Nasopharyngeal Cancer Screening

Methods, Target Populations, and Next Steps

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KEYWORDS

• Nasopharyngeal cancer screening • Plasma EBV DNA • EBV antibodies
• Liquid biopsy • Early cancer detection

KEY POINTS

- Screening of nasopharyngeal carcinoma (NPC) with plasma Epstein-Barr virus (EBV) DNA and EBV antibodies was shown to enable early cancer detection and impact cancer-specific survival.
- Recent advances, including the discovery of fragmentomics biomarkers of plasma EBV DNA and the development of antibodies against *Bam*HI fragment N leftward open reading frame 2b (anti-BNLF2b antibodies), enhance the specificity of NPC detection in the setting of screening.
- Although screening of NPC has been recommended in endemic regions, recent analyses revealed that screening was also cost-effective, in particular with EBV antibodies, for high-risk groups outside endemic areas.

INTRODUCTION

Nasopharyngeal carcinoma (NPC)[1] remains a significant health burden, particularly in endemic regions, such as Southern China and Southeast Asia, as well as among certain high-risk populations/ethnic subgroups in other areas.[2–4] In endemic regions, it ranks among the most common head and neck cancers, underscoring the importance of effective screening strategies. Evidence consistently shows that early-stage NPC is associated with markedly improved survival outcomes compared with

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Otolaryngol Clin N Am 59 (2026) 33–41
https://doi.org/10.1016/j.otc.2025.08.006 **oto.theclinics.com**

Abbreviations	
BNLF2b	*Bam*HI fragment N leftward open reading frame 2b
EBV	Epstein-Barr virus
EBNA1	Epstein-Barr nuclear antigen 1
IgA	immunoglobulin A
NGS	next-generation sequencing
NPC	nasopharyngeal carcinoma
PCR	polymerase chain reaction
PPV	positive predictive value
VCA	viral capsid antigen

advanced disease.[5,6] Unfortunately, most cases are diagnosed at an advanced stage, necessitating more intensive treatment regimens that often lead to substantial treatment-related toxicities and reduced quality of life. To address these challenges, NPC screening has been strongly advocated with the goal of detecting cancer at an earlier, more treatable phase. Solid evidence supports the benefits of screening in reducing mortality and improving patient outcomes. This review aims to discuss strategies for adopting NPC screening in clinical practice, aiming to optimize early diagnosis and enhance patient outcomes.

CURRENT EVIDENCE

How to Screen

Plasma Epstein-Barr virus DNA

NPC found in endemic regions exhibits a strong association with Epstein-Barr virus (EBV), with nearly all NPC tumor cells harboring the viral genome.[7] Consequently, EBV-based biomarkers have been extensively investigated for screening purposes. Among these, circulating EBV DNA and EBV antibodies in blood are the two most studied modalities. The application of plasma EBV DNA for NPC screening has been rigorously evaluated in a large-scale prospective study.[8] The study tested plasma EBV DNA using real-time polymerase chain reaction (PCR)[9] in more than 20,000 asymptomatic, middle-aged men—an age group with the highest incidence. The findings revealed that plasma EBV DNA screening could effectively identify early-stage NPC (stages I and II),[8] which is often asymptomatic and thus frequently diagnosed at an advanced stage—greater than 70% of NPC cases in endemic regions are initially detected at an advanced stage (stages III and IV) based on local cancer registry data. Conversely, in the screening cohort, approximately 70% of detected NPC cases were at an early stage, with only 30% at advanced stages. Importantly, patients diagnosed through screening exhibited significantly better outcomes, with a 5-year progression-free survival exceeding 95%,[8] compared with age- and sex-matched historical controls diagnosed clinically. These results underscore the utility of plasma EBV DNA as a valuable tool for early detection and improved prognosis of NPC in high-risk populations.

It has been reported that approximately 5% of the general population will test positive for plasma EBV DNA by PCR,[10] a figure consistent with findings from the above-mentioned prospective study cohort,[8] where 5.5% (~1100 individuals) of participants exhibited detectable EBV DNA levels at initial testing. (In these studies, any detectable level of EBV DNA was classified as a positive result.) However, these initial positives are regarded as "false positives" within the screening setting, as not all individuals with detectable EBV DNA have NPC. To address this, the study used a sequential testing approach to refine the definition of "screen-positivity." Participants who tested positive initially underwent a repeat test 4 weeks later using the same PCR protocol.[8]

Only those with persistent EBV DNA detection at both time points were classified as "screen-positive." Conversely, individuals with undetectable EBV DNA at the first test, or those with positive results initially but negative upon retesting (termed "transient positives"), were categorized as "screen-negative." This strategy was based on the observation that patients with NPC typically have persistently elevated plasma EBV DNA levels before treatment, whereas non-NPC individuals tend to exhibit transient levels[11]—approximately two-thirds of whom revert to negative within 4 weeks. The proportion of participants with persistent positivity in the screening cohort on repeat testing was around 1.5%. Although this sequential testing approach effectively reduces false positives and conserves health care resources—such as confirmatory diagnostics and clinic visits—several challenges remain. These include logistical issues, particularly when scaling to large populations, and the psychological burden on individuals who must wait weeks for definitive results.

Recent advances in molecular profiling of plasma EBV DNA using next-generation sequencing (NGS) have shed light on its potential to enhance NPC detection. Studies have revealed distinct molecular features of plasma EBV DNA that differentiate NPC from non-NPC subjects,[12] including differences in fragment size distribution[13] and end motif properties,[14] which reflect underlying biological processes. In addition, researchers have identified disease-associated methylation profiles of plasma EBV DNA across various EBV-related diseases,[15] providing further avenues for improving diagnostic specificity. The development of innovative technologies, such as FRAGMA (FRAGmentomics-based Methylation Analysis), enables the inference of methylation status directly from sequencing data.[16] Applying these molecular analyses to plasma EBV DNA can synergistically facilitate more precise discrimination of NPC samples, thereby enhancing the specificity and positive predictive value (PPV) of NPC detection.[13,15] These molecular features and advanced analytical methods hold promise for refining screening strategies and are recommended for adoption in the clinical setting.[17]

Epstein-Barr virus antibody

The use of EBV antibody testing has shown promise for NPC screening, particularly the dual antibody detection. Such an approach involves measuring immunoglobulin A (IgA) antibodies against the viral capsid antigen (anti-VCA IgA) and Epstein-Barr nuclear antigen 1 (anti-EBNA1 IgA).[18] Based on logistic regression analysis in a training cohort of patients with NPC and controls, individuals can be stratified into high-, medium-, and low-risk categories for NPC with reference to the anti-VCA IgA and anti-EBNA1 IgA quantitative levels.[18] A randomized controlled trial demonstrated that early cancer detection was significantly improved through antibody screening.[19] A higher proportion of NPC cases identified in the screening group were diagnosed at early stages.[19] This shift toward earlier detection translated into a clear survival benefit, with improved NPC-specific mortalities. A recent follow-up report further underscored the impact of antibody screening on mortality reduction.[20] Over a 12-year period (2008–2019), approximately 50,000 individuals participated in screening, with about 120,000 nonparticipants, although also being randomized to the screening group, and a control cohort of roughly 180,000 individuals. The relative risk of NPC-related mortality among screened individuals was 0.46 compared with controls, with a notable 30% reduction in NPC-specific mortality when considering all individuals in the screening group.[20] Of note, the risk reduction was particularly pronounced in those older than 50 years of age, with a relative risk of 0.56. These findings highlight that antibody-based screening can substantially reduce NPC mortality, especially in older high-risk populations.

Recent research has explored the potential of a novel EBV antibody, targeting the protein known as *Bam*HI fragment N leftward open reading frame 2b (BNLF2b), for NPC screening.[21] This antibody, referred to as P85-Ab, measures the total serum anti-BNLF2b antibodies, encompassing IgA, IgG, and other immunoglobulin classes. In a large prospective cohort analysis involving more than 20,000 participants, the anti-BNLF2b antibody demonstrated superior diagnostic performance compared with the conventional anti-VCA and anti-EBNA1 IgA's protocol.[21] Notably, the anti-BNLF2b antibody exhibited significantly higher sensitivity (97.9% vs 72.3%) and specificity (98.3% vs 97.0%), therefore, an improved PPV of 10.0% compared with 4.3% for the standard two-antibody panel. These findings suggest that incorporating anti-BNLF2b antibody testing could enhance the accuracy of NPC screening, potentially enabling earlier detection and better risk stratification.[22]

Other biomarkers

Recent advances in NPC screening have also focused on analyzing high-risk EBV variants, including specific genotypes or viral mutations associated with increased oncogenic potential. Notably, variants in EBV-encoded small RNA[23] and *Bam*HI-A left frame transcript-2 genes[24] have been identified to be linked to a higher risk of NPC development. These high-risk EBV variants are increasingly incorporated into composite NPC risk models,[25] alongside other human variants (such as human leukocyte antigen),[26] to improve the accuracy of screening algorithms. By integrating genetic variations of EBV with serologic and viral DNA markers, these models aim to enhance early detection, particularly the specificity, and refine risk stratification for targeted surveillance and intervention.

Transoral brushing has previously been evaluated as a noninvasive, ambulatory method for detecting NPC through EBV DNA analysis.[27,28] One multicenter study involving slightly fewer than 100 patients with NPC and 500 non-NPC subjects evaluated the diagnostic performance of NP Screen, which uses a single-use transoral brush to collect nasopharyngeal epithelial cells.[27] The collected samples were preserved and shipped for quantitative PCR analysis of EBV DNA. The study reported remarkable sensitivity and specificity of greater than 95% for detecting NPC. Alternatively, a similar strategy of quantifying the EBV DNA load in a nasopharyngeal swab has been proposed to triage subjects[29] deemed high risk by the anti-VCA and anti-EBNA1 IgA analysis, with the goal to enhance the specificity and PPV of NPC detection.

Whom to Screen?

Screening for NPC is primarily recommended for populations residing in endemic regions,[17] where both plasma EBV DNA-based and EBV antibody-based protocols have demonstrated cost-effectiveness.[30,31] The highest age-specific incidence occurs during middle age,[4] leading to a general consensus to initiate screening starting between ages 30 and 40 in these areas.[17] Although NPC is less prevalent among women, screening remains cost-effective for both sexes[30] because of the potential for early detection and improved outcomes. Targeted screening for the selected high-risk subgroup of individuals with familial predisposition has been specifically evaluated.[32–34] In contrast, routine screening is not advised in nonendemic regions, where the low prevalence does not justify the costs and resource utilization. Recent studies have also emphasized the importance of screening high-risk individuals outside endemic areas, such as Asian Americans, Native Hawaiians, and other Pacific Islanders residing in the United States,[35] where NPC incidence is relatively elevated.

What to Do Next if Screen-Positive?

Upon a positive screening result for NPC, confirmatory investigations are essential to establish the diagnosis and determine the disease stage. Endoscopy[36] remains the primary tool for direct visualization of the nasopharynx, allowing for targeted biopsies to confirm malignancy. Concurrently, MRI has demonstrated higher sensitivity than endoscopy in detecting nasopharyngeal tumors,[37–39] particularly in identifying submucosal or extracapsular spread. Although MRI is more resource-intensive, offering it to all screen-positive individuals has been shown to be cost-effective by significantly improving diagnostic accuracy.[30,31] Incorporating MRI into the diagnostic pathway for screen-positive cases ensures a comprehensive evaluation, balancing clinical effectiveness with economic considerations in high-risk screening programs. Short contrast-free MRI recently showed promise in NPC screening, with both high sensitivity and specificity illustrated.[40] Such findings support the integration of MRI as a confirmatory test, whereas a contrast-free protocol offers the advantage of reduced costs.

If initial confirmatory investigations, such as endoscopy and MRI, do not identify NPC, follow-up surveillance for screen-positive subjects remains crucial. For plasma EBV DNA-based screening, such surveillance is supported by findings from the second round of screening, which was conducted approximately 4 years after the initial plasma EBV DNA testing for the entire screening cohort.[41] In this follow-up, the same protocol of two-time-point, real-time PCR-based plasma EBV DNA testing was used. Similar to the first round, this rescreening process has demonstrated its value in enabling early detection of NPC, with most (67%) of the screen-detected cases identified at early stages (I and II), and these patients exhibited significantly better progression-free survival compared with age- and sex-matched symptomatic patients.[17] Importantly, analysis of the initial plasma EBV DNA status revealed that subjects with detectable EBV DNA at the first screening (about 4 years ago) were at a markedly increased risk of developing NPC in the future. Specifically, individuals with transiently positive results had a relative risk of 4.4, whereas those with persistently positive results faced a substantially higher risk of 16.8, compared with baseline subjects with negative first-round results.[17] The risk prediction could be further enhanced through analysis of fragmentomics profile of plasma EBV DNA by NGS.[14] In addition, surveillance is equally necessary for screen-positive individuals identified through EBV antibody testing, as about 40% of NPC cases were detected during annual retesting in follow-up assessments.[19] These findings underscore the importance of continued surveillance and repeated testing in high-risk individuals, emphasizing that even negative initial investigations do not eliminate the potential for future NPC development.

Frequency of screening

The optimal frequency of screening involves balancing the mortality benefits gained through early detection against the associated costs,[30] ensuring sustainable and effective screening programs for high-risk populations.

CHALLENGES

Successfully implementing NPC screening programs hinges on achieving high participation rates, which remain a significant hurdle despite the accessibility of plasma EBV DNA and EBV antibodies as blood-based biomarkers—both minimally invasive and easy to collect. For example, the recent study on EBV antibody-based screening reported that only about 30% of eligible participants in the screening arm

actually underwent testing.[20] Nonetheless, this study observed a 30% reduction in NPC-specific mortality among the screened participants compared with controls, suggesting that higher participation could potentially lead to even greater mortality reductions. The low participation rate is likely influenced by multiple factors, including limited awareness of screening benefits, barriers to health care access, and emotional distress from positive testing results. Addressing these issues requires comprehensive educational initiatives to increase awareness, improve health care infrastructure, and clear communication to manage expectations and alleviate fears. Further research into individual attitudes and preferences is essential to develop tailored strategies that encourage participation. Separately, the analytical performances of plasma EBV DNA and EBV antibody assays across different laboratories need to be validated[42,43] to ensure satisfactory sensitivity for early NPC detection.

SUMMARY

In conclusion, NPC screening in high-risk populations offers a promising avenue for early detection and improved patient outcomes, particularly through plasma EBV DNA and EBV antibodies. Advances in molecular profiling and novel antibody markers like BNLF2b enhance screening accuracy, facilitating more effective risk stratification. Targeted screening strategies, focusing on endemic regions and high-risk groups outside these areas, are essential to maximize benefits while managing resource utilization. However, significant challenges remain, notably low participation rates driven by limited awareness and access barriers. Addressing these issues through public education, health care infrastructure improvements, and validation of diagnostic assays across laboratories is crucial. Continued research and collaborative efforts are vital to optimize screening protocols, increase participation, and ultimately, reduce NPC-related mortality worldwide.

CLINICS CARE POINTS

- Plasma EBV DNA and EBV antibodies (including dual anti-VCA IgA and anti-EBNA1 IgA testing and total anti-BNLF2b antibodies) are validated screening modalities of NPC.
- There is variable performance of different PCR assays for detection and quantification of EBV DNA in plasma, which affects its performance as a screening biomarker.
- Both endoscopy and MRI are recommended as investigations for test-positive individuals undergoing screening for NPC.

FUNDING

This work was supported by the Innovation and Technology Fund under the InnoHK Initiative, a major initiative of the Hong Kong Special Administrative Region Government.

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