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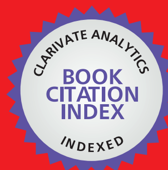

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Chapter

Screening of Nasopharyngeal Carcinoma

Yanping Yang and Yonglin Cai

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Abstract

Nasopharyngeal carcinoma (NPC) is one of head and neck cancer. It has a complicated etiology involving Epstein–Barr virus (EBV) infection, environment changes, and genetic susceptibility. Early symptoms of NPC are unspecific, so most NPC patients are diagnosed at a late stage. An effective population screening strategy could increase the early detection and early diagnosis rate. As far, serological detection of EBV antibodies such as VCA-IgA, EA-IgA, and EBNA1-IgA, are widely used in NPC mass screening; EBV DNA load detection in plasma or nasopharyngeal swab was applied to screen in endemic populations for assessing the feasibility. However, the current screening schemes still have disadvantages such as lowly positive predictive value, unclear effectiveness of screening and cost-effectiveness. In the present chapter, we try to review the different screening strategies for NPC to understand the future direction of development.

Keywords: Nasopharyngeal carcinoma, Screening, Early diagnosis, Biomarker, Epstein–Barr virus, Antibody, DNA load

1. Introduction

Screening is primary measure of secondary prevention of cancer. It mainly regularly monitor the asymptomatic high-risk population to achieve the purpose of early detection and early treatment of malignant tumors. Cancer carring out the secondary prevention should have the following conditions: 1) great harm to the health and life of the population; 2) long enough pre-clinical period; 3) better effect of both the early treatment and intervention of precancerous lesions; 4) screening methods with effective sensitivity and specificity.

Nasopharyngeal carcinoma (NPC) is a one of head and neck cancer. At present, the etiology of NPC has not remained completely elucidated, generally considered involving Epstein–Barr virus (EBV) infection, environment changes, and genetic susceptibility. There are no feasible preventive measures for NPC. However, it has secondary prevention. First, NPC exhibiting marked racial and geographical differences, is epidemic in the population of Southern China, Southeast Asia, and North Africa, which is extremely harmful to human life and health [1]. In 2014, the incidence and mortality of NPC were 2.48 per 100,000 and 1.23 per 100,000 in China respectively [2]. At the same time, the morbidity and mortality of males were higher than females. The morbidity was mainly in the young while the mortality was mainly in middle and old age. Besides, the occurrence and development of NPC is a multi-stage process, which includes initiation, promotion, malignant transformation, and advanced stage of disease [3]. It takes a certain amount of

time to develop into a malignant tumor. In addition, the clinical stage of NPC is an important factor affecting therapeutic outcome. The 10-year survival rate for NPC with stages I and II can reach up to over 90%, whereas for patients with stage III and IV is less than 50% [4]. At last, there are effective methods to detect pre-clinical patients, and the level of antibody against EBV antigens is significantly related to the risk of NPC [5, 6].

NPC first occurs in the epithelium of the nasopharynx, which can invade the base of the skull and metastasize to the cervical lymph nodes. It has the characteristics of complex manifestations, hidden onset, non-specificity of the initial symptoms, and difficulty in early diagnosis. According to statistics, patients with NPC at an early stage who came to the hospital accounted for only about 20% of the total [7]. Strengthening secondary prevention - early detection, early diagnosis, and early treatment - is an important part of the prevention and treatment of NPC. At the same time it is the key to improve the cure rate and obtain a better prognosis of NPC patients.

2. EBV specific antibodies-based serologic testing

EBV belonging to γ-herpesvirus is a human herpesvirus with B lymphocytes. Nearly 95% of adults worldwide are infected with this virus. EBV in infected cells can be divided into two states: EBV latent infection and EBV lytic infection. Only a few virus genes are expressed in EBV latent infection, which can ensure the basic replication function of the virus but lossing infection ability. In EBV lytic infection, EBV needs to be activated about 80 ~ 100 viral genes to complete host-to-host propagation, and finally produce and form infectious virions (or viral particles). After the initial infection, EBV can establish a lifelong latent infection in the host, and persistent EBV lysis replication state infection can lead to a series of human malignant tumors [8].

EBV infection is closely related to the occurrence and development of NPC. EBV latent infection of nasopharyngeal epithelial cells is considered to be a key step in the carcinogenesis of epithelial cells. After EBV infection, the expressed virus genes can produce different antigens, such as EBV nuclear antigen (EBNA), membrane antigen (MA), early antigen (EA), viral capsid antigen (VCA), BZLF1 transcription activator protein (Zta), BRLF1 transcription activator protein (Rta), etc. [9].

The detection of antibodies against EBV antigens in the sera of NPC patients was reported as early as 1966. Helen W et al. first proposed the view that immunoglobulin A (IgA) antibodies against EBV can be used for the diagnosis of NPC [10]. Studies also confirmed that the expression of IgA antibodies against VCA (VCA-IgA) in NPC patients was higher than in healthy people and the antibody titer was related to the stage of NPC. The idea of using this antibody for NPC screening also was proposed [11]. In 1977, Y Zeng et al. established a prospective prevention and treatment site for NPC in Cangwu, Guangxi province China, in order to carry out research on early diagnosis and etiology analysis. The first NPC mass screening was carried out in Cangwu County by the application of the immunoenzymatic (IE) method to detect VCA-IgA and EA-IgA [12]. Therefore, the NPC screening model suitable for the population in the high-risk areas was established by Zeng's team. Then this mass screening model was promoted to three high-risk areas in China, including Guangxi, Guangdong, and Hainan province; and more than four hundred thousand people were screened for NPC [13–16]. In the 1980s, similar methods were used to screen and follow up large populations in Guangdong and Taiwan provinces [17, 18]. SM Cao et al. performed a prospective screening study of 18,986 subjects with a 20-year follow-up in Guangdong province using the same method [6].

This study showed that both VCA-IgA and EA-IgA antibodies were effective serum markers for NPC screening in high-risk areas. Thus, this method was considered as the standard tool for NPC mass screening in China. However, the IE method for detecting EBV antibodies also has disadvantages, such as tedious operation, long time consuming, no quality control standard, and subjective influence on manual interpretation results. These characteristics made it difficult to consistently perform in a large population.

Enzyme-linked immunoassay (ELISA) with many advantages compared with the IE method, such as simple operation, automatic detection, and interpretation of results by a microplate reader, which have subsequently been applied in NPC screening. Many studies about using ELISA for NPC screening were reported. The detection of ZEBRA-IgG by ELISA was applied to screen NPC, but it's specificity and sensitivity were lower compared with the detection of VCA-IgA based on IE [19]. ELISA-based detection of EBV-related antibodies, such as VCA-IgA, EBNA1-IgA have also found to be a marker for NPC screening [20–22]. The detection rate of one single marker was found to be not ideal, and issues such as the combination of indicators for joint detection, the setting of thresholds, and the strategy of screening intervals were discussed.

In Indonesia, the two-step approach employed the EBV IgA ELISA based on a combination of VCA p18- and EBNA1-derived synthetic peptides as an initial screening test and the EA-IgA ELISA as a confirmation test. The sensitivity and specificity for diagnosing NPC using it significantly increased, as well as positive predictive value and negative predictive value [23].

JY Guo et al. evaluated the diagnostic effect of VCA-IgA, EA-IgA and Rta-IgG antibody detection alone or Combiningly in NPC. The triple-positive of VCA-IgA, EA-IgA and Rta-IgG antibodies suggested the highest risk of NPC, and the triple-negative of them showed the lowest risk [24].

In Taiwan, the ability of anti-EBV-IgA antibody to detect NPC in a high-risk population was evaluated. These markers targeted at the following EBV peptides including EBNA1, VCAp18, EAp138, Ead_p47 and VCAp18 + EBNA1 peptide mixture. The result showed that EBNA1-IgA was a sensitive biomarker for differential diagnosis of NPC. At the same time they identified 80% of the high-risk individuals who developed to NPC during follow-up (80% sensitivity) during measuring at baseline [25].

SM Cao's team developed a prediction formula to calculate Logit P-value with VCA-IgA and EBNA1-IgA as variables ($\text{Logit P} = -3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA}$). The specificity of the new screening scheme is equivalent to traditional screening scheme with the IE method (estimated at 98.5%), but the sensitivity of former (75.0%) is significantly higher than the latter (25.0%) [26]. A total of 28,688 Guangdong residents aged 30–59 years were screened by the combination of two EBV antibodies tests in addition to indirect mirror examination in the nasopharynx and/or lymphatic palpation (IMLP) in Sihui and Zhongshan, Guangdong province China. After one year of follow-up, the total detection rate of NPC was 0.14% (41/28,688), and the early diagnosis rate was as high as 68.3% (28/41) [27]. After six-year follow-up, the sensitivity of the new scheme was 95.7%, with AUC = 0.926 (95% CI: 0.885–0.966). The new screening scheme for NPC is verified to be the preferred serum diagnostic strategy for long-term screening in high-incidence areas of NPC [28]. For the best interval, studies have shown that the incidence of NPC was low in the first few years after the negative screening and then it would increase to the general population level. Therefore, the screening interval of 4–5 years may be more appropriate than 9–10 years after VCA-IgA negative detection in NPC screening [29]. The above research results were adopted by the Chinese Technical Program of Cancer Early

Diagnosis and Early Treatment --Technical Scheme of NPC Screening to guide the annual routine population screening in NPC high-risk areas (**Figure 1**).

However, there are still limitations in NPC screening using EBV antibodies as tumor markers. The false-positive rate of EBV serological screening is relatively high. The positive rate of EBV antibody in the high-risk areas of NPC is 3% ~ 10%. High-risk groups require further examinations, such as nasopharyngeal fibroscopy

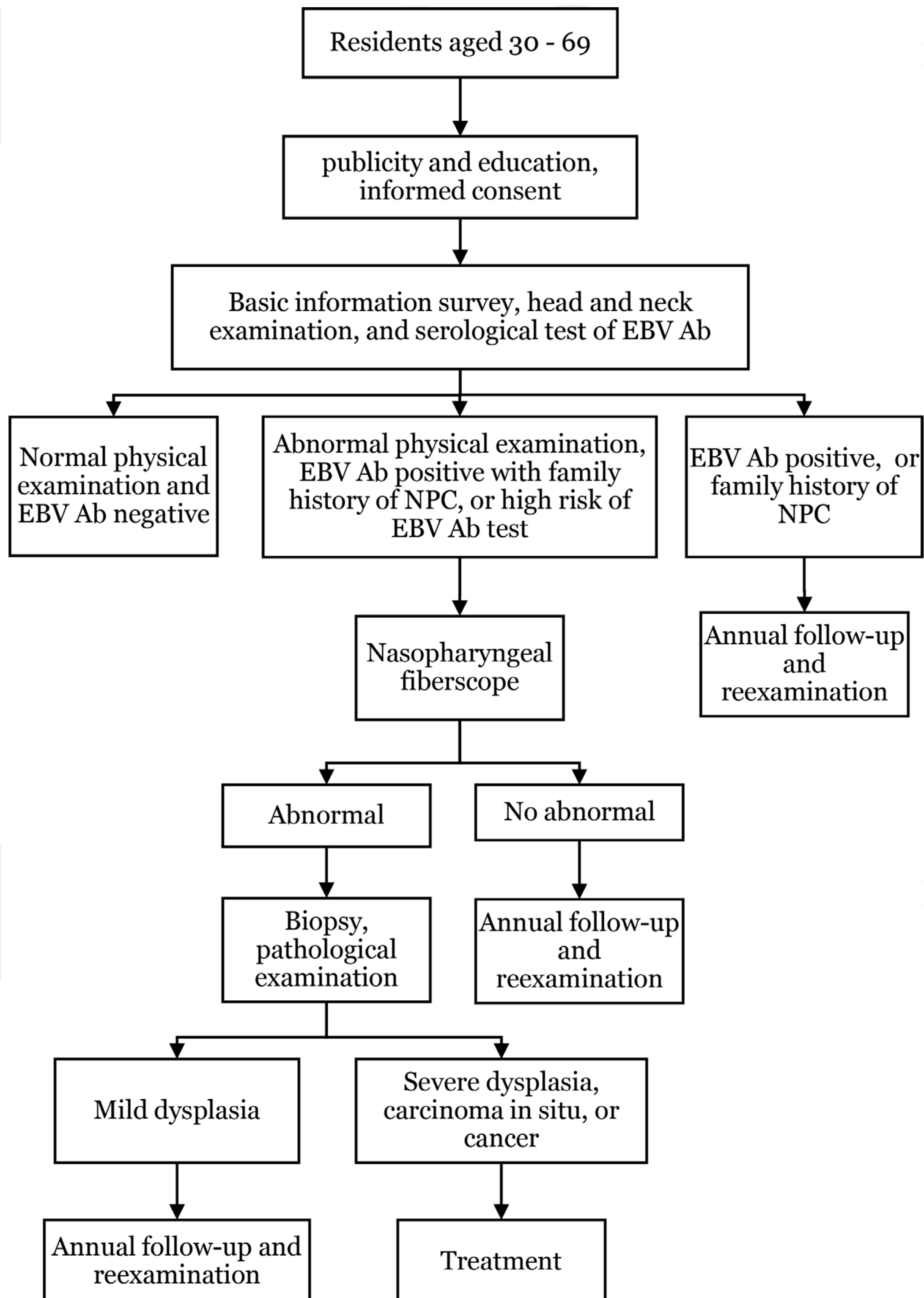

Figure 1.
Flowchart of NPC screening procedure for nasopharyngeal carcinoma (NPC). EBV, Epstein–Barr virus; ab, antibody. Cited by expert group of NPC screening project, expert committee on early diagnosis and treatment of cancer project, China. The technical scheme of NPC screening, early diagnosis and early treatment (2015 edition).

and nasopharyngeal tissue biopsy. Pathologically diagnosed NPC only accounts for 1.5% ~ 3.3% of the high-risk population at the initial screening, which further leads to a higher false positive rate [27, 30, 31].

3. EBV DNA load detection in plasma or nasopharyngeal swab/brushing

Plasma EBV DNA load detected with polymerase chain reaction (PCR) has been also explored to detect early-stage NPC in asymptomatic patients. Research has demonstrated that EBV DNA could be quantitatively measured in the blood of NPC patients by PCR [32]. A study of 175 patients in New York City found that EBV DNA test had much higher specificity and positive predictive value than IgA test alone [33]. A systematic review reported that the EBV DNA load test had the largest area of 0.932 under the summary receiver operator curve with high sensitivity (73%) and specificity (89%), which suggested that EBV DNA detection in plasma could be an efficient marker in NPC screening [34].

In Hong Kong, a prospective study of 20,174 participants revealed that EBV DNA load in plasma samples was particularly useful in screening for early asymptomatic NPC [35]. The participants were ethnically Chinese men at 40 to 60 years of age. The subjects with initial positive results were detected again after about four weeks, while the subjects with persistent positive EBV DNA in plasma were performed to check by nasal endoscopy and magnetic resonance imaging (MRI). The median duration of follow-up was 22 months (range, 12 to 44 months). This study showed that the sensitivity and specificity of this method in NPC screening were 97.1% and 98.6% respectively.

However, a study reported that EBV DNA load had a little poor sensitivity and specificity for NPC screening among high-risk family members compared with EBV-IgA serology [36].

The patients with early NPC may only release a limited amount of viral DNA to the blood, making it impossible to detect blood circulation. The potential value of plasma EBV DNA detection in screening for early NPC remains controversial. NPC mainly originates from epithelial cells in the nasopharynx fossa or posterior wall of the nasopharynx. EBV genome can be detected in almost all tumor cells of NPC cases [37]. Clonal EBV genome can be continuously detected in invasive cancer and precancerous high-grade dysplasia [38]. It is suggested that direct detection of EBV genome from nasopharyngeal brushing or swab specimens had highly predictive value for screening asymptomatic NPC.

In the 1990s, a prospective study was designed to assess the feasibility of a new method for NPC screening by using of PCR coupled with nasopharyngeal swab [39]. In this study, 55 patients were enrolled. The result showed that this method had a similar sensitivity to serological methods, indicating this new method was a good supplement to NPC screening. Nasopharyngeal swab is a quite simple procedure with little discomfort. SP Hao et al. detected the expression of EBV-derived latent membrane protein 1 (LMP-1) by nasopharyngeal swab, and he found that this strategy could serve as part of a screening program for high-risk populations with a sensitivity of 87.3% and a specificity of 98.4% [40]. Raymond's study has also confirmed the effectiveness of this new method of screening for NPC. This study performed on 578 patients yielded a sensitivity of 98.9% and a specificity of 99.3% with a positive predictive value of 96.9% and a negative predictive value of 99.7% [41]. In a prospective and population-based study, the detection of EBV load in the nasopharynx by nasopharyngeal swab was demonstrated to be a useful tool as a supplement to serological tests [42]. Studies of both Zheng and Zhang also verified the same conclusion [43, 44]. Notably, Nasopharyngeal swab detection of EBV load

alone should not be used as a mean of NPC screening because of its high false-positive rate [42]. However, nasopharyngeal swab serving as an applicable sampling method for NPC screening is great feasible, but more research will be needed in the future.

4. Novel biomarkers/technology for NPC screening

As mentioned above, EBV-related test has been widely used for early NPC screening, especially the combination of EBV-antibody VCA-IgA and EBNA1-IgA. With the development of research technologies, other biomarkers also develope for NPC screening. Liu et al. reported that a combination of PCR and MWCNT-Fe3O4 nanocomposites had the higher detection rate and higher sensitivity compared with the traditional ELISA method [45]. MWCNT-Fe3O4 nanocomposites are a combination of multi-walled carbon nanotubes and iron oxide nanoparticles, which can provide a large surface areas for antigen–antibody binding. A nested case–control study including 20 patients with NPC and 88 normal control showed that EBV microRNA BART2-5p had been proved to be a valuable biomarker for NPC screening with a sensitivity of 90.9% [46]. Thirteen genes including DNAAF1, PARPBP, TTC18, GSTA3, RCN1, MUC5AC, POU2AF1, FAM83B, SLC22A16, SPEF2, ERICH3, CCDC81, and IL33 have been associated with NPC detection based on comprehensive bioinformatics analyses [47]. A recent study showed that higher methylation rates of EGFR and ZNF6671 in circulating cell-free DNA (ccfDNA) could predict NPC, which was a potential novel molecular marker for NPC screening [48]. However, all these researches need more evidence and more data to demonstrate their effectiveness in NPC screening.

Many studies have shown that intestinal flora disruption was associated with malignant tumors. C. ramosum bacteria that promotes the secretion of 5-HT was found to be a strong risk factor for NPC. The establishment of a disease prediction model based on C. ramosum might be used for the prediction of disease risk in a high-risk population and early non-invasive screening of NPC [49].

Raman spectroscopy combined with multivariate analysis technology has been reported to analyze the sera of NPC patients and healthy individuals. In NPC samples, the lipid content, phenylalanine, and β-carotene decreased while amide III, tyrosine and tryptophan increased. The changes in these biomolecular concentrations may be applied for NPC diagnosis [50]. A unmodified nanotechnology based on surface-enhanced Raman spectroscopy was used to detect the blood circulating DNA; and the diagnostic sensitivity and specificity for differentiating the NPC patients from the normal control were 83.3% and 82.5% respeactively. Nanotechnology which was sensitive, rapid, and easy-to-use may have the potential to become a better method for NPC detection and screening based on liquid biopsy [51].

5. Cost-effectiveness of NPC screening

A Markov stimulation model was constructed to evaluate the cost-effectiveness of different screening strategies for serological tests in China. In this study, NPC detection rate, cost, quality-adjust life, and incremental cost-effectiveness ratio were considered. Results showed that strategy (annual screening for EBV-seropositive subject, triennial screening for seronegative subjects) was the economical and practical option [52]. In 2019, a Markov cohort model was also reported to use to estimate the screening for NPC with plasma EBV DNA for

50-year-old Asian American men in the United States. The study suggested that because of its high false-positive results in high-risk regions and its uncertain clinical value in non-endemic areas this method wasn't the most cost-effective, despite its specificity and sensitivity were high [53]. Therefore more research will be required in the future.

Studies about NPC screening based on EBV-related test have been widely reported, but there are few studies on the association between EBV-antibody screening and NPC mortality. Recently a study about prospective, cluster-randomized, controlled trial in southern China for NPC screening was revealed that the combination of EBV antibody EBNA1-IgA and VCA-IgA could effectively identify the high-risk population and improve diagnosis of NPC in the interim analysis. Although the mortality of the screening group was not significantly reduced, the specific mortality of NPC in the screening participants was significantly reduced [54]. That was the first report which presented a mortality reduction by NPC screening. It is expected to further improve the participation rate in the future, and finally confirm the effectiveness of NPC screening based on EBV detection.

6. Conclusions and future directions

Due to the hidden location of NPC, it is difficult to diagnose early. Strengthening the publicity of NPC prevention and control, popularizing basic knowledge of it, and making residents cooperate with screening projects will be way helpful to improve the accuracy of early diagnosis rate of NPC. At present, the above screening methods have positive significance, but they also have limitations regrettably. How to make better use of their advantages and disadvantages to carry out local screening schemes in different regions is worthy to further exploration. And developing faster, simpler, higher true-positive rate and lower false-positive rate screening methods and more effective treatment were important ways to improve the survival rate and life quality of NPC patients.

Currently, there are few reports from randomized controlled trials (RCT) and controlled clinical trials (CCT) to determine the efficacy of screening for NPC or the cost-effectiveness of a screening strategy. Future studies with long-term follow-up need to systematically assess the impact of the screening methods in mortality, assess their ability to detect NPC, evaluate the impact on quality of life and cost-effectiveness.

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Conflict of interest

The authors declare no conflict of interest.

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