Review Article

Epstein-Barr virus-based nasopharyngeal carcinoma population screening

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Abstract: Nasopharyngeal carcinoma (NPC) is characterized by its distinct geographical distribution and has a high incidence in southern China and Southeast Asia. Early diagnosis and treatment are the best strategies for individuals identified to have a high risk of NPC. The identification of high-risk populations and the use of appropriate screening methods are key factors when screening for NPC. Despite the discovery of the close association of Epstein-Barr virus (EBV) with NPC, the exact role of the virus in the development of NPC has not been completely elucidated. EBV serological antibody testing is of great significance while screening for NPC, and plasma EBV-DNA has been used for population screening. The screening also includes clinical examination (lymphatic palpation and indirect examination of the nasopharynx using a mirror) and obtaining the family history of patients with NPC. The main secondary screening methods include nasopharyngeal fiberscopy. Another approach to diagnose NPC includes cytological examination of the nasopharyngeal brush exfoliates. Evidence suggests that serological screening for NPC can increase the rate of early diagnosis and significantly improve the 5-year survival rate of the affected population. However, the NPC serological screening protocol has some shortcomings. For example, the positive predictive value needs to be improved. Further research into NPC should be focused on methods that will accurately identify high-risk individuals and optimize screening. Here, we have reviewed the latest progress in EBV-based NPC population screening.

Keywords: Nasopharyngeal carcinoma (NPC); Epstein-Barr virus (EBV); population screening

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Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor prevalent in southern China and Southeast Asia, especially among people living around the Xijiang river basin flowing through the Guangdong and Guangxi Provinces. The incidence of NPC can reach 40–60 per 100,000 individuals per year (1,2). The etiology of NPC remains largely unclear. The site of NPC is often concealed and the clinical symptoms in its early stage are usually not obvious. Most patients are diagnosed with NPC at the intermediate to late stages. The China National Cancer Centre recently reported that between 2012 and 2015, the 5-year overall survival rate of NPC was only 45.5% (3). Steps taken for the primary prevention of NPC are still lacking. Early diagnosis and treatment of NPC is the best choice for the management of NPC among individuals identified to be at high risk of NPC. Primary screening methods should satisfy several criteria and should be highly sensitive and specific, economical, and relatively simple to perform (4). Presently,

there are two main primary screening methods, namely, the serological determination of Epstein-Barr virus (EBV) and detection of serum EBV-DNA using real-time PCR. The screening also includes clinical examination of the head and neck (indirect mirror examination of the nasopharynx and lymphatic palpation) and obtaining the family history of patients with NPC. The main secondary screening methods include endoscopy. Other approaches to screen for NPC include cytological examination of nasopharyngeal brush exfoliates. Currently, the two-stage screening model (EBV serological antibody testing for primary screening and nasopharyngeal fiberscopy for secondary screening) is considered ideal for NPC screening in China. This model is not only simple and with high detection accuracy, but is also affordable. The latest progress in EBV-based NPC population screening will be described in the subsequent sections.

Screening method

Serological screening

Serological screening profile

EBV is a ubiquitous γ-herpesvirus (human herpesvirus type 4). Epithelial cells and B lymphocytes are the main targets for EBV infections (5). The detection of antibodies against EBV antigens in the sera of patients with NPC has been reported as early as 1966 (6). EBV-DNA and EBV nuclear antigen protein (EBNA) have also been detected in NPC cells (7). Henle *et al.* (8) were the first to detect IgA antibodies of EBV during the diagnosis of NPC. This finding has had significant implications in the diagnosis of NPC. Subsequent studies in 1976 confirmed the presence of viral capsid antigen (VCA)/immunoglobulin A (IgA) and early antigen (EA)/IgA antibodies in patients with NPC and that the positive rate of VCA/IgA (93%) was significantly higher than that in healthy individuals (9). The detection of EBV-specific IgA antibodies was proposed for the diagnosis and screening of NPC (10). The combined detection of EBV antibodies includes the primary screening index, mainly the "first-generation" immunoenzyme assay (IES) of VCA/IgA combined with EA/IgA and the "second-generation" enzyme-linked immunosorbent assay (ELISA) of VCA/IgA combined EBNA1/IgA.

Screening history

As early as 1980, Zeng *et al.* (11) were the first to perform EBV serological screening using the IES test in Cangwu County, an area with a high incidence of NPC. An IES was performed in 12,932 healthy individuals aged 40–59 years old in the Wuzhou City of Guangxi and 5.3% were found to have VCA/IgA antibodies. Thirteen cases of NPC were detected and the positive predictive value was 1.9% (12). In Zhongshan City, Sham *et al.* (13) randomly selected 130 of the 6,504 individuals with high VCA/IgA titer for nasopharyngeal fiberscopy and multisite biopsy and found 7 cases of asymptomatic NPC. Ji *et al.* (14) screened 42,048 individuals aged 30–59 years in Zhongshan City and their findings showed that the positive rate of VCA/IgA was 7.36%; 45 cases of NPC were found in the initial screening and the positive predictive value was 1.5%. After 13 years of follow-up, 159 cases of NPC were detected, including 97 cases of NPC in the VCA/IgA-positive population. The incidence rate of VCA/IgA-positive population was higher than that of the negative group in all years, and was about 20 times that of the negative population. This finding indicates that the incidence of NPC is higher in individuals with EBV infections who are seropositive. The presence of EBV antibodies in the serum is indicative of early asymptomatic NPC (15). At the same time, the dynamic monitoring of EBV antibody levels in 107 patients with NPC showed a period of strong antibody response to EBV before the diagnosis of NPC. Moreover, there is a serological window marked by the continuous increase in EBV antibody levels, which may be 10 years earlier than before the diagnosis, with an average of 3 years (16). This conclusion laid a theoretical foundation for EBV serological screening to detect NPC. From 1991 to 2001, Deng *et al.* (17) reported the results of VCA/IgA and EA/IgA in 413,164 healthy individuals. The positivity rate of VCA/IgA was 3.06%; 174 cases of NPC were detected and the early diagnosis rate was 86.8% (Changsha staging in 1979). During the same period (18), 10,665 people in Sihui City of Guangdong Province were screened for EBV antibodies; 74 cases of NPC were detected and the early diagnosis rate was 55.1% (Changsha staging in 1979 and Fuzhou staging in 2008). Cao *et al.* (19) found that the increase in EBV antibody titer was closely related to the increased risk of NPC during the 20-year follow-up of the 18,986 individuals, who were screened, and that VCA/IgA was better than EA/IgA in predicting NPC. EA/IgA was expressed in the early stages of EBV infection and was found to exhibit excellent specificity but low sensitivity. Ji *et al.* (15) found that there were no significant changes in EA/IgA before the onset of NPC, and the titer was low, suggesting that it was suitable for the further detection of

the VCA/IgA-positive population to improve screening specificity.

Recent status of screening

The traditional immunoenzyme-labeling method is often used to detect serological EBV antibodies, including VCA/IgA (using B95-8 cells as antigen) and EA/IgA (using induced Raji cells as antigen), in patients with NPC. Serum dilution ≥1:5 is considered positive. As this method was complicated to operate and there were difficulties in standardizing the color intensity, resulting in errors and low accuracy, it was gradually replaced by ELISA. With the development of new techniques for the detection of dual and triple antibodies, the sensitivity and specificity of the serological diagnosis of NPC have been significantly improved. Fachiroh *et al.* (20) used ELISA to detect VCA/IgA and EBNA1/IgA and reported the sensitivity and specificity as 85.4% and 90.1%, respectively. Jiang *et al.* (21) found that when VCA/IgA (detected using IES method) and EBNA1/IgA (detected using ELISA) were combined, the sensitivity and specificity for the diagnosis of NPC could reach 100% and 84%, respectively. Using logistic regression to integrate the two indicators, the diagnostic efficiency was improved compared with conventional parallel experiments, and the sensitivity and specificity were found to be 98% and 88%, respectively. Coghill *et al.* (22) used ELISA to detect IgA antibodies against EBNA1, VCAp18, Eap138, Ead_p47, and VCAp18 + EBNA1 in NPC and the control populations and found that EBNA1/IgA had the best diagnostic performance and high sensitivity; however, there is the scope of improvement in specificity. During the transition period of the EBV antibody detection technology update, some scholars used two detection methods in large-scale research. A study in Hong Kong (23) has reported the results of screening for EBV antibodies in a total of 929 relatives over 18 years of age in families with a high incidence of NPC. A total of 12 cases of NPC were detected, and the early diagnosis rate was 58.3% (AJCC staging). Cangwu City (Guangxi Zhuang Autonomous Region, China) and Sihui City (Guangdong Province, China) (24) screened 22,623 and 16,773 individuals, respectively. A total of 41 cases and 33 cases of NPC were detected, and the early detection rates were 53.7% and 72.7%, respectively (Fuzhou staging in 2008). The early diagnosis rate of NPC in the above population-screening studies was higher than that of the outpatient study of early diagnosis in the same period.

With the continuous development in ELISA technology, various commercialized EBV antibodies have been increasingly used in the diagnosis of NPC. However, there have also been problems associated with these analyses, such as large differences in the detection efficiency when antibodies from different manufacturers and batches are used. To address this shortcoming, Liu *et al.* conducted a prospective study (25) and compared the results obtained using different detection methods, different antibodies, and different antibody combinations. They also used logistic regression analysis to compare the receiver operating characteristic (ROC) curve area, sensitivity, and specificity of different antibody combinations for the diagnosis of NPC and found that the method of combined detection of EBNA1/IgA and VCA/IgA had a sensitivity of 95.3%, specificity of 94.1%, and area under the curve (AUC) value of 0.97. Through further research, they derived a formula for a cancer risk-prediction model and developed a risk-assessment plan for NPC screening.

From 2008–2010, a total of 28,688 residents aged 30–59 years participated in the screening in Zhongshan City and Sihui City, Guangdong Province, an area with a high incidence of NPC. A total of 862 high-risk individuals was identified using the above risk-assessment program and 38 cases of NPC were detected. The positive predictive value was 4.41% (38/862) and the early diagnosis rate was 68.3% (Fuzhou staging in 2008). Their analysis revealed that the vast majority of early-stage NPC were detected in high-risk groups (accounting for about 3% of the total number of screened individuals), indicating the accuracy of the risk-assessment program and screening strategy. On the other hand, the positive predictive value increased to 4.4% from the previously reported value of less than 2%, and significant progress has been made since then (26) (*Table 1*). These large-scale screening studies show that EBV antibody testing can be used to detect early NPC. The serological method for NPC screening is not only simple and with high detection accuracy but also reasonable in cost; therefore, it is considered worthy of further popularization and use.

EBV-DNA detection

Since plasma EBV-DNA is mainly derived from NPC cells, the detection of blood EBV-DNA has received extensive attention as an auxiliary diagnostic method for NPC. In 1998, Mutirangura *et al.* (27) studied whether circulating EBV-DNA in peripheral blood could be a marker for the diagnosis of NPC. For the first time, ordinary PCR was used and it was found that 31% (13/42) of patients with

Table 1 Cohort studies of NPC serological screening in different periods

Diagnostic test	Region	Enrollment time	Enrollment age (year)	Screening population	Number of cases	Early diagnosis rate (%)	Standard staging
"First-generation" IES of VCA/IgA combined with EA/IgA	Zhongshan (14)	1986	30–59	42,048	97	86.5	Changsha staging in 1979
	Guangxi, Guangdong, and Hainan Provinces (17)	1991–2001	Natural population	413,164	174	86.8	Changsha staging in 1979
	Sihui (18)	1992	30–59	10,665	74	55.1	Changsha staging in 1979 and Fuzhou staging in 2008
"First-generation" and "second-generation"	Hong Kong (23)	1994–2001	Relatives (≥18)	929	12	58.3	AJCC staging in 1997
	Cangwu (24)	2007	30–59	22,623	41	53.7	Fuzhou staging in 2008
	Suhui (24)	2007	30–59	16,773	33	72.7	Fuzhou staging in 2008
"Second-generation" ELISA of VCA/IgA combined EBNA1/IgA	Zhongshan and Sihui (26)	2008–2010	30–59	28,688	38	68.3	Fuzhou staging in 2008

IES, immunoenzyme assay; VCA, viral capsid antigen; IgA, immunoglobulin A; EA, early antigen; ELISA, enzyme-linked immunosorbent assay; EBNA, EBV nuclear antigen.

NPC were positive for EBV-DNA, whereas 82 healthy controls were negative. Lit *et al.* (28) found that although 95% of healthy people had previously been infected with EBV, it persisted in B lymphocytes in the form of latent infection. However, EBV infection in healthy people rarely led to the release of free EBV-DNA in the plasma, thereby not leading to positive findings. In the above studies, EBV-DNA had high specificity, but owing to low sensitivity, its application and use was limited. Lo *et al.* (29) were the first to use real-time fluorescent quantitative PCR to detect EBV-DNA in 96% (55/57) of patients with NPC and in the plasma of 7% (3/43) of the healthy controls. Moreover, they found that the average copy number of plasma EBV-DNA in patients with NPC was significantly higher than that in normal controls, and that the copy number of EBV-DNA in patients in the advanced stage was significantly higher than that in patients in the early stage. Lin *et al.* (30) determined the plasma concentrations of EBV-DNA in patients with advanced NPC and found it to be useful in monitoring patients with NPC and predicting treatment outcomes. Subsequently, many research groups (31-36) studied the importance of EBV-DNA copy numbers in the diagnosis of NPC and found that the sensitivity and specificity of the detection of each study varied. The reason may be attributed to the method of EBV-DNA extraction, different cut-off values, the amount and type of specimens, and the research objects (*Table 2*). Liu *et al.* (40) compared the differences in EBV-DNA between plasma and serum and found that the sensitivity and specificity of the detection of EBV-DNA in plasma were higher than that in serum (91% *vs.* 84%, and 93% *vs.* 76%, respectively). Le *et al.* (41) standardized the method of EBV-DNA quantitative detection by collaborating with laboratories from four different countries, thereby reducing the differences in the detection across different laboratories. Ji *et al.* (37) performed serological EBV-DNA tests on 825 individuals at high risk for NPC in Zhongshan City and Sihui City, Guangdong Province, and initially screened 38 cases of NPC. The positivity rate of EBV-DNA was 12.2%, the sensitivity and specificity of EBV-DNA detection were 86.8% and 90.0%, respectively, and the positive predictive value was 30.0%. The sensitivity of EBV-DNA detection for early NPC was 81.5%, and 14 cases were diagnosed after a year of follow-up. Chan *et al.* (38) determined serum EBV-DNA and VCA/IgA levels in more than 1,300 healthy individuals. Nasopharyngeal fiberscopy was performed in patients with either of the two positive indicators, and three cases of early NPC were found (1 case in stage I, two cases in stage II). All three patients with NPC were positive for EBV-DNA, whereas only one was positive for VCA/IgA. At the same time, the positivity rate of EBV-DNA in the healthy population was found to fluctuate between

Table 2 Summary of NPC studies and detailed characteristics

Study	Sample size (case/control)	Sensitivity (%)	Specificity (%)	Target	Method	Volume of plasma analyzed (μL)
Taiwan (30)	99/40	95	100	BamHI-W	RQ-PCR, plasma	200–400
Hong Kong (31)	139/178	95	98	BamHI-W	RQ-PCR, plasma	400–800
Guangzhou (32)	150/75	92	88	BamHI-W	RQ-PCR, plasma	500
Malaysia (33)	390/72	90	90	BamHI-W	RQ-PCR, plasma	400–800
Guangzhou (34)	160/76	69	88	BamHI-W	RQ-PCR, plasma	No report
Japan and Taiwan (35)	64/65	86	89	BALF 5	RQ-PCR, serum	100–200
Tunisia (36)	66/93	53	100	BXLF1	RQ-PCR, serum	200
Zhongshan and Sihui (37)	825 NPC high-risk population (38/787)	86.8	90	BamHI-W	RQ-PCR, plasma	400
Hong Kong (38)	1,318 healthy population (3/13,15)	100	98.5	BamHI-W	RQ-PCR, plasma	800
Hong Kong (39)	20,174 healthy males (34/20,140)	97.1	98.6	BamHI-W	RQ-PCR, plasma	No report

NPC, nasopharyngeal carcinoma; RQ-PCR, real-time quantitative PCR.

5% and 9%. The plasma EBV-DNA of two out of three healthy people shows a transient or temporary presence for less than 2 weeks, whereas that of patients with NPC is persistent. Therefore, repeated testing of patients who test positive in the first EBV-DNA test is important to distinguish true positives from false positives. In about 0.2% of the healthy population, plasma EBV-DNA will continue to be detectable for more than a year, and this mechanism needs to be further studied. In a study by Chan *et al.* (39), a test positive was defined as a positive test for two samples of plasma EBV-DNA at an interval of about 4 weeks. They found that of the 20,174 men screened, the EBV-DNA positive rate was 5.5% (1,112 people). Subsequently, results of the second test revealed that 309 patients continued to be positive for EBV-DNA. Nasopharyngeal fiberscopy and MRI were performed on some of the subjects who tested positive for EBV-DNA twice, and 34 cases of NPC were diagnosed initially. The sensitivity and specificity of EBV-DNA screening were 97.1% and 98.6%, respectively, and the positive predictive value of the test was 11.0%. Among the 34 patients with a confirmed diagnosis of NPC, 24 were in the early stages (I and II) and the early diagnosis rate was 70.6%. The results indicated that although an EBV-DNA test has a high positive rate (5.5%), two consecutive tests can eliminate most false positives and increase the effectiveness of screening. However, it cannot be ignored that although the positive predictive value of this program has improved, the recruited population were all males (the prevalence of the male population is higher than in females), which may be one of the reasons for the higher positive predictive value observed in this study.

In addition, the diagnostic value of EBV-DNA detection in early NPC needs to be further improved. Ji *et al.* (37) showed that the sensitivity of EBV-DNA in the initial screening of early NPC was only 81.5%. Shao *et al.* (42) and Yang *et al.* (43) reported 5 and 2 cases of stage I NPC, respectively, with a median EBV-DNA of 0 copy/mL, but the serology was positive. Moreover, the cost of EBV-DNA testing is high. EBV serology performed in bulk using ELISA platforms is a more affordable option. EBV DNA testing requires more sophisticated equipment and standardization among laboratories that use this method, making it more difficult to promote EBV-DNA testing on a large scale in populations in all high-risk areas.

Use of nasopharyngeal brush for the detection of exfoliated cells

As EBV infection may result in a state of persistent latent infection in the nasal epithelium of patients with NPC and is closely related to cell morphology and DNA, the use of nasopharyngeal epithelial cells for EBV-DNA

Table 3 Sensitivities and specificities of nasopharyngeal brush and blood EBV-DNA and EBV-IgA antibody testing for NPC

Study location	Diagnostic index	Brush (DNA)	Blood (DNA)	VCA/IgA	EBNA1/IgA
Indonesia (45)	Sensitivity	0.94	0.71	0.65	0.74
	Specificity	0.90	0.50	0.60	0.72
Guangzhou (46)	Sensitivity	0.96	0.76	0.89	
	Specificity	0.97	0.87	0.77	
Guangxi (47)	Sensitivity	0.88		0.88	
	Specificity	0.87		0.75	

EBV, Epstein-Barr virus; NPC, nasopharyngeal carcinoma; VCA, viral capsid antigen; IgA, immunoglobulin A; EBNA, EBV nuclear antigen.

detection has a certain clinical value in the diagnosis of NPC. Nasopharyngeal brush sampling is a non-invasive, convenient, low-cost, and highly reproducible tissue-sampling method that can be used to directly obtain the shed epithelial cells or tumor cells from the tumor site (44). Adham *et al.* (45) performed three different tests on 289 patients with suspected NPC and in 53 healthy controls and indicated the diagnostic value of the nasopharyngeal brush EBV-DNA test that compared well with that of the blood EBV-DNA and VCA/IgA tests. Zheng *et al.* (46) found that the EBV-DNA levels in the nasopharyngeal brush samples of patients with NPC were higher than those in the non-NPC populations and high-risk NPC populations. Moreover, they reported that the diagnostic efficiency of the nasopharyngeal brush EBV-DNA test was better than that detected based on serum VCA/IgA and plasma EBV-DNA. However, the test sensitivity was lower in stage I NPC. In a prospective cohort study, Chen *et al.* (47) used the nasopharyngeal swab EBV-DNA method and detected high VCA/IgA titers in 905 patients. The specificity showed an increase from 75% to 86.5%, which greatly reduced the number of people who were to be advised close follow-up (*Table 3*). Zheng *et al.* (48) studied EBV miRNAs in nasopharyngeal brush samples from 215 patients with NPC and in 209 healthy controls and found that the sensitivity and specificity of mir-bart1-5p were 93.5% and 100%, respectively. Even in patients with early NPC with negative titers of EBV-DNA, VCA/IgA, and EA/IgA, the nasopharyngeal brush mir-bart1-5p test still showed high diagnostic performance. Some studies also (49,50) suggest that the detection of tumor gene promoter hypermethylation in nasopharyngeal brush samples can be used for early diagnosis of NPC; however, the sample size of related studies was small and further verification using a larger population is needed. Therefore, cytological analysis of the nasopharyngeal brush exfoliates is one promising test that needs to be further explored.

Investigation of family history of NPC and clinical examination of head and neck

Investigation of family history of NPC

NPC is closely related to genetic factors and has significant family history characteristics. Studies show that individuals from families with a high incidence of NPC from high-incidence areas have a 4–20 times higher risk of NPC than the general population. Among them, the risk of NPC in EBV antibody-positive individuals is 31 times higher than in those without a family history of NPC and those who are EBV antibody-negative (51). Therefore, individuals with a family history of NPC should be considered as high-risk groups and should be regularly followed up. However, although the family history of NPC is associated with low sensitivity and specificity, it may be added along with other risk factors and/or genetic factors to improve predictive efficiency.

Clinical examination of head and neck

Clinical examinations of the head and neck include indirect mirror examination in the nasopharynx and/or lymphatic palpation (IMLP). The technical plan for NPC screening in China introduced in 2008 utilized EBV serological testing and IMLP. Few studies have evaluated the diagnostic value of IMLP alone. In 2008, a study that was designed to screen 28,000 people in Zhongshan City and Sihui City, Guangdong Province, China found that the missed diagnosis rate of IMLP was as high as 83% (34/41) and the sensitivity was only 17%. However, 92.7% of the cases of NPC were found in high-risk serology. Moreover, only 28% of the cases detected using IMLP identified early-stage patients (26). Since the accuracy of IMLP

examination depends on the physicians' experience and has low sensitivity especially in early NPC, the use of IMLP to screen populations in high-risk areas is limited.

Nasopharyngeal fiberscopy

Nasopharyngeal fiberscopy is an important method for the in-depth screening of NPC. Individuals who have been identified to be at high risk based on serological EBV antibodies and/or on the basis of their immediate relatives diagnosed with NPC, as well as those suspected with NPC after clinical examination are candidates for further examination using nasopharyngeal fiberscopy. Sham *et al.* (13) randomly selected 130 of 6,504 individuals with high VCA/IgA titers in Zhongshan City for nasopharyngeal fiberscopy and multisite biopsy and found 7 patients with asymptomatic NPC. The nasopharyngeal fiberscope has a soft body and is easy to operate. It magnifies the nasopharyngeal field of vision and can be used to reach the site of suspicious tissue for taking biopsy more accurately. The detection rate of early-stage tumors is higher using this method than using indirect nasopharyngeal endoscopy. However, early-stage tumors in certain areas, such as pharyngeal recesses and submucosal microscopic lesions, are likely to cause missed diagnosis and, therefore, need careful evaluation by ENT specialists. Currently, this method has been widely used to screen for NPC.

Screening process

Risk stratification of the screening population

In addition to the continuous updating of the EBV-antibody indicators and detection methods, the screening process is also being constantly optimized. The most prominent feature is the risk stratification of the population that is to be screened. In the 1970s, individuals who were EBV IgA antibody-positive were screened regularly and were indicated for routine annual re-examinations. This program was not only time consuming and labor intensive, but was also associated with poor screening efficiency. In 2002, Cheng *et al.* (52) used ELISA to detect 121 cases of NPC and screen 332 healthy individuals, and reported the sensitivities of EBNA1/IgA, EBNA1/IgG, and Zta/IgG to be 85%, 83%, and 79%, respectively. The sensitivity of the three combinations was as high as 92%; the specificity was 86%, 86%, and 80% respectively; and the specificity of the three combinations reached up to 93%. For the first time, a study divided the risk of NPC in the studied population into low risk, intermediate risk, and high risk based on the odds ratio. Among these groups, 93% of healthy people were at low risk with an odds ratio of 0.0 to 0.3, whereas 0.4% of healthy people were at high risk with an odds ratio of 137.9. The proposed risk stratification of the population for NPC screening showed a considerable reduction in the number of people who needed to be closely followed. In 2008, ELISA was used to detect VCA/IgA and EBNA1/IgA simultaneously and the probability of individuals diagnosed with NPC was calculated using the logistic regression equation. Accordingly, the cancer risk of the population was divided into high risk, medium risk, and low risk. Different groups of people use different follow-up plans. Individuals who are at high risk of NPC are indicated for nasopharyngeal fiberscopy and those at medium risk are recommended for annual follow-up. This program was adopted by the 2011 version of China's "*Technical Program for Cancer Screening and Early Diagnosis and Treatment*" (53), which has high health economic benefits.

Optimization and exploration of the screening methods

New methods are constantly being developed to improve the detection and management of NPC. Coghill *et al.* (54) tested the IgA and IgG antibody responses of EBV in 607 Taiwanese residents (175 cases of NPC and 175 matched controls; 37 screened individuals and 117 matched controls; 26 patients with NPC diagnosed during the follow-up of high-incidence families and 77 controls without NPC), used a protein chip to detect 199 sequences of 86 EBV proteins, and developed an antibody-based predictive model to assess the risk of NPC. When this model was combined with the currently used VCA/IgA and EBNA1/IgA model involving the detection of antibodies, the accuracy of predicting NPC in the general population of Taiwan was found to increase to 93%. However, further validation is required in other high-risk populations based on larger clinical trials. Yu *et al.* (55) used chemiluminescent immunoassay (CLIA) to detect EBV VCA/IgA and EBNA1/IgA antibodies in the sera of 1,252 individuals from different regions of China and found that the repeatability and diagnostic performance of CLIA for NPC were slightly higher than those of ELISA. However, owing to the high cost, further validation on the suitability of CLIA over ELISA for large-scale populations remains to be further explored.

Screening interval

Owing to the geographical distribution of NPC and other factors, there is still a lack of corresponding prospective reports in terms of the screening cycles for NPC. Previous studies have mainly used the Markov model to analyze the screening cycle. Using this model, Choi *et al.* analyzed the data obtained from 1,072 NPC family members to determine the early diagnosis rate and screening costs. Their results showed that the negative population was screened once every 3 years and that the positive population was screened once a year, resulting in the best outcomes and the lowest costs (56). Based on relevant literature, Rao *et al.* (57) evaluated the cost-effectiveness of the six screening programs using the Markov model. Their findings were consistent with those of Choi *et al.* The model analysis mostly drew on the existing literature; most of the parameters were completely based on foreign data and there was a lack of debugging of the epidemiological data of the Chinese population. Thus, further verification of the results is warranted. Chen *et al.* (58) retrospectively compared the 4–5-year and 9–10-year screening programs conducted on the seronegative populations in Sihui City, Guangdong Province. Their results showed that the outcomes from the 4–5-year program were better than those of the latter. Ji *et al.* (59) conducted a 16-year prospective study and determined the VCA/IgA in individuals in Zhongshan City, Guangdong Province, which is considered a high-risk area for NPC. Their analysis revealed that the detection rate was the highest at the first screening ($1{,}390.24/10^5$) in the seropositive population and that the incidence was significantly reduced by the fourth follow-up ($96.99/10^5$). Chen (60) and Sheng (61) conducted long-term follow-up studies on VCA/IgA and EBNA1/IgA levels of the screened population and found that the incidence of NPC in the group that exhibited an increase in antibody titers was concentrated at 5 and 3 years, respectively. Lian *et al.* (62) studied the current screening cycles and found that NPC in high-risk groups was mainly detected during primary screening and the first year of follow-up and that the detection rate of NPC dropped sharply after follow-up. Most studies report that the detection of NPC in EBV-positive patients is concentrated in the first 3 years. The follow-up principles implemented in the "*Technical Program for Cancer Screening and Early Diagnosis and Treatment*" published in China in 2011 are as follows: EBV-positive cohorts will be reviewed every year, EBV-negative subjects will be eliminated, and EBV-positive people who have not developed cancer at the third year of follow-up will also be eliminated. The negative population or the excluded population will be rescreened after 3–5 years (53).

Evaluation of the effect of NPC screening: mortality and survival rate

Mortality

Currently, there are no standardized technical plans globally for NPC screening. Mortality is the only and most direct indicator to evaluate whether screening is effective. To date, only one randomized controlled trial has assessed whether NPC screening can reduce mortality. In 2019, Ji *et al.* (63) conducted a prospective cluster randomized controlled screening study and reported that by combining the findings of the EBV antibodies based on VCA/IgA and EBNA1/IgA during NPC screening, the early diagnosis rate of NPC increased to 79% and the risk of death was reduced by 78%. This study proved for the first time that EBV-related antibody screening can significantly reduce the specific mortality of NPC in the screened population. This study has also become a Class I recommendation and has been included in China's CSCO "*2020 Nasopharyngeal Carcinoma Diagnosis and Treatment Guidelines*". Wei *et al.* (64) followed up the screening of 41,728 individuals in Zhongshan City for 12 years and found that the NPC death rate in the screening group was roughly 0.46 times that in the control group. The standard mortality ratio of NPC in the screening group at different periods was significantly lower than that in the control group (0.20–0.44 *vs.* 0.95–1.10). Additionally, the relative risk value of death from NPC at different screening time points and in the control groups was between 0.23 and 0.40. The above findings confirm that screening can effectively reduce the mortality of patients with NPC from different angles.

Survival rate

Although only a few studies have explored the impact of NPC screening on mortality, it is amply clear that NPC screening can significantly improve survival outcomes. Liu *et al.* (18) compared the long-term survival rates of the screened and unscreened populations in Sihui City, Guangdong Province, and reported that the 10-year survival rates were 38% and 18%, respectively, and the early diagnosis rates were 55.1% and 31.0%, respectively, suggesting that the former was significantly better than the latter. Chan *et al.* (39) used EBV-DNA for screening. They diagnosed 34 cases of NPC with an early diagnosis rate of 70.6%,

which was significantly higher than that reported for NPC in an outpatient setting (20%). The 3-year progression-free survival rate of these early patients was 97% after treatment, indicating that regular screening is more likely to detect early NPC, thereby improving survival.

Conclusions

NPC is mainly diagnosed in young and middle-aged individuals aged 25–60 years. It is a malignant disease that affects individuals, especially those in high-incidence areas. Although new tumor markers are being constantly discovered, EBV serological antibody testing still holds great significance in NPC screening. Currently, there are no clear population-based NPC screening technical programs. In China, the two-stage screening model based on the double antibody method of ELISA to detect EBV is considered the best method for population NPC screening. This model is simple to use and is associated with low screening costs and high accuracy, and is suitable for large-scale screening in NPC endemic areas. Evidence suggests that serological screening for NPC can increase the opportunity of early diagnosis and significantly improve the 5-year survival rate of the affected population. Results of a cluster of controlled studies show that initial screening can reduce the specific mortality of NPC in the screened population. Additional randomized controlled studies are needed to evaluate screening programs and health economics. Moreover, approaches to expand the coverage of NPC screening and improve patient compliance are other research areas for continued focus.

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Footnote

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