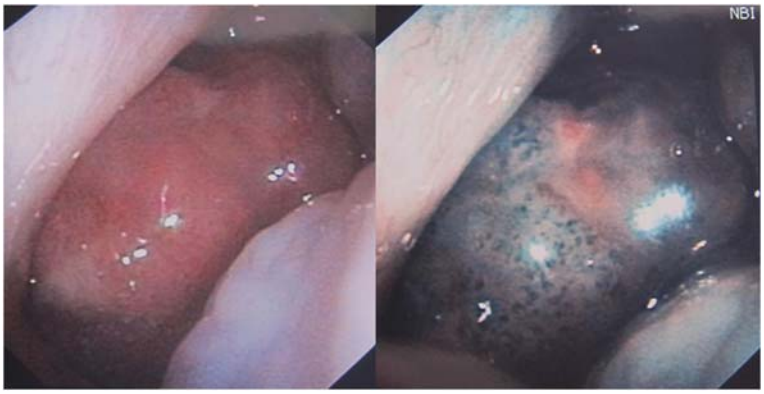

Figure 7.4 A submucosal mass in nasopharynx under white light covered by an unremarkable mucosa (*left*). A submucosal mass in nasopharynx under narrow-band imaging (NBI) with vascular tufts (*right*). Source*: Adapted from Vlantis, A. C., Woo, J. K. S., Tong, M. C. F., King, A. D., Goggins, W., & van Hasselt, C. A. (2016). Narrow band imaging endoscopy of the nasopharynx is not more useful than white light endoscopy for suspected nasopharyngeal carcinoma.* Head & Neck, *273(10), 3363–3369. Available from https://doi.org/10.1007/s00405-016-3940-6. Used with permission. Reprinted with permission from Springer Nature: European Archives of Oto-Rhino-Laryngology.*

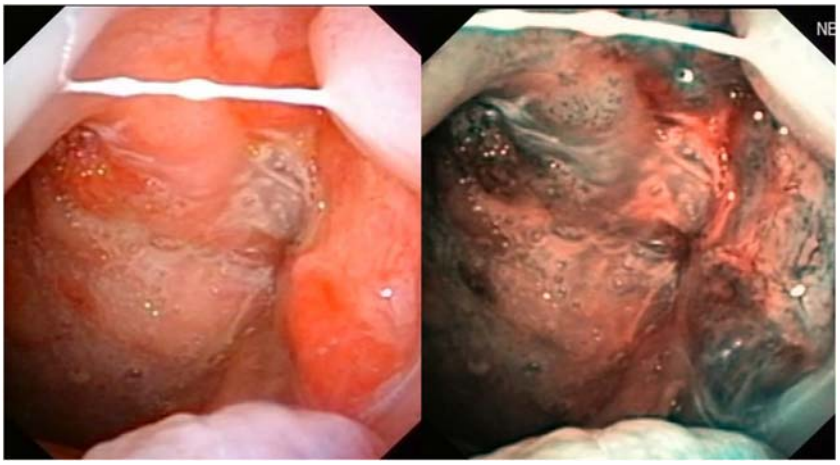

Figure 7.5 An extensively irregular nasopharyngeal mucosa with dilated vessels and vascular tufts under white light (*left*). An extensively irregular nasopharyngeal mucosa under narrow-band imaging (NBI) with both dilated and enlarged vessels and vascular tufts (*right*). Source*: Adapted from Vlantis, A. C., Woo, J. K. S., Tong, M. C. F., King, A. D., Goggins, W., & van Hasselt, C. A. (2016). Narrow band imaging endoscopy of the nasopharynx is not more useful than white light endoscopy for suspected nasopharyngeal carcinoma.* Head & Neck, *273(10), 3363–3369. Available from https://doi.org/10.1007/s00405-016-3940-6. Used with permission. Reprinted with permission from Springer Nature: European Archives of Oto-Rhino-Laryngology.*

NBI may be useful in the early detection of NPC. The distinctly different findings of NPC by NBI were identified and divided into five categories (Wang, Lin, Lee, & Weng, 2011). Type I (T1) refers to brownish spots, type II refers to an irregular microvascular pattern (IMVP), type III refers to LCs, type IV refers to side difference, and type V refers to either an IMVP or side difference. The false positive, false negative, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPP), and accuracy of NPC diagnosis by detecting type V (IMVP or side-difference) on NBI were 6.7%, 2.9%, 97.1%, 93.3%, 91.7%, 97.7%, and 94.9%, respectively. In T1 early stage NPC, there was a prevalence of brownish spots and side-difference. Nonkeratinizing undifferentiated NPC in the early stage showed brownish snake-like, winding microvessels to the left of the fossa of Rosenmüller under NBI endoscopy. It was found that early-stage superficial NPC lesions showed type IV microvessels under NBI endoscopy. Additionally, the superficial microvessels detected by NBI were positively correlated to tumor regression following radiotherapy. Therefore NBI endoscopy had predictive value in the treatment outcome of radiotherapy (Ni & Wang, 2016). NBI is useful in detecting NPC recurrence. A demarcated brownish area and typical brownish spots in the nasopharynx area under NBI can be seen in recurrent NPC (Lin & Wang, 2011).

NBI showed a higher sensitivity, specificity, and positive likelihood ratio for the diagnosis of NPC (Yeung, Vlantis, Wong, Tong, & Chan, 2018). Nasopharyngeal endoscopy coupled with NBI was able to provide a rapid, convenient, and highly reliable screening for high-risk populations.

Office-based ultrasound examination and fine-needle aspiration of the neck

Head and neck palpable lymph node enlargement is highly related to head and neck malignancy (Nasuti, Yu, Boudousquie, & Gupta, 2000). Metastatic cervical lymph nodes from head and neck cancers are site specific. In patients with a known primary tumor, the specific distribution of metastatic nodes in the neck helps to identify metastases and aids tumor staging. Enlargement of lymph nodes is usually the first presentation of NPC without other symptoms or the presence of a nasopharyngeal mass.

Ultrasound examination of the neck in the ENT/clinician's office is increasingly popular and in our own experience improves the clinical diagnosis of a neck mass. In a recent study, ultrasound images obtained with a linear-array echoendoscope at 7.5 MHz were used to identify a hypoechoic lymph node, and the cytological evaluation of the specimen was compatible with metastatic squamous cell carcinoma from a nasopharyngeal tumor (Sofferman & Ahuja, 2012).

Some features of metastatic nodes have been defined. Metastatic nodes tend to be hypoechoic compared with the adjacent muscles. Short axis to long axis

ratios (S/L ratio) of metastatic nodes tend to be greater than 0.5, suggesting metastatic nodes tend to be round. Sharp borders can usually be seen in metastatic nodes, whereas benign nodes tend to have less sharp borders (Ying, Bhatia, Lee, Yuen, & Ahuja, 2013).

Fine-needle aspiration cytology (FNAC), a minimally invasive technique, is utilized as part of the initial diagnostic investigation of NPC and has been reported to have high sensitivity and specificity. Endoscopic ultrasonography-guided fine-needle aspiration (FNA) biopsy is a minimally invasive and reliable technique and was applied to evaluate metastasis in mediastinal lymph nodes in a NPC patient (Ishikawa et al., 2005). Ultrasound-guided FNAC is an accurate method for the evaluation of neck nodes with a sensitivity of 89%, specificity of 98%, and overall accuracy of 95% (Knappe, Louw, & Gregor, 2000). Ultrasound-guided FNAC can correctly define the stage of cervical lymph nodes in 93% of patients with head and neck malignancy, contributing to further treatment planning and outcome prediction.

Further, a successful FNA requires a specimen with adequate cellularity, high-quality preparation, and an experienced aspirator and cytopathologist. When it is feasible, immediate on-site interpretation allows for decreased amount of artifact and limits the number of slides to be processed and examined, thereby increasing the likelihood of definitive and accurate final diagnosis. On-site cytopathologic evaluation of FNA specimens is accurate, cost-effective, and has improved patient care (Nasuti, Gupta, & Baloch, 2002).

Studies have been done to investigate the cytomorphological features and diagnostic problems of FNA. In a large set of metastatic NPC specimens, a dissociated or mixed architectural pattern of large, anaplastic cells and naked nuclei accompanied by an abundant lymphoid component was highly suggestive of undifferentiated NPC (Dey, Amir, Jogai, & Al Jussar, 2005). Cytology offers a rapid diagnosis, establishes the necessity of a complete cavum examination, and helps in avoiding unnecessary and harmful biopsies.

Preliminary investigations suggest that the presence of Epstein–Barr virus (EBV) genomes in neck metastases from an occult primary malignancy may be diagnostic and predictive of NPC. Using NPC diagnostic EBV gene amplification in FNA samples of neck metastases would help to confirm NPC as the primary disease (Macdonald et al., 1995).

However, diagnostic efficacy of FNA for residual or recurrent cervical lymph node metastasis in NPC is significantly reduced after previous radiotherapy. To ensure that the proper diagnosis is made early, triple assessment using FNA, plasma Epstein–Barr virus DNA, and MRI should be adopted in this scenario (Chan, Chan, Chow, To, & Wei, 2013).

FNAC biopsy needles are crucial for a successful lymph node biopsy. There are a few different kinds of biopsy needles including side-cutting biopsy needles

and end-cutting needles. Side-cutting biopsy needles have been commonly used in FNAC procedures with satisfactory outcomes. However, there is still failure due to various causes. It is not uncommon that there is inadequate tissue for cytological examination. End-cutting biopsy needles were found to be a better choice for use in obtaining both transcutaneous and intraoral biopsies under ultrasound guidance of neck lesions. In general 18G or 20G Franseen needles are adequate for biopsy of lymph nodes. End-cutting biopsy needles guarantee adequate tissue for cytological examination, leading to less failure of FNAC (Yuen, Lee, Bhatia, Wong, & Ahuja, 2012).

Laboratory test

The EBV (also called human herpesvirus 4), a double helix DNA virus, is one of the most common viruses in humans. Significant evidence supports that EBV infection is highly correlated with NPC. The current gold standard of clinical NPC detection is nasopharyngeal endoscopy combined with biopsy of suspicious lesions, but early diagnosis of NPC is sometimes difficult with endoscopy. A blood test for EBV antibodies is used to help diagnose early-stage NPC. In some cases, checking for EBV DNA may also help with screening.

Serological markers

Serological detection of EBV antibodies has been developed for many years and is frequently used in NPC mass screening and diagnosis. A specific subgroup of EBV-latent proteins, including EBV associated nuclear antigen 1 (EBNA-1) and two integral membrane proteins, latent membrane proteins (LMP) LMP-1 and LMP-2, along with the BamHI, a fragment of the EBV genome, were discovered in NPC patient's serum (Tam, 1999). Specific serologic responses were found to be induced against various gene products of EBV in patients with NPC.

EBV is associated with various diseases. The immune response of EBV infected patients to various viral antigens can be characteristic of the type of EBV-associated disease present and therefore the immune response is of diagnostic and prognostic significance. The antibody response to EBV-specific antigens: early antigen immunoglobulin A (EA/IgA), viral capsid antigen immunoglobulin A (VCA/IgA), and Epstein–Barr nuclear antigen immunoglobulin A are the most widely used biomarkers for NPC detection and are highly specific to NPC.

Serological detection of EBV antibodies has diagnostic value in NPC. Seroepidemiologic studies have proven that NPC patients have significantly higher levels of IgA antibodies against EBV against VCA/IgA and EA/IgA (Henle & Henle, 1976). EA IgA and IgG titers were elevated in most NPC patients regardless of whether their disease was active or in remission (Fan et al., 2004). An early study at the Prince of Wales Hospital showed VCA/IgA has higher sensitivity compared to EA/IgA, whereas EA/IgA has higher specificity compared to

VCA/IgA in the diagnosis of NPC. When EA/IgA and VCA/IgA are used together in diagnosis of NPC, higher sensitivity and specificity can be reached compared to either one of the serological markers used alone (Tam, 1999).

Serological detection of EBV antibodies has predictive value in NPC development. Serological levels of VCA/IgA and EA/IgA were significantly associated with increased risk for NPC, with a striking dose-response relationship that was most prominent during the first 5 years of follow-up (Cao et al., 2011). EBV antibody is an early marker of NPC. Taiwanese men who tested positive for VCA/IgA antibodies had a higher risk of developing NPC than did men who tested negative for VCA/IgA (hazard ratio = 22.0), even 5 or more years after antibodies were measured (hazard ratio = 13.9) (Chien et al., 2001). When preclinical serum VCA/IgA levels were elevated and maintained at high levels, it revealed a window of about 3 years immediately preceding clinical onset (Ji et al., 2007). Individuals who were positive for EBNA1/IgA at baseline experienced higher rates of NPC over follow-up (median, 6.5 years) than did persons who tested negative for EBNA1/IgA (Yu et al., 2011).

The traditional way of measuring the seromarkers of VCA/IgA and EA/IgA is the immunofluorescence assay. However, this method has its limitations. The relatively low PPV, lack of a standardized method, high intraobserver variation, and time-consuming protocols make it less applicable in large-scale population screenings. An enzyme-linked immunosorbent assay (ELISA) for EBV-related antibodies has been developed. It was reported that the combination of tests for EBV VCA/IgA and EBNA1/IgA by ELIZA had better sensitivity and specificity in detecting NPC (Liu et al., 2013).

Plasma Epstein–Barr virus DNA

Even though serological detection of EBV is a valuable marker to aid the diagnosis of NPC, it has limitations in terms of sensitivity, specificity, and titer-correlation with the tumor burden. Quantitative analysis of plasma EBV DNA has been reported to be a useful clinical and research tool in the screening and monitoring of NPC patients. An early study developed two regions of the EBV genome, the BamHI-W and the EBNA-1 regions, for real time quantitative polymerase chain reaction (RT-qPCR) assays and reported the rapidity and high accuracy of RT-qPCR assays of cell-free plasma EBV DNA (Lo et al., 1999). Further studies reported the sensitivity and specificity of using circulating EBV DNA for the detection of NPC by RT-qPCR analysis is 96% and 93%, respectively (Allen Chan & Dennis Lo, 2002).

Circulating EBV DNA can be detected and qualified in both plasma and serum. The sensitivity and specificity of detecting EBV DNA in the two different blood samples have been studied and the plasma group showed higher sensitivity and specificity than the serum group (Liu, Fang, Liu, Yang, & Zhang, 2011). EBV DNA detection in plasma samples offered a more powerful approach than

serum samples for the detection of NPC. Detection of circulating EBV DNA in plasma is promising and further refinement is required to improve the accuracy of the test for clinical NPC diagnosis.

Comparison of the two most sensitive peripheral blood markers of NPC, circulating EBV DNA and anti-EBV capsid antigen immunoglobulin A (VCA/IgA), found that circulating EBV DNA showed improved sensitivity, specificity, and accuracy. The sensitivities of EBV DNA and IgA-VCA for diagnosis of NPC were 95% and 81%, respectively. The specificities of EBV DNA and IgA-VCA were 98% and 96%, respectively. EBV DNA analysis could identify almost all false-negative IgA-VCA cases for diagnosis of NPC and could identify three-fourths of false-positive IgA-VCA cases for screening NPC. It also yields a 99% diagnostic sensitivity when combined with IgA VCA. The selective application of EBV DNA in an IgA-VCA-based screening protocol could improve screening accuracy with only moderate increases in cost (Leung et al., 2004).

For screening and early detection of NPC, studies done by the Chinese University of Hong Kong proved plasma EBV DNA was useful for detecting early NPC in individuals without a clinical suspicion of NPC (Chan, Hung, et al., 2013). A further study of plasma EBV DNA analysis on 20,174 people provided evidence that plasma EBV DNA analysis is a promising and useful technique for early screening of NPC (Chan et al., 2017). The sensitivity and specificity of EBV DNA in plasma samples in screening for NPC were 97.1% and 98.6%, respectively.

Studies have been carried out in recurrent NPC. Studies have shown plasma EBV DNA has predictive value for prognosis in metastatic/recurrent NPC patients undergoing palliative chemotherapy. Patients with low pretreatment plasma EBV DNA levels and undetectable posttreatment plasma EBV DNA showed a favorable prognosis, with 5-year overall survival (OS) rate of 50.6% and progression-free survival rate of 21.7% (An et al., 2011). EBV viral load was more informative than was serological assessments for distinguishing remission from relapsed disease (Fan et al., 2004).

NP screen

Transoral biopsy is an alternative sampling method, which is noninvasive and can be applied to large populations. One early study demonstrated the feasibility of detecting multiple molecular tumor markers including EBV DNA in nasopharyngeal (NP) brushing samples with a high sensitivity and specificity for NPC detection. It offers a powerful yet noninvasive approach for the diagnosis of NPC in high-risk populations (Tong et al., 2002). EBV DNA load in blood and NP brushes were correlated as a continuous variable with tumor stage, disease-free survival, and OS and might improve early detection of local failures posttreatment (Adham et al., 2013). A study conducted in the endemic region of South China using NP brush sampling of EBV load yielded a sensitivity of

98.9%, specificity of 99.3%, PPV of 96.9%, and NPP of 99.7% for NP Screen in detecting NPC (Zheng, Lu, Li, & Jia, 2015). An additional study demonstrated a statistically significant difference between EBV levels in the recurrence group of postreatment patients and controls. There was no correlation between EBV and the recurrence with different T classification (Lam, Chan, Ho, & Tsang, 2016). Therefore it may also provide an additional surveillance strategy for recurrence of NPC.

Radiological assessment

Radiological assessment is crucial in the assessment of NPC. It provides precise information about the tumor, including location, volume, invasion, metastasis, staging, and prognosis. As imaging for NPC will be discussed in detail elsewhere, reference here will be brief.

Ultrasonography

Ultrasonography could be a noninvasive and useful tool for the detection of endoscopically suspicious or endoscopically subclinical nasopharyngeal tumors. It was reported that thickened mucosa, asymmetry between the right and left sides of the nasopharynx, an obvious focal mass present in the nasopharynx, or invasion of the parapharyngeal space indicated the presence of NPC. Ultrasonography was compared to endoscopy and showed high sensitivity, specificity, and accuracy, with 90.1%, 84.8%, and 87.3%, respectively (Yong, Jun-Jie, Shang-Yong, & Xiang, 2014). Ultrasound examination of the neck in the ENT/clinician's office is increasingly popular and has been found to be improve the clinical diagnosis of a neck mass in our own experience. It is advisable to routinely employed ultrasound examination of the neck in the ENT/clinician's office to avoid a delay in diagnosis of cervical metastasis (Sofferman & Ahuja, 2012).

Computed tomography and magnetic resonance imaging

Radiological assessment is a more accurate and specific tool compared to serology. CT and MRI have excellent anatomic resolution and have been widely used in detection of NPC tumor, lymphadenopathy, and DM.

MRI is a far more useful and accurate tool compared to CT in detection of early NPC, staging of the primary tumor, and evaluation of associated retropharyngeal and cervical lymphadenopathy. This is due to its superior tissue contrast resolution and its ability to show soft tissues in exquisite detail. MRI has high sensitivity, specificity, and accuracy in detection of NPC, which are 100%, 95%, and 95%, respectively (King et al., 2006). The sensitivity and specificity rates of enhanced CT for the diagnosis of cervical lymphadenopathy are slightly lower compared to MRI (Castelijns & van den Brekel, 2002). MRI has an advantage over CT in the diagnosis of enlarged RLNs in patients

with NPC (Abdel Khalek Abdel Razek & King, 2012). MRI appears to be more sensitive in detecting bony involvement and is preferred in demonstrating skull base involvement (Chong & Fan, 1996). MRI is more sensitive than endoscopy as it could detect 12% of NPCs that were endoscopically invisible (King et al., 2015). MRI is the preferred imaging tool for NPC. However, high cost and time-consuming analysis of MRI are still the limiting factors of routine and repeat use of MRI.

Positron emission tomography/computed tomography

Positron emission tomography (PET)/CT is used to detect DM in various cancers. The added metabolic information of Fluorodeoxyglucose (^{18}F) combined with the anatomical structure can serve as prognostic indicators, helping to improve treatment planning and hence clinical outcome, especially in patients with locoregionally advanced disease (Lai & Khong, 2013). PET/CT is more accurate than MRI for determining cervical nodal metastasis and should be the better reference for the neck status (Ng et al., 2009). PET/CT imaging demonstrates higher diagnostic accuracy in the detection of distant metastases. FDG-PET/CT is the most sensitive, specific, and accurate modality for DM staging of endemic NPC. The sensitivity, specificity, and accuracy of FDG-PET/CT were 83.3%, 97.2%, and 96.2%, respectively (Chua et al., 2009).

Quality of life

Despite the implementation of intensity modulated RT (IMRT), NPC survivors still experience many physical symptoms which impact long-term quality of life (QoL) many years after treatment. Depression, anxiety, and fatigue remain common in long-term survivors and are highly correlated with QoL (McDowell et al., 2018). Assessments of QoL reflect the subjective perspective and specific concerns of NPC patients.

Functional assessment of cancer therapy-nasopharyngeal cancer

Functional Assessment of Cancer Therapy-General (FACT-G) was first developed to evaluate the subjective perceptive of cancer patients and was validated over a quarter of a century ago. The Functional Assessment of Cancer Therapy-Head and Neck (FACT-HN) consists of the FACT-G questionnaire (27 items assessing physical, social, emotional, and functional domains) and a 12-item inventory specific to HNC survivors (List et al., 1996). Based on FACT-G and FACT-HN, FACT-NP was designed to evaluate the QoL of NPC patients, as there are specific complications of NPC. FACT-NP consists of 27 items assessing physical, social, emotional, and functional domains from FACT-G, 12 items from FACT-HN, and seven items regarding specific concerns of patients with NPC including eating, voice quality, hearing, and olfaction (Tong et al., 2009). Higher scores reflect better QoL.

European Organisation for Research and Treatment of Cancer core quality-of-life questionnaire

The European Organisation for Research and Treatment of Cancer (EORTC) core QoL questionnaire assesses the QoL of cancer patients (EORTC quality of life questionnaire-C30, EORTC QLQ-C30). As head and neck malignancies and their treatments affect a variety of body functions, most notably breathing, swallowing, and speaking, EORTC quality of life questionnaire-H&N35 (EORTC QLQ-H&N35) was developed to evaluate significant physical, emotional, and social problems, caused by head and neck cancer that reduce QoL considerably. Studies have been reported to evaluate the QoL of NPC patients with a combination of EORTC QLQ-C30 and EORTC QLQ-H&N35 (Fang et al., 2010). The EORTC Head and Neck Cancer Group and the EORTC Quality of Life Group updated the EORTC QLQ-H&N35 in 2008 and the EORTC QLQ-H&N43 was developed and validated in a large international field study and confirmed the psychometric properties of its scales.

Quality of life-radiation therapy instrument-head and neck

The quality of life-radiation therapy instrument (QoL-RTI) is a general tool that assesses components of function (nine questions), emotion (seven questions), family/socioeconomics (six questions), and overall QoL (three questions) (Johnson, Casey, & Noriega, 1994). A reliable and valid subscale was developed for the QoL-RTI that specifically and concisely addresses the issues important to the head and neck radiotherapy patient (Trotti et al., 1998). QoL-RTI was validated in Chinese head and neck cancer patients by the Chinese University of Hong Kong, indicating that the Chinese version of QoL-RTI is a reliable and valid QoL measurement for postirradiated head and neck cancer patients. Patients reported the immediate side effects of radiotherapy, causing deterioration of the QoL measurement. The impact of radiotherapy was significant, especially in the aspect of functional and treatment-site related subscales (Lo et al., 2004).

Staging

American Joint Committee on Cancer tumor, node, and metastasis system

An accurate staging system is crucial for treatment guidance and prognosis prediction. The Union for International Cancer Control (UICC) and American Joint Committee on Cancer (AJCC) tumor, node, and metastasis (TNM) staging system for NPC based on the anatomical extent of the disease was first published in 1977 and remains the most widely used cancer staging system in the world. Regular updates of the AJCC TMN staging system are based on the development of diagnostic techniques and treatment strategies. The fifth edition, updated in 1997, was the most significant milestone in its development. In the fifth edition international experts were first able to reach consensus on a unified NPC staging classification.

The eighth edition (2017) of the UICC and AJCC TNM system is the most recent and the mainstream standard of NPC staging for appropriate diagnosis, treatment, and prognosis. We have highlighted a few updates from the most recent eighth edition of the AJCC TNM staging system. EBV positive lymph node involvement was designated as T0. The prevertebral muscle involvement was confirmed to be prognostic and designated as T2. Lateral pterygoid muscle (LP) is now down-staged as T2, which was previously staged as T4. T4 is now referred to as tumor with intracranial extension, involvement of CN, hypopharynx, orbit, parotid gland, and/or extensive soft tissue infiltration and soft tissue involvement. Regional lymph nodes classification adjustments include merging of N3A and N3B into N3, and the lower neck was included in the N category. In the staging system, the previous substages IVa (T4N0-2M0) and IVb (any T N3, M0) are now merged to form IVa, and the previous IVC (any T any N M1) is now upstaged to IVb (Amin et al., 2017).

Routine staging procedures include history and physical examinations such as CN examination, complete blood cell count, serum biochemistry, chest X-ray, nasopharyngoscopy, and CT or MRI of the nasopharynx and base of skull and neck. An accurate TNM staging system is crucial for treatment guidance and prognosis prediction in NPC patients.

The TNM staging system can predict DM and even precisely estimate clinical outcomes of NPC patients. The AJCC TNM staging system N classification and T classification were found to be significant and independent predictors for DM in NPC patients (Hui et al., 2004). NPC staging has been reported to be of prognostic value. A recent large-scale study on 868 patients with IMRT treatment staged with the 2002 AJCC TNM staging system reported that the 5-year OS rates in patients with stages I, II, III, and IVA–B were 100.0%, 94.3%, 83.6%, and 70.5%, respectively (Sun et al., 2014).

Updates of each edition of the AJCC TNM staging system have been proven to be more accurate than the previous edition. The seventh edition is more accurate than the fifth and sixth editions, with more even stage distribution, better distinction of prognosis between adjacent categories/stages, and more accurate identification of low-risk subgroups for refinement of treatment decisions (Lee et al., 2012). A recent validation study of the eighth edition of the UICC/AJCC staging system for NPC treated with IMRT demonstrated that the eighth edition staging system can predict the prognosis of NPC patients more accurately than the seventh edition (Kang et al., 2017).

RLN metastasis has been investigated and identified as a crucial prognostic factor of NPC. It was incorporated into the seventh edition of the AJCC staging system for NPC and classified as N1 disease in the TNM classification. Some studies have investigated the prognostic value of RLN metastasis in NPC patients. Before the inclusion of RLN metastasis into the AJCC staging system, a study showed the risk of DM was similar between patients

with RLN metastasis and patients with N1 disease, as classified by the sixth edition of AJCC staging system, regardless of its laterality (Tham et al., 2009). IMRT has greatly improved the treatment outcomes of NPC and RLN metastasis remains an independent prognostic factor in NPC. It is still reasonable for RLN metastasis to be classified as N1 disease, regardless of laterality (Ling-Long et al., 2014). MRI has an advantage over CT in being better able to separate the lateral retropharyngeal nodes from the primary tumor in the adjacent posterolateral nasopharynx. MRI is essential for detection of early NPC, staging of the primary tumor, and evaluation of associated retropharyngeal and cervical lymphadenopathy (Abdel Khalek Abdel Razek & King, 2012).

Although the AJCC staging system is the most widely used staging system and has been updated and optimized along with the development of medical assessments and interventions, whether the AJCC staging system is adequate for predicting the prognosis of patients with NPC still remains doubtful. According to the current AJCC TNM classification system, NPC is T-classified by anatomic extent, and no dimensional measurement is taken into account. NPC is often highly infiltrated and heterogeneous in all disease stages. Several studies have demonstrated primary tumor volume could improve the current staging system.

Gross tumor volume of primary tumor plus retropharyngeal nodes was associated with significantly poorer local control, lower DM-free rate, and poorer survival (Lee et al., 2010). Patients with a small primary volume disease had excellent local control, irrespective of T stage (Chua et al., 1997). Large primary tumor volume (>60 mL) was reported to be a predictive factor for treatment outcome and requires more aggressive treatment (Chen, Chen, Liu, Chang, & Chie, 2004). Measurement of the primary tumor volume in NPC offers important prognostic value in predicting local control. Some other studies have also suggested the volume of the tumor has a positive correlation with advancing T-classification groups and significant differences existed in the distribution of T-classification among various volume-based groups (Zhou, Chong, Khoo, Chan, & Huang, 2007). Primary tumor volume has been suggested for inclusion as an additional prognostic factor in the TNM framework for further refinement of the AJCC TNM staging system.

The AJCC TNM staging system is still the gold standard for staging and risk group classification. As mentioned in the radiological assessment session, radiological examination is crucial in NPC staging. MRI has proven to be a more valuable tool than CT in the diagnosis of NPC and, accordingly, is likely to have an impact on the NPC staging.

While significant advancements have been made in anatomical staging, we also see rapidly rising use of molecular stratification. The EVB DNA positive cervical lymph node involvement was designated as T0 in the most recent eighth

edition AJCC TNM staging system. It is thought that including an EBV DNA assay and gene molecular profiling into the staging system will allow for more accurate prediction of disease outcomes. A staging system based on all known prognostic factors including primary tumor volume and EBV DNA can be established, leading to a more accurate staging system and a more individualized treatment strategy, which would greatly benefit NPC patients.

Recurrent AJCC/UICC TNM stage system rTNM system

Of note 9%–40% of patients developed recurrent disease at the primary and/or regional site after definitive radiotherapy. The current recurrent AJCC/UICC TNM staging system (rTNM) uses the 'r' prefix to denote the TNM stage for relapse patients (You et al., 2015; Yu et al., 2005). Therefore rTNM staging ignores the striking differences between recurrent and primary patient populations, and the system may exhibit reduced accuracy when applied to recurrent diseases.

Other staging systems

Diverse staging systems were used for NPC staging before the AJCC/UICC TNM staging system was widely approved by international experts. Each staging system has its own advantages and disadvantages in different aspects, such as lymph nodes metastasis classification and stage distribution.

Ho staging system

The Ho staging system is rarely used clinically at present. It was developed by Ho in 1978 for NPC and was applied to Asian populations with reliably accurate prognostic separation of patients (Ho, 1978). There are two major differences between the AJCC system and the Ho staging system.

The Ho system compresses the four current AJCC T categories into three. All nasopharyngeal tumors confined within the nasopharynx are designated T1. The Ho system categorizes lymph node disease by its anatomic position. N1 refers to the upper cervical region, N2 refers to the lower cervical region, and N3 refers to the supraclavicular region, whereas in the AJCC system lymph node categories are based on the size of the largest lymph node, the number of involved lymph nodes, and the presence or absence of contralateral disease (Cooper, Cohen, & Stevens, 1998).

Chinese staging system

NPC is highly prevalent among Chinese people, especially those from the endemic region, South China. Clinical treatment started in China in the 1940s and the Chinese staging system was developed in 1965. The Chinese staging system has been updated regularly according the development of novel imaging techniques and treatment strategies. The Chinese staging system of 2008

has been commonly used in China. Compared with the seventh edition of the AJCC staging system, the N classification of the Chinese staging system was based on MRI as well as the lymph node criteria of the Radiation Therapy Oncology Group consensus guidelines. However, several studies showed that these two staging systems have limitations (Zong, Huang, Guo, & Pan, 2016). The most recent version of the Chinese staging system updated in 2017 is now consistent with the eighth edition of AJCC staging system.

Summary and concluding remarks

NPC is locally asymptomatic and is infrequently discovered until advanced stage. Physicians should be aware of NPC, especially when seeing a patient from Southern Asia with symptoms associated with NPC including neck mass, nasal symptoms, auditory symptoms, and neurological symptoms. Endoscopic examination with biopsy is the gold standard of NPC diagnosis. HD imaging and NBI have increased the sensitivity and specificity of endoscopic examination. A large body of evidence supports the role of EBV as a primary etiologic agent in the pathogenesis of NPC. EBV VCA/IgA and EA/IgA have been well studied and have been widely used for NPC screening. However, the specificity is relatively low. Analysis of EBV DNA levels is developing rapidly and has been proven to be efficient and accurate for screening, diagnosis, and prognosis of NPC in large-scale studies. Staging is crucial for treatment planning and outcome prediction. The most recent version of the AJCC/TNM NPC staging system has been reported to have more even stage distribution and to be more accurate in prediction of the clinical outcomes of NPC patients. Although there have been some other staging systems in the past, they are rarely used in clinical practice, and the AJCC remains the most widely used staging system in the world. Thorough assessment and accurate staging and grading are crucial for providing precise information about the disease and determining the next step of disease management.

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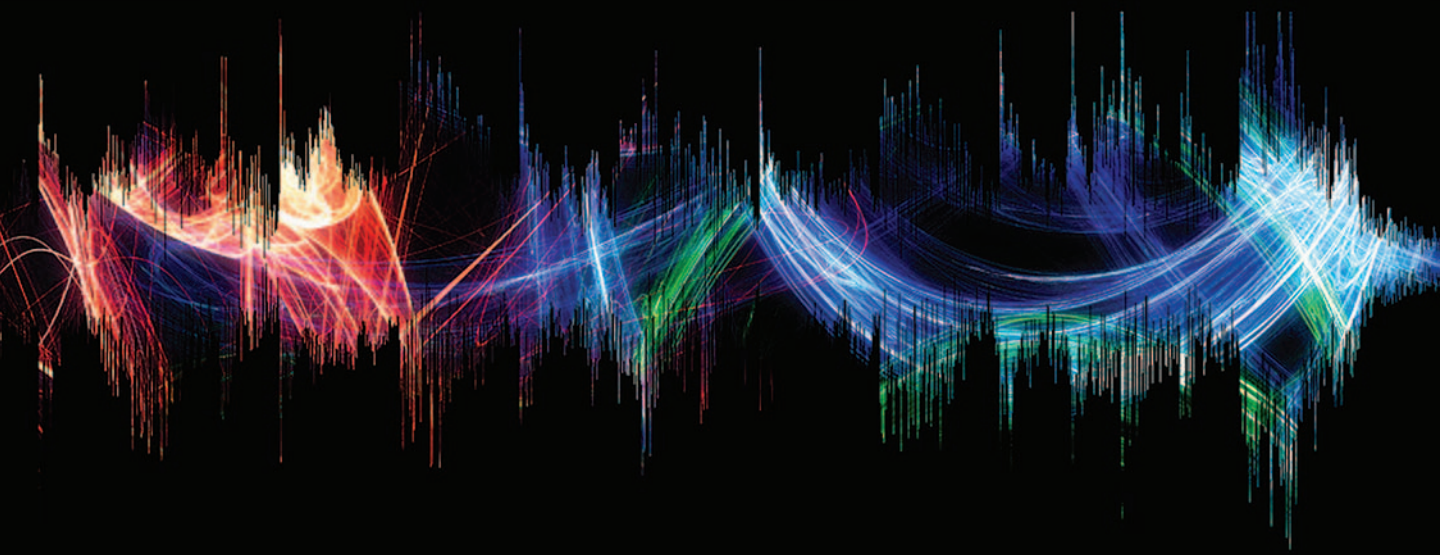

8

Laboratory investigation for nasopharyngeal carcinoma diagnosis

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Introduction

Nasopharyngeal carcinoma (NPC) is a nonlymphomatous squamous cell carcinoma that takes place within the epithelial lining of the nasopharynx. Its prominence is linked to viral, genetic, and environmental factors. Some of the symptoms of NPC include epistaxis, nasal obstruction, and discharge, hearing loss, headache, diplopia, facial pain, and numbness and neck masses. In early stages, the symptoms are ambiguous and nonspecific, which results in most patients afflicted with NPC being diagnosed when the disease is already in advanced stages (Tabuchi, Nakayama, Nishimura, Hayashi, & Hara, 2011).

The World Health Organization (WHO) has defined three subtypes of NPC: keratinizing squamous cell carcinoma (KSCC) (WHO type I), nonkeratinizing carcinoma (WHO type II), and undifferentiated carcinoma (WHO type III). A revised definition by the WHO stated that the histological type is keratinizing squamous cell, nonkeratinizing (differentiated and undifferentiated carcinomas), and basaloid squamous cell carcinoma (BSCC) (Barnes, Eveson, Reichart, & Sidransky, 2005). KSCC is uncommon within endemic areas, and has poor prognosis relative to nonkeratinizing carcinoma, which is common in endemic or nonendemic areas. It is also linked to the Epstein–Barr virus (EBV) (Yang, Wu, Zhou, & Chen, 2015).

The two-year survival rate for patients having stages III/IV or stages I/II NPC are 50% and $\geq$90%, respectively. The prognosis for stages III/IV [advanced nasopharyngeal carcinoma (NPC)] are noticeably poor. Early diagnosis and appropriate management/treatment will help ensure that the mortality rate due to NPC would decrease (Han et al., 2012; Tabuchi, et al., 2011).

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NPC can be detected via several approaches, such as elementary examination (complete blood count, liver, and kidney function tests, blood glucose levels, X-rays, and electrocardiograms) (Lin et al., 2000); physical examination; molecular test detection of EBV DNA or RNA such as quantitative polymerase chain reaction (PCR); cytology; immunohistochemical test; computed tomography (CT); magnetic resonance imaging (MRI); and evaluation of cranial nerve function and hearing. However, these methods need further confirmation by biopsies (Yang et al., 2015). A primary efficient screening protocol facilitates the early detection of NPC and effective curable treatment.

NPC is considered as unusual cancer due to it is strongly associated with EBV. Infections seem to come before malignant transformation since the presence of EBV was detected previously in preneoplastic lesions and the monoclonal EBV DNA in tumor samples(Fan et al., 2004; Han et al., 2012).

EBV can be detected via several methods (Table 8.1), such as PCR, serological tests, in situ hybridization (ISH), and immunohistochemistry (IHC) (Awan, Irfan, Zahid, Mirza, & Ali, 2017; Gulley, 2001; Hazelton & Gelderblom, 2003; Ocheni et al., 2010; Qi et al., 2013; Schramlová, Arientova, & Hulinska, 2010; Yates, Iliyasu, Ahmed, & Liman, 2017). Although ISH is the golden standard method in the case of detecting tumor-associated viral infection, the detection of EBV viral load via molecular assays are now being integrated for the clinical evaluation of tumors (Awan et al., 2017; Gulley, 2001).

ISH is a technique defined by the hybridization of DNA or RNA of a specific virus. An example of this is EBV encoded early RNA (EBER) hybridized with synthesized probes. During the process of the hybridization, a signal that can be traced by chromogenic (colorimetric) or fluorescent detection systems is generated. This signal appearance can either be positive or negative, as per the pathologist doing the visual examination. The detection of EBER via ISH helps diagnose latent infection present in the biopsy samples (Gulley & Tang, 2008; Hunt et al., 2014).

The detection of NPC can be performed via PCR and IHC. These methods discover the link between cancers and viruses. The former amplifies specific single or multiple copies DNA or RNA sequences, generating million copies of a particular genomic sequence that can be used to detect the low abundance of viruses and identify single genome copies, while the latter uses several antibodies (monoclonal and polyclonal) to verify and recognize tumor antigens present in the tissue samples. These methods are widely used by pathologists to pinpoint and diagnose many medical issues (Awan et al., 2017).

There are a few viable diagnosis or screenings for NPC, the most common ones being EBV serology and nasopharyngoscopy. The serologic assays can be used to determine the humoral immune response against EBV proteins, while EBV viral load assays can be used to determine the circulation of the

Table 8.1 Diagnostic method of Epstein–Barr virus (EBV) detection.

Method	Analyte, antigen, and substrate	Advantage	Disadvantage
Serology (VCA, EBNA, EA)	Titration of antibodies to viral proteins (VCA, EBNA, and EA) in serum samples	Inexpensive and simple; differential diagnostic between acute from remote infection; monitor disease status over time	Less sensitive and specific; not sufficient alone for diagnosis of EBV-related malignancy; not reliable in cases of immune system deficiency
Heterophile antibodies	Antibodies to Paul–Bunnell antigens (horse, goat, bovine, and sheep erythrocyte) in serum samples	Inexpensive and simple; useful in distinguishing late primary infection (transient) reactivation when positive	Less sensitive and less specific (especially in children); false-positive result in some cases of autoimmune disease; false-negative possibility is high in young children
Immunofluorescence assays (IFA)	EBV-transformed B cell lines (e.g., the P3HR-1 or Raj cell lines)	Gold classical standard method; highly specific; useful in staging of EBV infections	High degree of variability; lack of standardization; unequivocal diagnosis of acute EBV infection
Enzyme immunoassay (EIAs) and enzyme-linked immunosorbent assays (ELISA)	Purified native or recombinant proteins, synthetic peptides, or fusion proteins	Rapid; more sensitive than IFA; suitable for automation; inexpensive	Less specific; difficulty in staging of EBV infection (single patient's serum); lack of standardization; unequivocal diagnosis of acute E BV infection
In situ hybridization (ISH)	EBER transcripts or EBV DNA in tumor tissue (paraffin embedded tissue), cytology preparation	Confirmation of each diagnosis and its relation to EBV	Only applicable for cells; requires special skills; biopsy may be counterproductive because of histological overlap with Hodgkin and non-Hodgkin lymphoma; down-regulated of EBER in oral hairy leukoplakia
Immunoblotting analysis	Lysate of EBV-transformed cells line, recombinant antigen [as p72 (EBNA-1), p18 (VCA), p23 (VCA), p64 (EA), and p138 (EA)], combination of lysate and recombinant antigen	Highly specific; confirmatory method; useful in staging of EBV infection with single serum sample and detecting EBV-specific antibodies to multiple EBV-specific antigens simultaneously	Expensive; lack of standardization in terms of buffer conditions, the lysates from cell lines, and the combination of recombinant antigens
Chemiluminescence immunoassay (CLIA)	Synthetic peptides with different cut-off values for VCA IgM and EBNA-1 immunoglobulin G (IgG)	Sensitivity and specificity in distinguishing primary infection (transient) from past infection; can be used for screening	Requires further study
Viral cell culture	Lymphoblastiod cell line from patient lymphocytes	Accurate and semiquantitative measure of EBV in clinical samples	High costs and slow turnaround time (4–8 weeks): performed only in special laboratories
Electron microscopy	Morphological appearance of EBV from various clinical specimens	Identify whole virions representing replicative viral infection; Simple; rapidity, and nonselective nature	Not suitable for screening large numbers of sample; time-consuming; cannot be performed in an automated my low sensitivity.

(*Continued*)

Table 8.1 Continued

Method	Analyte, antigen, and substrate	Advantage	Disadvantage
Molecular methods: 1. PCR 2. Other nucleic amplification method	EBV DNA (viral load) in peripheral blood mononuclear cells (PBMCs), plasma, serum, cerebrospinal fluid, tissue	Sensitive, specific across a wide dynamic range; low risk of contamination (real-time PCR); rapid (1−2 days); differentiating between health carrier and EBV-related disease; screening and follow-up of high risk populations.	False-positive results due to uncorrected conservation of blood sample; false-negative results due to the presence of nucleases; expensive; need special equipment
Immunochemistry	EBV-related proteins including EBV nuclear antigen 1 (EBNA1) and the latent membrane proteins (LMP1, LIMP2a and LMP2b) in tumor tissue biopsy; paraffin-embedded sections	Rapid; cheap and effective method; LMP1 immunostains are more economic and rapid than EBER hybridization and more easily incorporated into routine clinical laboratories; distinguish latent from replicative infection based on expression profiles; identify EBV protein expression in specific cell types within histologic lesions	False-positive staining in specific cases such as poorly fixed tissues, cells of the nervous system, and in some uninfected hematopoietic. elements (eosinophils and plasma cells); not as effective as EBER−ISH in detecting EBV in nasopharyngeal carcinoma (NPC)

VCA, Viral capsid antigen; *EBNA*, EBV associated nuclear antigens; *EA*, early antigen; *EBER*, EBV encoded early RNA; *LMP*, latent membrane protein.

EBV DNA. Both assays have been found to be promising for use as tumor markers (Han et al., 2012).

NPC patients consistently report high serologic titers against viral antigens, which is attributed to the tumor burden, rendering the serologic titers suitable markers for the disease. Serologic tests are commonly carried out using enzyme-linked immunoassays (ELISA) or immunofluorescent assays. Multiple EBV-specific antibodies, such as anti-EBNA1, anti-EA (early antigen), and anti-VCA (viral capsid antigen) antibodies, especially those with distinct patterns, can be detected in healthy patients as well as in patients associated with EBV diseases. High titers of anti-EA and anti-VCA antibodies and diminished anti-EBNA titers are present and detectable in EBV-related neoplasia (Fan et al., 2004).

Antibodies of the immunoglobulin A (IgA) isotype are particularly significant in NPC patients, as per carcinoma's origin on a mucosal surface. However, serology tests cannot stand on its own as a diagnostic method of malignancy, due to the fact that the serologic pattern of EBV-related neoplasia are also present in patients suffering from autoimmune diseases, which confirms that serology methods suffer from reduced sensitivity and specificity, which subsequently limits their clinical application (Fan et al., 2004).

Molecular methods are also regarded as promising methods that can detect EBV viral loads. The accurate quantification of viral load and detecting specific DNA sequences was achieved via competitive PCR or real-time PCR. The latter

was proven to be superior in the turnaround time (about 1–2 days), sensitive, accurate, and less labor intensive, and also minimizes the risk of amplicon contamination as the tube remained closed post-DNA amplification parallel with the commercial competitive PCR assay (Fan et al., 2004). Previous studies confirmed EBV viral load marks of tumor burden in NPC patients, rendering it useful for diagnosis, prognosis, and treatment response evaluation (Fan et al., 2004; Han et al., 2012).

For a screening work-up, the serologic assays need to be made first, followed by EBV DNA PCR being performed if the patient tested positive to the serological tests, indicating high risk of NPC (Teresa, Yu, Hu, & Li, 2007). If the work-up is positive the patient need to follow it up with a specialist for nasopharyngoscopy, computed tomography (CT) scan, and MRI. This would help exclude low-risk patients, which ultimately reduces testing and treatment costs, and allows healthcare professionals to focus on high-risk patients (Liao & Lai, 2012).

A systematic review was conducted on 15 studies to determine the diagnostic accuracy of EBV DNA among NPC patients. Liu reported that this could be realized by looking for EBV DNA in the plasma or serum. The detection of EBV DNA was reported to be highly sensitive and specific at 0.92 (95% confidence interval (CI): 0.82–0.96) and 0.88 (95% CI: 0.78–0.94), respectively (Liu, Fang, Liu, Yang, & Zhang, 2011).

The early stages, regarded as the "occult primary" of NPC, remains a diagnostic challenge for the clinician. PCR is known to be an excellent tool for the early diagnosis of NPC and metastatic neck nodes via the detection of EBV DNA at a rate of 97.1%. This molecular tumor marker could be integrated into routine clinical use to quicken diagnosis and increase the rate of prognosis (Yap, Hassan, Chan, Choo, & Ravichandran, 2007).

The biopsy of nasopharynx is the preferred definitive diagnostic test for NPC, due to the limited sensitivity (70%–90%) of the nasopharyngeal cytology. Multiple biopsies are required from the nasopharynx in cases suspected to have NPC. In order to diagnose metastatic NPC, the fine needle aspiration cytology (FNAC) needs to be conducted on the enlarged cervical lymph node. Fine-needle aspiration can be used for the initial diagnosis/staging. Immunohistochemical staining and EBER ISH will instead be used when faced with cases having occult histological findings (Malaysia Health Technology Assessment Section (MaHTAS), MDD, & Ministry of Health Malaysia, 2016).

Epstein–Barr virus serology

EBV belongs to the subfamily of gamma herpes virus. It consists of linear double-strand DNA, resulting in infectious mononucleosis, which is the most common primary infection in adolescence and adults in the world. EBV can be

orally transmitted via direct contact with infected saliva. The rate of infection of EBV in adults worldwide is over 95%. Hong Kong reported the highest incidence rate of infection in children aged 6 and 10, at rates of 80% and 100%, respectively. The infection is usually asymptomatic in infants and children (Tabuchi et al., 2011). EBV can remain in latent stage in lymphocyte cells, especially B lymphocytes. It can manifest itself by contributing in several malignancies, such as NPC, but other factors can also do this (Tabuchi et al., 2011; Young & Dawson, 2014). In vitro studies confirmed the presence of viral DNA, as well as antigen expression, in lymphoid tissue and nasopharyngeal epithelium (Young & Dawson, 2014).

The aforementioned hypothesis positing that EBV's primary infection takes place several years prior to NPC agrees with the fact that EBV is present in premalignant epithelial lesion in the early stages of carcinogenesis. The EBV genome is made up of about 80 genes. EBV's latent genes are regarded as the most crucial, due to their link with carcinogenesis via the increased growth and survivability of infected cells, and its subsequent link to the development of NPC. The metastatic disease is the result of increased genetic/epigenetic changes to the post-NPC development (Liao & Lai, 2012).

Several EBV-associated antigens are applicable for the detection of immune response (antibodies) in acute infections, such as VCA, the EA, and the EBV nuclear antigen (EBNA). These methods are known to be effective in the detection of EBV infections (De Paschale & Clerici, 2012; Gulley & Tang, 2008). EBV antibodies (Ab) include VCA IgM, VCA IgG, VCA IgA, EBNA IgG, early D antigen (EA D) IgG, and heterophile antibodies (De Paschale & Clerici, 2012).

An acute infection results in the production of immunoglobulin M (IgM) and immunoglobulin G (IgG) anti-VCA. VCA IgM will be gone in 4–6 weeks, while IgG remain present at minute levels for a lifetime. In the case of NPC, IgG is present at levels that are higher in the case of an acute infection. Also, IgA anti-VCA, which is absent in an acute infection, is present, at significant levels in the case of NPC (De Paschale & Clerici, 2012). Otherwise, EA IgG is eliminated after 3–6 months. However, EBNA IgG antibodies will develop usually after 3-6 months of having the infection and remains for life (Fig. 8.1) (De Paschale & Clerici, 2012; Gulley & Tang, 2008).

Recent studies showed that increased levels of antibody, especially IgA antibodies against lytic and latent protein expression in epithelial cells, preceded the development of NPC (Coghill et al., 2014).

The strong link between EBV and NPC can be shown by the expression of EBV gene products in cancer cell and the associated immunological response to the EBV specific antigens. IgA serological response in NPC cases represent the mucosal origin in NPC, and its presence is linked to increased risk toward the development of NPC amongst the population, which could turn out to be

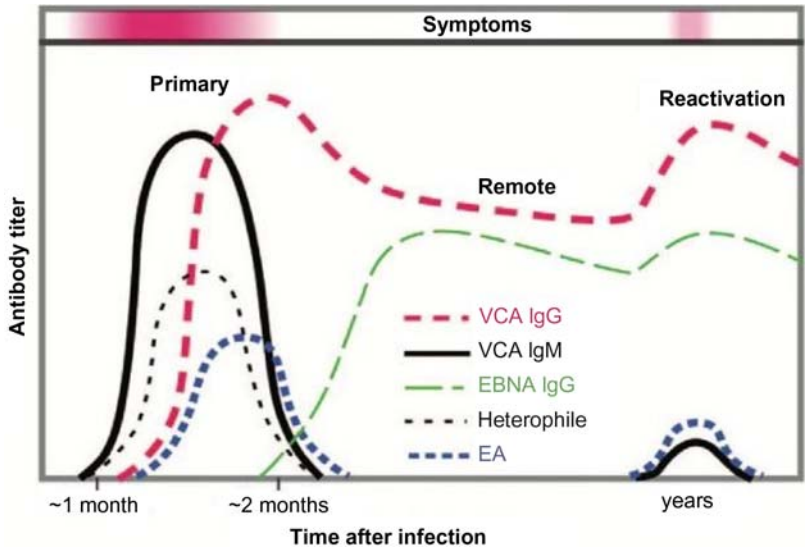

Figure 8.1 Serological titers distinguish primary infection from remote infection. IgG anti-VCA and IgM anti-VCA increase in concert with symptoms of primary infection and a positive heterophile test. After symptoms resolve, remote infection is characterized by EBV nuclear antigen (EBNA) and IgG anti-VCA without EA, although EA and IgM may reappear with or without symptoms on viral reactivation or EBV-related neoplasia. *IgG anti-VCA,* Immunoglobulin G anti-viral capsid antigen; *IgM anti-VCA,* immunoglobulin M anti-viral capsid antigen; *EA,* early antigen. Source: *Adopted from* Gulley et al., 2008.

effective as a diagnostic marker for the identification of early-stage NPC patients (Fachiroh et al., 2006).

Several latent gene products can be expressed within the NPC tumor cells. EBNA1 induces strong antibody responses with IgG and IgA, relative to other latent gene products, such as LMP1, LMP2, and BARF1 (Fachiroh et al., 2006). The EBV lytic cycle can be used to express multiple lytic gene products, subsequently inducing the development and maintenance of the malignancy. However, these genes are rarely prevalent in the NPC tumor cells (Fachiroh et al., 2006).

NPC can be diagnosed via detecting specific serological response against multiple EBV gene products, such as anti-EBNA1, anti-EA, and anti-VCA antibodies. NPC cases are distinctive in that they report high levels of VCA or EA IgA and EBNA1 IgA (collectively referred to as EBV IgA), although the response of EA or VCA IgG are unspecified in the case of NPC, due to the fact that they are revealed in other EBV diseases. NPC patients respond differently to different EBV lytic gene products, but they have shown increased antibody response, encompassing IgG and IgA, against small capsid protein VCA-p18 (Fachiroh et al., 2006).

The molecular diversity of EBV antigens recognized by antibody response (IgA and IgG) have shown different patterns when coming from different individuals and different stages of cancer (Fachiroh et al., 2006). It is also important that clinicians are able to discern antibody response patterns against NPCs in patients (Coghill et al., 2014).

The response level of EBV IgA, especially VCA IgA titers in NPC patients, could be useful for the prognosis, due to the fact that remission is linked to the lowered level of IgA and also when the tumor shrinks postradiotherapy. However, it should be pointed out that recurrent disease or enlargement of the tumor could be linked to stable/increased response level (Fachiroh et al., 2006; Lin et al., 2000).

Several EBV antibodies serological have been found to be helpful in NPC screening program including elevated titers of the following antibodies such as IgA antibodies against VCA, EA, EBNA 1 and 2, EBV-specific DNase and IgG antibodies against EBV transactivator protein (ZEBRA), especially EBV IgA against VCA and EA antigen (Tay et al., 2016). As of now, it is not known why the EBV-infected NPC cells proliferate quicker than EBV-negative NPC cells, which results in a clonal expansion of EBV-positive tumor cells within tumor masses (Lin et al., 2000).

EBNA-1 is an important latent EBV gene product which is required for EBV replication, development and initiation of cancers. In addition, EBNA- 1 is considered as a potential tumor marker that can be helpful and effective in the diagnosis and prognosis of NPC (Leight & Sugden, 2000; Sivachandran, Wang, & Frappier, 2012).

DNase assay is a method that is able to detect the presence of antibody-neutralizing EBV DNase activity. This antibody can be detected at increased levels of sera of NPC patients prior to the manifestation of clinical symptoms (Chen et al., 1989; Chen et al., 1987; Cheng, Chen, Glaser, & Henle, 1980; Chien et al., 2001). Also, IgG anti-ZEBRA is prevalent in high titers in young NPC patients relative to other markers such as VCA and EA, and is regarded as an excellent and sensitive marker toward the identification of early stage NPC. ZEBRA is an imperative EBV gene product that would require a transformation from a latent cycle to a productive cycle for it to work (Dardari et al., 2000; Yang et al., 2015; Zhang et al., 2006).

A high titer of EBV antibodies, such as VCA IgA, anti-EBV DNase, combined EBV EA IgA + EBNA-1 IgA, and EBNA-1 IgA has been detected prior to the development of tumors in the plasma of NPC patients in several serological studies (Chang et al., 2008; Cheng et al., 1980; Chien et al., 2001; Li et al., 2010; Liao & Lai, 2012; Ng et al., 2010; Teresa et al., 2007).

Several methods can be used for NPC serodiagnosis, such as cell-based indirect immunofluorescent assay (IF) and ELISA. The latter is the most commonly used

technique due to its sensitivity and specificity relative to the former but it is time-consuming method and unsuitable for large scale cases. Nevertheless, IF is considered as effective and the standard technique (Liao & Lai, 2012). It can also be used as a screening method for detecting NPC via the analysis of the IgA antibody titer against VCA, EA, and EBNA, although it requires different cell lines for specific analysis (Tabuchi et al., 2011).

ELISA encompass several EBV antigens, such as EBV cell extracts, purified native, or recombinant EBV proteins and synthetic peptides, all of which were previously reported EBV antigens, and other markers such as thymidine kinase, DNase, ribonucleotide reductase, ZEBRA, VCA-p18 (encoded by the BFRF3 reading frame), and EBNA1 (Fachiroh et al., 2006). The recent diversified antibody response against combined EBV antigens, such as ZEBRA and VCA-p18 IgA, or ZEBRA plus EBNA1 IgA tested for increased sensitive diagnosis of patients suffering from NPC. These tests are frequently conducted prior to the manifestation of NPC. Despite the fact that these tests are used to screen for NPC, it can also act as markers for the remission and relapses in NPC patients. However, it should be pointed out that these serologic screening tests are less sensitive and specific, and too expensive to run, all of which limit its subsequent clinical application (Fachiroh et al., 2006).

The results from the laboratory require skilled physicians to interpret. An initial diagnosis of NPC can be assumed in the presence of an increased of VCA IgA titer and lesion in the fossa of Rosenmuller detected by CT, which needs to be confirmed via biopsy and to be repeated if necessary (Tabuchi et al., 2011). A study conducted in southern China, an area prevalent with NPC, reported a total of 91% of NPC cases have increased and sustained levels of antibody prior to the diagnosis, and remained for the next decade at a mean duration 37 ± 28 months (Ji et al., 2007).

NPC oncogenesis is the result of interplay between events beside EBV reactivation, such as cellular genetic change due to environmental factors and/or immune deficiency. ISH is the standard technique for detecting EBER signal in all tumor cells in both histological and cytological materials, but EBER is absent from the adjacent normal tissue, with the exception of a few scattered lymphoid cells (Liao & Lai, 2012; Van Hasselt & Gibb, 1999).

Detection of EBERs using ISH with an EBER probe is preferred in the case of low EBV DNA. The expression of EBV antigens is limited, and in conventional IHC and ISH, it is rather inconvenient. It can also be used for a variety of tissues, encompassing old archive material, rendering it excellent for diagnostic pathology (Van Hasselt & Gibb, 1999).

Wide range of sensitivities and specificities. (56.4%–73.0%) and (88.2%–98.2%) has been reported previously of EBV DNA viral load. In addition, EBV

DNA viral load is considered as a useful marker for the early detection of NPC in a screening program (Tay et al., 2016).

Histopathological evaluation

Nasopharyngeal neoplasm was classified by the World Health Organization in 1978 into three histological types which are(WHO type I), nonkeratinizing carcinoma (WHO type II), and undifferentiated carcinoma (WHO type III) (Lin et al., 2000; Lu, Cooper, & Lee, 2010). In 2005, based on the second edition of the WHO NPC classification, the WHO defined the histological classification as splash cells, nonkeratinizing (differentiated and undifferentiated carcinoma), and BSCC. Lymph-epithelial carcinoma was considered a morphological variant of undifferentiated carcinoma (Table 8.2) (Li & Zong, 2014; Lin et al., 2000; Malaysia Health Technology Assessment Section (MaHTAS) et al., 2016; Yang et al., 2015).

Undifferentiated carcinomas reported a higher local tumor control rate during treatment and incidence of distant metastases relative to differentiated carcinomas. Squamous cell carcinoma (SCC) is common in older patients in nonendemic areas, who reported poor prognosis. Nonkeratinizing carcinoma is mostly prevalent in both endemic and nonendemic areas, and is linked with EBV infection (Boia et al., 2013; Yang et al., 2015).

Other studies have shown that SCC accounts for a quarter of NPC cases in North America, but only 1% occurred in endemic areas; while undifferentiated carcinoma accounts for 95% of all cases in high-incidence areas, with 60% of it taking place in North America (Tabuchi et al., 2011).

Risk has a strong genetic component to it, as per the increased risk of Chinese and North Africans and their descendants. EBV infection is linked to nonkeratinizing NPC, due to the increased levels of antibodies to EBV in patients with NPC, the presence of EBV DNA or RNA in all tumor cells, and the presence of

Table 8.2 Histopathological classification of nasopharyngeal carcinoma (NPC).

WHO Classification 2005	WHO Classification 1991	WHO Classification 1978
Keratinizing squamous cell carcinoma (KSCC)	Squamous cell carcinoma	WHO type I (well-differentiated keratinized SCC)
Nonkeratinizing carcinoma ● Differentiated ● Undifferentiated	Nonkeratinizing carcinoma ● Differentiated ● Undifferentiated	WHO type II (differentiated keratinized non-SCC)
Basaloid squamous cell carcinoma (BSCC)	No synonym exists (recently described)	WHO type III (undifferentiated carcinoma)

Source: Adopted from Malaysia Health Technology Assessment Section (MaHTAS) et al., 2016.

EBV in a clonal episomal result in the precursor damage of NPC (Lu et al., 2010; Yang et al., 2015).

Histopathology of nonkeratinizing carcinoma

Nonkeratinizing carcinoma of NPC involves over 95% of NPC in high-incidence areas, and about 75%–87% of NPC in low incidence areas. These tumors are commonly more radiosensitive relative to SCC, and are strongly linked to EBV (Li & Zong, 2014).

The biopsies differ in appearance, from the presence of an frank tumor containing ulcer, to the involvement of the mucosa with an intact surface epithelium. Tumors include solid sheets, irregular islands, and dyscohesive sheets and trabeculae of carcinomas intertwined with lymphocytes and plasma cells (Barnes et al., 2005).

Subclassifications into undifferentiated or differentiated subtypes are voluntary due to their differences lacking clinical or prognostic significance, and different regions of the same tumor or different biopsies taken at different time intervals from the same patient could share one type or more (Li & Zong, 2014). If both subtypes are present in the sample, the tumor can be classified based on the prominent subtype, or as a nonkeratinizing carcinoma possessing the features of both subtypes (Barnes et al., 2005).

Histopathology of undifferentiated and differentiated nonkeratinizing carcinoma

The undifferentiated subtype is the most important histopathological type of NPC, although its exact percentage differs from one patient to another. It accounts for 47%–92% in NPC cases of endemic populations. In a Western (nonendemic) population, this subtype of NPC was reported to be about 44% (Boia et al., 2013; Lu et al., 2010). The undifferentiated subtype is characterized by syncytial large tumor cells with undefined cellular borders. With a round-to-oval vesicular nuclei, the nucleoles exhibit scattered mitotic activity. The cells appear to be crowded, or in some cases, overlapping. In certain cases, the core of the cells could be more chromatin-rich than that of the vesicular. The sparse cytoplasm is either ampofophilic/eosinophilic (Fig. 8.2). The density of lymphocytes and plasma cells varies significantly. A desmoplastic is uncommon (Barnes et al., 2005; Gulley & Tang, 2008; Thompson, 2007).

The “Regaud” pattern denotes a solid platelet-growing tumor cell growth pattern which is usually with well-defined epithelial cell collection surrounded by infiltrated cells (solid type cells), while the “Schminke” pattern of the presence of apparently separate or loose tumor cells (sometimes referred to as reticulated pattern) with intermingled inflammatory cells (isolated cell types), which

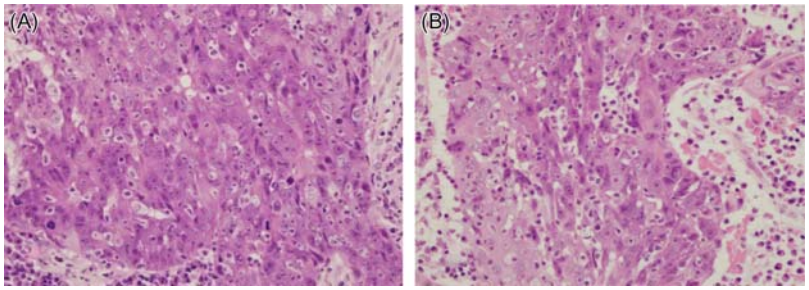

Figure 8.2 Cytological spectrum of nasopharyngeal nonkeratinizing carcinoma, undifferentiated subtype. (A) cells exhibit a syncytial quality, and possess vesicular nuclei, prominent nucleoli, and amphophilic cytoplasm ×400. (B) The syncytial-appearing cells have vesicular nuclei, distinct nucleoli, and lightly eosinophilic cytoplasm. There are some interwind lymphocytes ×400.

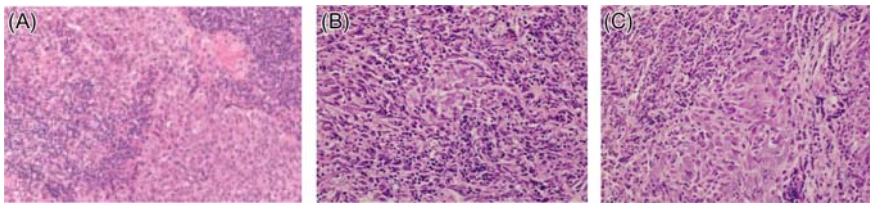

Figure 8.3 Nasopharyngeal nonkeratinizing carcinoma. (A) Example of differentiated subtype is characterized by sheets of tumor separated by a dense infiltrate of lymphocytes and plasma cells with plexiform arrangement. (B) Tumor Island in a lymphoid cell-rich stroma. Some lymphocytes are also seen within the tumor ×400. (C) this tumor shows an uncommon trabecular growth pattern ×200.

are the two growth pattern classically described (Brennan, 2006). This latter pattern illustrates the previous definition of NPC: "lymphoepithelial carcinoma or lymphoepithelioma." These histological patterns lack any prognostic significance (Boia et al., 2013; Lu et al., 2010).

Differentiated carcinoma is not common, representing ~7%–12% of all NPCs in southern China (Cao, Simons, & Qian, 2011). This value is similar to that of the undifferentiated type in Singapore, at about 41% of its NPC patients (Li & Zong, 2014).

The differentiated nonkeratinizing NPC is very similar to the histopathology of undifferentiated carcinoma, except that it differs from its undifferentiated counterpart via its display of cellular stratification and pavementing, often in a plexiform arrangement, which is a growth pattern similar to transient cell carcinoma in the urinary tract (Barnes et al., 2005; Peterson & Nelson, 2013). The tumor

cells exhibit fairly well-defined cell lines and vague intercellular bridges, and may, in exceptional cases, be temporary keratinized cells. Relative to its undifferentiated counterpart, the cells are often somewhat smaller, nuclear cytoplasmic relationships are smaller, the nuclei may be more chromatic, and nucleoli are not usually as prominent (Fig. 8.3) (Barnes et al., 2005).

In certain cases of nonkeratinizing carcinoma, the scattered epithelioid granulomas are prevalent, and so prominent that it engulfs the small carconomic islands. Many admixed eosinophil are present in a quarter of the cases. Certain specific cases exhibit the prominent infiltration of neutrophils despite the absence of ulceration (Barnes et al., 2005).

Immunoprofile and Epstein–Barr virus detection nonkeratinizing carcinoma

An immunoperoxidase reaction via antibodies to broad spectrum cytokeratins (e.g., AE1/3 and MNF-116) is usually effective (Lu et al., 2010). Cytokeratins are defined as intermediate filaments of proteins forming the cytoskeleton of epithelial cells that retain their expression in tumors originating from epithelial. Low-molecular-weight cytokines, including CK8, CK18, and CK19 recognized by antibodies CAM 5.2 or 35BH11, and a keratin (panceratin) cocktail, recognized by the antibody AE1/AE3, are all effective screening markers that can be used to detect epithelial differentiation (Bahrami, Truong, & Ro, 2008). Epithelial membrane antigen (EMA) can be used in place of CKs to detect epithelial differentiation, especially in sarcomatoid carcinomas or the undifferentiated carcinomas that are negative or focally positive for CK (Dabbs, 2013).

Almost all tumor cells exhibit significant staining for pancytokeratin (AE1/AE3, MNF-116). This uniform staining stands in contrast to the common focal staining in undifferentiated carcinoma, such as the lung and thyroid cartilage. High-molecular-weight cytokeratin staining (such as cytokeratin 5/6, 34ßE12) is significant, while low-molecular-weight cytokeratin staining (such as CAM5.2) is often weaker and uneven, and staining of Cytokeratin 7 and 20 are both negative (Barnes et al., 2005).

In nonkeratinizing NPCs with a significant lymphocytic infiltrate, the cytokeratin immunostain outlines the epithelial cells to show a pronounced meshwork pattern of staining. EMA staining is often focal in nature. The staining for p16 which is a tumor suppressor gene and also an inhibitor of the cell cycle, is usually negative in NPC, as opposed to the oropharyngeal carcinoma, which is characteristically positive for p16 (Mäkitie et al., 2003; Peterson & Nelson, 2013). Nonkeratinizing undifferentiated carcinoma exhibit cytokeratin-positive cells in cohesive groups or in meshwork reticulated pattern with interwind lymphocytes. Due to the division of cell clusters via lymphocyte infiltration, a distinct reticulated or meshwork pattern is generated, while the nonkeratinizing pattern differentiated NPC exhibit cellular sheets of closely related polygonal

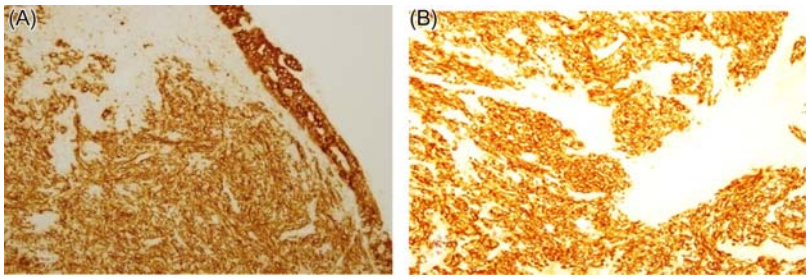

Figure 8.4 Nasopharyngeal nonkeratinizing carcinoma, undifferentiated subtype. (A) Immunostaining for pancytokeratin highlights the surface epithelium as well and irregular clusters and sheets of positive cells (carcinoma) in the stroma ×200. (B) Immunostaining for cytokeratin usually reveals a meshwork pattern of staining ×200.

tumor cells for immuno staining for cytokeratin (Fig. 8.4) (Barnes et al., 2005; Lu et al., 2010).

The immune reactivity of the EMA in NPC is often only focal. In the majority of the cases, tumors have a strong nuclear stain for P63, which is necessary for regenerative proliferation of the arms and legs and craniofacial and epithelial development. Overexpressions of p63 have been observed in many human cancers, especially in less differentiated tumors (Guo et al., 2006). A prominent feature of NPC is the presence of a massive lymphoid filtration in the primary tumor. This infiltrate is mostly made up of T lymphocytes and a small amount of B cells, monocytes, dendritic cells, scattered protein positive and eosinophils. The plasma cells are all polyclonal (Keryer-Bibens et al., 2006).

Characteristics that favor a diagnosis of carcinoma encompass the presence of contiguous cell groups in certain foci (estimated by mean size increase) and the poorly defined cell borders. The diagnosis can be easily confirmed via immune staining for cytokeratin. NPC with marked cellular spindle can resemble a high-grade sarcoma. In most cases, the diagnosis can be made by identifying in certain foci a component of typical NPC and can be further confirmed by cytokeratin immunoactivity (Barnes et al., 2005; Cohen & Scheimberg, 2015).

EBV detection of nonkeratinizing nasopharyngeal carcinoma is linked to EBV in 100% of cases, regardless of the ethnicity of the patients. EBV latent membrane protein 1 (LMP1) is generally positive in 30%–40% of cases, with its immunostaining uneven and weak, rendering it unreliable for exhibiting the presence of EBV. The ISH for EBV-encoded early RNA (EBER) is the most effective method of doing so. This makes it possible to clarify diagnostically challenging cases using conventional light microscopy (Peterson & Nelson, 2013).

Nonkeratinizing NPC exhibits nuclear labeling in almost all of its cells (Fig. 8.4). ISH of EBER can help diagnose NPC and distinguish between carcinoma and reactive epithelial atypia (Barnes et al., 2005; Lu et al., 2010; Peterson &

Nelson, 2013). A positive result signifies a nasopharyngeal origin (although not specific) in the case of a metastatic nonkeratinizing carcinoma of an unknown primary. Other methods of detecting EBV, such as PCR and staining for EBV LMP1, cannot be used for diagnosis, due to the smaller number of bystander EBV positive lymphocytes could affect a positive result (Lu et al., 2010; Peterson & Nelson, 2013).

Histopathology of keratinizing squamous cell carcinoma

KSCC is not prevalent within NPC endemic regions. In the case of NPC nonendemic regions, such as Singapore, it makes up 1%–20% of NPC cases (Tan & Putti, 2005). This stands in stark contrast with the western nonendemic regions, where it was reported that the proportion of NPC cases reached 67% (Shedd, von Essen, & Eisenberg, 1967).

The invasive carcinoma demonstrates obvious differentiation under a light microscope in the form of bridges and/or keratinization throughout the tumor, which are morphologically identical to KSCCs that are prevalent in the other head and neck mucosal regions. The level of differentiation can be further classified into well differentiated (most common), moderately differentiated, and poorly differentiated (Li & Zong, 2014).

The tumor grows to form irregular islands, alongside an abundance of Desmoplastic stroma and intertwined with lymphocytes, plasma cells, neutrophils, and eosinophils. The tumor cells exhibit features that are polygonal and layered, where the cell boundaries are separated and defined by intercellular bridges (Fig. 8.5) (Barnes et al., 2005; Li & Zong, 2014; Thompson, 2007). The cells at the core of the islands or close to the surface are often replete with more eosinophilic glassy cytoplasm, which can also be identified via cytoplasmic tonofibrils, indicative of cellular keratinization. In certain cases, the keratin form pearls. The nuclei often exhibit hyperchromia, while the level of nuclear pleomorphism varies between mild to prominent. Surface epithelium is

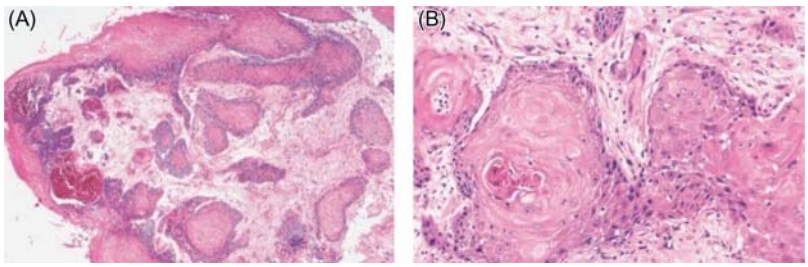

Figure 8.5 Nasopharyngeal keratinizing squamous cell carcinoma (KSCC), well differentiated. (A) Tumor shows invasion into the stroma. (B) Irregular islands of carcinoma infiltrate an abundant desmoplastic stroma. The tumor cells show obvious squamous differentiation and keratinization. Source: *Adopted from Barnes, L., Eveson, J. W., Reichart, P., & Sidransky, D. (2005).* Pathology and genetics of head and neck tumours *(Vol. 9). IARC.*

frequently evident in the samples, and it is assumed to represent in situ carcinoma (Barnes et al., 2005).

KSCC can be prevalent de novo or as per radiation-associated cancer that can be evident years after radiotherapy for nonkeratinizing NPC. The reported annual averages for postradiation SCC were 0.55%–1.0% (Chan, To, Wong, & Wei, 2014). Relative to its nonkeratinizing counterpart, KSCC exhibited a greater tendency for locally advanced tumor growth (76% vs 55%). The latter patient group reported a lower incidence (29%) of lymph node metastases relative to their previous counterparts (70%) (Reddy, Raslan, Gooneratne, Kathuria, & Marks, 1995). While certain studies proposed that this subtype of NPC is less sensitive to radiotherapy and a worse prognosis than its nonkeratinizing counterpart, others have reported that it does not differ much in the context of biological behavior (Li & Zong, 2014).

Immunoprofile and Epstein–Barr virus detection keratinizing squamous cell carcinoma

In keratinizing squamous cell carcinoma usually demonstrates immunoreactivity with strong diffuse staining for pancytokeratin, high-molecular-weight cytokeratin, and focal epithelial membrane antigen. In contrast, weak or scanty staining for low-molecular-weight cytokeratin. In the case of radiation-induced keratinizing squamous carcinoma, it is not linked to EBV. However, in the case of de novo KSCCs, the data pertaining to EBV remains murky (Barnes et al., 2005; Peterson & Nelson, 2013).

Apart from histological differences, latent infection of EBV is evident in most cases of NPC within endemic regions, but is absent in KSCCs (Breda et al., 2010). Usually, patients have lower or negative IgA titers against EBV compared to nonkeratinizing carcinomas. KSCC has lower loads of EBV relative to its nonkeratinizing counterpart. In the case of ISH, EBER tested positively in nonkeratinizing NPC, while it did not test positive nearly as often in KSCC (Li & Zong, 2014; Lu et al., 2010).

Basaloid squamous cell carcinoma

BSCC was first reported in 1986 in the tongue, larynx, and hypopharynx, marking itself as a distinctive histological variant of SCC. BSCC was defined back in 2005 in the WHO Blue Book as an aggressive high-grade variant of SCC that is made up of basaloid and squamous components. The upper aerodigestive tract is where this type of tumor is most prevalent, especially epiglottis, hypopharynx (piriform sinus), and the base of the tongue. Other sites for this type of tumor include floor of the mouth, oral mucosa, palate, tonsils, sinonasal tract, nasopharynx, and trachea. It has also been reported in the esophagus, lung, anus, cervix uteri, penis, and urinary bladder (Ereño et al., 2008; Wan, Chan, Lau, & Yip, 1995).

A predominantly basaloid histology is linked to distant metastases in 52% of patients. The lesions can develop distant metastases, deep invasion, local recurrence, and lymph node involvement. The lung and liver are the most common sites for distant metastatis (Ereño et al., 2008).

This type of cancer takes up about 0.2% of NPCs in southern China. There are some cases of BSCC that were analogous to tumors that are more common in other head and neck regions, and were reported to occur as the primary tumors of the nasopharynx (Barnes et al., 2005).

Basaloid cells are minuscule and possess hyperchromatic nuclei while lacking nucleoli, with scant cytoplasm. They remain closely packed and grow in a solid pattern dictated by a lobular configuration. In certain cases, there is a marked peripheral palisading Comedonecrosis, stromal hyalinization, and basophilic mucoid matrix (Li & Zong, 2014; Wan et al., 1995).

The tumor exhibits a lower clinical aggressiveness relative to BSCC occurring in other head and neck sites. These tumors, in Asians, tested positive for EBV, while in the case of Caucasians, only one case tested negative (Li & Zong, 2014).

Smaller numbers of EBV and nuclear signal is commonly detected in BSCC (Thompson, 2007). Via immune histochemistry, BSCC contains cytokeratins, EMA, and vimentin. Some authors have proposed a cocktail of keratins, made up of Cam 5.2, pankeratin AE/AE3, and CK7, while others recommend that the high-molecular-weight keratin of 34bE12 could be the most effective marker in the case of this tumor. Recent works confirmed the strong and diffuse staining for P63 immunomarker within the BSCC tumor (Radhi, 2012).

The IHC is crucial for recognizing and diagnosing endoscopic biopsies of BSCCs, since biopsies are superficial or lack the basaloid and squamous components that are common in this type of tumor. Diagnosing BSCC is indeed difficult (Ereño et al., 2008).

Application of brush cytology versus tissue biopsy

A definitive diagnosis of NPC usually will achieve by taking a tissue biopsy from the primary lesion or metastatic focus. This usually means the histological diagnosis of the biopsy material, but for the past 20 years, cytology in the neck and head cancer is being used more, including NPC (Van Hasselt & Gibb, 1999). There are two main branches of cytopathology: exfoliative and aspiration biopsy (Al-Abbadi, 2011).

Diagnostic cytology is noninvasive, simple, faster, cost-effective, has high acceptance among populations, and helpful in the field of cancer screening

(Ayele, Mohammed, & Yimer, 2017). It can be performed using methods such as brushing or scraping. Various types of samples can be extracted without anesthesia, such as vaginal scabs, sputum, urine, body fluids, etc., at low risk for healthy and critical patients (Ayele et al., 2017; Burkhard & Wellman, 2013).

Exfoliative cytology involves extracting (scraped) cells from the nasopharyngeal mucosa as a sample by special instrument. This approach is rather atraumatic, and fast and cheap. It is suitable for the diagnosis of a primary NPC, alongside recurrent and residual tumors (Kaur, Saxena, Samantha, Chawla, & Yadav, 2013; Van Hasselt & Gibb, 1999).

The FNAC is the most common method used for diagnosing head and neck cancers. It is the method used for detecting extranodal distant metastases, especially lymph node metastases in the cervix. It is safe, tolerable, cost-effective, and accurate, with minimal complications (Chang et al., 2001; Naqvi, Husain, Ansari, & Yousfani, 2004).

Exfoliative cytology was applied on the nasopharynx, due to the aforementioned benefits in the context of gynecology (Ablashi, Faggioni, Krueger, Pagano, & Pearson, 2012; Kumaresan & Jagannathan, 2014). The method collects cells that have been overthrown in the nasopharyngeal cavity, and the abraded cells from the surface of the nasopharynx.

Many devices can be used in exfoliative cytology such as cotton wool, swab, and uterobrush. However, these devices are not disposable, and need to be carefully sterilized for reuse, which is rather time consuming. After the sample is collected, it is spread evenly onto a glass slide. This slide is then wet fixed using spray fixative/immersed in 95% alcohol; however, some pathologists have reported a preference for air-dried smear. Then this slide will be stained by either Hematoxylin and Eosin, or the Papanicolaou stain, which will stain the nuclear parts of the cells, and also Gimsa stain will be used usually to stains cytoplasmic details. Hence, both staining methods complement one another (Lu et al., 2010; Van Hasselt & Gibb, 1999).

More smears can be tested in order to increase the diagnostic accuracy by detecting several EBV latent proteins, such as one to identify EBNA, while the other detects EBERs (Adham et al., 2013; Ramayanti et al., 2017; Van Hasselt & Gibb, 1999).

NPC can be diagnosed via the detection of Epstein–Barr virus distal non-diffuse (EBV DND) load and/or LMP1, which is a latent EBV gene product in carcinogenesis. Within the nasopharyngeal swab, sensitivity and specificity for the EBV DND load and LMP1 were 90% and 99% and 87.3% and 98.4%, respectively. Nasopharyngeal swab is a suitable diagnostic method in the screening workup, especially in the case of risky individuals (Abdullah, Alias, & Hassan, 2009; Hao, Tsang, & Chang, 2003; Tune et al., 1999). This method has been touted as an

accurate and noninvasive method, and as an alternative to biopsy, since the process is minimally traumatic to the patient and samples can be collected from an original lesion at the primary site of the nasopharynx (Adham et al., 2013; Zheng, Lu, Li, & Jia, 2015).

The nasopharyngeal swab can be used for cytological diagnosis of NPC by the detection of latent EBV viral proteins in infected cancer cells. However, cytology demonstrated limited sensitivity of 70%–90% for NPC diagnosis. Nevertheless, Hong Kong, Taiwan, Canada, and Indonesia reported high sensitivity and specificity using nasopharyngeal swab to diagnose NPC at 87.3%–96.4% and 90.0%–98.4%, respectively (Adham et al., 2013; Barnes et al., 2005; Zheng et al., 2015).

The detection of EBV DNA and RNA in NP brush/swab sample has been heavily researched for application in early-stage and recurrent/relapse NPC case diagnosis, due to its promising features as a prognostic tool for therapy and follow-up assessment. Detection of high levels of EBV DNA viral load in NP brush/swab samples indicate a recurrent disease/poor prognosis and considered as confirmatory tool in the case of serological screening tests in a NPC diagnosis program (Adham et al., 2013; Ramayanti et al., 2017; Zheng et al., 2015).

In the previous study by Ramayanti et al., 2017, was discovered that NPC brushing can be considered as a diagnostic tool for NPC, demonstrating increased loads of EBV DNA in NPC patients relative to the control samples. The pattern of expression profiles for both lytic and latent genes, as well as EBV DNA viral, was similar to the biopsy samples. The prevalence of viable tumor cells on the NP site is reflected by the increased EBV DNA loads in both NP brushing and biopsy section samples collected from NPC patients (Ramayanti et al., 2017).

However, nasopharyngeal biopsy is considered as a confirmatory diagnostic method for NPC cases by histopathological examination. The data provides the histopathologist with a diagnosis of benign (reactive lymphoid hyperplasia)/malignant (NPC) (Adham et al., 2013; Zheng et al., 2015). Biopsy is usually done in parallel with nasopharyngeal (NP) endoscopy of a suspected tumor lesion, followed by histopathological assessment and EBV evaluation using methods such as ISH for EBER and IHC for LMP1 and EBNA1 (Zheng et al., 2015).

Relative to the biopsy method, the NPC brushing technique has in the past reported false-negative rates, especially when the tumors are deeply embedded into the organ or body. The brushing procedure, going deep, could miss a few cells, resulting in negative rates (Adham et al., 2013; Stevens et al., 2006). Despite the pain, invasiveness, and difficulty of repetition associated with the biopsy method, biopsy considered a better choice, standard and more accurate

method compering with the NPC brushing technique especially at an early stage of cancer (Adham et al., 2013; Stevens et al., 2006).

Local anesthesia, accompanied with flexible and rigid endoscopes, are commonly used to extract biopsy samples. The rigid endoscope can easily visualize the nasopharynx, alongside small or submucosal lesion, sensitive at 95.1% and 95.6%, respectively (Waldron, Andrew Hasselt, & Wong, 1992).

With the presence of early-stage disease and indistinct tumor, the lateral pharyngeal recess, where it is most prevalent in early diagnoses, can be biopsied. Simple, safe, and noninvasive methods are needed for the early-stage NPC diagnosis (Adham et al., 2013).

Conclusion

An effective screening program involving NPC needs to be designed in order to achieve effective curable treatment, improve prognosis, and diagnose NPC early. Hence, the effective screening and diagnostic method (developed method) is needed recently that is simple, not time-consuming, noninvasive, low cost, and accurate. The diagnostic method also needs to be able to discern the prognosis of NPC and its corresponding stages.

Due to the link between EBV and NPC, screening tests such as the EBV antibody serological tests and methods that detect EBV DNA load and EBV antibodies have been developed. These methods have been determined to be effective at early diagnosis, treatment monitoring, and prognosis of NPC patients, especially in the case of endemic and high-risk populations. These tests have shown to be sensitive and specific, and EBV-EA IgA has been determined to be the best screening marker of NPC, since it can detect 70%–100% of patients afflicted with the disease (Lu et al., 2010).

Between the three histological types of NPC, nonkeratinizing carcinoma is the most common occurrence in endemic and nonendemic areas. It is also strongly linked to EBV. KSCC, relative to nonkeratinizing carcinoma, is known to be prevalent in nonendemic areas, and reported to have poor response to treatment, high incidence of advanced tumor growth, and a worse prognosis compared to nonkeratinizing carcinoma.

Immunohistochemical staining is most effective when differentiating between carcinoma and noncarcinoma cells, where NPC cells will induce a positive reaction relative to lymphoma, while atypical cells will exhibit a negative reaction.

Scanning for EBER using ISH for EBER is regarded as the standard diagnostic method for NPC, especially when other methods are not viable.

Nasopharyngeal brushing is regarded as the most suitable diagnostic method for NPC. It was posited as being easy, accurate, noninvasive, and minimally

traumatizing, and is the best alternative to an invasive biopsy. Nasopharyngeal brushing is also suitable for early diagnosis of NPC, detecting recurrences and relapses, and for monitoring treatments and prognosis. This technique is also flexible in that it can be used alongside other techniques for the confirmatory diagnosis of NPC.

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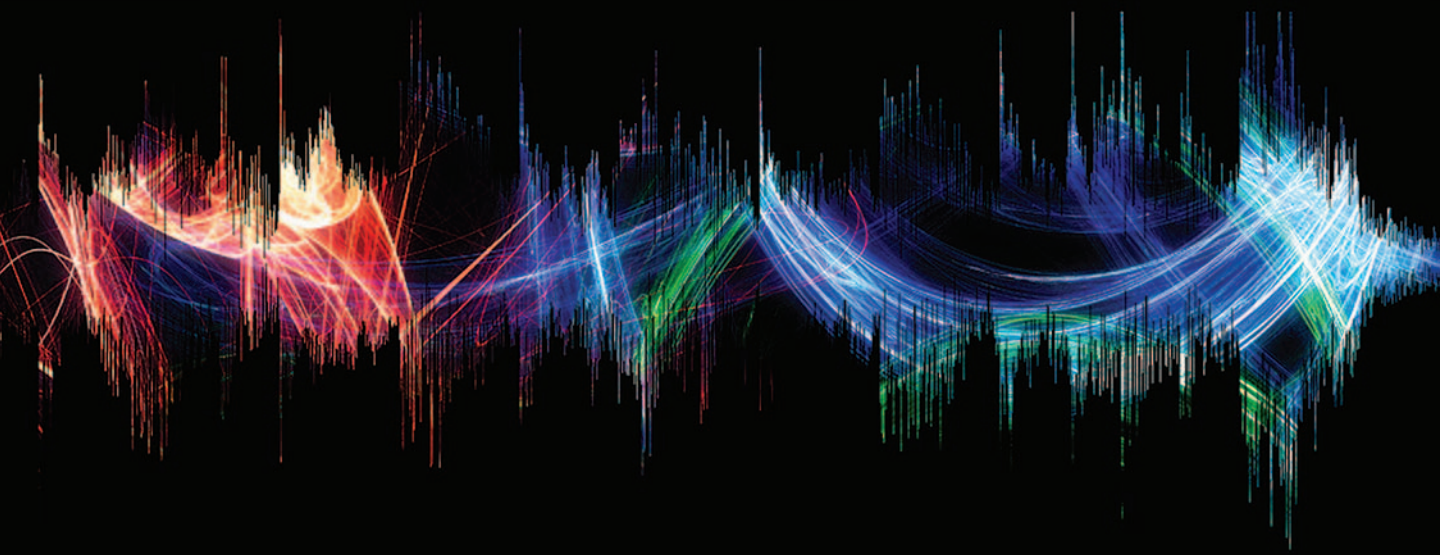

9

Open surgical salvage procedures for nasopharyngeal carcinoma

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Introduction

The nasopharynx is situated in the central part of the skull, a relatively inaccessible part of the body surrounded by critical structures. Surgeons in the late 19th century and early 20th century actually condemned the operation. The famous otolaryngologist Chevalier Jackson from Philadelphia commented that surgery in the nasopharynx did not offer any hopes of cure but instead increased the suffering of the patient (Jackson, 1901). By the mid 20th century, improvement in the understand of radiation therapy allowed precise delivery of curative dose of radiation to the nasopharynx and sparing surrounding critical structures from radiation damage. The endemic form of the cancer, Epstein–Barr virus-positive undifferentiated carcinoma, is also radiosensitive and radiotherapy as a single modality treatment will offer significant chance of cure. With ever-improving techniques of radiotherapy delivery, coupled with appropriate use of concurrent chemotherapy, the local control rate of early stage nasopharyngeal carcinoma (NPC) has approached 90% (Lee et al., 2002). On the other hand, open surgery to the nasopharynx needs to transgress a significant amount of normal tissue and that will cause significant morbidities to the patient. Therefore to date, surgical resection is still not an option for treatment of primary NPC and the standard of care for primary NPC is still radiotherapy with or without chemotherapy.

The situation is very different in the recurrent/salvage disease setting. The efficacy of second radiation in treating local failure is quite low, especially with the old two-dimensional radiotherapy technique (Lee et al., 1993). Moreover, the toxicity from second radiation is very high and can cause debilitating complications to patients even if the cancer is cured. Salvage surgery, even with the morbidities from transgressing normal tissue in the head and neck area, is more

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tolerable and offers high chance of cure in small local recurrences and resectable nodal recurrences. At present, the role of surgical treatment in NPC still confines to salvaging radiation failures.

Open nasopharyngectomy for salvaging local recurrence

With modern radiation techniques, the local control of early T stage NPC is well over 90% and only a small percentage of patients will develop local recurrences (Lee et al., 2005). For more advanced T stage diseases, the dosimetry to the tumor may be constrained by the maximum dose of the adjacent critical structures, and thus certain areas of the tumor may not be able to receive the ideal therapeutic radiation dose. In these cases, local recurrence may occur in these underdosed areas. Unfortunately, these underdosed areas are usually adjacent to critical structures like optic nerve, brainstem, or pituitary gland, making salvage surgery more difficult or even impossible.

Advances in endoscopic sinus surgery have expanded the indication to resection of malignant tumors in the paranasal sinuses and anterior skull base, including salvaging local recurrence of NPC. This does not mean that endoscopic surgery can replace open nasopharyngectomy completely. In cases where the internal carotid artery (ICA) is aberrant or the tumor is in close proximity to the ICA, open nasopharyngectomy still offers better protection of the vessel and easier vascular control in case the ICA is accidental damaged.

As the nasopharynx is deeply situated in the center of the head, the majority of the open surgical approaches require some form of osteotomy and all open approaches need to transgress a significant amount of normal tissue. It is not surprising that multiple approaches to the nasopharynx have been described in the literature, each with pros and cons. The following is a summary of the commonly employed open nasopharyngectomy approaches.

Transpalatal approach

The transpalatal approach was first described to resection a benign tumor of the nasopharynx, juvenile nasopharyngeal angiofibroma in 1956b by Wilson (1957). Professor Tu from Beijing started to employ the approach for salvaging NPC local recurrence in 1960 but not until 1988 did he publish his experience in the English literature (Tu, Hu, Xu, & Ye, 1988). Willard Fee of Stanford University also described his series of transpalatal NPC for recurrent NPC in the same year (Fee, Gilmer, & Goffinet, 1988). The exposure to the nasopharynx is achieved by a posteriorly based mucosal flap of the hard palate mucosa and detachment of the soft palate from the hard palate. The hard palate bone is then removed and the palatal mucosal flap retracted posteriorly and inferiorly, allowing exposure of the nasopharynx. After resection of the local recurrence, the hard palate mucosa is replaced and sutured back to the anterior palatal incision. There is a risk of palatal fistula if the palatal wound is not healed

completely. The main disadvantage of the procedure is the inability to extend the resection laterally as the pterygoid plates in both sides will limit the lateral reach of the surgical instruments.

The procedure fell out of favor as better approaches were later developed but reincarnated in the special form when the surgical robot was employed for nasopharyngectomy. A palatal flap or palatal split would be first performed then the surgical robot deployed transorally, with the telescope replacing the headlight and the robotic arms replacing the surgeon's hands (Ozer & Waltonen, 2008; Wei & Ho, 2010).

Transcervico-manibulo-palatal approach

In order to improve the exposure and lateral reach, the transpalatal approach has been modified to include a lip-split mandibulotomy. After a lip-split mandibulotomy and incision of the ipsilateral floor of mouth mucosa, the mandible is retracted laterally and the floor of mouth incision extended superiorly to the anterior pillar of the tonsil then upward to divide the soft palate from the hard palate. The nasopharynx and ipsilateral parapharyngeal space (PPS) can then be widely exposed. The ICA is then exposed in the neck and traced superiorly to the PPS, which can then be retracted laterally and protected before resecting the tumor in the nasopharynx and the PPS. Figs. 9.1 and 9.2 are schematic drawings illustrating this approach to the nasopharynx. This approach offers a wide exposure to the nasopharynx and ipsilateral PPS and allows the surgeon to dissect free the whole length of the ICA from the neck to the carotid canal. Morton described his cohort of seven patients treated with this approach with one early recurrence within 1 year and one late recurrence (Morton, Liavaag, McLean, & Freeman, 1996). However, this approach transgresses a significant amount of normal tissue and disrupts the muscles of swallowing, causing significant postoperative morbidities. In the 11-case cohort described by King et al., all patients suffered from at least one major morbidity, with eight patients developing palatal defects, six patients suffering from trismus, six patients had severe dysphagia, and 3 patients had malunion of the mandibulotomy site (King, Ku, Mok, & Teo, 2000). The procedure had fallen out of favor due to the high morbidities and newer approaches offering similar exposure with fewer morbidities.

Midfacial degloving approach

Casson et al. described the midfacial degloving approach in 1974 for excision of fibrous dysplasia of the midface (Casson, Bonanno, & Converse, 1974). Later, it was employed by Howard as an alternative to lateral rhinotomy for resection of juvenile nasopharyngeal angiofibroma (Howard & Lund, 1992). The procedure starts with detaching the columellar cartilage and lower lateral cartilages on both side from the upper lateral cartilage, nasal septum, and bony nasal framework, similar to performing closed rhinoplasty. Intercartilagenous incisions were placed between the upper and lower lateral cartilage inside the

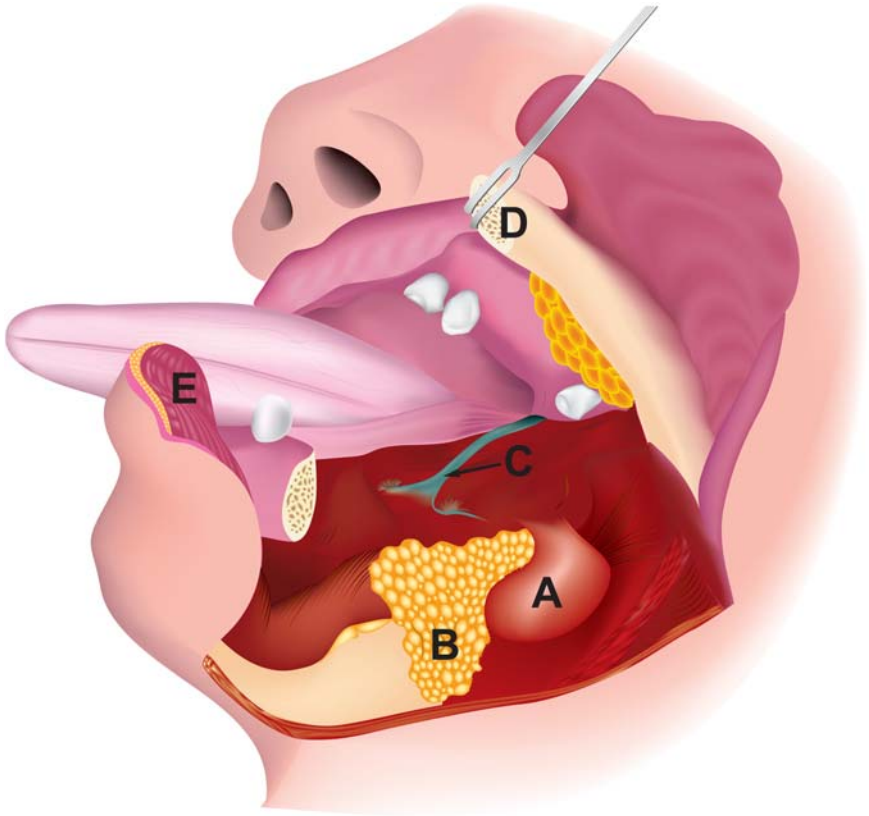

Figure 9.1 Diagram depicting the transcervico-mandibulo-palatal approach to the nasopharynx. *A*, Submandibular gland; *B*, cut edge of the mylohyoid muscle; *C*, lingual nerve; *D*, ipsilateral mandible swung laterally; *E*, lip split incision. *Source: Adapted from Morton, R. P., Liavaag, P. G., McLean, & M., et al. (1996). Transcervico-mandibulo-palatal approach for surgical salvage of recurrent nasopharyngeal cancer.* Head Neck, 18, *352–358.*

nasal vestibule on both sides. These incisions are then extended medially to form a transfixation incision of the nasal columellar. The soft tissue of the nasal skin is then freed from the underlying bony skeleton by creating a tunnel up to the nasion from the transcartilagenous incisions. The columellar cartilage is freed from the caudal end of the nasal septum. Then, a sublabial incision on the gingivobuccal sulcus is made from the last molar on one side to the contralateral side. The sublabial incision is dissected down to the premaxilla and in the midline, connected with the transfixation incision of the nasal columellar. The soft tissue of the midface is then freed up the infraorbital nerves on both sides. The soft tissue of the midface with the upper lip and nasal skin can then be retracted superiorly to expose the bony anterior midface. The nasal septum can then be cut at the inferior end and deflected to one side with a speculum.

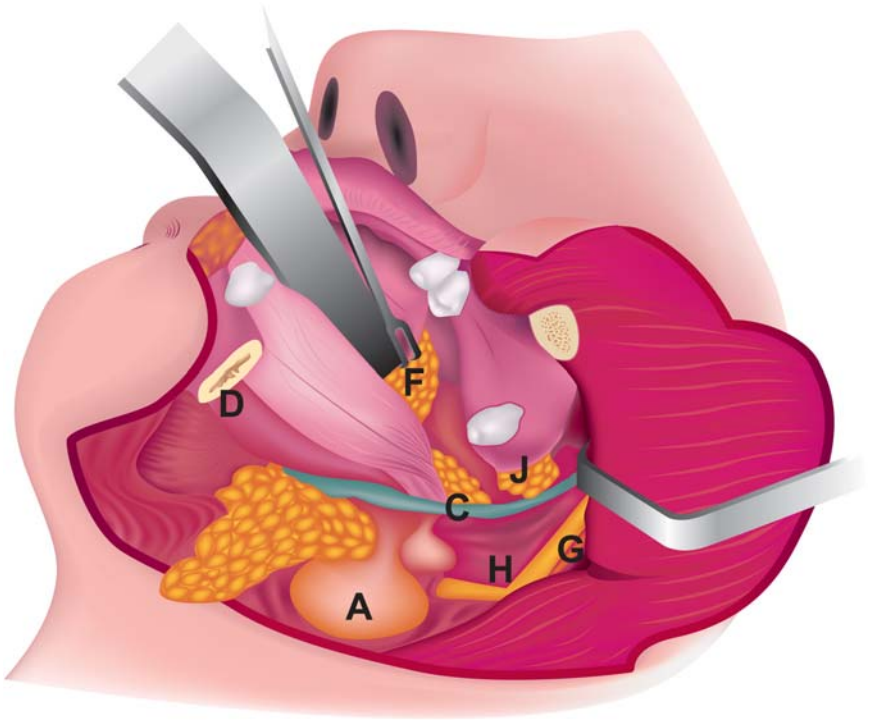

Figure 9.2 Diagram showing the nasopharynx after division of the medial pterygoid muscle. *A*, Submandibular gland; *C*, lingual nerve; *D*, mandibular osteotomy; *F*, soft palate detached from hard palate and retracted laterally; *G*, parapharyngeal internal carotid artery; *H*, hypoglossal nerve; *J*, cut edge of the medial pterygoid muscle attached to the lateral pterygoid plate. *Source: Adapted from Morton, R. P., Liavaag, P. G., McLean, & M., et al. (1996). Transcervico-mandibulo-palatal approach for surgical salvage of recurrent nasopharyngeal cancer.* Head Neck, 18, *352–358.*

Both inferior turbinates are then removed. The nasopharynx can then be visualized with illumination from headlights. Fig. 9.3 illustrates the incisions for the midfacial degloving approach.

The major advantage of this approach is the lack of facial incision and minimal disruption to the swallowing mechanism. Unfortunately, the exposure of the approach is limited and also lateral extension of the dissection is hampered by the pterygoid process and pterygoid plate. A medial maxillectomy and drilling down the pterygoid process can marginally improve the access to the PPS but visualization is still limited once surgical instruments are placed inside the nasal cavity and maxillary sinus. NPC commonly arises from the fossa of Rosenmuller and frequently has lateral extension to the PPS. The limitation in lateral extension has made this approach less suitable for salvaging recurrent NPC. Vlantis et al. compared the midfacial degloving approach to the maxillary swing

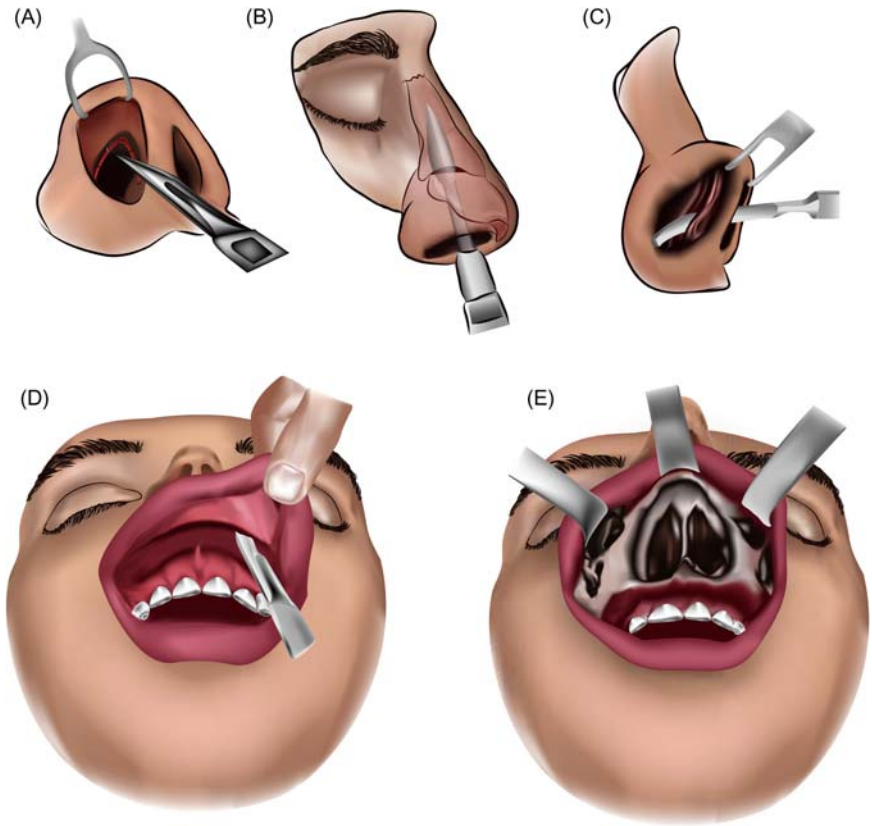

Figure 9.3 Incisions for the mid-facial degloving approach. *A*, Transcartilagenous incision; *B*, elevation of nasal skin from nasal bone and upper lateral cartilage; *C*, transfixation incision; *D*, sublabial incision; *E*, exposure of the mid-facial skeleton after connecting the sublabial incisions with the nasal incisions and retracting the mid-facial skin superiorly. The nasopharynx can be exposed after bilateral medial maxillectomies to remove the medial maxillary wall, then dividing the nasal septum at the inferior attachment and flipping it to one side. *Source: Adapted from Casson, P. R., Bonanno, P. C., & Converse, J. M. (1974). The midface degloving procedure.* PlastReconstrSurg *53, 102–103.*

approach in salvaging rT1 and rT2 local recurrences and found that the 5-year local-progression free survival for maxillary swing approach was 88.2%, superior to the 50.4% for the midfacial degloving approach (Vlantis et al., 2008). With improvement in endoscopic instruments and maturity of the endoscopic surgical techniques, this approach has been mostly superseded by the endoscopic nasopharyngectomy approaches described in the next chapter.

Maxillary swing approach

It was found that after total maxillectomy, the ipsilateral nasopharynx can be visualized directly and open operations in the nasopharynx can be performed

with good instrumentation (Wei, Lam, & Sham, 1991). It would not be practical to remove the patient's ipsilateral disease-free maxillary bone for salvage of NPC. However, if the ipsilateral maxillary bone was left attached to the cheek skin flap, there would be enough blood supply to the maxillary bone to keep it viable. This forms the basis of the maxillary swing approach.

A tracheostomy is advised before the start of the resection and general anaesthesia can be administered through the tracheostomy tube. Absence of the tube in the oral cavity or nasal cavity makes osteotomies easier. The tracheostomy can also protect the upper airway if there is any swelling to the oral cavity and oropharynx after the procedure.

The maxillary swing approach first starts with a maxillectomy incision, the Weber–Ferguson incision, with the subciliary part extended laterally and inferiorly for 1–1.5 cm reaching the anterior part of the zygomatic arch. Inferiorly, the lip-split incision is continued inferiorly in the midline of the hard palate until the hard palate and soft palate junction and then extend laterally toward the maxillary tuberosity. A sublabial incision is made from the lip-split incision extending along the gingivobuccal sulcus reaching the maxillary tuberosity. The soft palate is then detached from the hard palate. Alternatively, the hard palate mucosa is incised just medial to the ipsilateral alveolar process from incisor to molar. The ipsilateral hard palate mucosa is then elevated beyond midline. With this hard palate mucosal flap, the mucosal incision would not be placed directly over the hard palate osteotomy, reducing the chance of palatal fistula formation. Fig. 9.4 shows the facial incisions and osteotomies.

Before commencing the osteotomies, plates should be placed in the osteotomy sites to reapproximate the maxilla back to the rest of the facial skeleton. This is easier to perform before the osteotomy than at the conclusion of the resection. Titanium plates are placed over the osteostomies in the premaxilla, zygoma and between the superiomedial anterior maxillary wall and the nasal bone. Usually 2 mm miniplates for midface reconstruction are used for the premaxilla and zygoma while 1.5 mm microplate is used for the nasal bone and anterior maxillary wall. After ensuring the correct holes are drilled, screws placed, and plates are well fit, the screws and plates are removed and placed on the back table for use at the conclusion of the procedure. Osteotomies are performed similar to a radical maxillectomy, with the anterior maxilla, zygoma, and hard palate osteotomies performed with an oscillating saw and the posterior maxillary wall separated from the pterygoid plates by a curved osteotome. Fig. 9.5 shows the maxilla left attached to the cheek flap after completion of the anterior osteotomies and the placement of the curve osteotome for separating the posterior maxillary wall from the pterygoid process. The only difference between the maxillary swing and a radical maxillectomy is that the anterior cheek skin flap is not elevated from the bony wall of the anterior maxilla; instead, the maxilla bone is left attached to the anterior cheek skin, where the

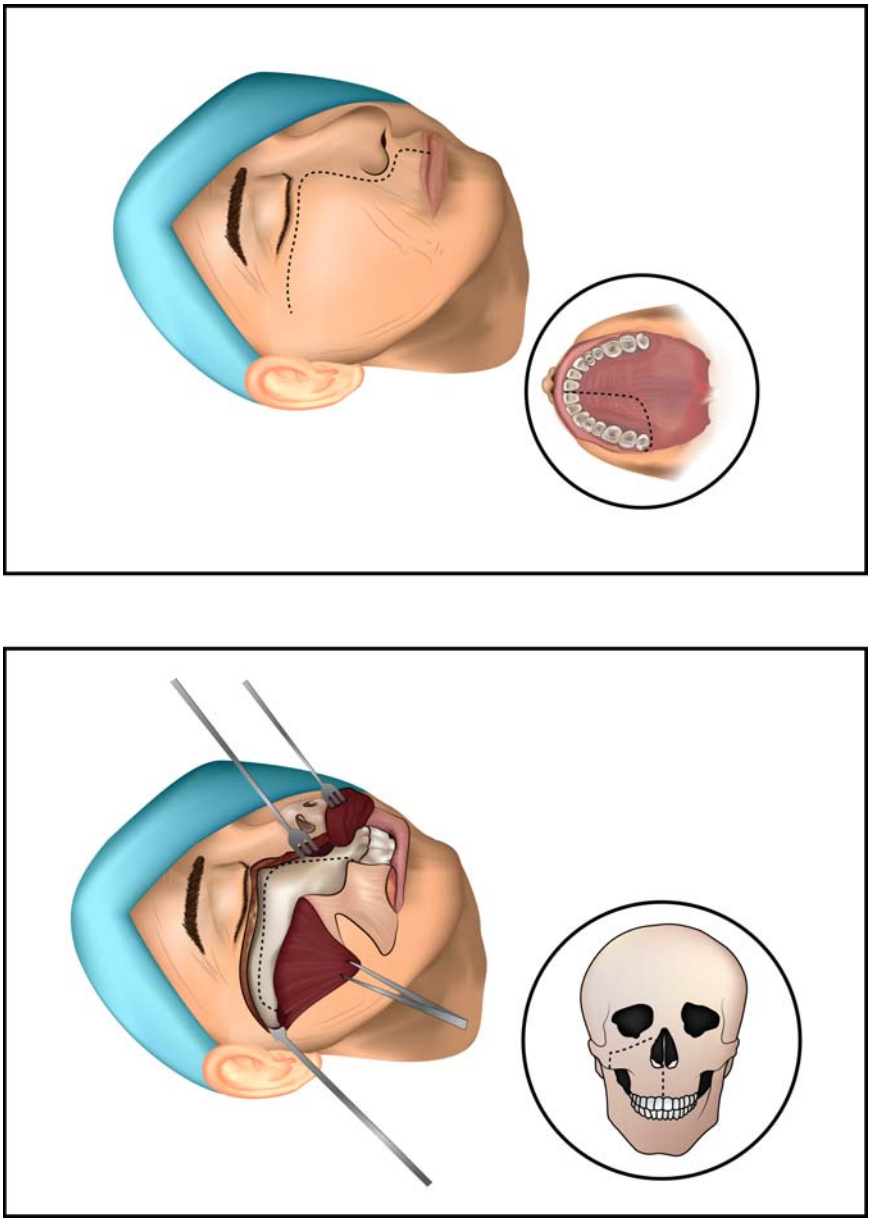

Figure 9.4
Upper diagram, facial palatal incisions for maxillary swing approach. Lower diagram, osteotomies for the maxillary swing approach. *Source: Adapted from Wei, W., Lam, K., & Sham, J. (1991). New approach to the nasopharynx: The maxillary swing approach.* Head Neck, 13, *200–207.*

maxillary bone will then derives all its blood supply. After the osteotomies, the maxilla and cheek skin are both swing laterally to expose the nasopharynx. The internal maxillary artery needs to be ligated. A posterior septectomy should be performed to improve the visualization of the contralateral

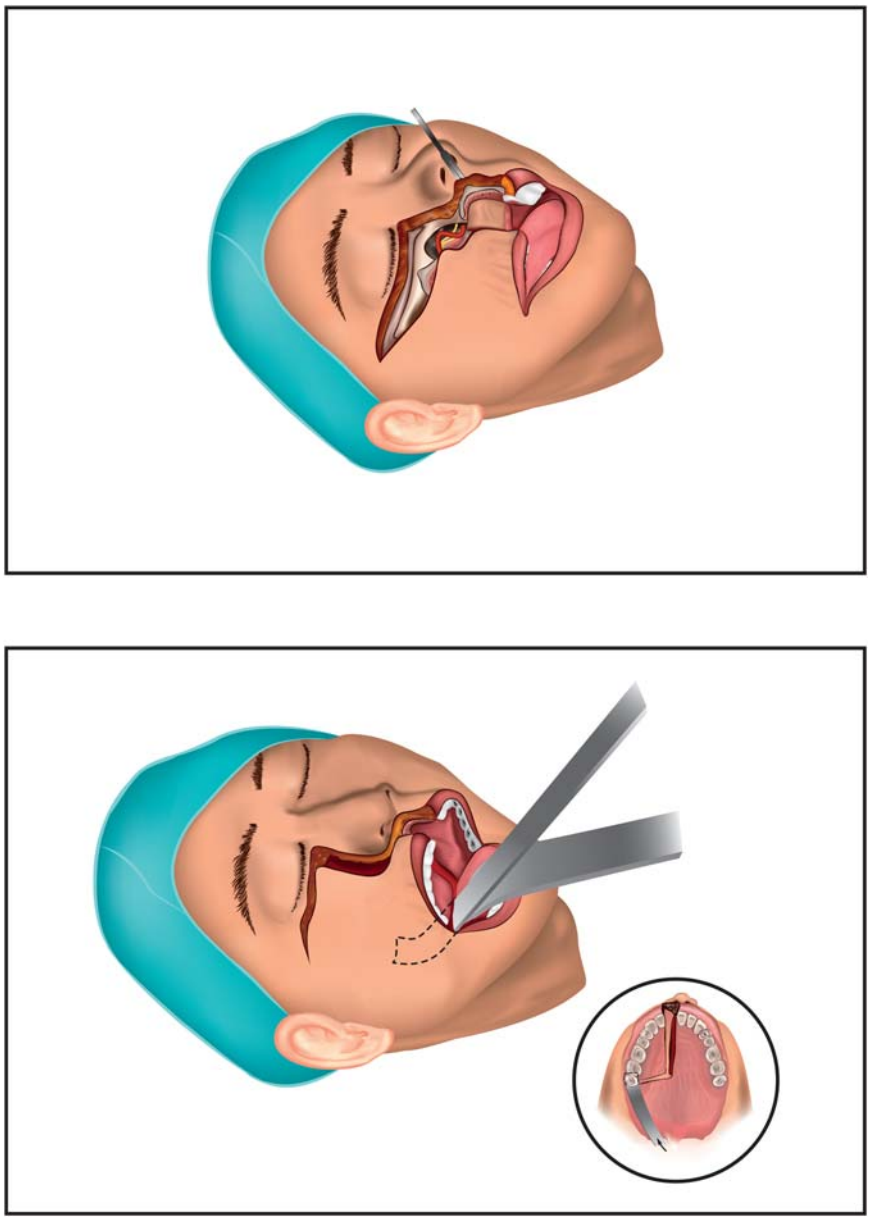

Figure 9.5
Maxillary swing approach. Upper diagram, the maxilla is left attached to the cheek flap after the anterior osteotomies. Lower diagram, the placement of the curved osteotome to separate the posterior maxillary wall from the pterygoid process. *Source: Wei, W., Lam, K., & Sham, J. (1991). New approach to the nasopharynx: The maxillary swing approach.* Head Neck, 13, *200–207.*

nasopharynx and Eustachian tube opening. Fig. 9.6 illustrates the view of the nasopharynx after swinging the ipsilateral maxilla laterally. To improve the exposure and access to the PPS, the pterygoid process and medial ptyergoid plate can be removed. Bleeding from the pterygoid venous plexus can be

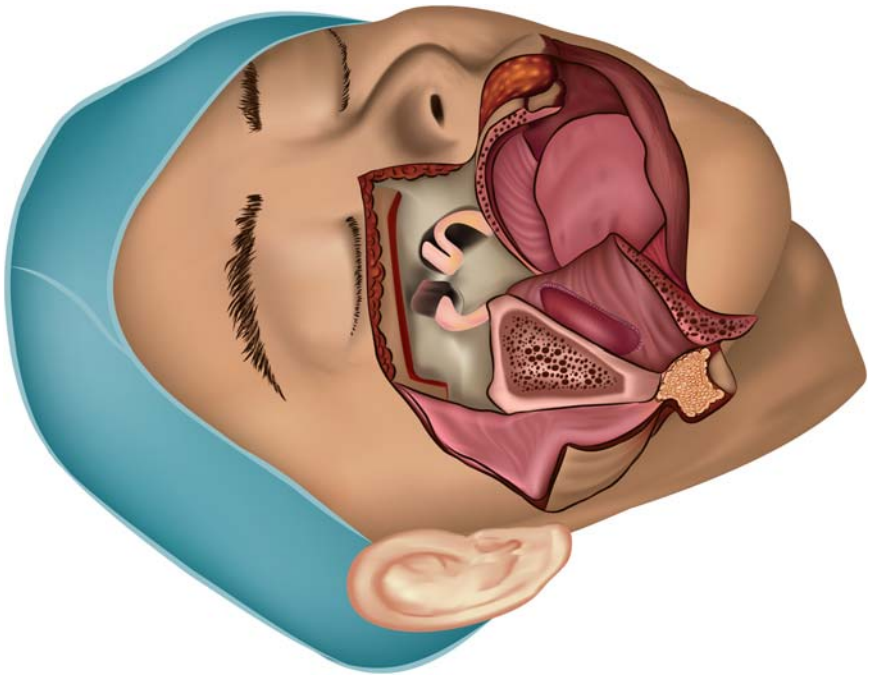

Figure 9.6 Maxillary swing approach. Exposure of the nasopharynx after swinging the maxilla bone laterally. The posterior septum is removed to improve the exposure to the contralateral nasopharynx. *Source: Adapted from Wei, W., Lam, K., & Sham, J. (1991). New approach to the nasopharynx: The maxillary swing approach.* Head Neck, 13, *200–207.*

controlled by plicating the pterygoid muscles with sutures and packing. The ICA in the PPS can be located by palpating a pulsatile structure posterior to the styloid process.

A 1–1.5 cm mucosal margin is marked around the tumor and resection of the recurrent tumor can then be performed. The resection should encompass the ipsilateral cartilaginous Eustachian tube. Studies have shown that tumors in the fossa of Rosenmuller frequently involve the Eustachian tube cartilage and the cartilaginous Eustachian tube should be resected to obtain a clear resection margin. After resection of the cartilaginous Eustachian tube, otitis media with effusion would be inevitable and the patient should be forewarned of this complication before the operation.

The prevertebral fascia should always be resected as the deep margin. Depending on the extent of tumor invasion, the prevertebral muscles may also need to be resected if preoperative imaging showed that the tumor had extended beyond the prevertebral fascia. The floor of the sphenoid bone and part of lower clivus bone can be removed for tumor clearance. During lateral dissection and division of the Eustachian tube from the bony canal, the position of the ICA should be

ascertained to prevent inadvertent injury of the vessel. Unless necessary, the ICA should not be exposed to prevent future rupture of the vessel. If the vessel is exposed, then coverage of the vessel by vascularized tissue must be performed. This will be discussed in the latter part of the chapter.

After conclusion of resection and frozen section has confirmed clear resection margins, the raw area after resection should be covered. A thin mucosal graft can be harvested from the inferior turbinate to cover the raw area or a contralateral nasoseptal mucosal flap harvested and rotated posteriorly to cover the raw area. If the area of exposed bone is too big for coverage by the nasoseptal flap or the ICA is exposed, the area can be covered by a free vastus lateralis muscle, with the vascular pedicle of the vastus muscle anastomosed to a branch of the external carotid artery and internal jugular vein (IJV) in the neck (Chan, Chow, Tsang, & Wei, 2012). Alternatively, a temporal-parietal fascial flap can be rotated in from the temporal area through the infratemporal fossa.

The maxilla can then be repositioned back to the facial skeleton and secured with plates and screws. The mucosal and facial incisions then closed with stitches. The nasal cavities are packed with nasal tampons. A prefabricated upper palate dental plate can facilitate the approximation of the hard palate mucosa to the maxilla bone. Patients can have a fluid diet the next day though chewing hard food is not recommended until 6 weeks after the procedure.

Infratemporal fossa approach

Unlike the previously described approaches, which are all anterior skull base approaches, the infratemporal fossa approach is a lateral skull base neurotological approach to the nasopharynx. The approach was first described by Ugo Fisch in 1979 (Fisch, 1983; Fisch & Pillsbury, 1979). The procedure first starts with a large C-shaped incision from temporal area extending to postauricular incision down to the upper neck. Then an extended mastoidectomy and blind sac closure of the external auditory canal are performed. A superficial parotidectomy is then performed to expose the upper branches of the facial nerve (Fig. 9.7). The upper branches of the facial nerve can then be rerouted and the masseter muscle is then detached from the zygomatic arch with the arch removed to gain access to the infratemporal fossa (Fig. 9.8A). The temporalis muscle is then detached from the insertion to the coronoid process of the mandible. The mandibular condyle is dislocated and retracted inferiorly (Fig. 9.8B). The ICA is exposed from the middle ear to foramen lacerum. Finally, the middle meningeal artery is ligated and the mandibular nerve divided as it exits the foramen ovale. The contents of the infratemporal fossa including the pterygoid muscles, muscles attached to Eustachian tube, PPS contents, Eustachian tube, and nasopharyngeal mucosa can then be resected. Fig. 9.9 illustrates the exposure of the nasopharynx and the adjacent anatomical structures of the approach. The pterygoid process and pterygoid plates can be removed to allow resection of tumor extending to the

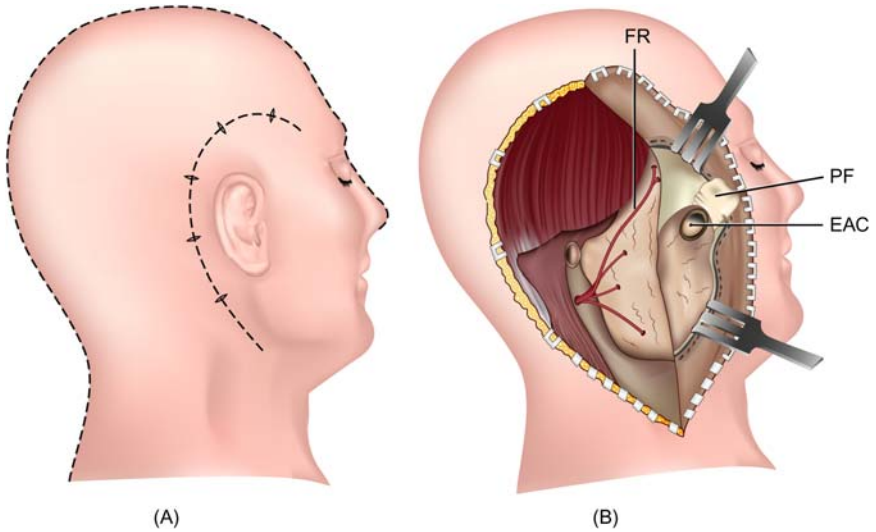

Figure 9.7 Infratemporal approach. Left, C-shaped incision for the approach. Right, Operative field after transection of the external auditory canal, superficial parotidectomy, and exposure of the facial nerve branches in the parotid region. *FR*, Frontal ramus of the facial nerve; *PF*, periosteal flap; *EAC*, cartilaginous external auditory canal. *Source: Adapted from Fisch, U. (1983). The infratemporal fossa approach for nasopharyngeal tumors.* Laryngoscope, *93, 36–44.*

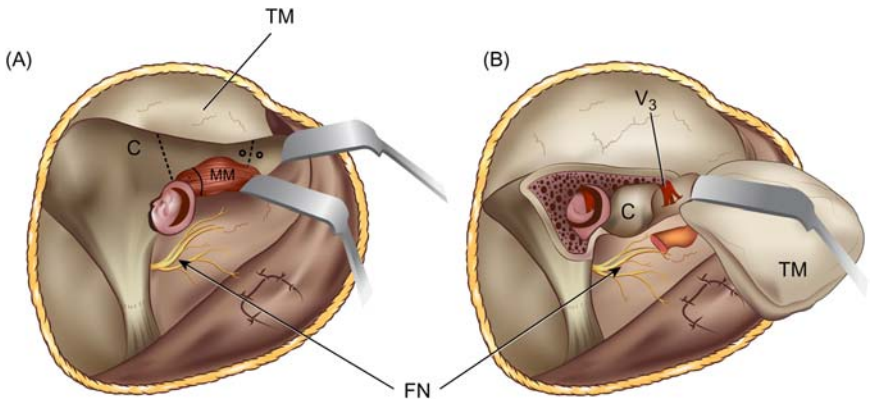

Figure 9.8 Infratemporal fossa approach. (A) Osteotomies on the zygomatic arch. (B) Temporalis muscle reflected inferiorly and the zygomatic arch removed exposing the mandibular condyle and mandibular nerve. *TM*, Temporalis muscle; *C*, mandibular condyle; *MM*, masseter muscle; *V3*, mandibular branch of trigeminal nerve. *Source: Adapted from Fisch, U. (1983). The infratemporal fossa approach for nasopharyngeal tumors.* Laryngoscope, *93, 36–44.*

pterygopalatine fossa. At the conclusion of the procedure, the temporalis muscle is mobilized to cover the mucosal defect of the lateral nasopharyngeal wall and abdominal fat is harvested to fill up the cavity before the skin incision is closed.

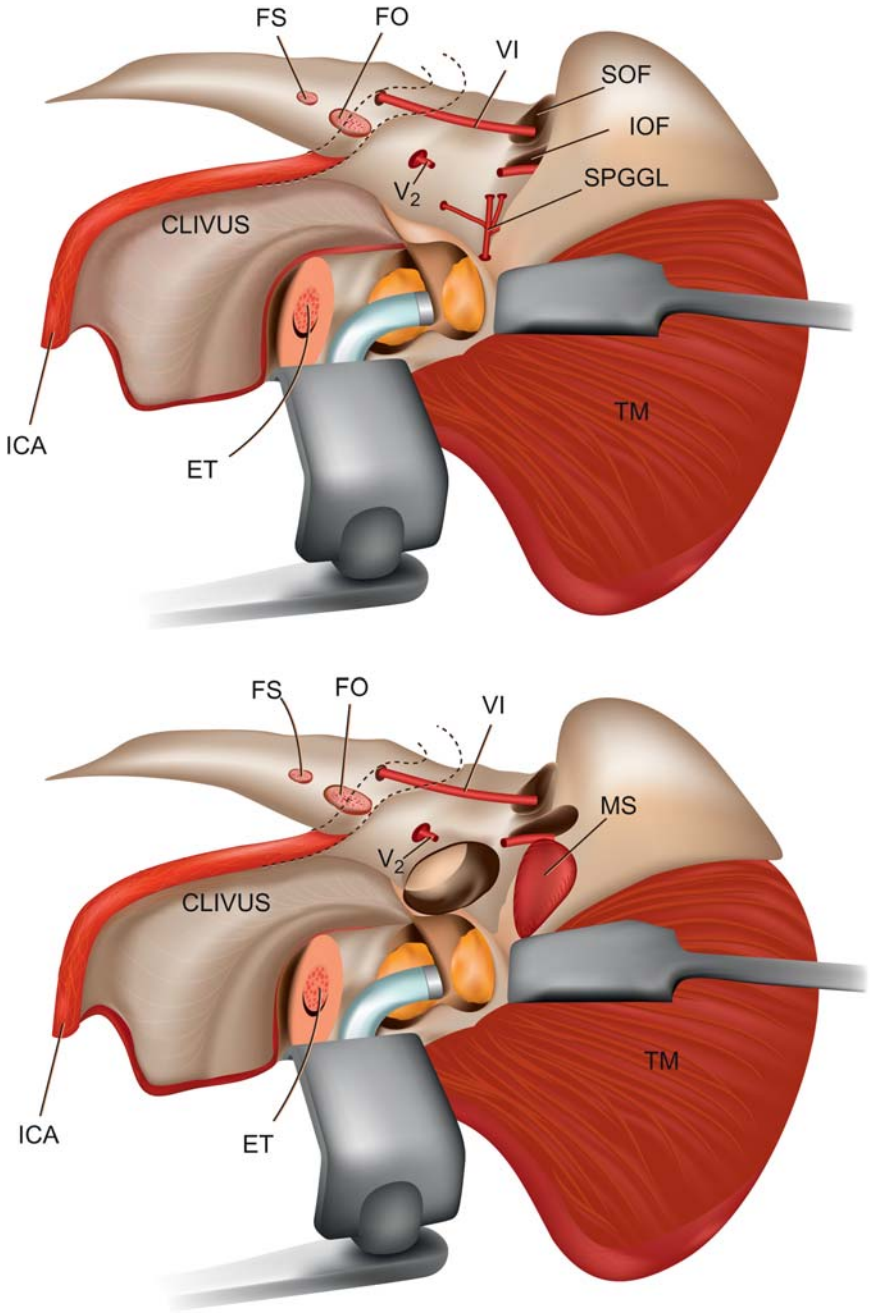

Figure 9.9
Exposure of the nasopharynx in the right infratemporal fossa approach after removal of the pterygoid process and pterygoid muscles. The middle meningeal artery and mandibular branch of trigeminal nerve have been divided and removed. The blue structure is the nasotracheal tube for ventilation. *ET*, Left Eustachian tube opening; *FS*, foramen spinosum; *FO*, foramen ovale; *V2*, maxillary branch of trigeminal nerve exiting the foramen rontondum; *VI*, abducens nerve; *SOF*; superior orbital fissure; *IOF*, inferior orbital fissure; *SPGGL*; sphenopalatine ganglion; *TM*, temporalis muscle reflected inferiorly; *MS*, maxillary sinus. *Source: Adapted from Fisch, U. (1983). The infratemporal fossa approach for nasopharyngeal tumors.* Laryngoscope, *93, 36–44.*

The major advantage of this procedure is that the ICA is identified early and protected during the resection of the infratemporal fossa and PPS contents. However, the procedure requires extensive complicated dissection of the lateral skull base and also damage to multiple cranial nerves including the facial nerve, mandibular branch of trigeminal nerve, and glossopharyngeal nerve. The mastication function would also be impaired after the procedure. The procedure is performed less commonly today as other approaches are simpler and less invasive.

Extended resection for advanced locally recurrent nasopharyngeal carcinoma

Traditionally, encasement of the ICA or extension of the disease intracranially are contraindications for nasopharyngectomy as the risk of injury to the ICA or intracranial structures are high and the extensive disease makes complete tumor resection difficult. With increasing experience of open nasopharyngectomy and good oncological outcomes, surgeons are starting to tackle more extensive tumors. In order to resect tumors that abut or encase the ICA, strategies must be devised to protect the vessel from injury and preserve the cerebral circulation. If the tumor is just abutting the ICA without gross encasement of the vessel, dissecting the tumor free from the vessel is possible without injuring the vessel. However, the ICA will be exposed after the resection and will need to be covered to prevent contamination from nasal secretions, subsequent infection, and rupture of the vessel wall. In these cases, coverage of the vessels should be planned with the techniques mentioned earlier in the chapter.

However, if preoperative imaging shows that safe dissection of the tumor from the ICA may not be possible, ICA scarification should be considered if the area of ICA involvement is the only area limiting a complete R0 resection. NPC patients with prior radiation to the neck frequently develop stenosis of the carotid artery, and ligation of one ICA may compromise the cerebral circulation. In order to preserve the cerebral circulation after resection of one ICA, an alternative route of revascularization should be considered. Prior to resection of the recurrent NPC and scarification of the ICA, a bypass grafting of the ipsilateral ICA circulation can be performed one to two weeks beforehand. A vascular graft using either the radial artery or long saphenous vein is used to perform an external carotid to internal carotid bypass. After performing a temporal craniotomy, the vascular graft is anastomosed to the ICA just proximal to the branching of the ophthalmic artery intracranially and the other end to the external carotid artery, distal to the facial artery. The vascular graft is placed in a subcutaneous tunnel behind the auricle. The ICA is ligated proximally at the level of carotid bifurcation and distally just proximal to the vascular graft. After recovery from the vascular bypass surgery, usually one to two weeks later, primary resection of the recurrent NPC can be performed, usually the craniofacial approach.

The recurrent cancer can then be resected with the ICA enbloc, including the part of skull base and dura involved by tumor. The defect can then be covered with a pedicle temporalis muscle flap or free vastus lateralis muscle flap described previously (Chan, Wong, Chan, & Wei, 2016).

Oncological results of salvage nasopharyngectomy

There has not been any randomized controlled trials comparing various modalities in the salvage of local failures of nasopharyngeal cancer and comparisons can only be done on case series, with the inherent limitations from confounding variables. A large review in 2005 on salvage of local failures showed that only patients with rT1−rT2 disease showed survival benefit with salvage treatment. With advances in surgical and radiotherapy techniques, selected patients with more advance disease also have prolonged survival after treatment.

Wei et al. concluded their 20 years' experience in managing NPC local failure with the maxillary swing technique had showed the 5-year local control was 74% and 5-year overall survival of 56%. A positive margin on frozen section and tumor size >1.5 cm were factors predicting poor local control and survival (Wei, Chan, Ng, & Ho, 2011). Hao et al.'s series of 53 patients with a mix of early and advance recurrent disease showed a 5-year local control rate of 53.6% and 5-year overall survival rate of 48.7% (Hao, Tsang, & Chang, 2002).

On the other hand, treatment results with conventional two-dimensional external beam reirradiation were much poorer. Lee et al. reported a 5-year local control rate of 23% with reirradiation with external beam with a small percentage with additional intracavity brachytherapy. The complication rate in the cohort was 47%. Teo et al. reported an even more dismal result with his cohort of 103 patients showing a 5-year local control of 15.2% and 5-year overall survival of 7.6%. For selected small local recurrence amenable to local intracavity reirradiation with iridium wire afterload brachytherapy, excellent results can be achieved with reported 5-year local control of 85% and 5-year overall survival of 47% (Law et al., 2002).

A large retrospective cohort comparing intensity-modulated radiation therapy (IMRT) versus endoscopic nasopharyngectomy showed that endoscopic nasopharyngectomy was more effective and less morbid than reirradiation with IMRT for treating NPC local failures (You et al., 2015). Whether these advantages would be still valid in open surgery, which typically addresses larger tumors, unfortunately is not known.

Surgery for salvaging persistent or recurrent disease in the neck

Since the introduction of concurrent chemotherapy in addition to radiotherapy in treating NPC, nodal recurrence or persistence has been a rare entity.

A review from Hong Kong in 2005 showed that isolated nodal failure was only 6% (Lee et al., 2005). Salvage treatment of nodal failures included reirradiation, surgery, or chemotherapy. Chemotherapy is not a curative intent treatment but can offer significant palliation and survival benefits in patients who are not able to tolerate surgery or reirradiation. Reirradiation for nodal failures in NPC has a poor efficacy of less than 20% 5-year survival (Sham & Choy, 1991). Moreover, reirradiation has significant toxicities to the structure of the neck, in patients that have already received high-dose radiation during the primary treatment. Surgery, in the form of a neck dissection, is usually tolerable and can offer more survival benefits.

Assessment of nodal failures of nasopharyngeal carcinoma for surgical salvage

Nodal persistent disease and nodal recurrence in NPC can be difficult to diagnose. Postradiation fibrosis makes the enlarged disease bearing lymph nodes difficult to be detected by palpation alone. Lymph nodes previously harboring metastatic cancer often retain the sonographic features of metastatic neck lymph nodes after radiation treatment, even if no disease is present. Fine needle aspiration also yields less cells for assessment and radiation and also induces atypia changes in the cells. Not uncommonly, confirmation of nodal failures relies on increasing size of the suspicious lymph nodes on serial imaging. Positron emission tomography–computer tomography (PET–CT) has emerged as a more sensitive method in detecting nodal failures. As cytological confirmation of nodal failures may be difficult, clinicians may need to make a diagnosis of nodal failures and embark on surgical salvage based on imaging findings. Therefore patients should be warned of the possibility of absence of viable cancer cells in the surgical specimen.

Significant fibrosis of the neck tissue can occur after radiotherapy and can cause relative fixation of the disease lymph node. Alternatively, these diseased lymph nodes often have extracapsular extension of the metastatic cancer, sometimes causing the lymph node to adhere to critical structures like the carotid artery or vagus nerve. Therefore it is recommended to perform a contrast computed tomography scan or magnetic resonance imaging scan to assess the anatomical relationship of the metastatic lymph node with these vital structures in planning the surgery. If the invasion of metastatic disease to adjacent structures likely will cause a R1 or even R2 resection, the feasibility of postoperative adjuvant radiotherapy in the form of brachytherapy should be assessed. The use of brachytherapy as postoperative adjuvant radiotherapy will be discussed later in the chapter.

Neck dissection for salvaging nodal failures in nasopharyngeal carcinoma

One must remember that the neck tissue in NPC patients with nodal failures has received high-dose radiation. In many cases, the neck node harboring the metastatic disease has received an additional radiation boost hoping to control the disease without resorting to surgical salvage. Therefore the traditional neck incision for neck dissection with a three-point junction should be avoided to prevent nonhealing of the skin wound. Instead, two parallel incisions devised by MacFee should be employed (MacFee, 1960). To avoid necrosis to the skin flap, the upper and lower neck incisions of the MacFee incisions should be separated by at least 7 cm or four finger breaths. Areas of skin suspicious of involvement by cancer should be resected with underlying metastatic lymph nodes and the skin defect repaired with a pectoralis major myocutaenous flap or a deltopectorial flap. Traditionally, a radical neck dissection was recommended for salvaging nodal failures in NPC. This was based on the fact that nodal failures in NPC usually involved multiple nodal levels and frequently had extracapsular spread. Extensive fibrosis of the neck tissue also made conserving it more difficult to preserve structures like the IJV and sternocledomastoid (SCM) muscle (Wei et al., 1992; Wei & Mok, 2007). Recently, this concept has been challenged (Khafif, Ferlito, Takes, & Thomas Robbins, 2010). Although NPC nodal failures frequently involve multiple nodal levels, it almost always spares one or two neck nodal levels. Improvement in imaging technology makes accurate preoperative identification of the involved lymph nodes possible and therefore uninvolved levels can be safely spared. Also, newer radiation techniques have reduced the radiation dose to adjacent normal structures and less fibrosis develops, making preservation of structures like IJV and SCM muscle feasible in selected cases.

Reirradiation of the neck with brachytherapy after neck dissection

If a R0 resection was not able to be achieved with conventional neck dissection, the area with residual microscopic or macroscopic cancer should be marked with surgical clips. A plastic tube can then be placed over the region 1 cm apart and secured with sutures to the underlying neck muscles. The overlying skin can be resected and the defect covered with a pectoralis myocutaneous flap to avoid radiation necrosis of the skin. After the operation, radioactive iridium wires are then delivered into plastic tubes to provide local brachytherapy radiation to treat the residual cancer (Figs. 9.10–9.12). With such surgery with reirradiation, the local control rate of the nodal disease is 66% and the 5-year survival rate is 38% (Wei et al., 2001).

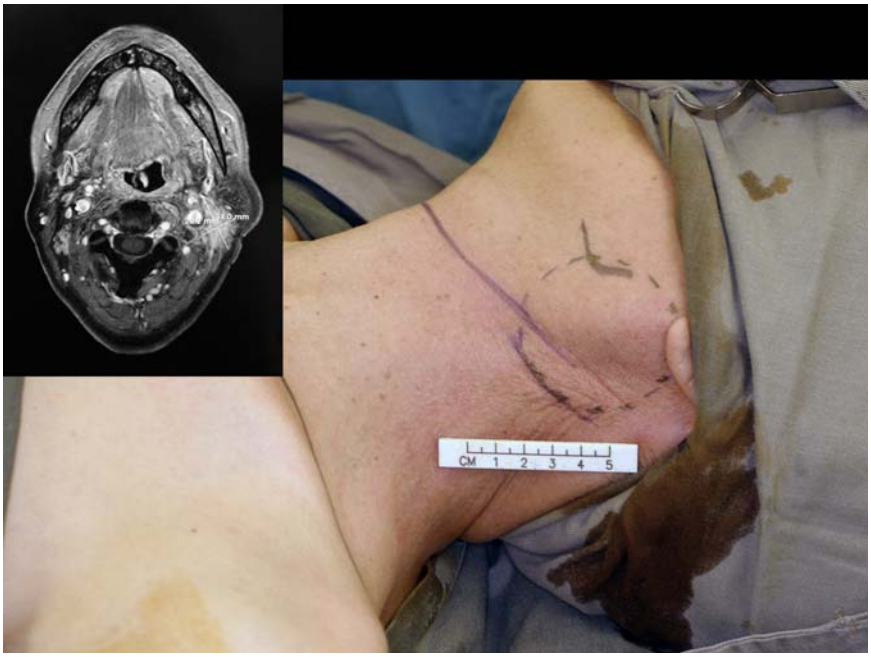

Figure 9.10
Left extended radical neck dissection for nodal recurrence of nasopharyngeal carcinoma. MRI scan showed extensive extracapsular extension of the recurrent disease partially encasing the carotid artery and also involving the overlying subcutaneous tissue. The area of subcutaneous soft tissue infiltration was resected enbloc with the neck dissection.

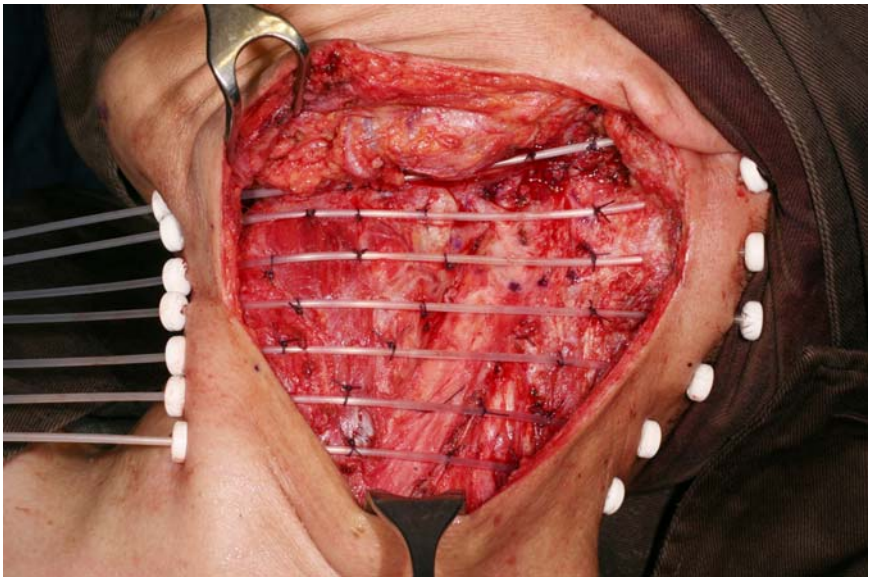

Figure 9.11
Placement of hollow plastic tubes 1 cm part on the neck soft tissue after completion of the extended radical neck dissection. The tubes would be afterloaded with radioactive iridium wires for brachytherapy treatment.

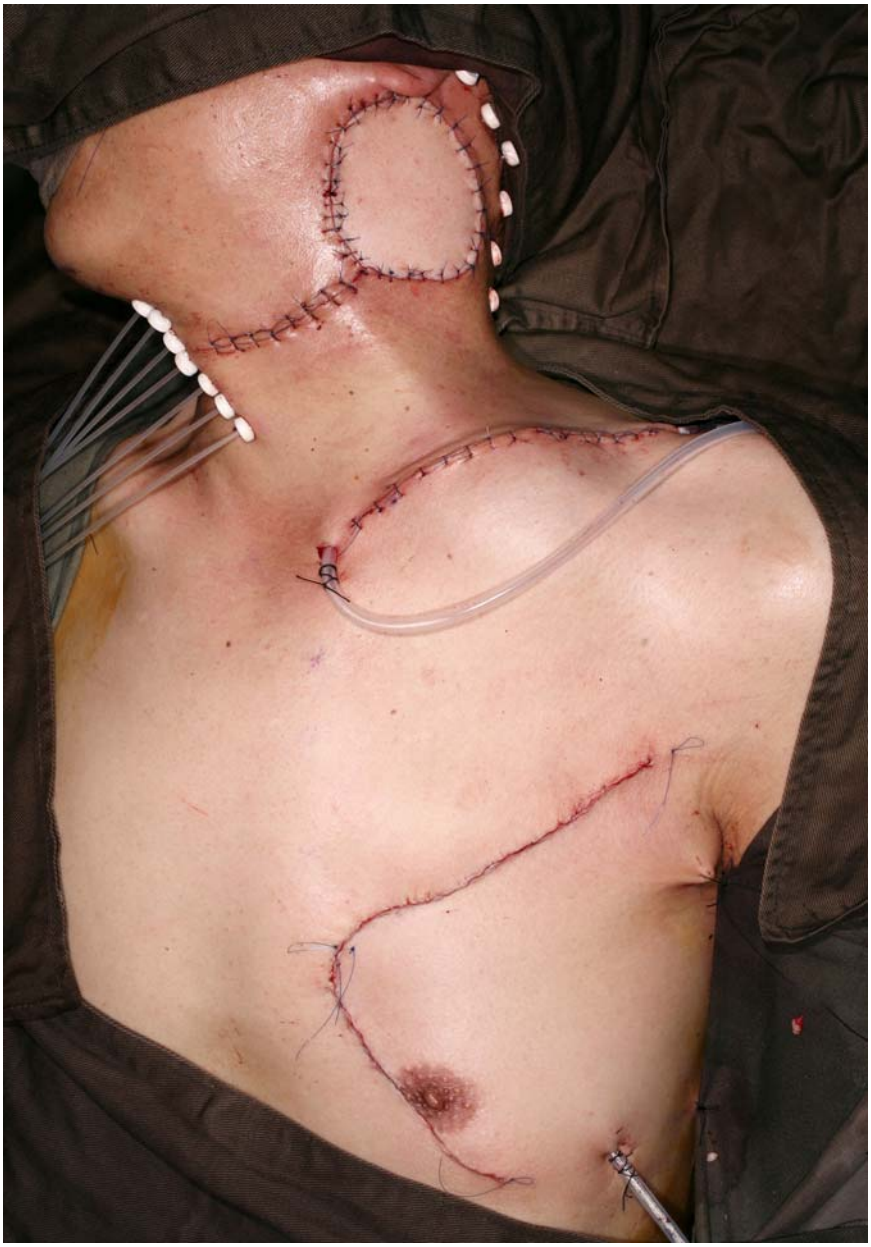

Figure 9.12
Clinical photo of the wounds after completion of the extended radical neck dissection and placement of brachytherapy tubes. A pectoralis major myocutaneous flap was used to close the skin defect.

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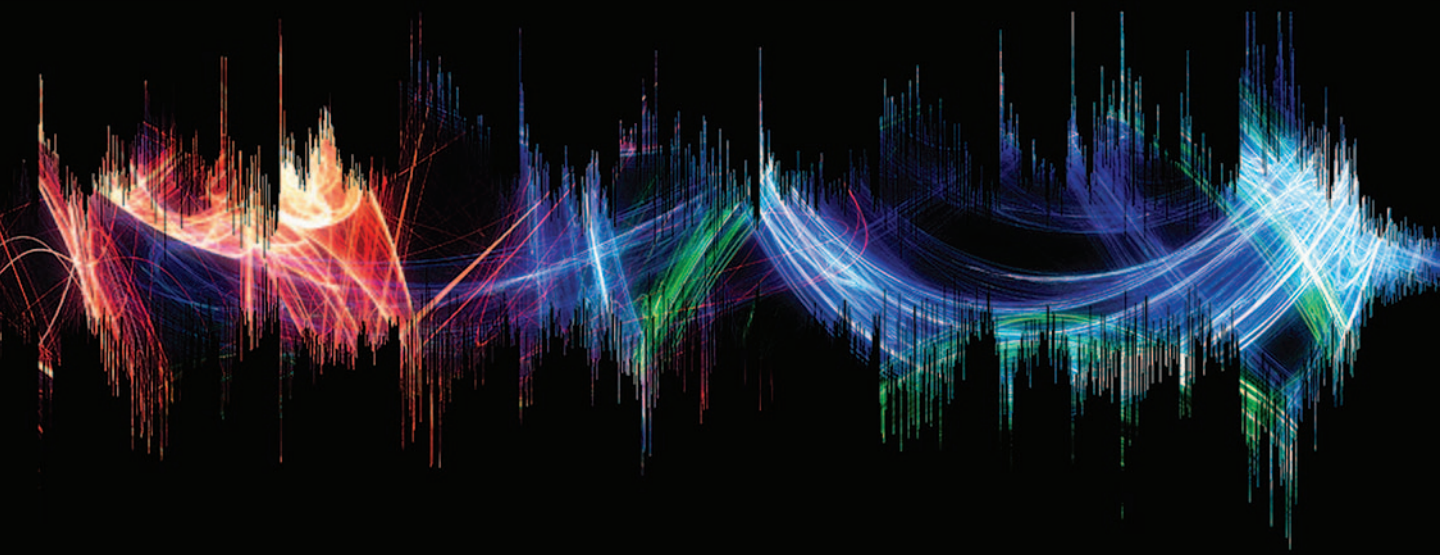

10

Radiation therapy and chemotherapy in early and advanced nasopharyngeal cancer

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Principles of radiation therapy

In the definitive treatment of nasopharyngeal carcinoma, radiation therapy (RT) has always been the mainstay of management strategy. The reasons are mainly because of its radiosensitive nature as a tumor, and the morbidity of surgery with regard to the anatomic location.

In addition, the use of radiation allows the management of both the primary and the draining lymph nodes, especially the retropharyngeal lymph nodes that can be challenging to be dealt with surgically. More importantly, over the years, the advances in radiation technology have enabled the clinical outcomes to improve significantly especially with regard to the toxicity profiles and improved local control.

The mechanism of RT in oncology has been well studied. Ionizing radiation imparts energy to tissues, releasing free radicals that causes DNA breaks. While some cells die an early death through apoptosis as a direct result of radiation, most do not die until mitosis occurs. As most nasopharyngeal cancers are radio responsive and have a short cellular doubling time of 3–5 days, tumors do show shrinkage within a range of a few days to weeks. The unit of radiation is Gray (Gy).

Radiation dosimetry

As with the radiation treatment of other squamous cell carcinomas (SCCs) of the head and neck, a dose of at approximately 70 Gy is delivered as definitive

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treatment. In NPC, this is typically delivered via external beam RT to the primary tumor and involved lymph nodes, 5 days per week over 6–7 weeks (Altun et al., 1995; Wei & Sham, 2005). However, unlike other head and neck sites, there is usually a high-risk clinical target volume (CTV) that is treated to 59.4 Gy. This would include the first echelon of draining lymph nodes namely the retropharyngeal lymph nodes and the cervical level II lymph nodes and also usually one additional echelon of cervical lymph node level from the involved nodal level. The low-risk CTV is treated to 54 Gy and includes the other uninvolved neck levels. For node-negative NPC, there is some evidence to just cover the upper neck and omit level IV and supraclavicular nodes. Nonetheless, as the morbidity of level IV lymph node irradiation is low, this approach has not yet gained widespread acceptance (Lee, Sze, & Ng, 2013).

There is a slight variation in the dose fractionation of radiotherapy regimes in different regions.

- United States, Singapore: 70/63/59.4/54 Gy in 33# over 6.5 weeks;
- Canada, Hong Kong: 70/63/56 Gy in 35# over 7 weeks.

Typically three dose levels in a concomitant boost regimen will be as follows:

- Gross disease planning target volume 70 Gy (PTV70): 70 Gy over 33 days (2.12 Gy per day);
- High risk subclinical planning target volume 59.4 Gy (PTV59.4): 59.4 Gy over 33 days (1.8 Gy per day);
- Lower risk subclinical planning target volume 54 Gy (PTV54): 54 Gy over 33 days (1.64 Gy per day).

Accelerated radiation therapy

The standard dose per fraction for conventional fractionation varies from 2 to 2.12 Gy, given as five daily fractions per week and is usually used together with concurrent chemotherapy. The concomitant boost of using 2.12 Gy is widely used in Radiation Therapy Oncology Group (RTOG) trials. In a bid to improve local control, Lee et al. studied the outcomes of accelerated RT in advanced NPC, delivering six fractions per week with concurrent chemotherapy in a randomized setting. Accelerated fractionation with chemotherapy resulted in a significantly higher 5-year failure-free rate of 88% compared with accelerated fractionation without chemotherapy (56%) or conventional fractionation with (65%) or without chemotherapy (63%). However, this did not translate to a statistically significant survival benefit (85% vs 66%, $p = 0.058$) (Lee, Tung, Chan, et al., 2011).

Further trials have also failed to prove a survival benefit despite having a larger sample size. As expected, accelerated fractionation is accompanied by increased toxicity in the form of mucositis and dehydration (Lee, Ngan, et al., 2015). Two other groups also confirmed the additional toxicity in separate trials

(Daoud et al., 2007). Teo et al. found accelerated fractionation to be associated with significantly increased radiation-induced neurological damage to temporal lobe, cranial nerves, optic nerve, and brainstem (Teo, Leung, Chan, et al., 2000). Dose per fraction to the temporal lobes should be kept to less than 2.12 Gy per day so as not to cause significant temporal lobe injury.

Image registration in radiotherapy planning

Magnetic resonance imaging (MRI) fusion is considered standard in most cancer centers treating nasopharyngeal cancer. MRI-based targets were 74% larger, more irregularly shaped, and did not always include the computed tomography (CT) targets (Emami, Sethi, & Petruzzelli, 2003). When CT-based plans were compared with those based on CT + MRI targets, 14% underdosing to target volume was found, and doses to the organs at risk (OAR) were significantly suboptimal. An approximately 20% dose reduction to OARs could be achieved using targets delineated based on CT and MRI fusion. MRI also allows superior assessment of skull base and soft tissue compared to CT (Abdel Razek & Kamal, 2013). Other potential benefits of MRI fusion include upstaging of disease with MRI, which may have a significant impact on the management and better differentiation of primary gross target volume (GTVp) from retropharyngeal lymph node gross target volume (GTVn). Additionally, MRI fusion also aids in OAR delineation.

Different MRI sequences are useful for delineating different target volumes and OAR, and hence different MRI sequences should be fused to optimize delineation. T2 fast spin echo is a sequence useful for delineating parapharyngeal space, sinus, retropharyngeal lymph node involvement. The T1 noncontrast sequence is useful for assessing clival and skull base involvement. T1 with contrast is a sequence ideal for delineating perineural invasion and intracranial involvement

Target volume and organs at risk delineation

The standard steps for delineation are as follows:

1. Review diagnostic images and radiology reports to determine extent of tumor involvement.
2. Review anatomy and lymph node atlas.
3. Contour OAR first, windowing tailored to respective OAR.
4. Start with GTV followed by clinical target volume 70 Gy (CTV70), then CTV59.4 and CTV54.
5. Expand CTVs. to respective planning target volume (PTVs.).

Organs at risk delineation

The neurological structures usually have the most variation in the contouring. As such, their anatomical borders on CT are described here.

Temporal lobes

Inferiorly: bounded by bony cranium boundaries;

Superiorly: extends to the effacement of the sylvan fissure;

Posteriorly: imaginary junction between the anterior 2/3 to posterior 1/3 of the brain;

Anteriorly: bounded by the bony cranium;

Medially: bounded inferiorly by the bony cranium, but bounder must include medial temporal including the hippocampus and move laterally thereafter to exclude basal ganglia;

Laterally: bounded by the bony cranium.

Brainstem

Inferiorly: level of C2 odontoid process

Superiorly: one CT slice below level of cerebral peduncles or bifurcation of basilar artery into posterior communicating artery

Anteriorly: up to the basilar artery

Optic apparatus

Globe and lens as visible on scans

Optic Chiasm and Optic Nerves: usually clearly identifiable; it is important to ensure it is contoured all the way to the posterior eye and optic foramen where visible.

Target volume delineation

Target volumes are described as per ICRU 50

GTV includes all gross disease including the primary tumor and all enlarged lymph nodes with a short axis more than 1 cm. The determination of GTV depends on clinical examination, CT, MRI, FDG positron emission tomography–computer tomography (PET–CT), or any other imaging used to stage the NPC.

CTV includes the gross tumor and the subclinical microscopic disease extension from the gross tumor. CTV70 includes GTV70 with a 3 mm margin. Some centers include the entire uninvolved nasopharyngeal mucosa. 1 mm margin is used if proximity to critical structures present an issue to allow for the full 3 mm expansion.

PTV is considered a 3D envelope in which gross tumor and any possible microscopic extension resides. It accounts for the effect of internal organ motion, patient movement, and setup errors. PTV70 includes CTV70 with a 3–5 mm

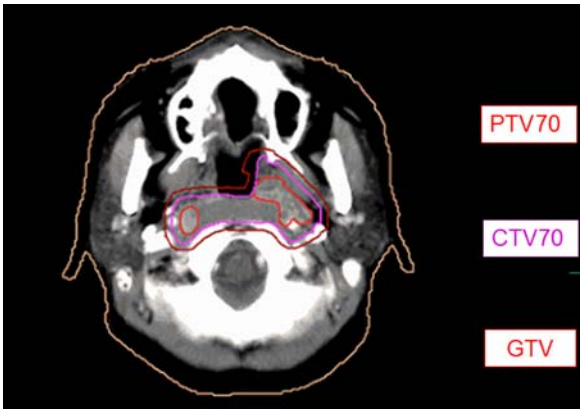

Figure 10.1 Representative computed tomography (CT) slice showing gross target volume (GTV), CTV70, and PTV70 in a patient with T2N1 nasopharyngeal cancer (NPC).

margin depending on setup and type of image guided radiotherapy (IGRT) utilized (Fig. 10.1). Again, a 1 mm margin is allowed if proximity to critical structures present an issue.

PTV63 is used for prominent lymph nodes with a short axis less than 1 cm; it is also a dose used for lymph nodes in close proximity to the brachial plexus.

CTV59.4 includes CTV70 with at least 5 mm margin. It is comprised of CTV59.4 Primary and CTV59.4 Neck.

For CTV59.4 Primary; this includes entire nasopharynx mucosa, clivus, skull base (foramen ovale), pterygopalatine fossae, parapharyngeal space, sphenoid sinus, posterior 1 cm of bilateral maxillary sinuses, and posterior 1 cm of the nasal cavity (Fig. 10.2). For advanced T3–T4 lesions, cavernous sinus and Meckel's cave will be included.

For CTV59.4 Neck; this includes retropharyngeal nodes and cervical lymph node levels IB to V. There is provision to consider omitting level IB for node-negative neck.

PTV59.4 includes CTV59.4 with 3–5 mm, depending on setup and type of IGRT utilized. Again, a 1 mm margin is allowed if proximity to critical structures present an issue.

The CTV54 and PTV54 is at physician discretion, and is usually used at the low anterior neck. It should start 2 cm distal of any GTV.

For CTV to PTV expansion, there is a role of differential expansion. Cheo et al. measured the magnitude of setup errors before and after cone beam computed tomography (CBCT) in 36 NPC patients and found that an appropriate differential expansion of PTV at clivus, C4, and C7 would be 2.33, 4.33, and 6.52 mm, respectively. In practical terms, this means that for most early stage NPC, the CTV70 to PTV70 expansion of at least 2.33 mm will be sufficient to overcome setup errors using CBCT as IGRT (Cheo, Loh, Chen, Lee, & Tham, 2015).

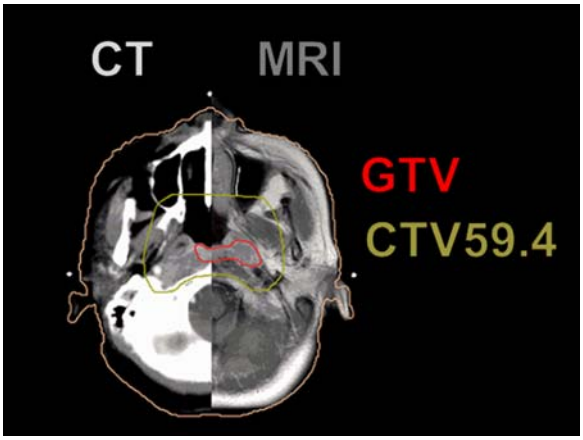

Figure 10.2 Representative computed tomography (CT) slice showing gross target volume (GTV) and CTV59.4 in a patient with T2N1 nasopharyngeal cancer (NPC).

Intensity modulated radiotherapy plan approval

The order of priorities in plan evaluation are as follows:

1. Critical normal structure constraints;
2. Dose specifications to target volumes;
3. Planning goals: Salivary glands;
4. Planning goals: Other normal structures.

The key principle in plan approval is to prioritize the neurological OAR over tumor coverage.

Organ preservation protocol

Tables 10.1–10.4 summarizes the plan approval steps and the details of each step. Table 10.1 summarizes the critical normal structure constraints for plan approval. Table 10.2 summarizes the planning goals for the target volumes. Table 10.3 in turn details the planning goals for salivary glands. Finally, Table 10.4 summarizes the planning goals for all other normal structures (Fig. 10.3).

Types of radiation therapy specifically including intensity modulated radiotherapy in nasopharyngeal cancer

Intensity-modulated radiotherapy

RT techniques have evolved from two-dimensional radiotherapy (2DRT) to three-dimensional conformal radiotherapy to intensity modulated radiotherapy

Table 10.1 Critical normal structure planning goals.

Structure	True structure constraint	Planning organ at risk volume (PRV) constraint (≥1 mm larger than true structure)
Brainstem	Maximum dose of 54 Gy	No more than 1% of PRV to exceed 60 Gy
Spinal cord	Maximum dose of 45 Gy	No more than 1% to exceed 50 Gy
Optic nerves and optic chiasm	Maximum dose of 50 Gy	Maximum dose of 54 Gy
Temporomandibular joint	Maximum dose of 70 Gy	No more than 1cc of PRV to exceed 75 Gy
Brachial plexus	Maximum dose of 66 Gy	

Table 10.2 Target volumes planning goals.

PTV 70/63/59.4/54	Major criteria	Minor criteria
Prescription dose	≥95% of PTV volume is covered by the prescribed dose	
Percentage of PTV receiving >110%	≤20% of PTV70	≤40% of PTV70
Percentage of PTV receiving ≥115%	≤5%	≤20%
PTV receiving <93% of the prescribed dose	<1%	<3%
Mean dose	≤74 Gy	≤74 Gy

PTV, Planning target volume.

Table 10.3 Salivary gland planning goals.

Structure	True structure constraint	Planning organ at risk volume (PRV) constraint (≥1 mm larger)
Parotid	Mean dose <26 Gy (at least in one gland)	V30Gy <50% (at least in one gland)

Table 10.4 Other normal structure planning goals.

Structure	Constraints
Oral cavity [excluding planning target volume (PTVs.)]	Mean dose less than 40 Gy
Each cochlea	No more than 5% receives 55 Gy or more
Eyes	Maximum dose less than 50 Gy
Lens	Maximum dose less than 25 Gy
Glottic larynx	Mean dose less than 45 Gy
Constrictors	Mean dose less than 45 Gy
Temporal lobe	Maximum dose of 60 Gy failing which no more than 1% to exceed 65 Gy

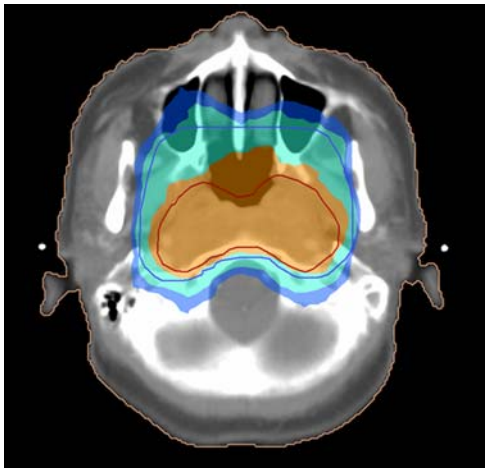

Figure 10.3 Representative slice dosimetry of the same patient with T2N1 nasopharyngeal cancer (NPC). PTV 70: *red solid line*; PTV 59.4: *blue solid line*; *orange colorwash*: 66.46 Gy dose cloud representing 95% prescribed dose coverage; *green colorwash*: 56.43 Gy dose cloud representing 95% prescribed dose coverage; *blue colorwash*: 51.3 Gy dose cloud representing 95% prescribed dose coverage.

(IMRT). Even from early days, 2DRT yielded excellent control rates in NPC albeit with significant toxicity. With the improvement in technology, not only has tumor control improved, toxicity has also become more tolerable. This has translated into a superior therapeutic ratio. Today, IMRT is the standard of care in most cancer centers in NPC treatment and Fig. 10.3 represents a typical IMRT generated radiotherapy plan.

A head-to-head phase 3 NPC trial compared 2DRT with IMRT involving 616 NPC patients. The results favored IMRT, demonstrating improved 5-year OS (79.6% vs 67.1%) and decreased toxicity with IMRT over 2DRT (Peng et al., 2012). Similar results were seen in a large single institutional experience of more than 1500 patients, demonstrating IMRT improving OS (Lee et al., 2014).

The key benefit of IMRT has been the ability to preserve parotid function. This was demonstrated in two randomized studies that showed hugely superior recovery in at least 25% of stimulated whole saliva flow in NPC patients receiving IMRT (83.3% vs 9.5%). There was also an improvement over time with xerostomia with IMRT vs conventional RT (Kam et al., 2007; Pow et al., 2006).

With recent advancements in proton and heavy ion therapy, there has been some interest in using intensity modulated proton therapy (IMPT) in head and neck cancer. Most of these studies have been dosimetric comparisons with IMRT. We still need to await long-term clinical data on IMPT on NPC (Taheri-Kadkhoda et al., 2008; Widesott et al., 2008).

Brachytherapy and stereotactic boost

In the few centers where head and neck brachytherapy expertise is available, certain groups have used brachytherapy as a way of delivering a boost dose after external beam RT. This modality has limited utility in early NPC and furthermore requires highly customized applicators and also high dose rate (HDR) Brachytherapy after loading system (Teo, Leung, Lee, & Zee, 2000).

Stereotactic radiotherapy in recurrent disease

More rigid immobilization of patient + IGRT allows a smaller treatment margin, and arc therapy allows for the dose to normal organs like the spinal cord to be kept to minimum. Most trials have utilized SRT as a boost after IMRT in a bid to improve local control. The Stanford series showed excellent 5-year local control (5YLC) rates of 98% and 5-year overall survival (5YOS) of 75%. The majority of the study subjects had locally advanced NPC receiving a 7–15 Gy SRT boost after 66 Gy of external beam radiotherapy (EBRT) (Hara et al., 2008). Similar studies have been conducted in Asia, and there are cases of significant neurological and mucosal toxicity resulting from SRT boost technique in NPC (Chen, Tsai, et al., 2006; Wu et al., 2007; Yau et al., 2004). SRT is now selectively employed at a limited number of centers for small-volume recurrence or palliation. A few selected studies involving stereotactic RT in NPC are listed in Table 10.5.

Role of radiation in early disease

Early-stage NPC refers to stage I disease using the American Joint Committee on Cancer (AJCC) eighth edition tumor, node, and metastasis (TNM) Staging System (Lydiatt et al., 2017). In early-stage NPC, radiotherapy is delivered alone with

Table 10.5 Studies involving stereotactic radiotherapy (SRT) in nasopharyngeal cancer (NPC).

Author/ year	Country	Patient group	Dose	Outcome	Toxicity
Hara (2008)	United States	82 NPC patients	7–15 Gy/1# after external beam radiotherapy (EBRT) 66 Gy	5-year local control (5YLC) 98% 5-year overall survival (5YOS) 75%	Retinopathy (3); temporal lobe necrosis (10)
Chen (2006)	Taiwan	54 NPC patients	12–15 Gy/1# boost after EBRT 68 Gy	3YLC 92% 3YOS 85%	Mortality from bleed (3)
Yau (2004)	China (Hong Kong SAR)	45 NPC patients	15 Gy/3# after EBRT 66 Gy	3YLC 82%	
Wu (2007)	China (Guangzhou)	90 NPC patients	18 Gy/3# after EBRT	3YLC 89.4%	Mucosal necrosis (6); brain stem/temporal lobe necrosis (9), and fatal hemorrhage (2)

excellent locoregional control, with 5YOS rates exceeding 90% (Lee et al., 2005). The disease is very limited in this group of patients, with the primary tumor confined to the nasopharynx, or adjacent oropharynx and/or nasal cavity but without posterolateral infiltration. There is also no involvement of lymph nodes or DM.

While this group of patients has a good prognosis, they belong to a minority. Due to the deep anatomical location of NPC, most patients present with cervical lymph node metastasis as the chief presenting symptom. Even from the 2D era of radiotherapy, control rates of early stage NPC have been very favorable. What has progressed significantly over the years is the reduction in toxicity of radiation, especially long-term toxicity. Intensity modulated radiation therapy (IMRT) has allowed xerostomia rates to drop significantly. With the advent of IMRT, practice has also shifted toward three dose levels, with elective irradiation further divided into high-risk and low-risk levels. The low-risk CTV has by far and large been at the discretion of the treating radiation oncologist with little consensus.

Management of the neck

The management of the neck has also been evolving in the management of NPC. It is a known fact that NPC has tremendous propensity for metastasis to bilateral neck nodes. In view of this, all patients generally receive bilateral neck irradiation, even for clinically node negative necks on staging (Hsu & Tu, 1983; Lee et al., 1992).

In keeping with the as low as reasonably achievable radiation safety principle, various groups have sought to investigate the role of omitting RT in the lower neck to further reduce toxicity. The worry was that there is always a risk of occult neck metastasis even in early stage NPC where the neck is staged clinically to be uninvolved. Ho et al. showed in their meta-analysis (MA) that the first echelon draining lymph nodes in NPC were the retropharyngeal lymph nodes and level II cervical lymph nodes (Ho, Tham, Earnest, Lee, & Lu, 2012). And the second echelon draining lymph nodes were cervical lymph nodes level III and V. The third echelon lymph nodes included level IV and supraclavicular fossa lymph nodes. More importantly the MA concluded that the risk of skip lymph node metastasis was only 7%. This meant that if the first echelon lymph nodes were not involved, making the second echelon and third echelon lymph node risk less than 7%. This risk is presumably lower in early NPC. A few groups have since investigated this approach of excluding radiotherapy to the lower neck in node-negative NPC patients. Li et al. conducted a randomized controlled trial of 301 node-negative NPC patients, with a standard arm irradiating whole neck versus an experimental arm irradiating upper neck only (level II and III). Rather encouragingly, there were no relapses seen in the lower neck for both arms of the trial (Li et al., 2013). In contrast, bilateral neck should be irradiated if there is any lymph node involvement in NPC.

Role of chemotherapy and radiation in intermediate stage nasopharyngeal cancer

Intermediate stage NPC refers to patients with stage II disease. This includes patients who have T2 staged primary tumor involvement with extension to the parapharyngeal space and/or adjacent soft tissue involvement (medial pterygoid, lateral pterygoid, prevertebral muscles). In addition, this includes patients staged as N1 lymph node involvement. The N1 stage is defined as patients with unilateral metastasis in cervical lymph node(s) and/or unilateral or bilateral metastasis in retropharyngeal lymph node(s), 6 cm or smaller in greatest dimension, above the caudal border of cricoid cartilage (no supraclavicular lymph node involvement).

This group of patients have had the most noticeable shift in the change of management with the addition of concurrent chemo that has allowed for superior survival benefit (Chen et al., 2011). Chen et al. conducted a phase III trial, randomizing stage II NPC patients (according to Chinese staging for NPC) to radiation alone versus chemoradiation. The concurrent chemotherapy used was weekly Cisplatin at a dose of 30 mg/m^2 instead of the usual 40 mg/m^2 used in locally advanced NPC. The caveat was that although all the included subjects had parapharyngeal involvement, a small proportion of the patients (13%) would be classified as stage III NPC based on the latest AJCC staging. The study concluded that concurrent Cisplatin improved 5YOS from 85.8% to 94.5%, fueled by a statistically significant improvement in DM-free survival (94.8% vs 83.9%). Of importance was the fact that the number of chemotherapy cycles delivered was the single independent factor associated with survival, progression, and distant control on multivariate analysis.

As expected, the concurrent Cisplatin was associated with greater acute toxicity. Concurrent chemotherapy was associated with increased Grade 3 or 4 leukopenia/neutropenia (12% vs 0%), nausea/vomiting (8.6% vs 0%), and mucositis (46% vs 33%). However, there was no such trend observed with regard to late toxicity. The caveat in this study is that the radiotherapy was delivered using 2DRT, which is not the standard of care today. Also, the patients were staged using conventional imaging without the benefit of PET–CT scan. Hence, the benefit of concurrent chemotherapy in intermediate stage NPC in the era of IMRT and PET–CT staging is an unanswered question.

The rationale for concurrent chemotherapy in intermediate stage NPC is twofold. Firstly, some of the older clinical trials investigating the role of concurrent chemotherapy in locally advanced NPC included a small number of currently staged intermediate stage NPC patients. This has led some to postulate the benefit of concurrent chemotherapy in intermediate stage NPC. Secondly, the other rationale for concurrent chemotherapy in intermediate stage NPC stems

from the higher distant relapse rate seen in this group of patients (Chan et al., 2012). Cheng et al. reviewed 149 stage II and III patients and found that despite both groups of patients receiving concurrent chemotherapy with radiation, there was very little difference in outcomes for stage II and III NPC patients, with a recurrence rate of approximately 18% (Cheng et al., 2000). There are many possible hypotheses for this. One hypothesis is that the stage II patients are understaged previously with the conventional CT thorax, liver, and bone scan. With the adoption of MRI of the postnasal space and whole-body PET–CT scan as the standard of care for staging NPC patients, the staging is now much more accurate and would likely have upstaged certain patients included in the older trials. Around this same time, there has also been adoption of chemoradiation for stage II NPC patients since 2011. We expect to see better outcomes for stage II NPC patients with more accurate staging and more aggressive management with chemoradiation.

The addition of chemotherapy in locally advanced NPC was a subject of an individual patient MA by Baujat et al. of 1753 patients. The addition of chemotherapy to radiotherapy provided a superior 5YOS of 6% (Baujat et al., 2006). The significant benefit to OS was only seen in concurrent chemotherapy, but not with induction or adjuvant chemotherapy. This was a study predominantly looking at the benefit of chemotherapy in locally advanced NPC and hence it cannot be applied to intermediate stage NPC patients. Another group performed a pooled analysis of two Phase III trials that addressed the benefit of induction chemotherapy (IC) in stage I–II NPC patients (Chua et al., 2006). However, this was a negative trial with the exception of the subgroup with T1-2N0-1 disease exhibiting significant improvement in OS of 79% versus 67% with the addition of IC. Song et al. conducted a retrospective study trying to answer the same question and concluded that neoadjuvant chemotherapy had a detrimental effect on local control and OS in stage I–II NPC. (Song et al., 2008) Hence, the evidence for neoadjuvant chemotherapy in NPC is limited compared to concurrent chemotherapy. Chemotherapy studies in early and intermediate stage NPC discussed earlier are summarized in Table 10.6.

Complications of radiation

Acute toxicity

The most prominent acute side effect seen during radiation or within 90 days of commencement of radiation for nasopharyngeal is acute mucositis. This manifests in the form of dysphagia and odynophagia. Most patients also suffer from xerostomia, and this can remain an issue with residual xerostomia persisting long term. Lethargy, radiation dermatitis, and dysgeusia make up the other common acute toxicities.

Table 10.6 Chemotherapy studies in early and intermediate stage nasopharyngeal cancer (NPC).

Author/ year	Patient numbers	Chemotherapy used	Chemotherapy sequence	Outcome [concurrent/ neoadjuvant chemo versus radiotherapy (RT) alone]	Toxicity
Chua (2006)	208 Stage I–II NPC patient	2–3 cycles of Cisplatin/Epirubicin or Cisplatin/ Bleomycin/5FU	Neoadjuvant	5-year local control (5YLC) 79 versus 73% 5YOS 79% versus 67%	Not reported
Cheng (2000)	44 Stage I–II NPC patients	Week 1 and 6 Cisplatin/5FU	Concurrent and adjuvant	3YLC 100% versus 91.7% 3YOS 68.5% versus 15.6%	Acute Grade 3 (G3) toxicity 68 versus 15.6%
Chen (2011)	230 Stage I–II NPC patients	Weekly Cisplatin at 30 mg/m^2	Concurrent	5YLC 94.8% versus 83.9% 5YOS 94.5% versus 85.8%	Acute G3 toxicity 63.8 versus 40.4%
Song (2008)	60 Stage I–II NPC patients	Three cycles of Cisplatin/5FU	Neoadjuvant	5YLC 77% versus 84% 5YOS 79% versus 84%	Not reported

Late toxicity

A wide range of other serious, late treatment-related complications can be seen after RT or concurrent chemoradiation (CRT) (Yeh, Tang, Lui, Huang, & Huang, 2005).

Damage to the temporal lobe causes significant deficits to the patient's cognition (Hsiao et al., 2010). Hsiao et al. demonstrated a deterioration of the NPC patient's short-term memory and language ability postradiation with the extent of damage related to the radiation dose to the temporal lobe. The more feared consequences of temporal lobe necrosis with its accompanied memory loss and complex partial seizures occur in up to 3% of patients. Again, this is more likely to happen in the face of higher radiation dose or accelerated fractionation. This manifests as a hypodense area in the temporal lobes on imaging (Lee et al., 2002).

In more advanced NPC, the dose to the pituitary gland is substantial especially when the sphenoid sinus is involved by tumor. This causes a higher risk of pituitary dysfunction. Hypothyroidism can also develop as a result of neck radiation, with a median onset of 18 months postradiation. Hence, monitoring of thyroid function tests postradiation is important (Siala et al., 2011).

Another uncommon but morbid complication is skull base osteoradionecrosis with bleeding from the internal carotid artery (Lam, Abdullah, Wormald, & Van Hasselt, 2001).

Table 10.7 Organs at risk (OAR) in nasopharyngeal cancer (NPC) and their corresponding toxicity.

OAR	Toxicity
Brainstem	Neuropathy
Spinal cord	Myelopathy
Optic nerve and chiasm	Optic neuropathy
Brachial plexus	Brachial plexopathy
Retina	Blindness
Cochlea	Sensorineural hearing loss
Temporomandibular joint	Joint dysfunction (trismus)
Parotid gland	Xerostomia
Thyroid gland	Thyroiditis
Larynx	Vocal dysfunction and aspiration
Neck muscles	Neck fibrosis
Pituitary gland	Hypopituitarism

A less well-known complication is delayed bulbar palsy. This can develop even as late as 18 years later. Surprisingly it has a incidence of up to 20% of NPC patients who have received radiation. This can manifest in many ways including, hearing impairment, speech impairment, swallowing dysfunction, and weakness of the neck and shoulder muscles (Chew et al., 2001).

Radiation-induced second malignancy is a well-documented albeit rare complication of radiation (Goh, Chong, & Low, 1999). A summary of the OAR and its corresponding toxicity is listed in Table 10.7.

Additional toxicity with concurrent chemotherapy

CRT has an increased risk of acute G3 or higher toxicities (Chen et al., 2008). The addition of chemotherapy carries and extra risks of neuropathy, emesis, neutropenia, nephrotoxicity, and ototoxicity.

The landmark Intergroup trial reported G3 or higher toxicity in 59 out of 74 patients (80%) in the CRT arm compared with 28 out of 68 patients (50%) in the RT-only arm. The most commonly reported acute G3 or higher toxicities in the CRT cohort included stomatitis, leukopenia, and nausea with rates as high as 37%, 29%, and 18%, respectively, compared with 28%, 1%, and 7% in the RT-only group, respectively (Al-Sarraf et al., 1998).

Carboplatin compared with Cisplatin has demonstrated some improvement in treatment-related G3 or higher acute toxicity with respect to anemia (2% vs

14%), nausea and vomiting (9% vs 20%), and weight loss of more than 10% (35% vs 17%) (Chitapanarux et al., 2007). This study will be discussed in detail in the latter part of this chapter.

Sensorineural hearing loss is also a prominent side effect, usually in the high-frequency range, with hearing impairment with rates of up to 42% in CRT patients (Chen, Jackson, et al., 2006).

Another side effect that is rare but more common with concurrent chemotherapy is Lhermitte's syndrome. This syndrome is also popularly known as the barber's chair syndrome, describing a sudden electric shock like sensation passing down the neck and spine often radiating to the limbs. This can be triggered by flexing the head forward, which is a position one adopts in the barber chair, hence the name.

Reirradiation in nasopharyngeal cancer recurrence

Localized recurrences in NPC can be dealt with surgically or with radiotherapy. With good local control, some of these patients can become long-term survivors.

For nonsurgical candidates, the challenge is to deliver a second dose of radiation to a previous irradiated field as majority of recurrences are in field recurrences. The limitation is that of the normal tissue tolerances, and the success of irradiation is often a function of the ability to safely deliver a high enough dose to bring the recurrent tumor under control (Lee et al., 1997). A second challenge is the fact that the recurrent tumor is possibly made up of a radioresistant clone of tumor cells having survived the first round of radiation.

Various approaches have been utilized in a bid to deliver the second dose of irradiation. These include, 3DRT, IMRT, brachytherapy, stereotactic surgery, SRT, and proton therapy.

The above radiation techniques in this setting have been covered in the earlier part of the chapter. None of these techniques are without a significantly high risk of morbidity. Leong et al. conducted a MA of the published data of long-term outcomes of reirradiation IMRT for recurrent NPC. The study included four comparative and eight noncomparative studies from 2005 to 2016. The authors found a 5-year local failure-free survival rate of 85%, 5-year distant failure-free survival of 85%, and 5YOS of 41% for locally recurrent NPC patients who received reirradiation. Of note, the pooled event rate for Grade 5 toxicities was 33%, leading the authors to conclude that while reirradiation can confer long-term local control and survival, one third of the treated patients died of treatment toxicity. Hence, reirradiation is hardly without its disadvantages (Leong et al., 2018).

To compensate for the lower dose used at reirradiation to meet OAR constraints, one strategy has been to employ chemotherapy either sequentially or concurrently (Chua, Sham, & Au, 2005; Poon et al., 2004). Nonetheless, this remains an area of controversy as there are no trials comparing radiation alone versus radiation with chemotherapy in this setting.

Even with long-term survival, up to 20% of patients can expect a significant risk of Grades 3–4 late toxicities of temporal lobe necrosis, multiple cranial nerve palsies, hearing impairment, hypopituitarism, trismus, and osteoradionecrosis (Liu et al., 2014).

Treatment of locally advanced disease

Locally advanced NPC refers to stages III, IVA, and IVB based on the TNM8/AJCC classification.

Stage III: T3 primary tumors (involving the bony structures of the base of skull and/or paranasal sinuses) or bilateral nodes in the neck, none ≥ 6 cm, and also no nodes in the lower neck/supraclavicular fossa.

Stage IVA: T4 primary tumors (tumors with intracranial extension and/or involving cranial nerves, hypopharynx, orbit, extension to the infratemporal fossa/masticator space).

Stage IVB: Patients with N3 disease (any lymph nodes >6 cm in the neck or lymph node in the supraclavicular fossa).

Both RT and chemotherapy have a strong and established role in the management of locally advanced NPC. Though concurrent chemoradiation (CRT) is the standard of care, significant controversies exist on the value of neoadjuvant, adjuvant, or sequential chemotherapy.

Concurrent chemoradiation

Al-Sarraf et al. in the US Intergroup study 0099 showed that adding chemotherapy in the concurrent and adjuvant setting improved progression-free survival (PFS) and OS. A total dose of 70 Gy in 35–39 fractions was used in this trial. The investigational arm received Cisplatin 100 mg/m^2 on days 1, 22, and 43 during radiotherapy, followed by further Cisplatin 80 mg/m^2 on days 1–4, every 4 weeks for three cycles. This very early trial in 1999 showed a 3-year survival of 46% versus 76% ($p < .001$) for chemoradiation group and affirmed the strong role of chemotherapy the treatment of nonmetastatic NPC. However, the true benefits of chemotherapy either in the neoadjuvant, concurrent, or adjuvant setting in isolation were not addressed in this trial (Al-Sarraf et al., 1998).

The updated 2015 Meta-Analysis of Chemotherapy in Nasopharynx Carcinoma Collaborative Group (MAC-NPC) consisting of 4806 patients from nine published trials addressed the issue of timing of the chemotherapy in combination with radiotherapy. Though addition of chemotherapy to radiotherapy is shown to significantly improve OS with absolute benefit of 6.3% at 5 years (95% CI 0.73−0.86, $p < .0001$), the effect is only seen in concurrent chemoradiation or concurrent chemoradiation followed by adjuvant radiotherapy group. There is no statistically significant benefit in the induction or adjuvant chemotherapy-only arms. This MA further strengthened the role of concurrent chemotherapy in the management of locally advanced NPC (Blanchard et al., 2015).

Adjuvant chemotherapy

With the improvement in the radiotherapy techniques such as IMRT and volumetric modulated arc therapy (VMAT) translating into excellent local control rates, distant metastatic disease has become the predominant mode of failure. Li et al. reported on the distant metastatic rates in the IMRT era at Sun Yat Sen Hospital. His team followed up on 576 patients who underwent IMRT for NPC from 2005 and with a median follow-up of 103.6 months. Not surprisingly, at 1, 3, 5, and 8 years, the rates of DM were 5.9%, 12.7%, 14.5%, and 16.4%, respectively. Though this study included NPC patients of all stages, the majority of the patients were stage III and IV. In fact, 68.4% of patients that failed treatment had DM. Of these patients with DM, locoregional control was achieved in 82.4%. The authors concluded that the main treatment failure pattern is DM in NPC treated with IMRT (A. C. Li et al., 2015).

The presence of subclinical micrometastases at the time of diagnosis may explain this finding. Hence, adjuvant chemotherapy has been employed in a few trials with the hope of replicating the results of other tumor sites such as breast in eliminating the subclinical microscopic disease and subsequently improving survival (Lin et al., 2002).

However, the results so far had been equivocal and most centers have not adopted adjuvant chemotherapy after definitive concurrent chemoradiation as a routine practice even in the high-risk group.

In an early multicenter trial by Rossi A et al., which recruited 229 patients from 1979 to 1983, further adjuvant chemotherapy with a regimen containing monthly Vincristine, Cyclophosphamide, and Adriamycin for 6 months after definitive radiotherapy alone did not improve the relapse-free survival or OS. The chemotherapy regimen that was employed is rarely used today in nasopharyngeal carcinoma (Rossi et al., 1988). In another trial of interest, Chi et al. published on aphase III study of adjuvant chemotherapy that followed radical radiotherapy between 1994 until 1999 which showed no OS or relapse free survival benefit (Chi et al., 2002). Both these trials were done at a time

when concurrent chemoradiation was not a standard practice in the definitive treatment of NPC. Also of note, both trials used older radiation techniques when IMRT was still in the infancy. Expectedly, a significant number of patients failed loco-regionally that could have biased the benefits of adjuvant chemotherapy in controlling the subclinical microscopic DM and eventually the OS or disease-free survival (DFS).

The results of these two early trials, the US Intergroup study by A-Sarraf et al. and also the updated 2015 MAC-NPC, in addition to strongly affirming the role of concurrent chemotherapy with radiotherapy, also points to an arguable benefit for adjuvant chemotherapy after radical radiotherapy in isolation. Hence, the focus of subsequent trials has shifted toward employing adjuvant chemotherapy after concurrent chemoradiation with the aim of improving the outcomes specifically in terms of DM disease that may eventually translate into OS benefit.

The largest phase III data so far looking at adjuvant chemotherapy after concurrent chemoradiation in nonmetastatic locally advanced NPC comes from a multicenter trial involving seven institutions in China. In the initial 2012 The Lancet publication, after a median follow-up of 37.8 months, Chen et al. reported no significant difference in terms of failure-free survival between 251 patients and 257 patients randomized into concurrent chemoradiation and concurrent chemoradiation followed by adjuvant chemotherapy, respectively (L. Chen et al., 2012). An updated report from this trial at a median follow-up of 68.4 months still failed to demonstrate failure-free survival rate or survival benefit for the adjuvant chemotherapy group (L. Chen et al., 2017).

Another important study is the "What Is the Best Treatment of Locally Advanced Nasopharyngeal Carcinoma? An Individual Patient Data Network Meta-Analysis" published in JCO in 2017. This MA of 5144 patients in over 20 trials by Ribassin-Majed et al. (2017) compared seven different timings of chemotherapy that included concurrent CRT, IC, adjuvant chemotherapy (AC), and radiotherapy in isolation (RT). The results of this complex MA showed that when compared to RT alone, CRT-AC has the highest survival benefit followed by CRT and IC-CRT, with absolute benefit at 5 years of 12%, 8%, and 6% respectively. Their corresponding HRs are 0.65 (0.56–0.75), 0.77 (0.64–0.92), and 0.81 (0.63–1.04). It is interesting to note that, when HRs of CRT-AC were directly compared to CRT or IC-CRT, it did not show significant difference with respective values of 0.85 (0.68–1.05) and 0.81 (0.61–1.07).

This MA also showed that the three best treatment regimens for locoregional control were IC-RT-AC followed by CRT-AC and RT-AC in order. When CRT-AC and CRT were individually compared, there was a nonsignificant difference trending toward CRT-AC. However, the analysis on the DM rates between the different treatment algorithms reported in this MA may cause doubts on the

true benefits of AC in controlling the distant microscopic disease. The three best treatments for distant control as reported were IC-CRT followed by IC-RT, and CRT-AC in order. Individual comparison between CRT-AC and CRT did not show statistically significant difference, HR (95% CI) of 0.87 (0.61–1.25). CRT was also reported as inferior to IC-CRT though not statistically significant, with an HR (95% CI) of 1.55 (0.94–2.56) (Ribassin-Majed et al., 2017). A summary of the results extracted from this MA is given in Tables 10.8 and 10.9.

This MA did not include in detail the type and quality of radiotherapy delivered at the sites selected to be included into the analysis. The doses and modality of radiotherapy used in the individual trials were also not detailed in this study. Older radiation techniques and skills is the likely explanation for the improved local control with more intense chemotherapy regimen in comparison to CRT alone, which may have eventually translated into survival benefit but not with regard to DM rates. The applicability of this MA in today's era of IMRT/VMAT in addition to the improvement in radiation oncologist skills and experience in treating NPC is surely arguable. The superior local control that is achieved today will mean that any future trials on more intense chemotherapy should be focussed on DM rates as the primary outcome while ensuring the quality of radiotherapy delivered to primary site.

With the evidence being equivocal, the decision on adjuvant chemotherapy following chemoradiation should be carefully discussed with the patient. The toxicities associated with further adjuvant chemotherapy needs to be balanced with the scarcity of strong evidence supporting it. Hence, adjuvant chemotherapy should not be the standard practice currently and its use be limited to clinical trials. Molecular markers or prognostic markers such as plasma EBV DNA levels, the subject of an ongoing NRG oncology trials, are some of the

Table 10.8 Hazard ratio's (HR) of six different timings of chemotherapy compared with radiotherapy (RT) alone.

	OS	PFS	LRC	DMFS
IC–RT	0.92 (0.75–1.12)	0.78 (0.66–0.93)	0.90 (0.70–1.15)	0.54 (0.37–0.79)
IC–CRT	0.81 (0.63–1.04)	0.68 (0.54–0.85)	0.80 (0.57–1.13)	0.44 (0.27–0.71)
CRT	0.77 (0.64–0.92)	0.77 (0.65–0.91)	0.85 (0.62–1.16)	0.68 (0.49–0.94)
CRT–AC	0.65 (0.56–0.75)	0.62 (0.54–0.71)	0.59 (0.46–0.76)	0.59 (0.46–0.77)
RT–AC	0.96 (0.71–1.29)	0.84 (0.63–1.11)	0.71 (0.44–1.17)	0.91 (0.54–1.54)
IC–RT–AC	0.87 (0.58–1.30)	0.83 (0.59–1.17)	0.50 (0.29–0.88)	1.13 (0.62–2.05)

OS, Overall survival; *PFS*, progression-free survival; *LRC*, locoregional control; *DMFS*, distant metastatic-free survival.

Reproduced with permission from ASCOpubs: Ribassin-Majed, L., Marguet, S., Lee, A. W. M., Ng, W. T., Ma, J., Chan, A. T. C., … Blanchard, P. (2017). What is the best treatment of locally advanced nasopharyngeal carcinoma? An individual patient data network meta-analysis. Journal of Clinical Oncology, *35*(5), 498–505.

Table 10.9 Estimated hazard ratios (HRs) of direct comparison of chemoradiation (CRT) with or without induction chemotherapy (IC) or adjuvant chemotherapy (AC).

	OS	PFS	LRC	DMFS
CRT-AC versus CRT	0.85 (0.68–1.06)	0.81 (0.66–0.98)	0.70 (0.48–1.02)	0.87 (0.61–1.25)
CRT-AC versus IC-CRT	0.81 (0.61–1.07)	0.92 (0.71–1.18)	0.74 (0.49–1.12)	1.35 (0.80–2.31)
CRT versus IC-CRT	0.95 (0.72–1.25)	1.13 (0.88–1.46)	1.05 (0.70–1.59)	1.55 (0.94–2.56)

OS, Overall survival; *PFS*, progression-free survival; *LRC*, locoregional control; *DMFS*, distant metastatic-free survival.
Reproduced with permission from ASCOpubs: Ribassin-Majed, L., Marguet, S., Lee, A. W. M., Ng, W. T., Ma, J., Chan, A. T. C., ... Blanchard, P. (2017). What is the best treatment of locally advanced nasopharyngeal carcinoma? An individual patient data network meta-analysis. *Journal of Clinical Oncology, 35*(5), 498–505.

approaches that may have potential role in identifying high-risk patients in whom a more intense chemotherapy approach may prove to be beneficial.

Induction chemotherapy followed by concurrent chemoradiation

IC is frequently employed as a treatment strategy in nasopharyngeal carcinomas with very large neck nodes as to avoid replanning after the expected significant shrinkage of the neck nodes halfway through the planned radiation treatment, or in cases where dose constraints of the OARs cannot be achieved due to significant intracranial extension of the disease. However, it must be noted that these two indications for IC is more of a standard clinical practice among clinicians rather than being guided by evidence in the literature. The true benefit of IC followed by chemoradiotherapy for OS, PFS or loco-regional control is another area of contention in the definitive treatment of locally advanced NPC.

Though the MAC-NPC MA (2015) did show statistically significant progression-free benefit of IC compared to RT alone or concurrent chemoradiation, this did not translate into OS benefit. Furthermore, this MA did not address the issue of IC followed by concurrent chemoradiation. The strong role established by concurrent chemoradiation in the management of locally advanced NPC means any future trials should not look into radiotherapy as the solo treatment modality in the trial protocol. Though the fore mentioned 2016 JCO paper by Laureen et al., showed the least benefit of IC-CRT compared to other sequencing, the applicability of the results in today's modern radiotherapy is arguable due to superior local tumor control that is achieved with today's modern radiation techniques.

There had been attempts by a few groups to reverse the order of the Intergroup trial by giving the chemotherapy in the induction setting rather than after chemoradiation. The rationale for reversing the sequence is that,

patients are likely to tolerate IC better as the toxicities of radiotherapy may mean poor compliance for further chemotherapy after completing the full course of radical radiotherapy. The strongest evidence for IC followed by concurrent chemoradiation come from two large prospective multicenter Chinese phase III trials affiliated to Sun Yat-Sen University Cancer Centre:

1. Cao (2017) addressed this issue in a prospective trial that randomly assigned 238 patients with TNM 6th/UICC stage III–IVB (excluding T3N0-1) nasopharyngeal carcinoma patients. In this two-arm trial, the control arm received induction IV Cisplatin 80 mg/m^2 every 3 weeks with concurrent radiotherapy and patients in the investigational arm on the other hand received two cycles of IV Cisplatin 80 mg/m^2 and Fluorouracil 800 mg/m^2 continuous infusion (civ) D1–D5 every 3 weeks for two cycles before concurrent chemoradiation. There was a trend toward improved 3-year distant metastatic-free survival (DMFS) in the induction arm (86% vs 82% $p = .056$). However, there were no significant differences with respect to OS or locoregional relapse-free survival (LRRFS). It is interesting to note that this trial despite including both 2DRT and IMRT (51.3% 2DRT in investigational arm and 62.6% 2DRT in observational arm, there were no significant statistical difference in terms of DFS, DMFS, OS, and most importantly LRRFS. The locoregional control of above 90% in both arms would imply that with good radiotherapy techniques, there is minimal effect of more chemotherapy in addition to concurrent chemoradiation in terms of locoregional control. However, long-term follow-up data from this trial is preferable to assess the effect of IC on OS and DMFS as NPC is known to recur at a later stage compared to head and neck SCCs. Authors also have highlighted two important issues with this trial that may have had an impact on the final statistical analysis namely; there were more patients in the chemoradiation-only arm who received 2DRT and also a significant number of patients in the chemoradiation-only arm received IC due to the long waiting period for RT. Doublet induction chemotherapy regimen consisting of Cisplatin and Fluorouracil (PF) was used in this trial, unlike in other trials where three drug combinations of Paclitaxel, Cisplatin, and Fluorouracil (TPF) were used. The reported toxicity did not differ significantly on individual analysis though on cumulative analysis it showed significantly higher Grade 3–4 toxicities ($p < .001$) in the IC arm. Grade III and IV adverse events of importance in this trial for clinical practice are summarized in Table 10.10 (Cao et al., 2017).
2. In a 2016 Lancet publication by Sun et al., 480 nonmetastatic node-positive NPC patients from 10 centers in China were enrolled into CRT only or IC followed by chemoradiation. The investigational arm received three cycles TPF regimen consisting of Docetaxel 60 mg/m^2, Cisplatin 60 mg/m^{2,} and Fluorouracil 600 mg/m^2 per day over 5 days every 3 weeks. The dosage of the TPF regimen used is considered lower than what is usually used in other head and neck IC regimens. Both the induction followed by concurrent

Table 10.10 Grade III and IV toxicity profile with and without IC; induction chemotherapy; CRT; concurrent chemoradiotherapy.

	IC-CRT (%)	CRT (%)	*p* value
Anemia	9.7	3.8	.10
Neutropenia	10.1	8.5	.681
Mucositis	6.7	5.0	.456
Dry mouth	2.1	0.8	.446
Thrombocytopenia	1.7	0.8	.681
Cumulative	66.3	49.1	$<.001$

Source: Data from Cao, S.M., Yang, Q., Guo, L., Mai, H.Q., Mo, H.Y., Cao, K.J., ... Hong, M.H. (2017). Neoadjuvant chemotherapy followed by concurrent chemoradiotherapy versus concurrent chemoradiotherapy alone in locoregionally advanced nasopharyngeal carcinoma: A phase III multicentre randomized controlled trial. European Journal of Cancer, *75*, 14–23.

chemoradiation and concurrent chemoradiation-only arms received concurrent Cisplatin 100 mg/m^2, every 3 weeks during the course of definitive radiotherapy.

This trial showed a better failure-free survival (FFS) in the investigational arm (80% vs 72%, HR0.68) despite the lower doses used in the TPF regimen as mentioned earlier. Moreover, 3-year OS (92% vs 86%, HR 0.59, 95% CI 0.36–0.95) and distant FFS were significantly superior in the IC arm. There was a trend toward improved outcomes in terms loco-regional free survival (LRFS). The reported Grade 3 or 4 adverse events that are significantly different in the investigational arm compared to the standard CRT arm are (neutropenia 42% vs 7%), leucopenia (41% vs 17%), and stomatitis (41% vs 35%). No late toxicities were reported in the paper (Sun et al., 2016).

Most other evidence for IC followed by chemoradiation are retrospective reviews. A large retrospective study, again from Sun Yat Sen University, reports a significant benefit of IC chemotherapy in locoregionally advanced NPC (except T3-4N0) in the era of IMRT. In the multivariate competing risk analysis model by Zhang. J et al. (2017) looking at IC as an independent prognostic factor in decreasing cancer-specific mortality, the HR for cancer-specific mortality is 0.654; 95% CI 0.488–0.951, $p = .016$, and overall mortality [hazard ratio (HR) 0.654; 95% CI 0.471–0.909; $p = .011$] were significantly in favour of IC. This is an important study in the era of IMRT and other advanced radiotherapy techniques (Zhang et al., 2017).

Another interesting recent study published in *Nature* by Lan et al. in early 2017 was on the benefit of IC for NPC patients with cervical nodal necrosis (CNN). CNN had been identified in a previous study, also by Lan et al. (2015), as a poor prognostic factor with 12% reduction in 5-year OS, DFS, and DMFS

compared to non-CNN patients. It is estimated that 20% of CNN patients will subsequently develop metastatic disease (Lan et al., 2015). This retrospective study with authors from three different countries reviewed the MRIs of 792 nonmetastatic node-positive NPC patients who had CNN at presentation. In fact, 508 patients were identified and propensity-matched on a 1:1 basis into two groups, namely the IC + CRT group and CRT-alone group. At 50 months of median follow-up, the reported 5-year disease-specific survival (DSS), DFS, and DM free survival showed statistically significant benefit in the IC + CCRT group as opposed to the propensity matched CRT-only group. At 5 years, 81.9% of patients in the IC group were distant metastatic-free as opposed to 67.3% in chemoradiotherapy-only group ($p < .001$). It is estimated that risk of DM can be reduced by more than 50% with the addition of IC. The failure pattern of this propensity matched study is summarized in Table 10.11. The early toxicities were reported as acceptable, but long-term toxicities are not reported. Some of the early toxicities of clinical importance are summarized in Table 10.12.

This study by Lan et al. (2017) did not show any statistical difference in terms of loco-regional recurrence rate, which conforms to other newer trials that emphasize quality of the radiation delivery. There was also no difference in OS rates between different chemotherapy regimens used [TPF, PF, or Paclitaxel and Cisplatin (TP)]. It is interesting to note that, while there is no difference observed in terms of DSS, DFS, and DMFS among the different IC regimens, the 5-year loco-regional RFS is significantly higher in patients receiving ≤ 2 cycles than those >2 cycles ($p = .017$) regardless of the regimens or the number of chemotherapeutic agents used. The authors were unable to come to a conclusion on this phenomenon due to the small number of patients receiving >2 cycles of IC (Lan et al., 2017). This effect is of particular interest because it has been postulated that there is a possibility of IC inducing more radioresistant clones of cancer cells leading to poorer local control rates.

A very recent MA from Singapore was conducted and published in the green journal in 2018. This MA by Tan et al. that used advanced statistical tools and

Table 10.11 Patterns of failure in the propensity-matched cohort of 508 patients with cervical nodal metastasis (CNN), with or without induction chemotherapy (IC).

Failure pattern	IC-CRT (%)	CRT (%)	*p* value
DM alone			.001
DM + LRR	25	75	
LRR alone			.530

CRT, Concurrent chemoradiation; *DM*, distant metastasis, *LRR*, locoregional recurrence.
Source: Data from Lan, M., Chen, C., Huang, Y., Tian, L., Duan, Z., Han, F., . . . Lu, T. (2017). Neoadjuvant chemotherapy followed by concurrent chemoradiotherapy versus concurrent chemoradiotherapy alone in nasopharyngeal carcinoma patients with cervical nodal necrosis. Scientific Reports, *7*, 42624.

Table 10.12 Grade 3–4 toxicities in the propensity-matched cohort of 508 patients with cervical nodal metastasis (CNN) with or without induction chemotherapy (IC).

	IC-CRT (%)	CRT (%)	*p* value
Anemia	2.36	0.39	.122
Neutropenia	16.9	11.8	.129
Thrombocytopenia	5.12	2.76	.254
Mucositis	28.3	20.9	.064
Dermatitis	9.45	11.4	.562

CRT, Concurrent chemoradiotherapy.
Source: Data from Lan, M., Chen, C., Huang, Y., Tian, L., Duan, Z., Han, F., ... Lu, T. (2017). Neoadjuvant chemotherapy followed by concurrent chemoradiation versus concurrent chemoradiation alone in nasopharyngeal carcinoma patients with cervical nodal necrosis. *Scientific Reports, 7*, 42624.

quality assessment methods to verify the included studies, had both randomized controlled trials (RCTs) and observational studies (OBS) in the analysis. Unlike most systematic reviews that view RCTs as the gold standard evidence, authors of this manuscript stressed on the fact that good quality OBS are usually good indicators of real life practice as they include representative populations. This is the first MA that specifically looked into answering the question of the benefits of IC followed by concurrent chemoradiation and also the first NPC systematic review/MA to include OBS. Statistical analysis of the six RCT's that included mostly stage III–IVB patients, demonstrated there was a statistically significant 23% reduction in the hazard for death (HR 0.77, 95% CI 0.60–0.98, $p = .03$) when IC is used. There was a trend toward improved OS with TPF against non-TPF regimen (HR 0.54 vs 0.90, interaction $p = .06$). For the four OBS in the analysis, there was a statistically significant 42% reduction in the hazard for death (HR 0.58, 95% CI 0.39–0.89, $p = .01$). Four RCTs reported DMFS that is currently seen as the most important outcome for the incorporation of IC and there was a statistically significant 37% reduction in the hazard for development of distant metastases (HR 0.63, 95% CI 0.47–0.83, $p = .001$) in favor of IC (Tan et al., 2018).

This study summarizes the current available evidence and suggests that there is a strong potential benefit of IC in LA NPC to survival in these patients by reducing DM in locally advanced NPC.

Systemic therapy

Commonly used systemic therapy regimen in the concurrent chemoradiation setting is either:

1. Cisplatin 100 mg/m^2 on D1, 22, and 43 as in the concurrent setting of Intergroup 0099 study;
2. Weekly Cisplatin 30–40 mg/m^2.

Historically the choice between these two regimens had been an institutional practice rather than "head on" trials on the efficacy. Most centers today are adopting weekly Cisplatin regimens as practiced in other head and neck concurrent regimens for logistic reasons and convenience. Weekly regimens are less resource intensive and can be given within shorter time as an outpatient procedure. A Korean phase II trial with 109 patients in 2016 compared the 3 weekly against weekly Cisplatin regimen for stage II–IVb NPC. This study showed the 3-year PFS, which was the primary end point, and Grade 3–4 toxicities were not of statistically significant difference. However, patients' quality of life at 3 weeks after completing the treatment was much better for those receiving weekly Cisplatin regimen (Lee et al., 2016). Evidence from clinical trials point to a minimum cumulative threshold dose of Cisplatin above 200 mg/m^2 in the concurrent chemoradiation regimen to be of benefit in terms of OS and also loco-regional control. In contrast, the Cisplatin regimen used has a seemingly less effect (Lee, Tung, Ngan, et al., 2011).

The other platinum agent that is frequently used after Cisplatin is Carboplatin. However, its use in general practice is usually limited to the setting of renal impairment, as the dose is calculated based on the area under curve, which corresponds to glomerular function. Chemotherapeutic regimens containing either of these two drugs were compared in a randomized, noninferiority, open trial from Thailand. The disease outcomes and the acute toxicity profiles of clinical importance at the median survival of 26.3 months (range 3–74.6 months) are summarized in Tables 10.13 and 10.14, respectively.

The Cisplatin-based chemotherapy regimen that was used in this trial follows the Intergroup 0099 study: Cisplatin 100 mg/m^2 on days 1, 22, and 43 with concurrent radiotherapy followed by adjuvant Cisplatin 80 mg/m^2 and 5-FU infusion at 1000 mg/m^2/day, days 1–4 given every 4 weeks for a total of three

Table 10.13 3-years disease free survival (DFS),overall survival (OS) and completion of three cycles of adjuvant chemotherapy between the Cisplatin and Carboplatin arm.

	Carboplatin (%)	Cisplatin (%)	Hazard ratio (HR)	*p* value
3-years DFS (as treated)	60.9	63.4	0.7 (95% CI 0.50–0.98)	.9613
3-years actuarial OS rates (as treated)	79.2	77.7	0.83 (95% CI 0.63–1.01)	.9884
Completion of three cycles of adjuvant chemotherapy	70	42		

Source: Data from Chitapanarux, I., Lorvidhaya, V., Kamnerdsupaphon, P., Sumitsawan, Y., Tharavichitkul, E., Sukthomya, V., & Ford, J. (2007). Chemoradiation comparing Cisplatin versus carboplatin in locally advanced nasopharyngeal cancer: randomized, non-inferiority, open trial. European Journal of Cancer, *43*(9), 1399–1406.

Table 10.14 Toxicities with statistical significance or trend toward statistical significance between Cisplatin and Carboplatin arm.

	Cisplatin (%)	Carboplatin (%)	*p* value
Anemia	47	18	< .0001
nausea/vomiting (WHO Grade 1–3)	59	34	.0537
Nephrotoxicity (WHO Grade 1–4)	26	0	.002
Weight loss (> 10%)	50	20	< .0001
Needing nasogastric tube	48	22	.0002
Mucositis (RTOG Grades 2–4)	47	63	.0514

Source: Data from Chitapanarux, I., Lorvidhaya, V., Kamnerdsupaphon, P., Sumitsawan, Y., Tharavichitkul, E., Sukthomya, V., & Ford, J. (2007). Chemoradiation comparing Cisplatin versus carboplatin in locally advanced nasopharyngeal cancer: randomized, non-inferiority, open trial. *European Journal of Cancer, 43*(9), 1399–1406.

cycles, 4 weeks after the completion of radiotherapy. Patients in the Carboplatin arm on the other hand had Cisplatin replaced by Carboplatin 100 mg/m^2 on days 1, 8, 15, 22, 29, and 36 with concurrent radiotherapy followed by adjuvant Carboplatin. Otherwise the 5-FU infusion and the frequency chemotherapy administration is similar to that of the Intergroup 0099 study.

The authors concluded that the Carboplatin arm was not inferior to the Cisplatin arm as the upper bounds of the 95% CIs for both end-points were less than 1.25. However, caution should be applied in advocating the Carboplatin in place of Cisplatin as currently long-term data on the efficacy and toxicity profile is not available (Chitapanarux et al., 2007).

There have been attempts to incorporate molecular targeted agents such as Cetuximab or Nimotuzumab in the induction, concurrent, or adjuvant setting of NPC. Though preliminary data is promising, the use of these agents in standard clinical practice should be limited until further evidence is available. Though some physicians extrapolate the concurrent Cetuximab with RT data from the head and neck SCC trials for patients who are unfit for chemotherapy, it should be cautioned that this practice is not based on robust phase III evidence from existing studies.

Metastatic nasopharyngeal cancer

As discussed earlier in the treatment of nonmetastatic NPC, with the improvement in the radiation techniques and skills, distant recurrence is becoming the predominant mode of failure in nasopharyngeal carcinomas. Compiled data from multiple studies show that up to 15%–30% of patients with NPC would have presented with metastatic disease at diagnosis or developed metastatic disease eventually (Lee, Ma, Ng, & Chan, 2015). Though there is no curative

treatment available at the moment, given the very chemo-sensitive nature of NPC, first-line palliative chemotherapy is known to induce a very high response rate in excess of 70% that translates to improved survival and also quality of life (Ngan et al., 2002). A median survival of 11–28 months can be expected as reported in first-line studies using platinum-based doublet chemotherapy regimens. Despite the improvement in the median survival, the median time to progression generally stagnated at around 7.3–10 months plus only a small number of patients with oligometastatic disease do eventually become long-term survivors of more than 5 years (Lo & Huang, 2002).

Platinum-based doublets are the standard of treatment and the use of three drug combinations failed to improve PFS or OS, while having higher toxicities. There is a lack of phase III data in the existing literature comparing platinum-based doublet chemotherapy and three-drug combinations. Jin et al. (2012) compared five different chemotherapy regimens for disseminated NPC in their retrospective review, and the results of the four commonly used combinations are summarized in Table 10.15.

The authors concluded that there was no statistically significant difference in terms of PFS or OS among the five groups compared, namely PF, TP, Gemcitabine and Cisplatin (GP), TPF, and Bleomycin, Cisplatin, and Fluorouracil (BPF). This is the largest study to date that showed that platinum-based three drug combination may not offer much advantage over doublet but has higher toxicities (Jin et al., 2012).

A large multicenter phase III trial from Sun Yat Sen University, published in The Lancet in 2016, showed GP to be superior to PF in metastatic NPC. Patients in the GP arm were given Gemcitabine 1 g/m^2 on days 1 and 8 and Cisplatin 80 mg/m^2 on day 1, whereas the PF arm received Fluorouracil 4 g/m^2 infusion over 96 hours and Cisplatin 80 mg/m^2 on day 1. The chemotherapy cycles were repeated every 3 weeks for a maximum of six cycles. The median PFS, which is the primary end-point of the study, is summarized in Table 10.16. The

Table 10.15 Response rates (RR) comparison for four different type of regimens, Gemcitabine and Cisplatin (GP), Cisplatin and Fluorouracil (PF), Paclitaxel, Cisplatin, and Fluorouracil (TPF), and Paclitaxel and Cisplatin (TP).

Regimen	X^2	*p* value
GP versus PF	4.57	.033
TPF versus PF	7.04	.008
TPF versus TP	5.579	.018

Source: Data from Jin, Y., Shi, Y.X., Cai, X.Y., Xia, X.Y., Cai, Y.C., Cao, Y., ... Jiang, W.Q. (2012). Comparison of five Cisplatin-based regimens frequently used as the first-line protocols in metastatic nasopharyngeal carcinoma. Journal of Cancer Research and Clinical Oncology, *138*(10), 1717–1725.

Table 10.16 Median progression free survival (PFS), interquartile range (IQR) and hazard ratio (HR) at a median follow-up time for PFS 19.4 months (IQR 12.1–35.6).

Chemotherapy regimen	Median PFS	Unstratified HR	*p* value
GP	7.0 months (IQR 4.4–10.9; 95% CI 6.3–7.6)	0.55 (95% CI 0.44–0.68)	<.0001
PF	5.6 months (IQR 3.0–7.0; 95% CI 4.9–6.2)		

Source: Data from Zhang et al., 2016.

Table 10.17 Statistically significant Grade 3 and 4 toxicities between the Gemcitabine plus Cisplatin arm (GP) and Cisplatin plus Fluorouracil arms (PF).

Toxicity	GP (Grade 3–4) (%)	PF (Grade 3–4) (%)	*p* value
Leukopenia	29	9	<.0001
Neutropenia	23	13	.0251
Thrombocytopenia	13	2	.0007
Mucosal inflammation	0	15	<.0001

Source: Data from Zhang et al., 2016.

preliminary data from the trial also suggest that GP regimen might improve OS in patients with recurrent or metastatic NPC.

The Gemcitabine arm had more adverse events as expected, especially blood-related events such as leukopenia, neutropenia, and thrombocytopenia. The PF arm had more mucositis. As most patients have had radiotherapy to primary site with the expected sequalae of radiation (mucositis), this might have likely resulted in more patients in the PF arm discontinuing the study drug (Zhang et al., 2016). The Grades 3 and 4 toxicities with statistical significance in the study are presented in Table 10.17.

To date this remains the strongest data available on the efficacy of first-line palliative chemotherapy in metastatic or recurrent NPC. As the earlier mentioned retrospective study did not show significant benefit of a three-drug regimen over doublet chemotherapy and this study by Zhang et al. (2016) showing significantly prolonged PFS with GP, the GP regimen should be the preferred regimen in the first-line metastatic setting.

The treatment algorithm in the second-line setting is much more complex. If significant time has lapsed between the initial platinum-based chemotherapy and recurrence, rechallenging with a platinum backbone would be a reasonable option provided neurotoxicity is not a major issue. For patients who have had single-agent platinum in the concurrent setting, adding another agent such as Gemcitabine to the platinum-based doublet is also a common practice at recurrence. Other alternative conventional chemotherapeutic agents with antitumor

activity in NPC include oral capecitabine, docetaxel, methotrexate, bleomycin, ifosfamide, epirubicin, doxorubicin, irinotecan, and vinorelbine.

Molecular-targeted therapies and immunotherapy/immunomodulation have been an area of active research in metastatic or recurrent NPC. Cetuximab, a chimeral monoclonal antibody inhibiting the extracellular domain of the epidermal growth factor receptor, has at present the strongest evidence of benefit. In a multicenter phase II clinical trial, Chan et al. (2005) reported on 60 heavily pretreated NPC patients who were progressing on platinum-based chemotherapy who subsequently received a regimen containing Cetuximab and Carboplatin. A response rate of 12% was seen in this group of patients with another 48% of them having stable disease (Chan et al., 2005). Clinical trials of other molecular targeted agents such as Gefitinib, Erlotinib, Sorafenib, Pazopanib, Famitinib, and Sunitinib are either saddled with poor response rates, drug-related toxicities, or poorly powered studies to detect a statistically significant effect on tumor control. A newer molecular targeted agent, Axitinib, has achieved a very high clinical benefit rates (CBR) of 78.4% (95% CI 65.6%–91.2%) at 3 months and the effect was still seen at 6 months after commencement of the single-agent therapy at a rate of 43.2% (95% CI 30.4%–56.1%) in 37 patients who can be evaluated for response. In this preclinical and phase II correlative study involving platinum-pretreated metastatic or recurrent NPC patients, the authors defined the CBR as the percentage of patients achieving complete response, partial response, or stable disease by RECIST criteria for more than 3 months. Interestingly, the authors also mention that patients who had elevated diastolic blood pressure in the first 3 months of treatment had better OS (Hui et al., 2018).

Immunotherapy is another area of intense research in metastatic or recurrent NPC, be it the Epstein–Barr virus (EBV)-directed adoptive immunotherapy by the transfer of cytotoxic T-cells, autologous dendritic cell vaccination, or immune check point inhibitors like programmed cell death ligand 1 (PD-L1) or programmed cell death protein 1 (PD-1). Promising results were seen in the KEYNOTE-028 nonrandomized phase Ib trial that enrolled 27 PD-L1 expression status-positive, heavily pretreated, metastatic, or recurrent NPC patients. These patients were treated with pembrolizumab for up to 2 years unless unacceptable toxicity developed or disease progressed. Statistical analysis at the median follow-up time of 20 months, 78% of patients either had partial responses or stable disease (Hsu et al., 2017). Further evidence from RCTs are needed to determine the role of immune checkpoint inhibitors in metastatic or recurrent NPC.

Role of radiation in metastatic disease

Patients presenting with metastatic disease at diagnosis is a unique clinical scenario that is not uncommon for clinicians treating NPC. The decision to

proceed with local radiotherapy to primary site with or without concurrent chemotherapy is a complex decision taking into account the life expectancy, presence of significant visceral disease, and disease response to palliative chemotherapy. Most radiation oncologists will proceed with either high-dose palliation or radical dose of radiotherapy to the nasopharynx and neck despite the known metastatic setting. The rationale behind this is that a significant number of metastatic NPC patients may carry on to become intermediate or long-term survivors and in this group of patients good control of the primary site will improve the quality of life. Lower doses of palliative radiotherapy may not be sufficient to achieve a long-term control and some patients may end up with complications of local disease progression such as facial disfigurement, carotid rupture, and lymphatic obstruction in the neck leading to edematous lips, face, and oral cavity on top of the poor oral hygiene that follows.

Three recent studies have also affirmed the role of radiotherapy to the primary site in the setting of metastatic NPC. Chen et al. (2013) from Sun Yat-sen Cancer Centre in a retrospective review of 408 patients identified locoregional radiotherapy and systemic chemotherapy as independent prognostic factors favoring OS in metastatic NPC patients. For patients undergoing systemic chemotherapy alone or locoregional radiotherapy alone, the calculated mortality risk is about the same. The mortality risk for both chemotherapy or radiotherapy-only groups were 60% lower when compared to patients who had no active treatment at all. However, the benefits of having both chemotherapy and locoregional radiotherapy in term of mortality are much higher than having either one in isolation. The benefit is estimated to be in the range of 130% when combination of both the treatments are compared to locoregional radiotherapy or chemotherapy in isolation. Hence, the authors concluded that locoregional therapy when combined with systemic chemotherapy is associated with improved survival in metastatic NPC patients (Chen et al., 2013).

In another retrospective review from Fujian Provincial Cancer Hospital, Zheng et al. (2016) investigated the effect of treating metastatic disease with palliative radiotherapy on survival. This study showed 57.7% of metastatic patients who received both chemotherapy and radiotherapy are alive at 2 years, compared to patients who received chemotherapy alone at 32.7% and the best supportive care at 1.6%. The disease-free interval from completion of initial therapy to the detection of metastatic disease, disease burden (number of metastases), total number of chemotherapy cycles received, and importantly the dose of palliative radiotherapy is shown to be independent factors affecting the survival (Zheng et al., 2016). Hu et al. utilized the Surveillance Epidemiology and End Results database to look at the role of radiotherapy in metastatic NPC involving 679 patients of that 448 patients received RT. These patients had a median follow-up of 13 months. The patients who received radiotherapy had statistically significant improvement in terms of OS and cancer specific survival. The

authors found a 50% mortality risk reduction in patients who received radiotherapy (Hu et al., 2017).

Rusthoven et al. conducted a retrospective review of the National Cancer Database and identified 718 cases metastatic NPC (Rusthoven et al., 2017). They concluded similarly that RT was associated with superior median survival of 21.4 months versus 15.5 months without radiotherapy. The 5YOS improved from 10% to 28% with radiotherapy. Of interest, the group also found that patients who received a dose of more than 50 Gy had better survival. Patients who had received radiotherapy are the only ones who had a chance to become long-term survivors exceeding 10 years. The benefits of RT are seen in either single or multiorgan metastases and also in different anatomical sites of metastasis.

The postulated mechanism by which the addition of radiotherapy improves survival is by reducing the tumor bulk and also through the immunomodulatory effect of radiotherapy.

Conclusion

RT remains the cornerstone of any first-line definitive treatment for NPC. For treatment with radical intent, concurrent chemotherapy with RT has a role to play in intermediate and locally advanced NPC.

The management of NPC is based primarily on the stage of the disease. In patients with early stage NPC, the standard of care should be radiation alone to a dose of 70 Gy. For patients with intermediate stage NPC, the standard of care should be low-dose weekly Cisplatin (30 mg/m^2) concurrent with RT to a dose of 70 Gy. Concurrent chemotherapy confers a survival advantage on top of local control benefit.

For patients with locally advanced NPC, both chemotherapy and radiotherapy have a strong established role. The standard of care should be CRT with a regimen similar to the intermediate stage but weekly Cisplatin dose of 40 mg/m^2 instead of 30 mg/m^2 as used in the intermediate stage disease. Concurrent Cisplatin confers both local control and survival benefit. In addition, it also decreases the rate of distant spread in comparison with radiation alone. However, significant controversy exists on the timing of chemotherapy with radiotherapy. The evidence from the literature is very strong for concurrent chemoradiation for both locoregional control and distant metastatic control, though the true benefit of concurrent chemotherapy for locoregional control in the era of modern and advanced radiotherapy techniques is arguable. As distant metastatic disease is becoming the predominant mode of failure, there had been attempts by many investigators to increase the dose intensity of chemotherapy by adding induction or adjuvant chemotherapy with concurrent chemoradiation. Although the evidence so far is in favor of IC, the extra toxicity

from IC is a significant concern. The same can also be said of adjuvant chemotherapy. Hence, most centers have not adopted the induction nor adjuvant chemotherapy approach in addition to concurrent chemoradiation in locally advanced NPC.

If patients are considered high risk for distant metastatic disease and additional chemotherapy is planned to improve distant control, it should preferably be given in the neoadjuvant setting as the evidence is stronger based on the 2016 trial by Sun et al. and another recent MA by Tan et al. Patients are also likely to tolerate the chemotherapy given in an induction setting better than in the adjuvant setting as the expected side effects of radiotherapy may in poor compliance to adjuvant chemotherapy. Hence, the decision to offer additional chemotherapy and the timing of it should be balanced against the increased toxicity, knowing the scarcity of data supporting its use.

Neoadjuvant chemotherapy should be strongly considered in patients with CNN where the risk of DM may be reduced by 50% based on the study by Lan et al. Since there is no statistically significant OS benefit between different chemotherapy regimens commonly used in the neoadjuvant setting (TPF, PF, or TP), doublet chemotherapy is preferable rather than three-drug combinations given the more favorable toxicity. Based on the same study by Lan et.al, if IC chemotherapy is given, it should be limited to no more than two cycles as higher number of cycles may contribute to poorer locoregional control, for reasons not well understood currently.

The most commonly used systemic chemotherapy in the concurrent setting is Cisplatin, given either weekly or three times weekly. Most centers adopt a weekly regimen due to logistic reasons and convenience rather than being guided by efficacy based on the evidence in the literature due to the lack of it. It is likely that a minimum cumulative dosage of 200 mg/m^2 of Cisplatin is needed for the benefits in terms of OS and locoregional control rather than the type of Cisplatin regimen used. In patients with renal impairment or poor performance status, Carboplatin can be considered instead of Cisplatin, which has shown similar efficacy in a phase II trial from Thailand. Otherwise, Cisplatin should remain the standard of care in concurrent chemoradiation setting due to the established long-term data on the efficacy. Strong phase III data is still lacking in the use of molecular targeted agents such as Cetuximab or Nimotuzumab in concurrent setting.

The CTV for irradiation includes elective bilateral necks in all definitive cases due to the high incidence of early spread to bilateral neck nodes in NPC. Consideration may be given to omit the lower neck irradiation in early stage NPC patients who are clinically staged as node negative.

The follow-up of NPC patients posttreatment should be for a minimum of 10 years due to the natural history of the disease. NPC tends to recur much later, either

local or distant, compared to other SCCs of the head and neck region. While there is no strong evidence for close follow-up in improving outcomes, most centers do follow up on patients every 3 months in the first year, every 4 months for the next 2 years, then 6 months for years 4 and 5, and annually from year 6 onward.

Patients who present with DM at diagnosis or who develop DM subsequently should be treated aggressively as response rates in excess of 70% can be expected in the first-line platinum-based palliative chemotherapy and a number of patients with oligometastatic disease can become long-term survivors. On the choice of systemic therapy agents, doublet chemotherapy of Cisplatin and Gemcitabine is likely the most optimal and three-drug combinations may not be offering extra benefit despite the increased toxicity.

Patients presenting with DM disease at diagnosis should be treated aggressively at the primary site and in some instances at the oligometastatic sites too as it has been shown to improve survival for reasons not well understood currently. In this group of patients, there is also a greater likelihood of completing the full course of radiotherapy if Cisplatin–Gemcitabine doublet chemotherapy is used in the preradiotherapy setting due to fewer issues with oral mucositis compared to regimens using Fluorouracil. Moreover, there is high level of evidence to suggest that this combination of Cisplatin and Gemcitabine confers superior PFS and OS as compared to Cisplatin + Fluorouracil regimens.

Clinical trials incorporating molecular targeted therapy or immunotherapy is a viable approach for patients who are refractory to conventional chemotherapy.

Reirradiation in recurrent NPC can also achieve good outcomes, but it often comes with a high price in terms of toxicity. For this reason, surgery is always given consideration whenever the disease is resectable.

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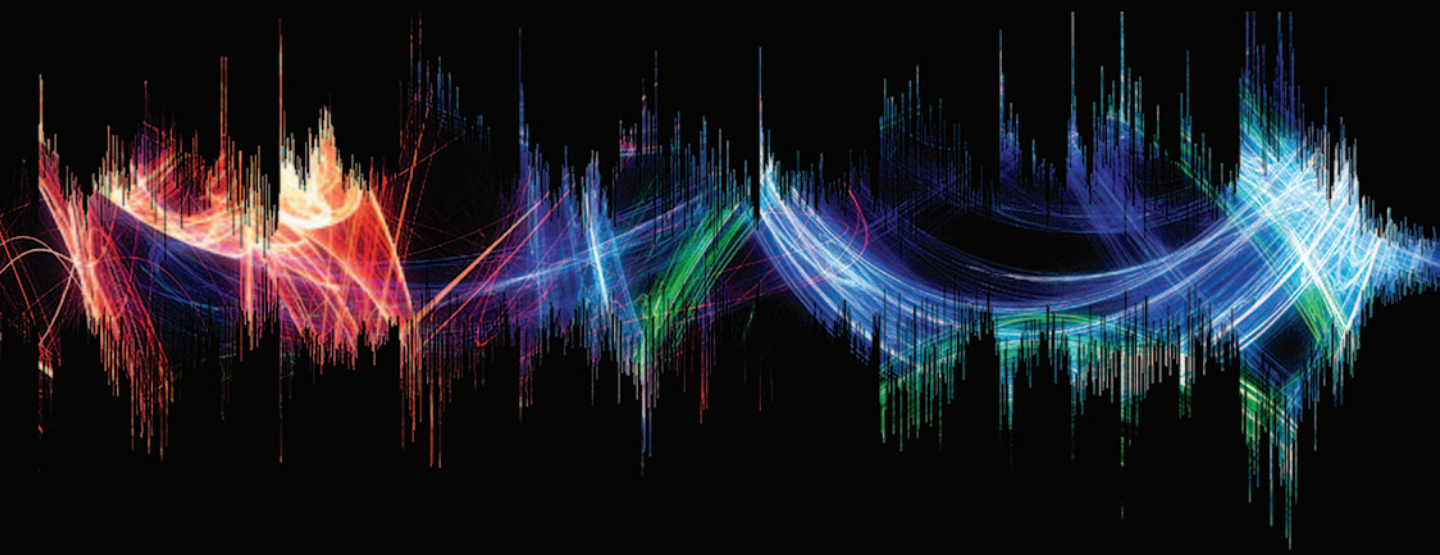

11

Role of endoscopic endonasal surgery in recurrent nasopharyngeal carcinoma: endoscopic endonasal transpterygoid nasopharyngectomy

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Introduction

The primary treatment for untreated nasopharyngeal carcinoma (NPC) is radiotherapy (RT) alone for early-stage lesion and concurrent chemo-radiation for advanced tumor. Suarez et al. (2010), Ma et al. (2001), and Lin, Liang, Jan, Jiang, and Lin (2004) reported that incidence of local recurrence was approximately 8% to 58%. You et al. (2015) mentioned locally recurrent NPC can be treated with salvage reirradiation or surgery. Ridge (1993) recommended that resection of locally discrete recurrent NPC should be considered unless the patient is unfit for surgery. Tumors that persist or recur after primary radiotherapy have demonstrated significant resistance to radiotherapy. Surgery should be considered for patients presenting with residual or recurrent tumor after radiotherapy or chemoradiotherapy, and for patients with glandular or mesenchymal differentiated tumors that are poorly responsive to radiotherapy as mentioned by Al-Sheibani et al. (2011). Furthermore, high dose reirradiation may still result in severe complications like osteoradionecrosis, brain necrosis, radiation induced myelitis, hypopituitarism and trismus as described by To et al. (2002) and Chang et al. (2000).

Nasopharyngectomy is a well-established surgical procedure for salvaging locally recurrent NPC; via various surgical approaches, that is, midfacial degloving,

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transpalatal, transmaxillary, maxillary swing, or transmandibular as mentioned by King, Ku, Mok, and Teo (2000). The common complications for open nasopharyngectomy are middle ear effusion, palatal fistula, nasal regurgitation and trismus.

Ideally, a surgical approach to the nasopharynx should provide adequate visualization of the tumor margins, allow complete oncologic resection with negative margins, allow the possibility to extend the resection margins if necessary, and allow the identification and protection of important neurovascular structures. In addition, it should avoid facial scarring or deformity, preserve neurologic and masticatory functions, and facilitate the reconstruction of the surgical defect. Over the past decade, the evolution of these approaches has incorporated endoscopic endonasal techniques to complement conventional skull base approaches, and in certain patients, as the sole approach.

Endoscopic endonasal transpterygoid nasopharyngectomy (EETN) has then emerged as a viable treatment option for locally recurrent NPC with minimal invasiveness, avoiding morbidity from external approaches and the absence of facial scar. A literature review by Emanuelli et al. (2014) showed that endoscopic method attained a higher negative surgical margin of 93.75% than external approach (71.6%).

Radical neck dissection or modified radical neck dissection should be performed for regional recurrence of NPC at the neck, provided there is no distant metastasis. Either unilateral or bilateral neck dissections are performed depending on the extent of the nodal disease.

Patient selection

Patient selection is perhaps the most important aspect of effectively treating patients with EETN. Generally speaking, patients categorized as rT1, those categorized as rT2 with minimal parapharyngeal extension, and select patients categorized as rT3 (Involvement of floor of sphenoid sinus) can be treated with EETN as described by Chen et al. (2012). Exclusion criteria for patients are based on disease factors and patient factors. Disease factors include significant parapharyngeal space extension, internal carotid artery (ICA) involvement, cavernous sinus with multiple cranial nerves involvement, brain parenchymal involvement and presence of distant metastasis. Patient factors include patients who are medically unfit to tolerate surgery and undergo general anesthesia.

Preoperative workup

All patients who undergo EETN will have thorough preoperative workup. The preoperative workup includes clinical factors, radiological factors, and pathological factors.

For clinical factors, patients require a thorough medical examination including nasopharyngoscopy to delineate the tumor extension. The patient's full medical

status must also be investigated to determine eligibility for general anesthesia. For radiological factors, radiological examination is essential to ascertain the extent of the primary tumor, assess for regional lymph node involvement, and rule out distant metastatic disease. Investigational techniques include computed tomography and magnetic resonance imaging (MRI), and occasionally positron emission tomography scan. For pathological factors, histopathological examination of the suspected local recurrence or residual tumor and type of tumor must be confirmed before any surgical intervention.

Special attention has to be given to ascertain the relationship of the tumor to the parapharyngeal and petrous segments of the ICA. Generally, patients with tumors that are encasing the ICA and, extensive dura or intradural involvement are not suitable candidates for EETN. Other important preoperative preparations depending on the individual case include computed tomography angiography (CTA) with computed tomography and MRI fusion for intraoperative navigation. An acoustic Doppler ultrasound probe can complement the surgical navigation device for the identification of critical vessels, especially ICA. The preferred prophylactic perioperative antibiotic regimen should include a third-generation cephalosporin with cerebral spinal fluid penetration.

Surgical technique

The surgery is done under general anesthesia with the patient lying in supine position. The nasal cavities are decongested with Moffett's solution for 30 minutes as illustrated by Benjamin, Wong, and Choa (2004). The solution contains 1 mL adrenaline 1:1000, 2 mL of 10% cocaine, 4 mL of 8.4% sodium bicarbonate, mixed together with 13 mL of water for injection. Following general anesthesia, there is widespread vasodilation, hence producing hyperemic mucosa. A topical decongestant of Moffett's solution could reduce nasal blood flow, optimizing the surgical field. Infiltration of both middle turbinates and nasal septum with a solution of lidocaine 1% and epinephrine 1/100,000 enhances the hemostasis. Surgery proceeds via a purely endoscopic endonasal approach using a 0- and 30-degree rod lens endoscope. A fundamental premise of the endonasal endoscopic approach is that two surgeons work concomitantly, using a bimanual, three-/four-handed technique via both nostrils and nasal cavities. This facilitates dynamic visualization as well as bimanual dissection, which is vital for depth perception, traction, countertraction, and for maintenance of a blood-free surgical field.

Sinonasal corridor

Firstly, surgery is initiated by enlarging the natural sinonasal corridor ipsilateral to the lesion by removing the inferior half of the right middle turbinate and completing an uncinectomy, large midmeatal nasomaxillary window, and anterior and posterior ethmoidectomies. This increases the working space and

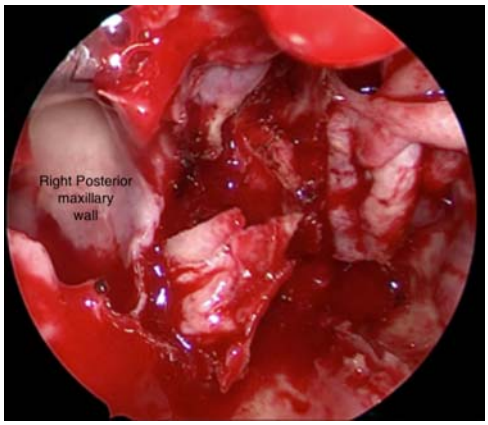

Figure 11.1
Right medial maxillectomy performed as part of the transpterygoid approach.

expose the entire posterior wall of the antrum. A medial maxillectomy is performed to expose the entire height of the posterior wall of the maxillary sinus and to allow an extended dissection of the pterygopalatine fossa. This medial maxillectomy is limited anteriorly by the nasolacrimal duct, which acts like a fulcrum point, preventing free movement of the scope laterally. Endoscopic Denker's approach (also known as the Sturman–Canfield approach) can be performed to further increase the lateral angle of exposure and optimize instrument maneuverability. Endoscopic Denker's approach is a procedure to remove the piriform aperture, as well as the anterior maxillary wall, until the lateral wall of the antrum is in direct and full view especially viewing the entire infratemporal fossa (Fig. 11.1).

Additional lateral control is obtained by bringing the instruments from the contralateral side of the nose through a posterior septectomy. A generous posterior bony septectomy allows a bimanual technique traversing both sides of the nasal cavity. This extensive posterior septectomy allows visualization of the entire posterior wall of the maxillary sinus using a 0-degree endoscope that crosses over to the contralateral side of the nose.

Nasoseptal flap

The Hadad–Bassagasteguy nasoseptal flap (HBF) should be harvested from the contralateral side of the tumor as illustrated by Al-Sheibani et al. (2011). It is critical to harvest the HBF from the contralateral side because its pedicle and proximal blood supply would be surrendered ipsilateral to the transpterygoid dissection. Later, a Caicedo reverse flap is transposed from the contralateral side to cover the HBF donor defect as illustrated by Prosser, Figueroa, Carrau, Ong, and Solares (2011). Clinical harvesting of these septal flaps presumes that the tumor does not involve this area. If tumor involves the nasal septum, other vascularized flaps can be considered (Figs. 11.2–11.5).

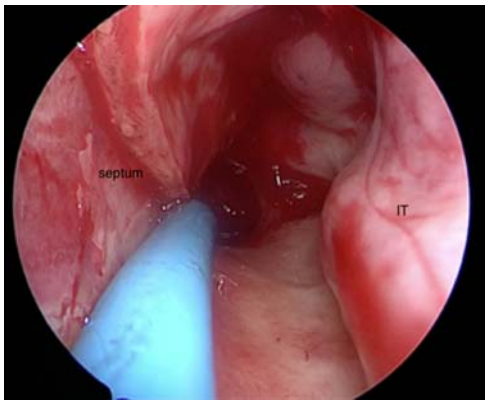

Figure 11.2
Creating Hadad–Bassagasteguy nasoseptal flap (HBF).

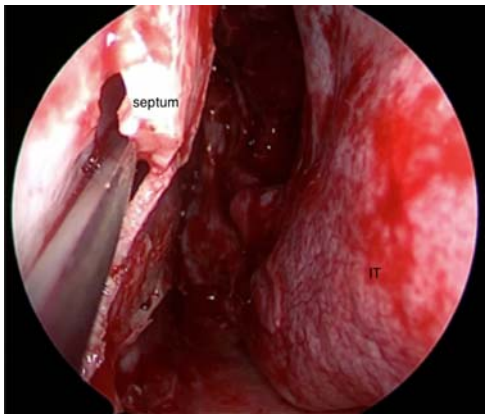

Figure 11.3
Posterior septectomy.

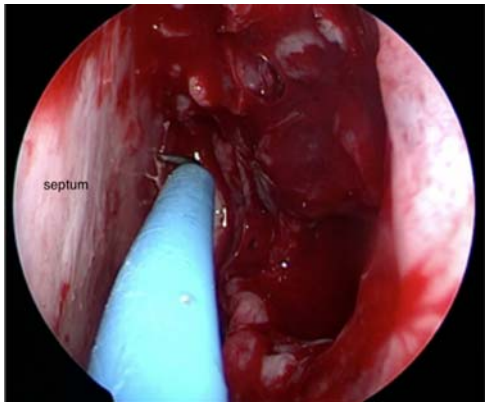

Figure 11.4
Creating Caicedo's flap.

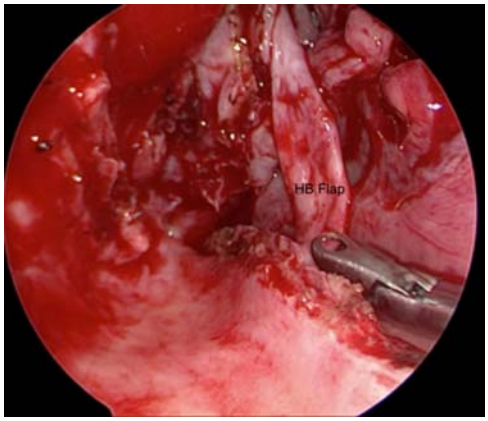

Figure 11.5
Two nostrils and four hands technique after removing posterior septum.

Inferior sphenoidectomy

The anterior face of the sphenoid sinus is often opened early during the approach, enlarging the sphenoid ostium after completing the ethmoidectomies. As the superior part of the sphenoid crest is removed, the sella turcica's floor and intersinus and intrasinus septations, as well as the lateral walls of the sphenoid sinus, come into direct view. Prior to removing the inferior component of the sphenoid rostrum, its mucoperiosteum is dissected laterally and inferiorly to identify key anatomic landmarks as this area comprises the pedicle of the HBF as described by Hadad et al. (2006).

The lateral walls of the sphenoid and the medial pterygoid plates (lateral wall of the posterior choana) form a vertical strut that intersects the floor of the sphenoid sinus. The junction of the vertical medial pterygoid plate with the horizontal floor of the sphenoid sinus forms a wedge-shaped area ("pterygoid wedge") that contains both the vidian canal and the palatovaginal canal. After complete removal of the vomer, the intersinus septum, and the sphenoid sinus floor, the sphenoidectomy should extend superiorly to be in plane with the roof of the nose and laterally to be in plane with the laminae papyracea bilaterally as described by Caicedo-Granados et al. (2010). Complete removal of the sphenoid sinus floor is performed until the cavity is flush with the clivus (Fig. 11.6).

Pterygopalatine fossa dissection

Dissection of the soft tissue contents of the pterygopalatine fossa is a prerequisite for the transpterygoid approach. After exposing the entire posterior maxillary sinus wall via medial maxillectomy, the sphenopalatine and posterior nasal arteries are divided at the level of the sphenopalatine foramen. Removal of the posterior wall of the antrum and the ascending process of the palatine bone exposes the pterygopalatine fossa with its intrinsic and bordering foramina and fissures as mentioned by Hosseini et al. (2012).

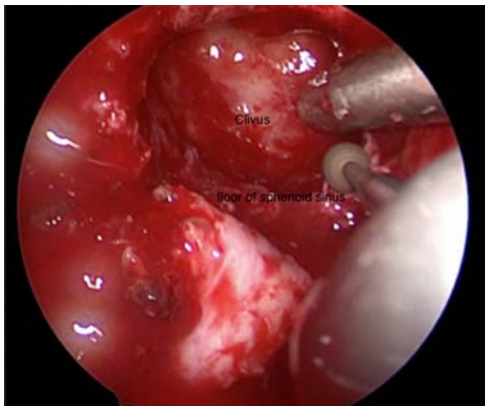

Figure 11.6
Drilling anterior wall and floor of the sphenoid sinus.

Transpterygoid dissection

A transpterygoid dissection starts with the identification of the vidian nerve proximal to the pterygopalatine ganglion, as it exits from the vidian canal. The vidian nerve can be localized following the palatovaginal canal laterally or by drilling its canal starting at the pterygoid wedge. The vidian nerve, within the pterygoid canal, courses toward the second genu of the ICA between the horizontal and vertical segments. For the most part, the vidian canal remains inferior to the second genu of the ICA; therefore initial drilling in a 3–9 o'clock orientation, helps to prevent injury to the ICA. In some cases, the superior aspect of the canal is covered just with a very thin bone or may even be dehiscent, thus exposing the nerve in the floor of the sphenoid sinus.

The maxillary division of the trigeminal nerve passes through the foramen rotundum as it courses from Meckel's cave into the pterygopalatine fossa as described by Fortes et al. (2008). The maxillary nerve can also be identified in its canal coursing the lateral wall of the sphenoid sinus. The pharyngeal end of the Eustachian tube or torus tubarius is just posterior to the pterygoid process. Removal of the pterygoid process exposes the cartilaginous Eustachian tube. The parapharyngeal segment of the ICA is posterior to the Eustachian tube in most of the cases.

All these landmarks are crucial to identify during the transpterygoid approach before tumor extirpation (Figs. 11.7 and 11.8).

Tumor extirpation

Tumor removal begins by marking out at least 1 cm margin if technically possible around the tumor. The mucosal cuts are made with needlepoint electrocautery, which helps with hemostasis. The superior and posterior dissection occurs by elevating the mucoperiosteum from the floor of the sphenoid sinus and the clivus posteriorly. Monteiro and Witterick (2014) mentioned the dissection proceeds inferiorly to the level of the soft palate, until the prevertebral musculature deep to

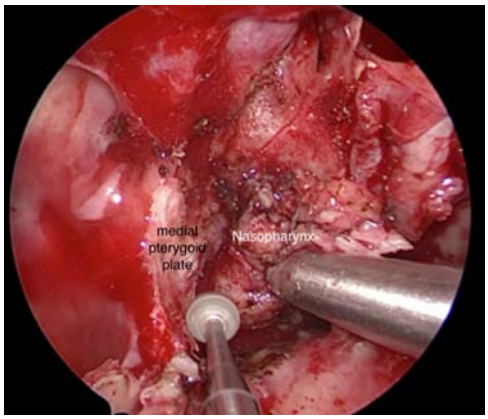

Figure 11.7
Drilling pterygoid base and medial pterygoid plate.

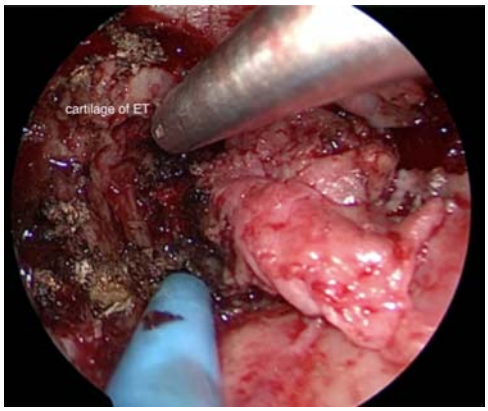

Figure 11.8
Resecting of cartilage part of eustachian tube.

the pharyngobasilar fascia and prevertebral fascia is encountered. Electrocautery or Kerrison rongeurs both can be used, and are effective at removing portions of the prevertebral muscle and fascia, as these structures are quite resilient. The muscles are included in the en-bloc resection if involved with malignancy.

Laterally, the medial pterygoid plate and pterygoid process are exposed, above which lies the sinus of Morgagni, through which passes the Eustachian tube and tensor veli palatine muscle. These structures can be excised along with the levator palatini muscle to expose the parapharyngeal tissues as mentioned by Kassam et al. (2009). The mucosa is elevated off the base of the foramen lacerum and continues anteriorly toward the posterior aspect of the fossa of Rosenmuller. The Eustachian tube cartilage laterally is identified and included in the specimen. As the dissection proceeds laterally, the Doppler probe is used to map out the ICA, thereby reducing the likelihood of inadvertent injury. Following complete tumor removal, margin status is confirmed by sending circumferential and deep margins for frozen section analysis (Figs. 11.9 and 11.10).

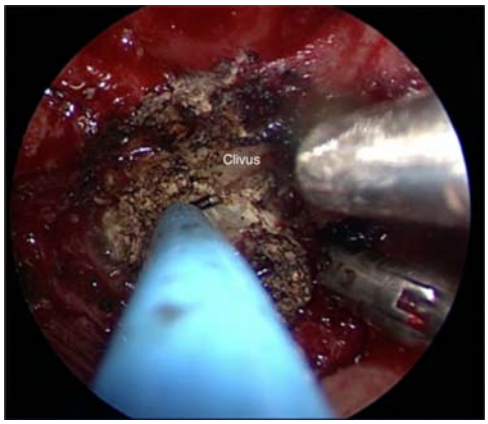

Figure 11.9 Resecting posteriorly at prevertebral muscle.

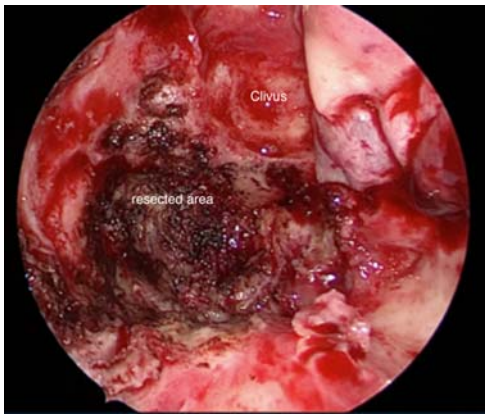

Figure 11.10 Final view of resection of right fossa of Rosenmuller (FOR) of nasopharynx.

Nasopharyngeal reconstruction

Yip et al. (2013) described reconstruction with a vascularized pedicle flap at the nasopharyngeal defect facilitates the healing of the defect, resists irradiation, and protects the ICA against exposure and blowout. The nasoseptal flap or HBF is rotated into the nasopharyngeal defect. The edges of the flap are allied well to cover the bare area especially exposed bony portion at the clivus. Absorbable gelatin sponges are placed on the flap, and a Foley catheter is used to support the nasoseptal flap against the nasopharyngeal defect. In cases where the nasoseptal flap is unavailable, a lateral nasal wall flap can be harvested for reconstruction. Other reconstructive options include healing by secondary intention or the use of regional flaps such as pedicled temporoparietal fascia flap (Figs. 11.11 and 11.12).

Following reconstruction, the nasal cavity is thoroughly inspected for hemostasis followed by careful suction of the nasopharyngeal cavity. The patient is then

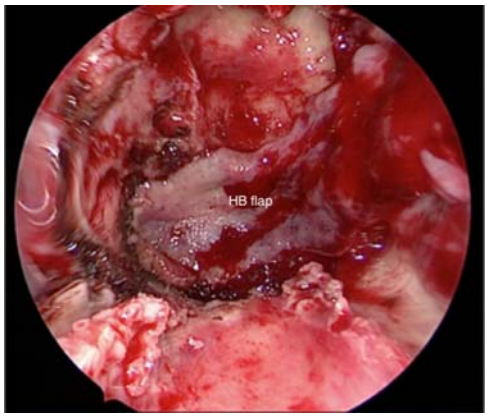

Figure 11.11
Placing of Hadad–Bassagasteguy flap (HBF) to the resected area.

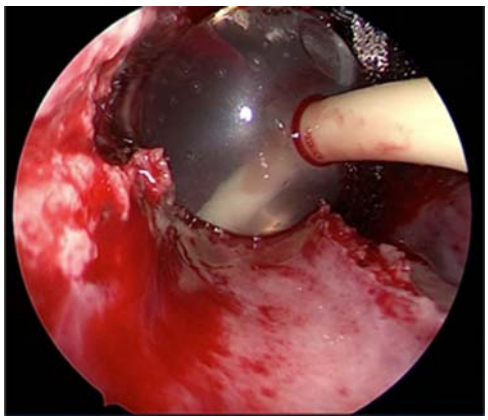

Figure 11.12
Catheter tube with balloon to hold the flap at place.

reversed from general anesthesia and transferred to the postoperative care unit for recovery. The patient is admitted to a standard ward unit and is usually discharged from hospital within 2–3 days.

Postoperative care and complications

Patients are generally seen every 2–3 weeks to undergo endoscopic examination. During these clinic procedures, decrusting is performed, paying careful attention not to disrupt the nasoseptal flap or any other flaps that are laid on. Patients are asked to liberally use sodium bicarbonate mixed with mupirocin

nasal rinses 3–4 times per day. They may experience a significant amount of postoperative crusting requiring frequent endoscopic debridement until reepithelialization occurs. This process can take up to 3 or more months, even with a nasoseptal flap. Postoperative headache is a common complaint following EETN due to exposed bone. Exposed bone covered with a pedicled nasoseptal flap reduces this incidence significantly.

Serous otitis media is another common complication encountered in the postoperative period. This can be managed by myringotomy with tympanostomy tube insertion or amplification with hearing aids.

Postoperative epistaxis can occur secondary to bleeders that are not secured during the surgery especially when the nasoseptal flap is not in used, and the bleeding is from the posterior septal artery. Other possible surgical sequelae include xerophthalmia secondary to injury of the vidian nerve, numbness in the V2 distribution, skull base injury including cerebrospinal fluid leak, and injury to the ICA in either its parapharyngeal, petrous, or clival portions as described by Valentine and Wormald (2011).

Conclusion

In conclusion, EETN is a feasible approach for the surgical treatment of selected primary and recurrent nasopharyngeal tumors. The surgical technique requires a trained and experienced team with specialized instruments. This technique shows relatively low morbidity with promising preliminary outcomes and local control of the disease that is comparable to conventional techniques.

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12

Nasopharyngeal carcinoma screening and prevention programs

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Introduction to screening

The World Health Organization (WHO) definition of screening is identification of unrecognized disease in an apparently healthy individual population, based on presumption. It involves the utilization of rapid, effective, low-cost tests or examinations that can be applied on the population at large. Implementation of effective screening programs involves mobilization of infrastructure, financial, and human resources.

Upon confirmation of a diagnosis of NPC in a patient, both patient and family go through the initial phase of shock, subsequent bargaining, depression, and acceptance. Once they have come to terms with the condition and treatment begins, thoughts and attention turn toward the rest of the family. The usual questions include: "Can my son/daughter/sibling/develop the disease too? Is there a screening method to detect the disease in advance? Is there a method to prevent the disease? How frequently and for how long should one keep checking to ensure that they will not develop the disease?" To answer these questions, it is imperative to quantify the risk of NPC among first-degree relatives, as well as to identify screening tools and prevention methods for individuals of high-risk families and the population at large.

Rationale for screening

As mentioned, screening is relevant within endemic high-risk populations or in first-degree relatives of patients with NPC. The incidence of NPC in people

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from the Southern province of China, particularly among the Cantonese from Guangdong, is higher than Chinese from other provinces. The incidence is 10–30 times greater in people of this descendance than in the local population, even if one has migrated and settled elsewhere (Mousavi, Sundquist, & Hemminki, 2010; Parkin & Iscovich, 1997). Evidence shows that the risk of developing NPC is 4-to-8 folds higher among first-degree relatives of a person with NPC (Petersson, 2015; Yuan et al., 2000).

Liu et al. (2017) performed a large population-based case-control study involving a cohort of 40,781 first-degree relatives of NPC patients. They demonstrated that the risk of developing NPC was more than 4-fold in first-degree relatives of patients (OR = 4.6, 95% CI 3.5–6.1), compared to those without a history of NPC in the pedigree. The excess risk was significantly higher for a maternal history and sibling history as compared to paternal or parent history. The lifetime cumulative risk of developing NPC was 3.7% (95% CI 3.3% to 4.2%) up to the age of 74 (Liu et al., 2017). In Hong Kong, for example, which is an endemic area with high prevalence of NPC, the incidentrate of NPC among cancer families is 7.2% (Yu et al., 1986). This evidence suggests the necessity of screening for early detection especially in high-risk populations in endemic areas.

The pertinent problem with NPC is delay in diagnosis. Tumors in early stages usually are asymptomatic or present with vague non-troubling symptoms. Hence, more often than not, patients tend to present at an advanced stage that results in a poor prognosis. Effective screening methods not only provide early detection of the disease, but can also indirectly result in a decrease in the morbidity and mortality rate as a whole.

Epstein–Barr virus serology as a screening tool

Epstein–Barr virus (EBV) infection has classically been associated with NPC. Antibodies against EBV are usually present and can be detected in serum long after the infection with EBV, and well before the development of cancer. Hence the convenience of testing the presence of such antibodies for screening purposes. People with elevated antibody levels have a 5-to-30 times higher risk of developing NPC (Chien et al., 2001). The various antibodies used in a clinical setting are immunoglobulin A (IgA) antibodies against viral capsid antigen (VCA), early antigen (EA), and EBV nuclear antigen 1 (EBNA1) (Ji et al., 2014). Antibodies measured by ELISA have higher diagnostic accuracy than pure immunofluorescence assays. IgA/VCA and IgA/EA are used in combination as the former is more sensitive (89%), whereas the latter is more specific (97%) (Tsang et al., 2004).

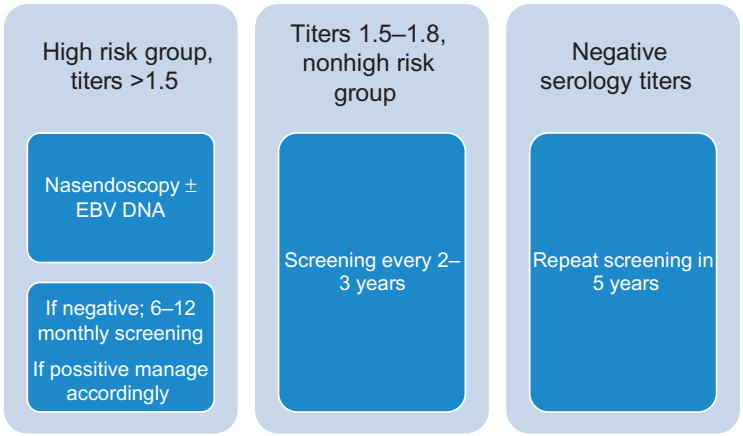

Figure 12.1 Suggested screening strategies using serology titers.

Cao, Simons, and Qian (2011) described the screening interval for persons with positive EBV serology. A regular screening interval and follow-up of every 6−12 months was recommended for a person from a high-risk population with significantly elevated titers, significant here meaning combination titers of 1.5 or more. On the other hand, a person with a negative titer may be screened once every 5 years. Whereas a population with elevated titers of between 1.5 and 1.8 but who do not meet the high-risk criteria may be screened at an interval of 2−3 years (Cao et al., 2011). Patients with positive titers proceed to have a nasendoscopy, and biopsies are performed for suspected lesions. Fig. 12.1 depicts the screening strategy as described above.

The limitations of antibody testing for screening purposes are as follows.

A low percentage of the population with positive antibodies goes on to develop NPC later on in life. The specificity of serology studies, especially IgA/VCA, is reduced because these antibodies may be detected in normal healthy individuals as well (Wu, Li, & Pan, 2018). Another limitation of antibody testing is the persistently elevated levels of antibodies in apparently healthy individuals that makes differentiating them from potential patients challenging.

A more accurate tool for NPC detection is the measurement of the EBV DNA load in the population. EBV DNA and IgA/VCA have a concordance rate of 84.4%, making EBV DNA a better predictor of disease in the presence of raised antibody titers (Chen et al., 2015). Hence patients with raised antibody titers can be tested further for their plasma EBV DNA load or, high-risk patients with strong family history may benefit from direct testing of EBV DNA instead of serology. Cost issues would be a potential hurdle when EBV DNA testing is considered at a large scale for population-based screening purposes.

Epstein–Barr virus DNA for screening

p53, "the guardian of the human genome," is the most investigated marker in head and neck cancer. The p53 pathway is activated to arrest the cell cycle for DNA repair to commence once damage has been detected (Dahiya & Dhankhar, 2016). Unlike other squamous cell carcinomas of the head and neck region, overexpression of cyclin D1 protein is the main molecular event in NPC. Just as human papilloma virus (HPV) presence can be detected in oropharyngeal cancer via p16 immunohistochemistry staining or DNA in situ hybridization (ISH), so is detection of EBV in NPC.

EBV infection may be demonstrated by the presence of EBV DNA in tumor cells or by the presence of EBV in clonal episomal form (Han et al., 2012). Numerous studies have emerged on the role of circulating EBV DNA as a marker of the disease for diagnostic purposes, for population screening, as a prognosticator, or for post-treatment surveillance (Lo et al., 1999). In 2004 Lin et al. published the first such quantitative findings (Lin et al., 2004). The key underlying principle is that since EBV DNA is constantly shed from tumor cells into the circulating bloodstream, it makes sense to test for its elevated levels.

The problem with EBV DNA is that it is detected in both individuals with the disease and healthy persons (Kanakry & Ambinder, 2015). The principal differentiating factor is in the molecular characteristics of the DNA, wherein the fragment lengths were found to be longer and higher in quantity compared to that in healthy individuals (Lam et al., 2018).

The role of EBV DNA as a screening tool is questionable due to the length time and lead time biases. However, a meta-analysis by Han et al. in 2012 demonstrated a statistically significant role of EBV in such circumstances. The accuracy of EBV detection in plasma was found to be higher than in serum. The pooled specificity, sensitivity, positive likelihood (+LR), and negative likelihood (−LR) were 0.94 (0.92−0.96), 0.78 (0.75−0.81), 12.51 (7.88−19.86), and 0.14 (0.05−0.37), respectively, for plasma EBV DNA detection (Han et al., 2012). Chan and Lo (2002) demonstrated the positive role of EBV DNA as a screening tool in the high-risk Chinese population (Chan & Lo, 2002). Plasma EBV DNA has a sensitivity rate of 86.8% with a negative predictive value of 99.3%, making it a highly feasible tool to diagnose NPC (Ji et al., 2014). But it becomes less accurate beyond 1 year from baseline testing.

Nasendoscopy

Nasendoscopy with biopsy remains the gold standard for diagnosis of NPC. This is true if a lesion is present. The act of performing an invasive procedure in an asymptomatic healthy individual for screening purposes may be

questionable. Nasendoscopy is recommended for screening purposes in people from high-risk populations, with significant family history and elevated antibody or DNA levels. In doubtful cases that may harbor a submucosal lesion, narrow band imaging may prove useful to detect the tumor, owing to the subtle abnormal changes in the mucosal vascularity.

Circulating biomarkers

New molecular markers have mushroomed with the advent of gene sequencing technology and ISH techniques. As a result, mutation of a gene in the form of over-expression or deletion can be identified in certain cancer types. The presence of certain markers in high quantities in the circulating blood levels can also be detected in such patients. These findings in turn are applied from bench to bedside in the form of genetic testing, screening, surveillance post-treatment, targeted therapy, and vaccines. Chemokine CCL27 is one such marker that can differentiate a potential NPC patient from a healthy individual in a group of seropositive individuals to EBV IgA/VCA antibodies (Mao et al., 2018). Further larger studies are still needed to explore the potentials of molecular markers as screening tools.

Effectiveness of screening

Screening strategies in high-risk population groups have demonstrated improved survival rates attributable to early detection of the disease (Ng et al., 2009). Ng et al. (2009) analyzed 1200 patients over a period of 11 years. Those with first-degree relatives with NPC at the time of diagnosis were 2 years younger than their counterparts with a negative family history (47.9 vs 49.8, $p = .04$). Patients with a positive family history were detected at an earlier stage (37% vs 23%, $p < .01$) and had a 5-year overall survival (OS) rate that was better than those without a first-degree relative with the disease (79% vs 69%, $p < .01$). Their study essentially emphasized the need for screening of first-degree relatives of patients who hail especially from endemic areas.

Ng et al reemphasized this point in 2010 by publishing another retrospective review on annual screening outcomes of asymptomatic relatives of NPC patients over the same 11-year period. EBV serology and nasendoscopy were utilized as modes of screening. The sensitivity and specificity of EBV serology in this program were 88.9% and 87.0%, respectively. They demonstrated that the screening program resulted in earlier detection of cancer with 59% of people detected in early stages ($p < .001$), (Ng et al., 2010). Although statistically insignificant, disease-specific, and OS rates were higher in the screened group.

In Malaysia, NPC is the third most common cancer among men. The Ministry of Health Malaysia published a Health Technology Assessment Report on NPC

screening in 2011. No recommendations were made for a population-based screening as there were insufficient evidence. Neither the risk-benefit ratio nor cost-effectiveness of a screening program could be justified (Ministry of Health Malaysia, 2011).

Yang, Wu, Zhou, and Chen (2015) attempted to analyze the effectiveness of screening in terms of mortality reduction while looking at the impact of screening on quality of life and cost effectiveness among others. This Cochrane review suggested that there was insufficient level I evidence to recommend any solid population-based screening programs (Yang et al., 2015). Large high-quality controlled trials are warranted to establish definite conclusions.

Rationale for prevention

NPC is attributed to significant environmental risk factors and hence is a preventable disease. The environmental risk factors that are well known to NPC include high levels of volatile nitrosamines and butyrate derivatives in preserved food, especially in salty-preserved fish, pickled vegetables and dried meat, oncogenic viruses, alcohol, and smoking. The effort to increase public awareness on NPC and its associated risk factors will help in detecting the disease at early stage. Subsequently, correct and proper treatment can be instituted.

In the endemic region of NPC including Malaysia prior studies have shown that the lack of awareness and knowledge of primary healthcare workers is one of the main reasons for delayed diagnosis. Given that the presenting stage is the most important prognostic factor, appropriate training of all physicians treating NPC and seeing patients with NPC is critical. Importantly, the risk of death in this group was significantly higher when compared with patients who were immediately referred or received a follow-up appointment, proving that screening and early detection is crucial in the management of NPC.

High-risk group

Targeting high-risk groups for screening and prevention programs is effective at combating NPC. The risk factors for NPC include EBV virus, excessive consumption of salted fish and pickled vegetables, and long-term smoking.

First-degree relatives represent the closest genetic association to an affected NPC patient. Thus it is prudent to follow first-degree relatives of affected patients. First-degree relatives of NPC patients are at a higher risk of developing the disease.

This statement is best highlighted by a study by Loh, Goh, Lu, Hsieh, and Tan (2006), which involved 200 consecutive patients in Singapore that were diagnosed with NPC from 1998 to 2003. They only included patients newly

diagnosed as having undifferentiated carcinoma (World Health Organization type 2b) in the study. All patients who were unable to provide information about the existence of malignancy in their first-degree relatives were excluded. The information provided on the first-degree relatives was confirmed with the Singapore Cancer Registry. The patients were divided into two groups. Group 1 consisted of patients with familial NPC. Group 2 was composed of the remaining patients (those with nonfamilial NPC). Familial NPC is defined as NPC occurring in two or more first-degree relatives within the same family. Hence, the patients in group 1 had at least one first-degree relative with NPC. First-degree relatives include parents, siblings, or children. The rate of familial NPC in this study was 15.5%. Applying the definition of familial NPC to other studies, the rates vary from 5.8% to 19% in endemic regions (Loh et al., 2006).

Nasopharyngeal carcinoma is an infection-related cancer strongly related to Epstein–Barr virus (EBV). EBV is a well-recognized carcinogen that has not only been implicated in the etiology of NPC but several malignancies of both epithelial and lymphoid origins, including gastric cancer, non-Hodgkin's lymphoma, and Hodgkin's lymphoma. EBV is strongly associated with the etiology of NPC, Hodgkin's and Non Hodgkin's lymphoma and gastric carcinoma. Now EBV-associated cancers account for approximately 1.5% of all cancers worldwide and represent 1.8% of all cancer deaths (Cao, 2017). Thus it is imperative to developed an effective screening and prevention program globally focusing, in particular, on the endemic region of NPC.

Of note, at this juncture, the early detection and screening of the lytic proteins and EBV DNA have been applied to clinical and high-risk populations. For EBV-based secondary cancer prevention, Cao et al. suggested further confirming the effect of population-based NPC screening by EBV-related serum EBV VCA/IgA antibodies and quantitative EBV DNA loading methods, which can be used to identify high-risk individuals and diagnose early-stage patients (Cao, 2017).

Apart from China, Hong Kong, and Taiwan, Indonesia also has a large number of NPC patients and the majority of them present with late-stage disease. Additionally, physicians and clinicians often fail to identify and diagnose NPC early in the disease process, hence contributing to the late-stage disease at initial presentation. Implementation of educational and training programs on the risk factors, clinical presentation, and necessary tests required is vital to increase the detection rate of NPC.

The first multicenter study conducted in Indonesia by Fles, Wildeman, Sulistiono, Haryana, and Tan (2010), demonstrating that the effect of additional training for NPC symptoms and diagnosis,can be of value as knowledge of the healthcare workers working in the primary healthcare centers (PHCC) was still increased after 1½ years. They stated that the long-term effect was only tested in Yogyakarta since the area is more transparent and easy to visit. They found that there were no differences in short-term knowledge increase

between participants in the different cities and the results obtained in Yogyakarta are representative of those for the other cities where the NPC awareness study was conducted. Nevertheless in the long term, their knowledge consistently improved, suggesting that the program is effective for different health professions working in the PHCC. In addition they revealed that the nurses and midwives had less knowledge than general practitioners (GPs), but after the Train–The–Trainer program it was increased to more than the knowledge of the GPs, suggesting that different professions have different learning curves (Fles et al., 2016).

Education programs targeting dietary habits

The nonviral exposure most consistently and strongly associated with risk of NPC is consumption of salt-preserved fish, a traditional staple food in several NPC-endemic areas. In studies of Chinese populations, the relative risk of NPC associated with weekly consumption, compared with no or rare consumption, generally ranged from 1.4 to 3.2, whereas that for daily consumption ranged from 1.8 to 7.5. NPC risk is also increased with consumption of other preserved foods, including meats, eggs, fruits, and vegetables, in southern Chinese, Southeast Asians, North Africans/Middle Easterners, and Arctic natives as well as in low-incidence northern Chinese and the US population Saltpreserved foods are a dietary staple in all NPC-endemic populations. This dietary pattern may explain part of the international distribution of NPC incidence (Chang & Adami, 2006).

The dietary habit can be an ideal prevention program for the local population. For instance, education on healthy dietary habits should start at primary school years, targeting both public and private schools. Sessions on related diseases and cancers can be introduced to students and teachers in staggered manner so that the knowledge and awareness will be maintained over longer periods. It is important to cultivate a culture of healthy eating in young. Multimedia, brochures, campaigns, workshops can be jointly organized to promote the awareness about NPC.

In southern China, intake of salted fish and other preserved foods is particularly high among boat-dwelling fishermen and their families, the population subgroup at the highest risk of developing NPC. Salted fish is consumed at early in infancy and frequent feeding of infants especially in the Cantonese population and in families of lower socioeconomic status. Childhood exposure, especially at weaning, appears to be more strongly related to NPC risk than adulthood exposure. Further, increasing duration and frequency of consumption are independently associated with elevated risk of NPC. Comparing persons who were weaned on salt-preserved fish to those who were not, the relative risk of NPC ranged from 1.7 to 7.5 (Chang & Adami, 2006).

In contrast to preserved foods, frequent consumption of fresh fruits and/or vegetables, especially during childhood, has been associated with a lower risk of NPC. Some studies have found inverse associations with intake of specific fruits or vegetables including carrots, Chinese flowering cabbage, green leafy vegetables, fresh soybean products, citrus fruit such as oranges or tangerines, or with dietary intake of vitamin E or C or serum levels of carotene. The apparent protective effect of fruits and vegetables may be attributed to its antioxidant effects by preventing the nitrosamine formation and its other anticarcinogenic properties (Chang & Adami, 2006).

Low-risk groups

NPC awareness programs are effective at increasing the knowledge of healthcare workers and patients. Programs like this help to diagnose NPC suspects at an earlier stage through referral at the onset of the disease. Once the medical specialists are trained, public awareness should be expected to improve. Patients' attitudes toward the health care system, resulting in a delay in diagnosis, should be improved by educational and awareness programs. The risk factors of smoking and alcohol consumption is vital to be addressed as these factors are preventable. The campaign of stop smoking, reduce alcohol intake and live a healthy life can be easily conducted and is effective in preventing the disease and tumor, not only NPC but other head and neck cancers too.

In contrast, there was no evidence that undifferentiated or nonkeratinizing carcinomas were associated with cigarette smoking. Similarly, a significant increase in risk was observed for the heaviest alcohol consumers (21 or more drinks/week) only for differentiated squamous cell carcinomas (OR = 2.9; 95% CI 1.2–6.9). The association with cigarettes and alcohol appeared to be stronger among persons 50 years or older.

There was a substantial and rapid decrease in risk with cessation. When the type of cigarette was examined, we found little difference in risk between persons who were lifetime nonfilter cigarette smokers and those who began smoking nonfilter cigarettes and switched to filter cigarettes. Both groups experienced approximately twice the risk as persons who were lifetime filter cigarette users after controlling for the frequency and duration of cigarette smoking. There was no risk differential between persons who usually smoked filter cigarettes versus those who usually smoked nonfilter cigarettes.

General population-based programs

GPs working in a PHCC are the firstline of care for patients in need of medical attention. For correct and early diagnosis of NPC these GPs need to be able

to recognize early-stage NPC symptoms. Wildeman et al. (2012) conducted a study in Yogyakarta, Central Java, and showed that knowledge of NPC and related symptoms among GPs working in PHCCs is insufficient for recognition of NPC and to initiate the appropriate referral. Their other on-going studies in the Yogyakarta province and the study from the Jakarta region revealed that similar to the Yogyakarta region, insufficient knowledge to refer NPC suspects to the hospital. Besides confirmation on the lack of knowledge, they investigated if improvement of knowledge is possible with the introduction of a focused education program. This is the first study that has examined the effect of different teaching methods on Indonesian GPs about NPC (Wildeman et al., 2012).

The study by Fles et al. (2010) revealed that knowledge on essential aspects of NPC among GPs in the Puskesmas in Yogyakarta, Indonesia is poor. This lack of basic knowledge of NPC may be a fundamental cause for late-stage discovery of the disease by GPs. The majority of doctors believed that the incidence of NPC in their region was at least a tenfold lower than the estimated incidence. In addition with the lack of knowledge about early NPC symptoms and risk factors, they concluded that the knowledge about NPC of these doctors is not sufficient (Fles et al., 2010).

Enlarged lymph nodes in the neck is the most common symptom of NPC presentation. The study by Fles et al. regularly see patients with enlarged neck lymph nodes, but stated they see very few cases of NPC. Combining the overall answers given regarding early symptoms and incidence, the authors concluded that GPs often do not consider NPC when they see a patient with enlarged neck lymph nodes or other symptoms pointing toward NPC (Fles et al., 2010). This again contributes to delay in diagnosis of NPC.

Additional training sessions can increase knowledge of key symptoms, in particular early-stage symptoms. By providing additional training on NPC and its early symptoms, it will increase the diagnosis and referral of patients with early stage NPC. An early detection program for NPC was proven to be effective for down staging the cancer, but the training was not sufficient. Thus more training programs should be implemented with the assistance of government agencies and nongovernmental organizations, in order to improve awareness and facilitate early diagnosis of NPC.

Conclusion

NPC is generally uncommon except in certain endemic areas around the world. As is known, it may not be possible to change the non-modifiable risk factors to reduce the risk of NPC in the population at large. However, targeted prevention programs aimed at reducing the modifiable risk factors (i.e., tobacco smoking and consumption of salted fish) may prove beneficial on a

broader population-based scale. Such programs are already in place in countries like China and Taiwan and could be adopted by other places with a high rate of NPC (e.g., Malaysia and northeast India).

A structured awareness program tailored to the general public, as well as medical education on early detection for medical practitioners may encourage higher pick-up rates of the disease at an early stage, if not prevent it.

With regard to screening, various known screening modalities have stood the test of time be it nasal endoscopy, serum IgA VCA, and EA or EBV DNA. The future of diagnosis, treatment, and surveillance lies in the use of molecular markers, as these not only prove beneficial for screening, but for surveillance post-treatment, and for the development of targeted therapies and vaccines. The potential limitations anticipated are the availability of such tests, the expertise for interpretation, the time factor for specimen processing and obtaining verified results, as well as the biggest hurdle being the high cost factor that will eventually be absorbed by the patient. These may in turn discourage patients from spending on such tests. Quick, effective, pocket-friendly, highly specific, and sensitive screening tools may be more easily accepted by the population.

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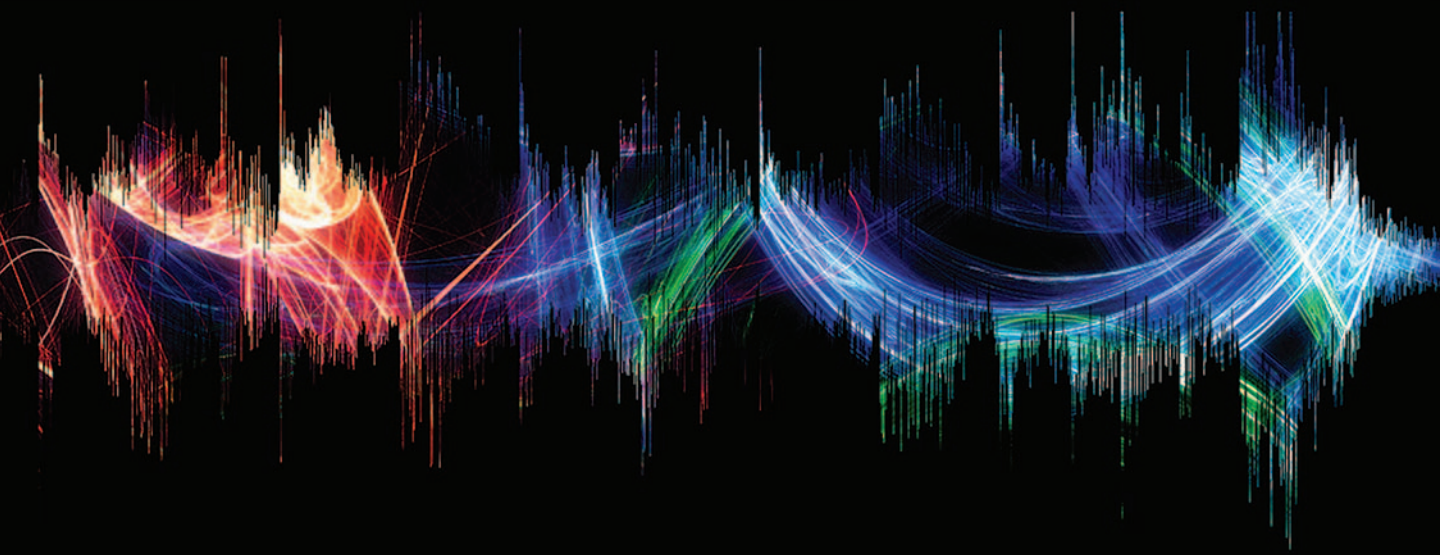

13

Survival rates and quality of life of nasopharyngeal carcinoma patients

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Introduction

Nasopharyngeal carcinoma (NPC) is well known as an endemic disease in the East Asia region and certain geographic locations such as North Africa, Arctic, and Middle East countries and the incidence continues to rise. At present, NPC not only affects older age groups but also younger age groups have been afflicted with this disease. Despite the increase in incidence, the overall survival (OS) of NPC patients remains poor despite multimodality treatment and newer methods of assessment. The quality of life (QoL) of NPC patients requires additional attention as the majority of these patients suffer from numerous treatment-related morbidities and sequalae either from chemoradiation or salvage surgery for its treatment as well as from the disease itself.

QoL and its assessment has emerged as an important key issue in healthcare especially in the setting of chronic diseases and cancers. Traditionally, the medical care of cancer patients has mainly focused on survival rates. However, the survival rate assessments do not provide knowledge and understanding of the patient's mental health and emotional well-being, which is of primary importance for comprehensive cancer patient care and should be regarded as high priority. At this juncture, there is a paradigm shift of evaluation of treatments effects in chronic diseases and cancers that not only focuses on survival rates but more importantly on the QoL of these unfortunate patients.

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The spectrum of QoL of any given cancer patients encompass the personal feelings in the domains of physiology, psychology, and the society. These domains are heterogenous and overlap with each other and require understanding by the managing team. The QoL of cancer patients not only reflects the treatment effects but also the overall rehabilitation effects. The issues of survival and QoL among NPC patients require special attention from all health professionals and workers as more young patients are being diagnosed with the disease and may suffer from a myriad complications from its treatment and the disease itself. This in turn will add significant health and economic burden to any given institution.

There are multiple factors that can influence the survival and QoL of NPC patients and can be factors that exist before starting treatment, during treatment as well as after treatment. Pretreatment symptoms such as loss of appetite, difficulty in opening the mouth, and the presence of feeding tubes are related to advance-stage tumors and can adversely affect patient survival and QoL. The presence of fatigue, depression, xerostomia, and other unwanted complications due to treatment-related side effects are well-known factors that impair patient QoL and cause significant economic burden not only to institutions but also to a country as a whole.

Survival rates of nasopharyngeal carcinoma patients

Due to significant number of NPC cases diagnosed every year, survival has been the topic of discussion among scientists and healthcare providers over the past decade. This can be seen by the many articles published on this issue, the majority of which originate from China due to a significant number of cases being reported as compared to other countries. Survival rate will reflect prognosis of a certain tumor as well as treatment efficacy. By knowing the survival rate of NPC patients, optimum effort can be made to improve patient survival. There are many factors that can significantly affect survival, and these factors can be categorized as tumor-, treatment-, and patient-related factors. Of all these three factors, the treatment factor has been widely studied and with the advancement of technologies and better research quality, more targeted treatment modalities can be developed.

In the research field, there are many abbreviations used to indicate survival. Among them are OS; disease-free survival (DFS); disease-specific survival (DSS); progression-free survival (PFS); distant metastasis-free survival (DMFS); and many others. OS is defined as the time from diagnosis to death resulting from any cause. DFS is defined as the time from diagnosis to local failure, nodal failure, systemic failure, or death resulting from any cause, whichever occurred first. DSS is defined as the time from diagnosis to death from NPC. In research, survival estimations have been performed using the method of Kaplan and Meier, univariate analysis using the log rank test, and multivariate analysis using the Cox proportional hazards model.

Tumor stage is strongly related to prognosis and survival. A new staging system was developed by Kang et al. based on Magnetic resonance imaging (MRI) and intensity-modulated radiotherapy (IMRT) treatment in an effort to optimize the management of NPC. The proposed new staging is as follows: stage I (T1N0M0); stage II (T1N1M0 and T2N0M0); stage III (T1N2M0 and T2N1-2M0); stage IVa (T1-2N3M0); and stage IVb (TxNxM1) (Kang et al., 2017). Advanced stage NPC was known to have poorer survival as compared to early stage. Most patients are in advanced stage NPC at the time of diagnosis and it accounts for 60%–70% of all NPC patient (Chen, Hu, et al., 2012; Chen, Mao, et al., 2012). Public awareness is crucial to detecting an early stage tumor because treatment outcome and survival is usually excellent for this group of patients. Advanced stage NPC can be in two forms: locoregionally advanced without distant metastasis or with distant metastasis. A large study cohort consists of 720 patients of NPC with stage 1 disease showed a remarkable improvement in OS and DFS over 20 years. This is the longest follow-up that has been carried out by any study. The author attributed these findings to role IMRT played in reducing the locoregional failure rates.

Advances in imaging technologies have also played a role in changing survival trends among NPC patients and other head and neck cancers (HNCs). The computed tomography scan was the earlier method of staging the disease but has been largely replaced by MRI and positron emission tomography scan, which is highly sensitive in picking up bony and soft tissue disease in difficult areas like parapharyngeal space, skull base, and deep cervical nodes. Subsequently, this methods enhances tumour surveillance and delineates the tunour tissues and its surrounding structures better, which allows for a precise targeted treatment and improved therapeutic ratio.

Treatment is another important factor that significantly affects survival in NPC. Due to its location and high sensitivity to radiation (more than 95% are nonkeratinizing), radiotherapy (RT) is the primary treatment modality for NPC. The radiation is used as the primary treatment modality either for early or advanced staged NPC. IMRT has slowly replaced the two-dimensional radiotherapy (2DRT) and three-dimensional conformal radiotherapy in most centers around the world due to its efficacy and reduced morbidity. It allowed slightly higher radiation dose to be delivered to the primary tumor while protecting normal tissues, which significantly improves the local control rate and increases the 5-year survival rate to 80% (Kang et al., 2017). The addition of chemotherapy drugs to the standard RT treatment can improve patient survival particularly for some stage II NPC and most of stage III and IV NPC. Studies have shown that the addition of concurrent chemotherapy to RT is the recommended treatment approach for locoregional advanced NPC because it can significantly improve the survival of this group of patients (Blanchard et al., 2015) and has been shown to benefit OS either with or without adjuvant chemotherapy (Chen, Hu, et al., 2012; Chen, Mao, et al., 2012; Langendijk, Leemans, Buter, Berkhof, & Slotman, 2004).

Studies have also shown that combination of RT and chemotherapy reduces the risk of mortality by 18% and increases 5-year survival by 4% to 6% (Al-Sarraf, & Reddy, 2002). Concurrent chemoradiotherapy (CCRT) also has survival benefits for selected patients with distant metastasis at first diagnosis (Yin, Zhang, Wang, Wang, & Yuan, 2017).

Induction chemotherapy given before the start of CCRT has become the standard of treatment in locoregionally advanced NPC with or without metastasis in some centers. A meta-analysis showed that compared with CCRT alone, the addition of neoadjuvant chemotherapy to CCRT reduces distant failure in locoregionally advanced NPC patients (OuYang et al., 2013). Another meta-analysis confirmed that neoadjuvant chemotherapy followed by CCRT improves PFS and OS (Wang et al., 2016).

In cases of metastatic NPC treated with palliative chemotherapy, the prognosis of patients remains poor. However, subset of patients treated with chemotherapy plus the definitive dose of radiation to the primary diseases showed favorable outcomes. Patients with single-organ and tissue-region metastases showed better outcomes with the addition of radiation especially IMRT than those with extensive disease metastasized to multiple organs. Yin et al. reported that for metastatic NPC treated with chemotherapy and radiation (especially IMRT) has survival benefits especially, those with single metastases (Yin et al., 2017). In addition, the literature also documents sites of metastases associated with survival, where the presence of liver metastases carries poor survival compared to lung and bone metastases.

Another factor that affects NPC patient survival is the age of the patient at the initial diagnosis. There has been substantial controversy in the literature on the age at diagnosis as a factor in patient survival rates. Some reports have claimed that younger patients tend to have better survival than those diagnosed at an older age. However, other characterization and details of the NPC should not be underestimated as it also imparts significant prognostic factors. These may include the tumor bulk or the T stage, the presence of neck nodes, and the size of nodes.

Wu et al. showed in a multivariate analysis that age was an independent prognostic factor for NPC-related mortality. Increasing age at diagnosis of NPC was associated with a significantly higher risk of NPC-related mortality. Compared to patients aged 1–19, the hazard ratios for patients aged 20–39, 40–59, 60–79, and 80–99 were 2.030 [95% confidence interval (CI) 1.004–4.104], 2.871 (95% CI 1.474–5.590), 4.443 (95% CI 2.273–8.683), 12.024 (95% CI 5.855–24.695), respectively. The T category and N category were also independent prognostic factors for CSS; gender, ethnicity, grade, and chemotherapy status were not associated with CSS in univariate analyses (Yin et al., 2017). The effect of age on survival can be related to other factors. For example, younger patients can better tolerate the treatment-related morbidities.

Multiples studies have investigated the role of systemic inflammatory response markers and have shown their potential role in predicting the prognosis of NPC patients. Systemic inflammatory response markers have been shown to play a vital role in tumor formation as well as tumor progression and can influence the prognosis of many types of cancers. These markers include monocytes, leukocytes, lymphocytes, neutrophils, platelet count, neutrophil-to-lymphocyte ratios (NLR), and platelet-to-lymphocyte ratio (PLR). Lymphocytes predominantly involved in the host immune response to tumor whereas neutrophils responsible for mediating the angiogenesis growth factors, chemokines, and proteases that play roles in carcinogenesis and tumor progression. In short, lymphocytes eliminates the tumor but neutrophil promotes tumor formation, thus high lymphocytes and low neutrophils count carries good prognosis of any given cancers. These markers have drawn the interest of researchers who are continuously trying to find a novel biomarker that can be effectively used in treating NPC.

Additionally, the NLR and PLR can also be used to predict the recurrence of NPC. A study by Jiang et al. showed that high NLR and PL Rare associated with poor prognosis factors for NPC (Jiang, Qu, Pan, Huang, & Zhu, 2018). Several other studies have also reported that the lymphocyte counts the NLR and PLR were associated with survival outcomes of South East Asian population who had NPC. These studies further showed that patients with high NLR or low lymphocyte counts have poor prognosis.

The QoL of NPC patients deserves special attention as more individuals are being diagnosed with the disease. In certain geographic areas, the disease may be diagnosed early but in other regions in South East Asia such as in Malaysia, Indonesia, Thailand, and Philipines, patients are commonly diagnosed at stage IV disease. The primary treatment modality for patients with advanced disease is mainly palliative with chemoradiation and occasionally a salvage neck dissection is necessary. All these treatments can result in both positive and negative effects on patient survival and QoL.

Sun et al. (2018) highlighted in his study of 186 patients with locally advanced NPC with either IMRT alone or IMRT in combination with chemotherapy and reported that chemotherapy was identified as the only factor that significantly correlated with patient survival. The other clinical factors included in the analysis were age, gender, radiation doses, radiation treatment time, tumor classification and histopathological types, cigarette and alcohol use as well as concomitant diseases. They also noted that the inclusion of chemotherapy in the treatment plan delayed the occurrence of distant metastases and local recurrence of NPC. Importantly, they further elucidated that both static and dynamic IMRT were used in the study as monotherapy or used in combination with chemotherapy but no statistical difference was found between the two types of IMRTs in short-term response, disease progression, and patient survival (Sun et al., 2018).

In the study of 164 patients with NPC, the advantage of combining chemotherapy with high-dose IMRT, became gradually more apparent with time, with a 3-year OS rate of 92.1%, compared with an OS rate of 72.7% for patients treated with IMRT alone ($p = .013$). The 5-year OS rate was 90.9% compared with an OS rate of 68.2% for patients treated with IMRT monotherapy ($p = .006$). This finding correlated with significantly prolonged local recurrence-free survival (LRFS) rates and DMFS rates for patients treated with chemotherapy. Patients treated with IMRT and induction, concurrent, and adjuvant chemotherapy also showed a trend of an increased 5-year OS, LRFS, and DMFS rates, despite the lack of statistical significance, due to the limited number of patients in this group. The results of this study highlight the importance of combining chemotherapy with high-dose IMRT in the management of locally advanced NPC (Sun et al., 2018).

Guo et al. documented that the presence of plasma Epstein–Barr virus (EBV) DNA in patients with NPC was associated with apoptosis of tumor tissues and also had the same polymorphism as the primary site indicating that plasma EBV DNA is derived from cancer cells rather than from inflammatory cells (Guo et al., 2019). They further noted that the available current data establishes that the plasma EBV DNA is closely related to the extent of tumor, it has almost become a tumor marker for predicting prognosis in patients with NPC and was also identified as an independent prognostic factor for patients with NPC in their current study. However, they found that highly varied EBV DNA cut-off levels are used in different studies. They reported that other studies have observed that <1500 copies/mL EBV DNA before treatment had prognostic significance for poorer disease recurrence and OS. In contrast, another study reported that a pretreatment EBV DNA load of 8000 copies/mL was a more powerful prognosticator for OS. Guo and colleagues reported that <4000 copies/mL (vs ≤ 4000 copies/mL) was associated with improved survival (Guo et al., 2019).

The study by Chen et al. highlighted that the univariate and multivariate analyses confirmed that tumor volume (TV) is a significant prognostic factor for survival rate in NPC, with TV >50 mL predicting poor treatment outcome. T stage and N stage were also identified as adverse prognostic factors for 5-year OS rate. In the univariate analyses, NV >25 mL was also shown to predict poor prognosis, but it did not maintain statistical significance in their multivariate analysis. The 5-year locoregional relapse-free survival, distant failure-free rate, DFS, and OS rates exhibited a distinct downward trend. They further stated that, the cumulative survival curves becomes distinct during follow-up period. These results indicate that this new prognostic indicator has a significant prognostic value in IMRT therapy for NPC. Furthermore, the prognostic value for tumor burden was found to be comparable to that of clinical staging by the American Joint Committee on Cancer (AJCC) tumor, node, and metastasis (AJCC TNM) staging system (Chen, Fei, Huang, Ding, & Chen, 2018)

Wu et al. conducted a study comprises of 614 patients with newly diagnosed, nondisseminated NPC treated by IMRT between 2004 and 2008 and stratify survival outcomes with tumor stage. They found that the median follow-up duration was 112.7 months, and the 10-year local relapse-free survival rates for T1, T2, and T3 were 94.2%, 92.5% and 91.4% ($p > .05$), respectively, and significantly higher than that of T4 disease (79.3%, $p < .05$ for all rates). They further noted that as N category increased from N0 to N3, the 10-year distant metastasis-free survival rates significantly decreased accordingly ($p < .01$ for all rates). Furthermore, the 10-year OS rates were 100%, 87.1%, 75.5%, and 55.6% for stages I, II, III, and IV, respectively ($p < .05$ except stages I and II) (Wu, Liu, et al., 2017; Wu, Liao, et al., 2017).

A study by Zhao et al. showed that the proportion of NPC patients with phase III-IVa-b disease was approximately 75.5%, and the 5-year OS, LRFS, regional recurrence-free survival (RRFS), DMFS, and PFS rates were 80.9%, 91.7%, 96.2%, 91.7%, and 75.6%, respectively. The study also reported that IMRT significantly improved the patient's OS, LRFS, and PFS and the distant metastasis was the most common cause of treatment failure in patients with NPC, followed by local recurrence and regional lymph node recurrence. They stated that although previous studies on IMRT have reported similar results studies on conventional radiotherapy of NPC have indicated that local recurrence is the main reason for treatment failure. In this study, they observed that 12 patients with NPC developed SPTs after treatment. In addition, in studies on conventional radiotherapy, the incidence of SPTs was approximately 2.0%−5.2%. Hence, the incidence of SPTs induced by IMRT and conventional radiotherapy is similar. However, as the follow-up duration of the preset study was relatively short and the incidence of SPTs would increase with an increase in the follow-up duration, physicians should carefully consider the development of SPTs after treatment with IMRT (Zhao et al., 2016).

Peng et al. conducted a study on the impact of EBV status on the prognosis of patients with stage III−IVB NPC in a population with a high prevalence of EBV infection and NPC. They found that distant metastasis was the main treatment failure pattern for EBV-positive patients. The results of univariate analysis in their study may be less meaningful because of the unbalanced distribution of tumor stage and treatment intensity. In addition, the outcomes of multivariate analysis showed that patients with positive EBV have an obviously poorer prognosis than EBV-negative patients, and overall stage still remains the most important prognostic factor. Moreover, they noted that the subgroup analysis revealed that this difference was mainly observed in patients with T3, N1 disease, and overall stage III. EBV-associated NPC follows a stepwise malignant transformation and consists of latent EBV infection, evasion of host immune surveillance, loss of heterogeneity at specific chromosomal regions, genetic mutations and activation of oncogenic signaling pathways, and epigenetic silencing of tumor suppressor genes. However, human papilloma virus (HPV) has been considered as a contributing factor in EBV-negative NPC in nonendemic regions and is correlated to poor prognosis (Peng et al., 2016).

Health-related quality of life in nasopharyngeal cancer

QoL and its assessment have become increasingly important in healthcare, especially in the field of chronic and terminal diseases. Conventionally, medical care for cancer patients has focused on survival rate. However, survival rate assessments provide no knowledge and understanding of the patient's mental and emotional well-being. Evaluations of treatment effect in chronic diseases have therefore been changed to depend not only on survival duration, but also on patient QoL. An individual's QoL encompasses personal feelings in the domains of physiology, psychology, and society. The QoL of cancer patients reflects not only treatment effect, but also rehabilitation effect. Few studies have reported the QoL of NPC survivors with a survival time of more than 2 years. The QoL in this population should not be ignored, because NPC affects young people, and NPC survivors can have problems with swallowing, speech, and hearing, as well as psychological effects from loss of function (Hong, Tian, Han, & Ni, 2015).

NPC is a malignancy with a high incidence in several geographic areas, especially southern China and Hong Kong. Because NPC is found in close proximity to the base of the skull and is sensitive to radiation, the primary treatment is RT alone or in combination with chemotherapy. RT causes various side effects, such as xerostomia, dysphagia, and hearing loss. These side effects obviously have a serious impact on health-related QoL in NPC patients (Su et al., 2016). These side effects may also result in certain emotional responses, thereby influencing the patient QoL. Psychological distress is caused by multifaceted adverse emotional experiences that have psychological (cognitive, behavioral, and emotional) and social side effects. In addition, the nature of the spirit may affect an individual's ability to respond effectively to cancer, physical symptoms, and treatment side effects. Psychological distress is widespread in many cancer patients and previous studies have suggested that the diagnosis and treatment of NPC can lead to the occurrence of psychological distress (Wang et al., 2018).

Until recently, improved treatment methodologies and the changing epidemiology, most notably the rise in HPV-associated oropharyngeal cancers, have resulted in a rapid increase in the number of HNC survivors. Accordingly, this expanding survivor population has generated a surge of interest in the late effects of HNC therapy. Evolving data demonstrate that acute toxicities may persist long-term and develop into late effects. In addition, late effects may manifest months or years after completion of therapy, persisting for years or even lifelong, far longer than previously believed. When severe, late effects may profoundly affect function and QoL. The most frequently studied late effects of therapy are those that are due to *local* tissue damage from cancer or its therapy. However, late *systemic* symptoms, which may have a more ubiquitous and profound impact on long-term function, have remained elusive from the

standpoint of both research and management. Systemic symptoms, also known as sickness behaviors, include fatigue, central pain, neurocognitive dysfunction, mood disorders, thermal discomfort, sweating, gastrointestinal symptoms, and sleep disturbances. Systemic symptoms tend to occur in clusters, which is felt to be due in part a common underlying pathobiology. While the mechanisms and pathways that contribute to systemic symptoms have yet to be fully elucidated, neuroinflammation is believed to be one of the important connective threads (Wulff-Burchfield, Dietrich, Ridner, & Murphy, 2018).

The QoL of NPC patients has been widely studied with the following inventories: the European Organization for the Research and Treatment of Cancer Core QoL Questionnaire (QLQ-C30) and Head and Neck Module (QLQ-H&N35) the MOS 36-Item Short-Form Health Survey (SF-36) The University of Washington Quality of Life, the Functional Assessment of Cancer Therapy-General Scale (FACT-G) and Head and Neck Module (FACT-H&N) and the Functional Assessment of Cancer Therapy-Nasopharyngeal (FACT-NP) (Su et al., 2016).

The impact of diagnosis and treatment on QoL in patients with NPC has been the subject of several studies and controversies over the past decade. The different treatment strategies and the introduction of new treatment protocols have produced many studies on QoL, and resulted in the publications of several cross-sectional studies and a smaller number of longitudinal prospective studies. The 5-year OS rate of 90% for stage early stage disease and around 60% for late-stage disease makes QoL issues in patients with NPC quite important.

1. *QoL instruments*
   The most widespread and used questionnaires in the literature are the EORTC QOL-C30 and QOL-H&N35 modules from the Quality of Life Unit, EORTC Data Center in Brussel, Belgium. The EORT Quality of Life Questionnaire-Core 30 (QLQ-C30) incorporates 30 items and consists of five functional scales (physical, role, cognitive, emotional, and social functioning); three symptom scales (fatigue, pain, and nausea/vomiting), a global QoL scale; and six single items (dyspnea, insomnia, appetite, constipation, diarrhea, and financial impact) (Aaronson et al., 1993). The EORTC Quality of Life Questionnaire-Head and Neck 35 (QLQ-H&N35) is a supplement module to the QLQ-C30 and consists of items used for assessing QoL for HNC patients. It incorporates 35 questions making up 7 multiple-item symptom scales (pain, swallowing ability, taste/smell, speech, social eating, social contact, and sexuality) and 11 single-item scales, which assess the presence of symptomatic problems related to the teeth, mouth opening, dry mouth, sticky saliva, coughing, feeling ill, use of painkillers, use of nutritional supplements, feeding tube, weight loss, and weight gain (Bjordal et al., 1999). All scales pertaining to the EORTC QLQ-C30 and QLQ-H&N35 range from 0 to 100. A high score for a functional or global QoL scale represents a relatively high/healthy level of functioning or global QoL, whereas a

high score for a symptom scale indicates a higher level of symptoms or problems. Other QOL studies have used different instrument such as the MOS 36-item short form health survey (SF-36); The University of Washington Quality of Life Questionnaire (UW-QOL); the FACT-G including the FACT-H&N; and the FACT-NP (Su et al., 2016).

2. *Baseline and temporal trends of QoL*
   Prospective studies suggest that most QoL issues in HNC patients progressively deteriorate during treatment, improve in the first year, and then remain stable but with exception of xerostomia (Ackerstaff et al., 2012). Pain, activity, and anxiety were prevalent issues at baseline in patients with HNC (Rathod, Livergant, Klein, Witterick, & Ringash, 2015). Longitudinal studies conducted in patients with NPC suggest that QoL progressively improves throughout the first year after diagnosis. Particularly, the physical capability , vitality, body pain, social function, and role emotional scores declined during the chemoradiation and rose again after treatment with those measured at 6 months after treatment being significantly higher than those pretreatment (Chen, Liu, Wang, Wu, & Zhang, 2015). During a course of radiation treatment, most symptoms escalated at week 3 and continued to worsen throughout the course of treatment. There is evidence that symptoms may occur in clusters, thus exacerbating the overall symptoms experienced and showing a synergistic effect on important patient outcomes, including functional status, QOL, emotional status, and even survival (Xiao et al., 2017). However, analysis of trajectories of QoL in a population of Chinese patients diagnosed with NPC unveiled significant subgroup differences in adaptation: most NPC patients showed high stable patterns in both physical and emotional scores with only 10% and 7% of patients showing a recovery trajectory in physical and emotional scores, respectively. Interestingly, an optimistic outlook played a substantial role in maintaining QoL, suggesting that patients who reported negative future expectations adopt unsuccessful coping strategies. Thus interventions helping patients to achieve and maintain an optimistic view should be implemented (Lam, Ye, & Fielding, 2012).
3. *Posttreatment QoL*
   QoL assessment in posttreatment survivors reveal a considerable prevalence of xerostomia, difficulty in chewing and swallowing ability, and hearing loss (Fang, Tsai, Chien, et al., 2010; Fang, Tsai, Lee, et al., 2010; Jang-Chun et al., 2014; Lastrucci et al., 2017; Lovell, Wong, Loh, Ngo, & Wilson, 2005; Talmi et al., 2002; Teguh et al., 2008; Wu et al., 2007). Factors related to patients, disease, and treatment may impact on these symptoms.

Effect of patient-related factors

Elderly, female gender, low household income, body mass index, and a high number of comorbidities are significant predictors of poor QoL in NPC patients

(Cengiz et al., 2005; Lam et al., 2012; Lastrucci et al., 2017; Ng & Wei, 2006; Qiu, Yang, Tian, & Liu, 2011; Wu et al., 2007). Also, unmarried status adversely affects QoL, suggesting patients who are single are at risk from insufficient social support (Lam et al., 2012).

Effect of tumor-related factors

Advanced T and N stage NPC had worse specific symptoms including xerostomia (Lee et al., 2014) and significant weight loss (Qiu et al., 2011) and were found to be independent prognostic factors for poor global QoL (Cengiz et al., 2005; Wu et al., 2007) in patients with NPC.

Effect of treatment-related factors

Due to the complex anatomy of the nasopharynx and its location adjacent to vital organs, treatment of NPC remains a challenge. NPC is highly sensitive to RT and moderately sensitive to chemotherapy. Therefore RT is the first choice of treatment for early NPC. In addition, chemotherapy can be used to improve the treatment results in patients with intermediate and advanced stage disease. More recently, the development of endoscopic sinus surgery opened a new treatment choice in select cases (Si et al., 2017). With the advent of image-guided radiation therapy, techniques have moved from conventional 2DRT to 3DCRT or IMRT and the curative effect of treatment increased with the 5-year OS rate of NPC patients reaching 70%–80%. However, significant treatment-related symptoms such as taste change, dry mouth, sticky, sore throat, lack of appetite, and difficulty in swallowing remain of major concern to NPC patients undergoing treatment. Swallowing dysfunction and xerostomia are the two most common side effects of RT. It is not surprising therefore that dysphagia negatively influences QoL (Bian, Song, & Wu, 2015). Dysphagia and poor appetite affects family and social relationships (Lam et al., 2012). Thus successful oral care during treatment is critical to prevent unfavorable effects on QoL. Lang et al. showed that using mouthwash six times daily with recombinant human granulocyte colony-stimulating factor prevented and treated RT-induced mucositis, related pain, and xerostomia, thus improving the QoL in patients with locally advanced NPC (Si et al., 2017).

Compared to conventional RT, intensity-modulated or conformal RT was shown to significantly be better at parotid sparing (Fang, Tsai, Chien, et al., 2010; Fang, Tsai, Lee, et al., 2010). In addition, conformal RT significantly improves the scales of global QOL, pain, appetite loss, senses, speech, social eating, teeth, opening mouth, xerostomia, sticky saliva, and feeling ill (Fang et al., 2007).

Proton therapy is the most advanced form of radiation treatment. Proton therapy is associated with significantly reduced radiation dose to nontarget tissues. More recently, in a series of patients with nasopharynx and paranasal sinus

cancers, proton therapy was associated with a lower opioid pain requirement at the conclusion of radiation and a lower rate of gastrostomy tube dependence compared to IMRT (McDonald, Liu, Moore, & Johnstone, 2016). To repeat a CT scan and replanning during the course of IMRT can improve the QoL as well as locoregional control.

It has been known that IMRT has significantly lower radiation-induced toxicity than two-dimensional conventional radiotherapy (2D-CRT), but the change in the patient-reported xerostomia scores or QoL may not be statistically different between the two groups. This inconsistency may be explained as follows: (1) QoL assessment may contain questions that are not specific to RT-induced toxicities. (2) The criteria used to differentiate between grade 1 and grade 2 of QoL is rather vague and subjective. (3) Physician and patient bias may exist in an unblinded randomization setting. (4) Previous studies used a small sample size and a relatively shorter follow-up time. However, this study shows that IMRT has better QoL with or without concurrent chemotherapy in a longer follow-up time. The result further confirms that lower radiation-induced toxicities of IMRT may produce better QoL compared to 2D-CRT (Pan et al., 2017a, 2017b).

IMRT increases the cost of NPC treatment and eventually increases the financial difficulties of individuals in developing countries such as China. Some studies found that financial difficulties adversely affected QoL. Consequently, IMRT would adversely affect QoL. However, we found that patients receiving 2D-CRT suffered from greater financial difficulties than those receiving IMRT. This may mean that patients received 2D-CRT because of financial difficulties. Financial burden after treatment gave patients receiving 2D-CRT worse QoL, but the relationship between financial problems and QoL is still unclear (Pan et al., 2017a, 2017b). Further controlled studies should be performed to test the interference of financial difficulties on QoL.

Effect of addition of chemotherapy on quality of life

RT combined with chemotherapy is the primary treatment modality for advanced locoregionally NPC. It has been suggested that concurrent chemotherapy adversely affects QoL of NPC patients. In order to exclude the interference of chemotherapy, Pan et al. conducted a subgroup analysis to compare the QoL of IMRT versus 2D-CRT without concurrent chemotherapy. The results revealed that IMRT alone significantly improved the QoL compared to 2D-CRT alone. Moreover,their subgroup results also suggested that IMRT had better QoL than 2D-CRT with concurrent chemotherapy (Pan et al., 2017a, 2017b)

Concurrent chemotherapy (CT) and RT increases the effectiveness of RT. The interaction between CT and RT results in radiation "sensitization," which is either additive or supra-additive (Seiwert, Salama, & Vokes, 2007). Unfortunately, this

also increases radiation-induced toxicity. The severity and duration of symptoms increase when CT is combined with RT, which may lead to treatment interruption, psychological distress, decreased functional status, and poor QoL (Pan et al., 2017a, 2017b). Concurrent CT resulted in significantly more cases of grade 3–5 oral mucositis compared with RT alone (Wee et al., 2005).

While in stage III and IV NPC the addition of CT to RT is the standard of treatment, in patients with stage II evidence showing that the addition of CT results in a better outcome is weak. Thus when deciding to use concurrent CT–RT in patients with intermediate stage disease, QoL issues are important. Pan et al. compared the QoL of stage II NPC patients treated with RT versus CT–RT and found that RT group had better outcomes than the CT–RT group for global QoL, functional scales, symptom scales of fatigue, insomnia, financial problems, and weight gain (Pan et al., 2017a, 2017b).

Xu et al. evaluated the effectiveness and toxicities of induction CT followed by concomitant cisplatin–CT or cetuximab–RT in locally advanced NPC (Xu et al., 2015). Cetuximab was more likely to cause seriously acute adverse events. Pain, swallowing, speech, eating, feeling ill, and weight loss subscale scores were significantly higher in patients enrolled in receiving cetuximab–RT, while the effectiveness was not significantly different between the treatment arms. Lee et al. conducted a randomized phase II study to compare weekly and triweekly cisplatin delivery during RT with respect to efficacy, toxicity profiles, and QoL (Lee et al., 2016). While effectiveness and toxicity profile were similar between groups, patients on the weekly regimen showed better physical, emotional, and social functioning as well as less appetite loss than those on the triweekly regimen.

Effect of surgery on quality of life

A surgical approach to early stage NPC tumors can be a therapeutic alternative. Several studies show that resection of the primary lesions via the hard palate approach and administered a reduced dose of RT and chemotherapy for patients after surgery, achieving relatively satisfactory results, however, the hard palate approach is difficult to popularize due to large trauma, small surgical field, and long postoperative recovery time. Si et al. investigated the impact of endoscopic sinus surgery on the QoL and survival in patients with early NPC (Si et al., 2017). Patients receiving endoscopic sinus surgery were matched with those received concurrent CT–RT. The surgery group had a significantly lower pain score than CT–RT group. Also, long-term dry mouth scores were significantly lower in the surgery group. Furthermore, surgical treatment positively impacted the physical condition and weight gain.

Salvage reirradiation and surgery are the most commonly used treatments for locally recurrent or persistent NPC. The QoL of patients who underwent maxillary swing salvage nasopharyngectomy for residual or recurrent NPC was

evaluated by Chan, Chow, and Wei (2012). This study showed that the QoL of patients after salvage nasopharyngectomy was satisfactory with no significant changes in mean global health system scores after surgery. You et al. evaluated 683 patients diagnosed with a local recurrence of NPC and tried to identify whether IMRT or endoscopic nasopharyngectomy was the more effective treatment modality through a case-matched study (You et al., 2015). IMRT was associated with a worse global health status score as well as in all functional scales and with significantly more severe financial difficulties and insomnia than endoscopic nasopharyngectomy. Patients undergoing salvage IMRT complained of more severe weight loss, pain, dysphagia, sensory problems, and tooth difficulties than those receiving endoscopic nasopharyngectomy.

Quality of life as prognostic marker in patients with nasopharyngeal cancer

QoL measurements is an important tool as a supplemental treatment endpoint in oncology as well as a potential prognostic predictor in cancer patients. In a large study including 347 patients with NPC curatively treated by conformal radiotherapy, a 10-point increase in the pretreatment physical functioning score was associated with a 23% reduction in the likelihood of death and a 22% reduction in the likelihood of distant metastasis (Fang, Tsai, Chien, et al., 2010; Fang, Tsai, Lee, et al., 2010). In a series of 273 patients with NPC, posttreatment physical functioning, fatigue, and appetite loss were significant predictors of both disease-specific and OS in multivariate analysis (Tsai, Chien, Huang, Liao, & Fang, 2013).

Survival rates and QoL of NPC patients are intimately related as effects of each can adversely or positively affect one another. At this juncture, the emergence of new treatment techniques had made most of the NPC patient attain better QoL for an extended duration. Nevertheless, some of the chemoradiation side effects are chronic and progressive and can adversely affect NPC patient QoL. Several studies have shown the global health status significantly correlates with EBV DNA where high pretreatment EBV DNA levels are associated with large tumors or multiple lymph nodes. This represent patients with advanced stage tumor who generally had poor QoL.

Anxiety and depression are common symptoms among cancer patients and those with other chronic illnesses. This can be a result from the disease or cancer itself or from its treatment. It is imperative to identify these sequelae as it can cause significant dysfunction and impairment of patient QoL if it is left untreated or treatment is suboptimal. In fact, numerous papers have showed that anxiety and depression can last long after treatment has been completed. For example, a study by Hong et al. documented that the prevalence of self-reported fatigue in NPC survivors was 18.52% and suggested that this higher prevalence might be due to the

method that was used for fatigue assessment (Hong, Tian, Han, & Ni, 2015). The participants may have reduced understanding to differentiate between the fatigue and depression. This is due to the fact that fatigue and depression are both heterogenous with mixture of physical, cognitive, and emotional dimensions. There is a high degree of overlap across these dimensions.

The QoL and prognosis of cancer patients are significantly affected by psychological distress. Cancer patients often experience psychological problems such as severe depression and anxiety. Physical, social, cognitive, psychological, and emotional issues, as well as physical symptoms such as pain, nausea, vomiting, and fatigue, all affect QoL. Several studies have found that patients with NPC have psychological distress after RT, with approximately 13% of patients suffering from severe psychological distress. Buchmann et al. found that patients with HNC, including NPC, had a higher incidence of psychological distress, including a self-reported history of depression, concerned relatives and friends, and emotional and physical problems. NPC patients often experience head and neck pain, skin and mucous membrane reactions, language issues and dysphagia, hearing loss, social difficulties, dry mouth, cough, phlegm, sensory discomfort, and other problems that decrease QoL during radiation therapy. However, there have been no studies focusing on the correlation between psychological distress and QoL in patients with NPC after RT (Wang et al., 2018)

Anxiety and depression are the psychological problems most commonly seen in cancer patients. The reported prevalence of anxiety and depression in cancer patients ranges from 25% to 54%. In a study using the Self-Rating Anxiety Scale and Self-Rating Depression Scale, researchers reported that the prevalence rates of anxiety and depression in 46 NPC survivors (mean survival duration: 5.7 ± 3.1 years) were 82.6% and 78.3%, respectively. Those studies indicated that psychological disorders, such as depression and anxiety, are apparent as early as the start of RT and can last long after RT. Our study also suggested that anxiety and depression can continue for many years after RT. Psychological problems in cancer survivors should not be ignored, because depression and anxiety can influence cancer survival and QoL. Data from several studies indicate that poor psychological status can influence a patient's immune status and illness duration and that untreated depression can result in significant morbidity and mortality. Close attention and psychological care should therefore be given to patients not only during the period of RT but also during follow-up (Hong et al., 2015).

Dysphagia is another significant morbidity experienced by the majority of NPC patients. Post-RT dysphagia is often caused by dysfunction of the swallowing muscles. Of the various muscle groups involved in swallowing, the suprahyoid group of muscles plays an important role. This group includes the geniohyoid, mylohyoid, digastric, and stylohyoid, and is responsible for moving the hyoid bone, which then causes other physiological changes during swallowing. These changes include upper esophageal sphincter opening,

which allows pharyngeal clearance of the bolus into the esophagus, and laryngeal vestibule closure and epiglottic retroflexion, which are important for airway protection. They stated that due to possible muscle damage by radiation, the anterior hyoid displacement in irradiated NPC patients was found to be significantly reduced compared to healthy individuals (Cheng, Lee, Ahuja, & Tong, 2018).

Of note, reducing deglutition disorders-related symptoms such as oropharyngeal pain, dry mouth, food stuck in the throat, and choking as well as deglutition disorder-related complications like pulmonary complications can both result in a significant improvement of patient QoL together with a reduction in hospitalization costs. The radiation-induced dysphagia, as a final multifactorial side effect often requiring enteral nutrition, represents a real "Achille's heel" that occurs in more than 50% of patients and can lead to a malnutritional status and an increased risk of aspiration pneumonia. The 1- and 2-year rates of percutaneous endoscopic gastrostomy tube dependence is reported, respectively, in 24% and 14%, whereas clinical aspiration pneumonia is reported in 3% of cases (Ursino et al., 2016).

Tsai et al. reported that radiation-induced dysphagia in HNC plays an important role in QoL domains and highlighted the importance of not only parotid sparing by modern IMRT techniques, but also preserving the pharyngeal muscles that are involved in swallowing function during irradiation. However, another report it was observed that dysphagia was tumor site-specific, and that NPC patients suffered from less dysphagia than oropharyngeal cancer patients. They also found that, in contrast to other anatomic sites of HNC, NPC survivors presented some specific but common late sequelae related to the irradiation field, such as otitis media, hypothalamic-pituitary-thyroid dysfunction, and neuropathy related from temporal lobe necrosis, cranial nerve palsy, or carotid arterial stenosis (Tsai et al., 2014).

Besides parotid sparing for the prevention of xerostomia or dysphagia, the modern conformal radiation technique should place more emphasis on the anatomic structures involved in these late complications from radiation (e.g., cochlea, thyroid and pituitary gland, temporal lobe, and carotid artery). Furthermore, regular examinations such as carotid duplex scanning or evaluation of thyroid function for early detection and possibly intervention of these potential late complications should be kept in mind in routine clinical practice especially for those with high-risk factors and long-term survival (Tsai et al., 2014). This is prevalent years after completing chemoradiation and is partly due to the presence of mucositis or xerostomia as well as fibrotic changes over the pharyngolaryngeal musculoskeletal structures. Numerous studies worldwide have highlighted the different incidence of dysphagia among HNCs especially in NPC patients who received chemoradiation and the percentage is varies but mostly were on the higher side. A study by Lovell et al. showed a high prevalence of dysphagia of 84% that was similar to other published reports

(Lovell et al., 2005). This study showed that dysphagia has a negative impact on QoL among patients with NPC where respondents with dysphagia reported a worse health-related QoL than those without dysphagia or swallowing difficulties. In addition, they agreed that self-reported swallowing difficulty was a significant predictor of a lower health-related QoL.

Fatigue is a significant symptom experienced by patients either due to disease or treatment and can significantly impair patient QoL. The term cancer-related fatigue (CRF) is a common disease experienced by HNC patients, especially those who underwent chemoradiation therapy. CRF is defined as a persistent subjective sense of physical, emotional, and/or cognitive tiredness related to cancer or its treatment that is not due to recent activity and significantly interferes with normal functioning and causing distress. Most cancer patients will struggle with some amount of fatigue during the course of treatment. About one third will have persistent fatigue for a number of years posttreatment. The prevalence of fatigue is reported to be between 59% and 96% in patients undergoing chemotherapy, 65%–100% in patients receiving radiation therapy, and 30% in long-term survivors. Patients who have completed primary treatment and are undergoing posttreatment surveillance should be monitored continuously because fatigue may persist beyond the time of active treatment.

Conclusion

As discussed in this chapter exploring all vital factors related to QoL is crucial and helpful not only for the clinician to make accurate diagnosis and treatment decision but it will also help all other healthcare workers provide NPC survivors with the best adequate health services in their respective communities. Advancement and refinement in treatment techniques and instrumentation in the coming decades may increase patient survival and improve their QoL. However, the treating team understand that patient QoL is compromised not only by the disease but also by the treatment intended to cure the disease. As the burden of NPC patients has increased over recent years, and they are part of our communities, it is our responsibility to ensure their lives our as good as ours.

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14

New developments in nasopharyngeal cancer

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Early diagnosis by Epstein–Barr virus biomarkers

Undifferentiated and poorly differentiated nasopharyngeal carcinoma (NPC) has a strong correlation with Epstein–Barr virus (EBV), whereas differentiated NPC is more related to smoking and drinking. In the high incidence NPC regions, the EBV genome and several actively expressed gene products are found in all tumor cells in up to 100% of the cases. In the low incidence regions this percentage is lower, although still more than half of the cases are related to EBV (Wei & Sham, 2005). Research in NPC has focused on the use of EBV-related biomarkers for risk screening, (early stage) diagnosis, predicting treatment outcome and early detection of recurrent disease. These markers include EBV [immunoglobulin A (IgA)] serology and EBV DNA load since NPC patients have elevated Immunoglobulin G (IgG) and IgA antibody titers to several EBV-encoded antigens as well as increased EBV DNA derived from shed (apoptotic) fragments from the tumor into the circulation. Increased IgA antibody levels are found against early antigen (EA), viral capsid antigen (VCA), and the latent Epstein–Barr nuclear antigen 1 (EBNA1) as well as inhibitory antibodies to the EBV-specific DNase (Cevenini et al., 1986; Chen et al., 1987). These antibody responses against defined viral antigens are the basis of a proposed screening test for NPC in high-risk populations (De Vathaire et al., 1988; Ji et al., 2007; Tamada et al., 1984). Recent insight in the molecular basis and diversity of

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anti-EBV IgA and IgG responses allowed the development of more defined serological tools (Fachiroh et al., 2004, 2006, 2008; Paramita et al., 2007; Paramita, Fachiroh, Haryana, & Middeldorp, 2009). EBV DNA load in the circulation and in nasopharyngeal brushings can be used in addition as independent NPC-related EBV markers, since both have been detected in a higher proportion of NPC patients than controls (Lin et al., 2004; Paramita et al., 2009; Stevens et al., 2005, 2006; Tong et al., 2002; Tune et al., 1999). EBV IgA serology testing in particular appears to fulfill criteria as a possible screening tool, since the cost is relatively low and it is easy to use when combined with finger-prick blood sampling (Fachiroh et al., 2008; Ji et al., 2011). Patients with elevated EBV IgA serology plus defined chronic symptoms suggestive of NPC may be selected for more costly EBV DNA testing in the nasopharyngeal brushing to make early stage NPC diagnosis possible (Hutajulu, 2012).

IgA–VCA or IgA–EBNA1 serology has no (or limited) prognostic value in detecting persistent or recurrent disease. This is probably due to the continued antigenic triggering of memory IgA responses in nasopharyngeal mucosa and the long half-life of antibodies in general. Multiple studies have explored the correlation between EBV and prognosis of NPC. It is suggested that a high EBV DNA load at diagnosis is related to poor outcome. Some authors have proposed to include the DNA load into the tumor, node, and metastasis (TNM) staging (Leung et al., 2006; Ng, Yuen, Au, Chan, & Lee, 2014). Leung et al. confirmed that early stage NPC and a high EBV DNA load in blood at diagnosis are related to poor outcome (Leung et al., 2006). They used a cut-off value of 4000 copies/mL in plasma/serum, measured by a real-time quantitative reverse transcription system that amplified a DNA segment in the BamHI-W fragment region of the EBV genome. Comments to this multiinstitute analysis stated that their method was poorly standardized, giving considerable variation between institutes (Le et al., 2013). A few others confirmed the correlation, but it is not consistently found. This might be due to the different method of the polymerase chain reaction technique and amplicon chosen. Standardization and confirmation of the correlation should first be assessed before EBV DNA load will be included in the TNM. When this is completed, it will be of great value to decide on radiotherapy alone or combined with (an extended course of) chemotherapy. Patients with the expected good clinical outcome can eliminate chemotherapy from their treatment and are thus spared from the heavy side effects. For patients with early stage disease and an expected poor outcome based on EBV DNA load, concurrent chemotherapy can be added to their treatment, giving them a much better change on complete response and survival than when treated by radiotherapy alone.

Posttreatment levels of the EBV DNA load are also explored. A high EBV DNA load in blood after complete treatment is suggested to be related to poor prognosis. It suggests that the tumor is still present. Unfortunately, this hypothesis is not as easy as it is thought. Not all patients with a persistently

high level of EBV DNA load turn out to have a poor prognosis. This suggests that tumor might still be in regression, or that dead tumor cells are detected. Also for this application of the DNA load standardization is necessary, since it could also be of great value to know who needs another course of adjuvant chemotherapy.

Close monitoring of patients with a high EBV DNA load posttreatment is probably the best available EBV method for screening high-risk patients. If the load decreases after treatment and then increases at a certain point, this suggests tumor growth and thus recurrent disease (or progression of residual disease). An increasing level of DNA load justifies thoughtful examination, with imaging.

For local residual and recurrent diseases, the DNA load in the mucus from the nasopharynx, obtained by a nasopharyngeal swab, showed promising results in one study (Stoker et al., 2015). The specificity and sensitivity for local disease were higher than the EBV DNA load in blood. Further study, to compare the nasopharyngeal swab with fiber endoscopy, would be of great interest. In particular, during the first month after treatment distinguishing between tumor and postradiation damage is difficult, and treatment of local residual disease is still feasible at that time with a relatively good outcome (see the following section "Local failure").

Imaging modality of nasopharyngeal cancer

Many studies have investigated the roles and efficacy of computed tomography (CT) scan, magnetic resonance imaging (MRI), positron emission tomography (PET) scan, or any of those combinations for assessment and management of NPC and reported different results. Chen et al. documented that MRI has a higher sensitivity than CT and FDG PET/CT in local tumor size evaluation (Cheng, Li, Xing, Wang, & Li, 2016). MRI can identify the retropharyngeal nodes (RLN) that would have been missed on the CT scan and has a good capacity to depict the detailed anatomic location. Therefore MRI has been widely used in NPC assessment and management. Another study by Sheng et al. showed that 18F-FDG PET/CT was highly accurate for detecting metastasis in MRI-negative cervical lymph nodes and could provide valuable information for the delineation of target volumes in intensity-modulated radiotherapy (IMRT) treatment of NPC (Sheng et al., 2017). Chen et al. also documented that the combination of PET and CT scan is more sensitive in picking up the nodal diseases in NPC patients compared to MRI alone (Cheng et al., 2016). Based on these and many more studies, the combinations of different modalities appear to give the best view of the complete extent of the local tumor and the spread or regional and distant metastases. In the following, these studies and others are described in more detail.

Local disease

The role of imaging in the management of NPC has changed in recent years. Traditionally the CT scan has been the mainstay method of imaging for staging of NPC. A CT scan of the head and neck can be used for delineating the local tumor volume, the extent of involvement of surrounding critical structures as well as the presence of neck metastases. The radiation treatment plan is based on the radiological findings and thus adequate tumor staging is crucial. These days many clinics use MRI for locoregional staging of NPC. MRI is better than CT in the detection of the submucosal disease. According to Gamba et al., MRI provides better resolution than CT in terms of assessing parapharyngeal spaces, marrow infiltration of the skull base, intracranial disease, and deep cervical nodes (Gamba et al., 2018).

Regional nodes

The presence of neck metastases carries poor prognosis, which typically depends on the size of the nodes and numbers of nodes involved by the disease. The location of neck nodes also has been shown to influence the prognosis and survival. Several authors have described the specific patterns of lymph nodes metastases in NPC and agreed that nodes metastases occur in an orderly fashioned pattern with infrequent skip metastases. Skipping of levels is apparent in a violated neck either due to previous surgery or chemoradiation, where significant fibrosis and scarring will disrupt the normal pattern of the lymphatic drainage.

According to Sheng et al. metastases could exist in MRI-negative cervical lymph nodes, and 40% of cervical lymph nodes metastases occur in lymph nodes with diameter less than 10 mm (Sheng et al., 2017). Another study by Xu et al. highlighted the role of small lymph nodes in the survival rate and disease-free survival of NPC patients. They defined the small lymph nodes as those nodes smaller than 1.0 cm. They concluded that the presence of small nodes is not an independent prognostic factor and should not influence the nodal contouring or the dose delivered to nodal areas (Xu et al., 2017).

The involvement of retropharyngeal lymphnodes has significant implications in the management of NPC. Typically, the positive retropharyngeal (Rouviere) nodes carry poor prognoses, and this is particularly true in the presence of central necrosis. Tang et al. stated that retropharyngeal lymphnode necrosis should be adopted as a factor for individualized treatment planning (Tang et al., 2014). Ho et al. documented that MRI has better soft tissue contrast than CT scan for assessment of retropharyngeal lymphnode and other cervical lymphadenopathy (Ho, Tham, Earnest, Lee, & Lu, 2012). The nodal status was changed from negative to positive when patient is reassessed with the MRI. Numerous studies have shown the superiority of MRI compared to CT scan in the assessment of NPC. This is vital as the subsequent treatment strategy will largely depend on the presence or absence of these nodal disease. A study by

Liu et al. showed the specific pattern of retropharyngeal lymphnode involvement in relation to the surrounding vital structural metastases. The incidence of retropharyngeal metastases is higher if there is muscular involvement or levels II, III, and V lymph nodes are involved. This study also concluded that retropharyngeal lymphnode metastases is equal to the cervical lymph node metastases (Liu et al., 2006).

New evidence has emerged on the importance of central necrosis in the prognosis and survival of NPC patients. Central necrosis tends to occur in nodes larger than 2.0 cm and in the case of massive tumor infiltration (Nishikawa et al., 2017). Tumor necrosis represent severe tumor hypoxia. Central necrosis generally occurs as a late event in the process of carcinogenesis. Tumor hypoxia make it difficult to deliver full radiation dose to the target volume, making the therapeutic ratio of radiation suboptimal. Lu et al. in his study used the MRI characteristic findings of the nodes that are presence of a focal area of high signal intensity on T2 weighted images and a focal area of low signal intensity on T1-weighted images with or without surrounding enhancement (Lu, Wei, Li, & Li, 2017). They confirmed that the presence of central necrosis is highly related to poor prognosis.

Distant metastases

PET scan (18F-FDG) is sensitive and accurate in the detection of nodal metastasis but lacks soft tissue resolution and thus cannot be used without MRI or CT scan. For staging of distant metastases, several studies have noted that 18F-FDG PET is more sensitive and accurate than the conventional workup of chest radiography, abdominal ultrasound, or skeletal scintigraphy (Gamba et al., 2018). Currently, the majority of head and neck surgical oncology centers use PET scan as the primary modality for assessing distant metastasis and locoregional recurrence. With the advent of technology and instrumentation, the new development of imaging techniques, supplemented by new reagents and modifiers, will certainly push the assessment and management of NPC to yet another level for overall better treatment outcomes and quality of life.

Treatment of nasopharyngeal cancer

The imaging techniques also play an important role in planning for radiation of NPC. Several centers use a combination of CT scan and MRI in delineating the target volume while others resort to PET scan, which can be more sensitive in highlighting the tumor. The complementary role of the CT scan and MRI is that the CT provides the geometry and electron density of the anatomical structures for treatment planning whereas MRI is sensitive in differentiating malignant tumor from soft tissues. A study by Wu et al. did not show any significant advantageous of adding PET scan as an imaging modality for treatment planning before IMRT

(Wu et al., 2016). Other literature has shown different results on the efficacy of using PET scan in radiation treatment planning and the majority of the research concludes that the role of PET remains controversial in the management armamentarium of NPC (Wu et al., 2016).

At present, radiation oncology has also shown improvement in parallel with advancements in imaging techniques. The IMRT with upfront dose escalation to the hypoxic subvolumes is an excellent alternative method to overcome tumor hypoxia. Today, it is possible to target the small subvolumes in the primary tumors with dose escalation, termed as dose painting (Tang et al., 2014). Newer techniques that have been implemented includes the hypoxia imaging like F-fluoromisonidazole positron emission tomography (FMISO–PET), which can sensitively detect presence of tumor hypoxia. This sophisticated system can also be registered with the CT scan as well as MRI. The other newer technique is diffusion weighted MR imaging (DWI) is on the basis of intravoxel incoherent imaging, which make it more sensitive to the subtle abnormalities, and to provide the diagnostic information by different pathological tissues. The apparent diffusion coefficient value determined from the DWI is useful in differentiating the benign and malignant lymph nodes in selected cancers (Tang et al., 2014).

Primary treatment for nasopharyngeal cancer

NPC is responsive to both radiotherapy and chemotherapy. In recent decades many studies have been performed to optimize the treatment for NPC, and have done so with success. In the early days, treatment for NPC consisted of radiotherapy alone. The median 5-year survival was 24%–62%, with a high locoregional failure rate of 40%–80% and a high incidence of distant metastasis (15%–50%) (Ali & Al Sarraf, 2000; Al Sarraf & Reddy, 2002; Baujat et al., 2006). Through the introduction of chemotherapy, these statistics have improved considerably.

In 1998, The Intergroup Study 0099 was the first of its kind that proved the benefit of chemotherapy on progression-free survival and overall survival (Al Sarraf et al., 1998). Since then, the addition of chemotherapy has become the standard for treatment of advanced NPC. Multiple chemotherapeutics have been studied. Effective single agents are methotrexate, bleomycin, doxorubicin, adriamycin, carboplatin, and taxanes (Ali & Al Sarraf, 2000; Al Sarraf & Reddy, 2002). Combined therapies are more effective than single-agent therapy, and in particular cisplatinum-based regimens are effective. Chemotherapy can be given in a neo-adjuvant (induction chemotherapy followed by radiation), concurrent (chemotherapy and radiation concomitantly), or adjuvant (chemotherapy after radiotherapy) schedule. Also, combinations of these are possible.

All combinations have been studied, with varying results. The meta-analysis by Langendijk et al. showed an absolute increase in overall survival when chemotherapy was added to radiotherapy (Langendijk, Leemans, Buter, Berkhof, & Slotman, 2004). Subgroup analysis showed that this effect only remained significant when concurrent chemotherapy was used. The absolute benefit in survival was 20% in 5 years. To date, the National Comprehensive Cancer Network recommends concurrent chemo-radiotherapy with cisplatinum-based regimens for stage II and higher (Lee, Ma, Ng, & Chan, 2015). The actual benefit of additional adjuvant and/or neo-adjuvant chemotherapy is still up for discussion. Besides the uncertainty of better tumor control, the addition of another course of chemotherapy may be too toxic. In The Intergroup Study 0099 adjuvant chemotherapy was added to concurrent therapy, which resulted in a decrease in compliance rate of 55%, due to additional toxicity (Chen et al., 2015).

Chemotherapy and radiotherapy have gone through some changes in recent years. In the beginning, radiation was only possible in two dimensions. Later this was changed to three-dimensional radiotherapy. Today multidirection radiation planning is the standard of treatment in most well-equipped hospitals. The advantage of IMRT above two-dimensional (2D) and three-dimensional (3D) radiation is a higher dose on the targeted tumor while minimizing the dose to the surrounding structures, aiming for better tumor control and fewer side effects. In particular, the salivary glands are spared by IMRT treatment (Kong et al., 2014; Moretto et al., 2014). A disadvantage of IMRT is the risk of geographic misses. Peng et al. (2012) ($n = 616$) found a benefit in 5-year survival in NPC in favor of IMRT; 80% and 67% for IMRT and 2D, respectively. Local control and regional control were not significantly different. Radiation-induced toxicities were lower in the IMRT group compared to those in the 2D radiotherapy group (Peng et al., 2012). In the next section, this will be discussed in more detail. With the development of new radiotherapy techniques like IMRT, the treatment, as well as the prognosis of NPC patients has improved.

The majority of NPC patients who undergo radiation therapy have some anatomic and structural changes during the course of treatment. Thus the radiation dose needs to be modulated in order to maintain the optimal radiation dose on the tumor volume. Globally, this has been achieved by utilization of IMRT. Typically, IMRT can deliver a high dose of radiation to the targeted area while minimizing the dose to the normal surrounding organs and tissues and subsequently can increase the therapeutic ratio of radiation therapy. The majority of studies have documented that IMRT has reduced the local recurrence rates as well as regional recurrence but not the distant metastases, especially in early stage NPC. In case of T4 disease, the role of IMRT is limited due to the presence of a very steep dose gradient that contributes to the inadequate tumor dose and increased risk of marginal failures (Fung, Wu, & Teo, 2014). This risk can be reduced by using precision treatment of increased adequate and tight dose

coverage of the targeted volume. This so-called image-guided radiotherapy, can potentially increase the accuracy of the radiation delivered to the desired area.

Sun et al. reported that IMRT produces excellent survival rates in T1-3 NO disease and thus it is vital to observe the trends of long-term complications of treatment in T1-3 N0 NPC patients (Sun et al., 2012). However, for T4 disease with no neck nodes, it remains a challenge with poor survival rates despite availability and usage of IMRT.

Adaptive radiotherapy (ART) has recently been introduced in an effort to maximize tumor response to radiation over the specified treatment duration (Sun et al., 2012). The concept of ART is to modify the treatment plan so as to cater for the tumor response and organ deformations during a radiation course. This in turn will restore the initial planned dose distribution and subsequently minimize the unintended normal tissue toxicity and tumor coverage. In order to implement ART, committed staff and instruments are needed in order to monitor the treatment plan and make treatment changes accordingly depending on the tumor shrinkage. This means repeated replanning has to be performed over the course of treatment in order to ensure an effective radiation dose is delivered to the targeted disease areas.

Today, there is increasing interest in the use of proton radiotherapy (PRT) for treating nasopharyngeal cancer (Holliday & Frank, 2014; Holliday & Frank, 2016). Due to the characteristic sharp dose fall-off of protons, PRT can deliver a high therapeutic radiation dose to the tumor in the nasopharynx with minimal exit dose (van de Water, Bijl, Schilstra, & Langendijk, 2011). Improvements have been made recently in the dose delivery of protons, including intensity-modulated proton therapy (IMPT). This will further reduce toxicity without compromising efficacy. Comparing dosimetric analyses of IMPT and IMRT have revealed advantages in the planning and have suggested that with IMPT lower doses can be delivered to nontarget tissues in patients with NPC (Taheri-Kadkhoda, Björk-Eriksson, Nill, & Munter, 2008). This is in line with early findings from Loma Linda University Medical Center and MD Anderson Cancer Center where also good clinical outcomes of PRT were described for patients with NPC (Holliday et al., 2015). However, proton therapy is available in only a few centers around the world and still has to prove its benefits over traditional techniques.

Accurate assessment of tumor response is necessary in order to quantify the disease volume so as to deliver proper treatment planning. This is particularly true during the treatment as the tumor is shrinking and repeated planning is necessary. This is important in ensuring an optimal tumor regression in response to the treatment. Zhang et al. showed that the primary tumor regression speed at the end of radiation was an independent prognostic factor of overall survival, failure free survival, and distant metastasis-free survival (DMFS). Generally, the

prognosis was significantly more favorable when an advanced tumor had disappeared at the end of treatment duration (Zhang et al., 2014).

The adverse events and complications from radiation are well known, especially pertaining to the development of second primary tumor. From 2003 to 2011, Chow et al. studied 700 patients and found that the occurrence of tongue carcinoma and sarcoma of head and neck were the most common second primary tumors, and constituted about 7% and 22% of these cases occurs within the radiation field (Chow, Au, Mang, Cheung, & Ngan, 2018). These unwanted complications can be hindered with widespread use of IMRT and new radiation techniques in the near future in treating NPC patient so as to achieve better overall treatment outcomes and quality of life.

New emerging treatments in nasopharyngeal cancer

The prediction of the prognosis of NPC patients mainly is based on the clinical TNM staging, but patients with the same clinical stage display different clinical outcomes, suggesting that the TNM stage is insufficient as a prognostic guide (Liu, Chen, Huang, & Huang, 2015). Several authors suggested that the biological behavior and the prognosis of NPC patients could be different even though they belong to same classification category.

One of the fundamental factors that governs the biological behavior of NPC is hypothesed to be the alteration of molecular signaling pathways. Such signaling pathways of NPC are crucial for cell survival, growth, and metastases. Continous effort in searching for a novel molecular biomarker that can provide a more accurate prognostication and aid as a effective guide to develop a specific therapeutic intervention for these NPC patients remains crucial. In this context, whole exome and genome sequencing studies of NPC have highlighted several promising therapeutic targets, and a wide range of investigational drugs are being investigated in clinical trials. For example, drugs targeting tumor angiogenesis, kinase signaling pathways as well as immunotherapy.

It is well known that NPC is endemic in certain geographic region and is etiologically associated with EBV. At the beginning of this chapter, the possibilities of EBV as a biomarker in screening and treatment evaluation was discussed. Also, in the treatment itself of NPC EBV is a major topic of interest. The latest studies on the pathogenesis of EBV, in both NPC cells latently infected with EBV and B cells reveal alterations in the cell metabolism that support the pathogenesis of the persistent and latent EBV infection. However, the molecular aspects underlying the switching of lytic to latent EBV infection in NPC cells are largely unclear and still under intense research focus. Molecular events and alterations of the cell metabolism are likely to play critical roles in switching EBV infection from lytic to latent in NPC cells. Of note is that the expression of viral genes

including LMP1, LMP2, and possibly EBV-encoded micro-RNAs (MiRNAs), may play essential roles in alterations of cell metabolism to support NPC pathogenesis (Zhang, Jia, Tsang, & Tsao, 2017). Subsequently, further studies and investigation in this area will eventually lead to discovery of potent biomarkers that can target the EBV from the very beginning.

It is envisioned that regulatory approval for new drugs for advanced NPC will occur in the near future. Recent findings suggest that the epidermal growth factor receptors (EGFRs) may be a good molecular target for various malignancies including NPC. It has been shown that the EGFR plays a critical role in the development of invasive NPC, which is a significant finding for treatment planning. In addition, multiple studies have also demonstrated that the EGFR is highly expressed in the majority of NPC patients. These EGFR-targeted treatment agents have emerged and are expected to form a new strategy for NPC treatment. For instance, at his juncture, numerous clinical trials have shown that gefitinib has different patient responses to specific treatment protocols (Li et al., 2018). There is a need for indicators that can regulate and predict the sensitivity of NPC to gefitinib. One such example is MiRNAs, which play a critical role in cancer development. This needs further research and studies in order to come to a consensus on the therapeutic effects of gefitinib.

Liu et al. reported that there are two main trials of adding the targeted agents to the current Cisplatin and IMRT as part of NPC treatment. The first one was a trial in Hong Kong by Ma and collegues who used cetuximab to target EGFR whereas the second trial was performed by the Radiation Therapy Oncology Group who used bevacizumab to exploit specifically the angiogenesis pathway involvement in NPC. Both of these trials used IMRT and Cisplatin in combination with targeted therapy. Both of these regimens seem to be well tolerated and the result is promising as both showed 90% rates of 2 years DMFS. There are other numerous studies that have showed efficacy of EGFR-targeted therapy especially in cases of recurrent or metastatic NPC.

The other new finding includes Coronarin D (CD), a natural product extracted from the rhizomes of *Hedychium coronarium*, that has been reported to possess anticancer potential. It has been shown that the CD potently suppressed cell viability in various NPC cell lines. Treatment of cells with CD induced G2/M arrest, apoptosis, and autophagy, which is important in halting the process of carcinogenesis. Further studies showed that CD increased the production of reactive oxygen species and subsequently activated both autophagy and apoptosis. Further research on this new biomarker is necessary for quantifying and confirming its true effects in the carcinogenesis process and tumor cycles.

Other molecules of interest are the regenerating gene IA that has been investigated on the effects of its expression and impact on NPC patient survivals. Xing et al. found an expression of 30.5% in stage T2, 44.44% in stage T3, and 47.83% in stage T4 disease (Xing, Chen, & Han, 2017). This study showed that

patients with high regenerating genes IA (REGIA) have a higher stage than REGIA negative patients. They further conclude that there was no significant correlation between REGIA expression and age, gender, and WHO classification. Several transcription factors have also been demonstrated to play crucial roles in the regulation of NPC.

Zinc-finger protein X-linked (ZFX), a novel transcription factor, has been implicated in the initiation and progression of several malignancies including NPC. A study by Li et al. showed that ZFX expression was significantly elevated in NPC tissues compared with that of normal nasopharyngeal tissues (Li et al., 2018). They further showed that the expression of ZFX was significantly correlated with lymph node stage and clinical stage. Imperatively, high expression of ZFX in patients results in a lower 5-year survival rate, progression-free survival rates, locoregional relapse-free survival rates, and distant metastasis-free survival rates. These markers could serve as effective prognostic biomarkers for NPC patients. In addition, targeting this molecule might also be a novel therapeutic strategy in order to prevent the NPC progression and metastases.

Emerging evidence has also demonstrated that cyclooxygenase-2 (COX-2) plays crucial roles in head and neck cancer pathogenesis. This includes in NPC where the COX-2 expression has been shown to be associated with lymph node metastases and progression of the disease (Li et al., 2015). Further research in this area is needed in order to elucidate its true effects as the COX-2 inhibitors are widely available and inexpensive. In addition, these latest findings will lead to its potential role for use as a prognosis marker and evaluation as well as it can be the key factors in deciding a refine and efficacious treatment protocol.

Local failures in nasopharyngeal cancer

Local failure of nasopharyngeal cancer occurs in approximately 5%–30% of patients (Chua, Sham, Hung, Kwong, Kwong, Leung, 1999; Yu et al., 2005; Levendag et al., 2013; Zou et al., 2014). This is probably an underestimation, especially in developing countries, since these statistics are derived from centers of excellence. A recent study in an academic hospital in Indonesia, where NPC has an incidence of at least 6.2:100,000 (14,000 cases a year), revealed that only in 29% of the patients treated with curative intent a complete response directly after treatment was obtained. Local persistent disease was seen in 31% of patients who were evaluable for therapy assessment (Wildeman et al., 2013).

The treatment of local failures is challenging. Surgeons dedicated to NPC specialized in NPC recommend surgery (open and/or endoscopic) (Chen et al., 2009; Gondhowiardjo, Prajogi, & Sekarutami, 2008; Hao & Tsang, 2010).

However, extensive experience is required to get access to the nasopharyngeal space and to achieve free tumor margins, which is a prerequisite for successful surgery. Radiotherapy options are also limited, due to the dose-related toxicity. Sometimes brachytherapy or stereotactic radiotherapy is given. For selective cases good tumor response rates are achieved with surgery or reirradiation (or a combination), although the side effects can be significant.

Eighty-five percent of NPC patients are diagnosed in countries with poor access to radiotherapy facilities. One study showed a correlation between survival and access to radiotherapy. Limited access was related to poor survival outcome. In Indonesia local failure is a common problem and the required surgical and radiotherapy equipment is often not available. In Indonesia, it is not exceptional to wait for more than 6 months for initial radiotherapy causing the high rate of local failures (Gondhowiardjo et al., 2008; Stoker et al., 2013). Therefore readily available, easy-to-perform, and preferably low-cost alternative modalities are needed.

Surgical treatment of recurrent or persistent nasopharyngeal cancer

Conventional treatments for small local failures of NPC are surgery and reirradiation. Hao et al. showed that surgery for T1 lesions gave a 5-year local control rate of 58% and an OS of 65% (Hao & Tsang, 2010). Wei, Chan, Ng, and Ho (2011) performed the maxillary swing procedure with curative intent on 246 patients (Wei et al., 2011). Radical margins were achieved in 78% and the 5-year disease-free survival was 56%. Vlantis et al. retrospectively reviewed open surgery of 80 patients (Vlantis et al., 2008). The majority (95%) had rT1-2 stage. In 69% pathological clean margins were achieved. In case of positive margins, patients were treated with brachytherapy. The 5-year overall survival was 43–59%. Chen et al. treated 37 patients with endoscopic surgery; radical margins were achieved in 97% (Chen et al., 2009). During follow-up, no recurrences were seen for the rT1. In summary, the 5-year disease-free survival after surgery for small local failures is approximately >55%.

Radiation as salvage treatment for local failures is disputable since a full dose (60–70 Gy) was unsuccessful in the primary treatment, suggesting that the residual tumor cells are insensitive for radiation. Moreover, the cumulative dose toxicity makes side effects the limiting factor (i.e., brain necrosis, cranial nerve palsy, and endocrine dysfunction; Ren et al., 2013). Brachytherapy is the most commonly used modality for small local failures. Cheah et al. later reviewed the outcome of 33 patients treated with brachytherapy (Cheah, Lau, Yusof, & Phua, 2014). The 5-year local disease-free interval was 45% and overall survival was 28%. Major complications were seen in 35% of patients. Recently, Ren et al. (2013) published very promising results of 3D-image-guided high-dose-rate

intracavitary brachytherapy in 32 patients (Ren et al., 2013). They had a 100% complete response rate for the small local failures and a 5-year overall survival of 97%. Xerostomia and mucositis were frequently seen, but limited to Grade 1–2. There were no neurological complications.

Photodynamic therapy in nasopharyngeal cancer

Photodynamic therapy (PDT) is a treatment based on the activation of an intravenously administered photosensitizer by a light source with a wavelength of 652 nm (Hopper, 2000; Nyst, Tan, Stewart, & Balm, 2009). During light exposure, reactive oxygen species are generated that harm and destroy the tumor cells. For the treatment of recurrent or residual NPC, the only requirements are a photosensitizer, a laser, a nasopharyngeal light-applicator, and a lux-meter (Nyst et al., 2007). PDT can be performed under local anesthesia. The treatment procedure is easy to learn and can be performed in the outpatient clinic. Although a number of studies with head and neck carcinomas including NPC showed good response rates with acceptable side effects PDT has not yet been integrated in routine clinical practice (Abbas, Jerjes, Upile, Vaz, & Hopper, 2012; Kulapaditharom & Boonkitticharoen, 1999; Kulapaditharom & Boonkitticharoen, 2000; Lofgren et al., 1995; Tong, van Hasselt, & Woo, 1996; Wildeman, Nyst, Karakullukcu, & Tan, 2009).

Several clinical trials with first-generation hematoporphorin-derived photosensitizers (HpD or Photofrin) have shown that PDT is effective in destroying NPC. Local tumor control and complete responses were found in the majority of patients with limited recurrent or persistent disease, while achieving long-term palliation in cases with extensive recurrence (Hopper, 2000; Lofgren et al., 1995; Sun, 1992; Tong et al., 1996). The disadvantages in these studies were the light delivery and the selection of the photosensitizer. Complete illumination of the tumor is difficult, due to the location in the nasopharynx, which is deeply hidden in the head with vulnerable structures like the soft palate. The photosensitizers used (first generation) have a depth penetration of a maximum of 5 mm and a light hypersensitivity of several months.

To overcome these challenges, a special nasopharyngeal applicator has been developed and a new (second generation) photosensitizer Temoporfin (Foscan) was introduced. This applicator allows one-stage illumination of the entire nasopharynx (Fig. 14.1) (Nyst et al., 2007) and the new sensitizer reaches a depth penetration of 10 mm with a light hypersensitivity of only a few weeks. Research in NPC cell lines confirmed a much better efficiency of this sensitizer as compared with HpD (Yow, Chen, Mak, Cheung, & Leung, 2000).

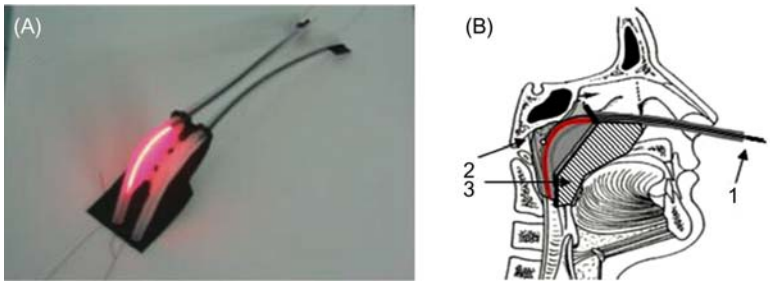

Figure 14.1 Nasopharyngeal applicator for photodynamic therapy (PDT). (A) Applicator; (B) Schematic view of positioning and illumination. 1. Cylindrical diffuser in shielding tube. 2. Target area. 3. Soft pallet is shielded. Source: *Nyst, H. J., van Veen, R. L., Tan, I. B., Peters, R., Spaniol, S., Robinson, D. J., Sterenborg, H. J. C. M. (2007). Performance of a dedicated light delivery and dosimetry device for photodynamic therapy of nasopharyngeal carcinoma: Phantom and volunteer experiments. Lasers in Surgery and Medicine, 39(8), 647–653.*

Photodynamic therapy versus surgery and radiotherapy for local failures of nasopharyngeal cancer

In a phase I study and later in the phase II study, in Yogyakarta, Indonesia, the combination of the second-generation photosensitizer and the applicator was used for the treatment of 22 and respectively 21 patients with recurrent and persistent NPC restricted to the nasopharynx (Nyst et al., 2012; Stoker et al., 2015). The therapy is easy to perform and well tolerable under local anesthesia. Clinical benefit was evident. In the phase I trial the 2-year disease-specific survival was 80% and overall survival 70%. In the phase II trial the 2-year local control rate was 75%, and overall survival was 65%. Pain (headache) was the most common side effect. No treatment-related deaths or other serious adverse events where seen. During the intervention no complications were encountered and all patients were treated under local anesthesia.

A few others have studied the role of PDT for local failures in the nasopharynx. Both recurrent and persistent disease were included in these studies. Kulapaditharom et al. treated six patients with rT1-2 disease; all of them had a complete response and a mean disease-free survival of 26 months (Kulapaditharom & Boonkitticharoen, 2000). Lofgren et al. treated five patients with a tumor depth $<$10 mm; three out of five patients remained local disease-free for over 4 years (Lofgren et al., 1995). Succo et al. published the results of six NPC patients with small local disease [T1 or T2a (American Joint Committee on Cancer, AJCC 2007)]; 5 (83%) had a complete response (Succo, Rosso, Fadda, Fantini, & Crosetti, 2014). The 2-year disease-free survival was $>$50%.

In summary, PDT has shown to be effective for limited local failures with a 2-year local disease-free survival of at least 50%.

Comparing PDT with reirradiation and surgery is difficult due to the small number of patients, the heterogeneity of the patient population, the indistinctness of residual and recurrent disease, and the use of different study outcome parameters. Nevertheless, it appears that PDT is not underachieving in clinical efficacy. The advantages of PDT include: it is availability and the easy procedure, makes it a very good option for treatment of persistent and recurrent disease in low incidence and low-income countries. Also for more advanced lesions PDT has been successfully used. PDT showed clinical benefit in a number of patients suffering from a locally advanced recurrent NPC (Abbas et al., 2012; Indrasari et al., 2012; Kulapaditharom Boonkitticharoen, 2000). The exact mechanism of these outstanding response is not fully understood because entire illumination of the tumor was not possible. It suggests that an immunological response might have controlled tumor progression. Future studies can also focus on the role of PDT in combination with immunotherapy for NPC.

Immunotherapy for nasopharyngeal cancer

Immunotherapy can involve a broad range of strategies. Roughly, their goals can be categorized in increased tumor antigenicity (making the tumor cells more recognizable to the immune system), increased immunological response (for a stronger and longer lasting immune response), and a decreased immune escape of tumor cells. The presence of EBV in NPC makes it a potential target for immunotherapy. One of the strategies to improve tumor antigenicity is EBV cytolytic virus activation therapy. In the lytic phase of EBV, more immunogenic proteins are expressed, making the tumor more susceptible for immunological elimination and susceptible for antiviral drugs, like valganciclovir. Despite initial promising results, a phase I/II trial did not result in a clinical breakthrough, probably due to a more complex strategy of tumor escape (Wildeman et al., 2012). A strategy for a more direct immune effect is the administration of or expansion of EBV-specific cytotoxic T lymphocytes (CTL). Expansion of the EBV-specific CTL in NPC has been achieved, but the lack of actual clinical effects on tumor response means more research is needed. Hypothesized problems are that NPC tumor cells are not recognized by the CTL and that the CTL might not reach their target place (nasopharynx or place of metastatic disease) in an active state (Jain, Chia, & Toh, 2016; Tsang, Lee, & Kwong, 2014). Currently, several clinical trials are being performed with the aim of improving the strategy of EBV-specific CTL therapy in NPC (Smith et al., 2016). In immune-checkpoint blockades, one of the promising targets is the programmed cell death pathway. Programmed death-1 (PD-1) is a cell-surface receptor that is expressed on lymphocytes. After it binds with programmed death-1 ligands

(PD-L1) located on the cell surface of the tumor, the lymphocyte proliferation program and its effector functions are inhibited. It is assumed that in this way PD-1 plays an important role in the immune escape of tumor cells. In a "normal" immune response, this pathway prevents damage to collateral tissue during an inflammatory response. In NPC, both PD-L1 and PD-1 are highly expressed, and associations have been found between the extent of expression and the stage of disease, and the interval to recurrent disease and overall survival. This implies its role in tumor growth and supports its potential as a target in treatment (Chen et al., 2013; Hsu et al., 2010; Lee et al., 2015). Studies with anti-PD-1 in the treatment of melanomas showed promising results (Ribas et al., 2016). Currently, two anti-PD-1 agents are being studied for patients with recurrent and/or metastatic NPC (i.e., Nivolumab and Pembrolizumab). Preliminary results of Pembrolizumab showed tumor reduction in two-thirds of the patients. Additionally, the influence of the extent to which PD-1 and PD-L1 are expressed in the tumor microenvironment on clinical outcome has been studied (Hsu et al., 2010, 2015; Lee et al., 2015; Ribas et al., 2016; Schumacher, 2016).

Conclusion

Radiotherapy in combination with chemotherapy is the standard of treatment for NPC and gives relatively good survival rates. Nevertheless, a significant number of patients experience side effects of the treatment itself or will end up with residual or recurrent disease or distant metastasis. Extensive research is happening and making progress. Early diagnosis and follow-up will be improved by the use of EBV biomarkers. The standard treatment is enhanced by better imaging and radiation techniques for higher radiation dose to the target volume and less to the surrounding tissues. In the case of tumor relapse advanced surgery and radiation techniques are explored. Also, relatively new treatment modalities like PDT and immunotherapy can contribute significantly in the prognosis of patients with NPC.

Even though sophisticated equipment and effective treatment modalities are available in the high-end centers, the majority of patients with NPC will not have access to these facilities due to scarceness and lack of financial resources, especially in low- to middle-income countries. Hence it remains our responsibility to ensure that all NPC patients have access to well-equipped treatment facilities in the near future.

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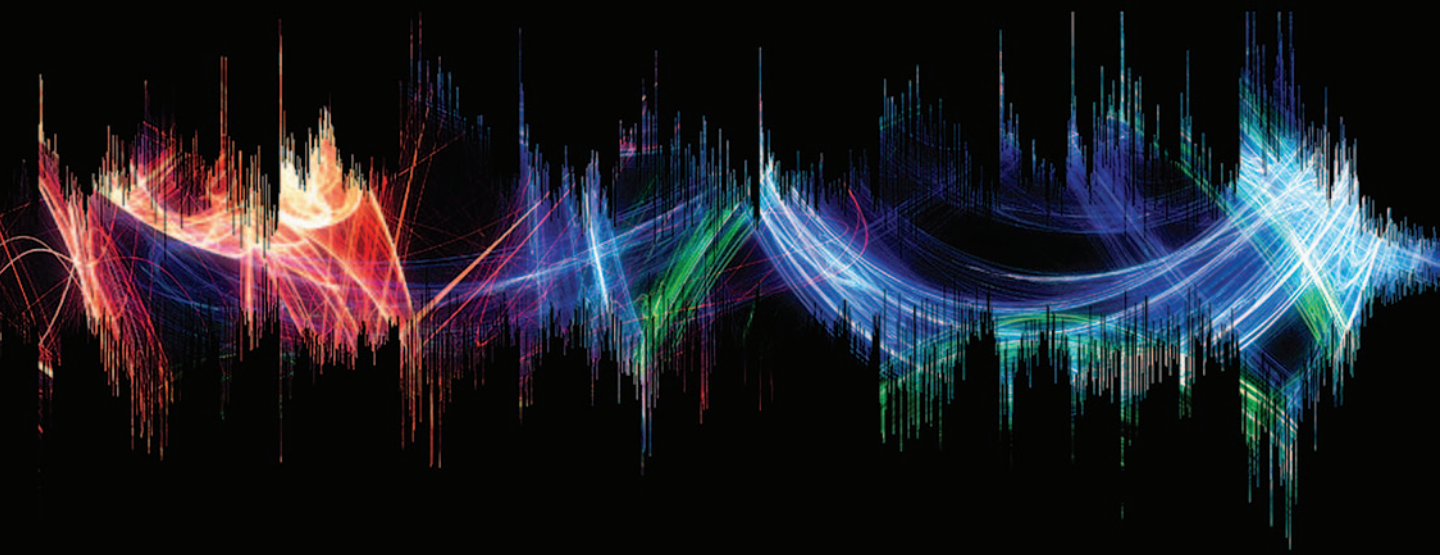

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An Evidence-Based Approach to the Management of Nasopharyngeal Cancer

From Basic Science to Clinical Presentation and Treatment

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An Evidence-Based Approach to the Management of Nasopharyngeal Cancer: From Basic Science to Clinical Presentation and Treatment is a comprehensive overview with updated management procedures for nasopharyngeal carcinoma. Written by experts on the subject, it is organized in a simple yet comprehensive manner to aid in the understanding of this complex condition.

This book discusses several topics related to NPC such as epidemiology, pathophysiology, risk factors, and treatment (surgical and nonsurgical). Moreover, it discusses the key features of clinical presentation of NPC and recent advances and future promising therapies. The book is a valuable source for clinicians, graduate students, oncologists, and several members of biomedical field who are interested in understanding nasopharyngeal cancer in a practical and applicable way.

Key Features

- Discusses current trends in surgery with use of endoscopy, robotic, and navigation technology in the management of NPC
- Encompasses colorful figures of pathology, clinical cases, endoscopic findings, surgical approach, resection of tumors, brachytherapy, robotic, and navigation technology to help fully comprehend the content

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