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Diagnostic value of BNLF2b antibody, dual-antibody testing and Epstein–Barr virus DNA in nasopharyngeal carcinoma: a prospective cohort study in Hunan Province, China

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ABSTRACT

Objectives This study evaluates the diagnostic value of the BNLF2b antibody, dual antibody testing and Epstein-Barr virus DNA (EBV-DNA) individually and in combination for nasopharyngeal carcinoma (NPC) detection.

Design A prospective cohort study.

Setting The study was conducted at Hunan Cancer Hospital, in a region in China with a high incidence of NPC, between January 2024 and June 2024.

Participants A total of 350 patients with suspected NPC were enrolled based on clinical suspicion (eg, metastatic cervical lymph nodes or nasopharyngeal abnormalities with non-specific symptoms). The inclusion criteria included age ≥18 years, residency in Hunan Province, and provision of informed consent. The exclusion criteria included prior history of NPC or other head and neck malignancies, severe immunological/systemic diseases and inability to complete diagnostic evaluations.

Primary and secondary outcome measures Demographic, clinical and biomarker data were collected, including BNLF2b antibody, EBV-DNA and dual antibody testing. Diagnostic performance metrics were calculated against histopathological confirmation as the gold standard. Follow-up assessments were conducted for non-NPC cases.

Results Among 350 suspected NPC participants, 74 were diagnosed with NPC through biopsy. BNLF2b antibody exhibited the highest sensitivity (83.78%) and specificity (95.65%) among single biomarkers in NPC diagnosis, outperforming dual-antibody testing and EBV-DNA. Combining BNLF2b with dual-antibody testing improved specificity to 99.64%, although with reduced sensitivity (67.57%). NPC-diagnosed participants and those testing positive for BNLF2b or dual antibody biomarkers had a significantly higher prevalence of family history of NPC ($p<0.05$). Alcohol consumption was significantly more common among dual antibody-positive participants compared with antibody-negative participants ($p<0.05$). One NPC case was identified during follow-up, which tested positive for BNLF2b antibody and the dual antibody method at baseline and follow-up, underscoring the predictive value of these biomarkers.

Conclusion The BNLF2b antibody is a highly sensitive and specific biomarker for NPC detection, particularly for early-stage disease. Combining BNLF2b with the dual antibody method enhances specificity, making it valuable for identifying at-risk individuals and guiding early interventions.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This was a prospective cohort study, reducing selection and recall biases.
⇒ Multiple biomarkers were systematically evaluated both individually and in combination.
⇒ Standardised methods were used for biomarker detection and histopathological confirmation.
⇒ The single-centre design may limit the generalisability of the findings.
⇒ The relatively short follow-up duration might not capture late diagnoses of nasopharyngeal carcinoma.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a malignant epithelial tumour arising from the mucosal lining of the nasopharynx, marked by both a distinct geographic distribution and a strong etiological linkage to Epstein–Barr virus (EBV) infection.[1 2] NPC is most prevalent in southern China, Southeast Asia and parts of North Africa, but remains relatively rare in Western populations.[1 2] Numerous epidemiological and molecular studies have established EBV as a key factor in NPC pathogenesis, with viral DNA and related proteins detectable in nearly all tumour cells, especially in endemic regions.[1 2] Recent global data indicate that the incidence of NPC among middle-aged and older adults rose by approximately 58.2% from 1990 to 2021, although some high-incidence regions have observed simultaneous declines in both incidence and mortality.[3] Nonetheless, Bayesian age–period–cohort analyses suggest that age-standardised incidence and prevalence could

continue to rise in China through 2030, highlighting persistent challenges in disease control.[4]

In clinical practice, NPC frequently presents with non-specific or asymptomatic manifestations, such as nasal obstruction, epistaxis or cervical lymph node enlargement, leading many patients to receive diagnoses only after the disease has progressed to locally advanced or metastatic stages.[5] The late presentation of NPC in many patients underscores the need for more effective screening and early detection methods. Current diagnostic strategies for NPC often incorporate imaging techniques in conjunction with serological and molecular tests targeting EBV.[6] Among these serological tests, the viral capsid antigen IgA (VCA-IgA) and EBV nuclear antigen 1 IgA (EBNA1-IgA) have been the cornerstone of screening protocols, although with varied diagnostic performance across disease stages.[7] Additionally, EBV-DNA quantification has demonstrated considerable value in identifying individuals at risk for NPC, although its accuracy may also fluctuate with tumour burden.[8 9] While these methods are useful, their sensitivity and specificity can vary by disease stage, prompting researchers to seek additional biomarkers that could facilitate earlier and more accurate NPC identification.

BNLF2b, a protein implicated in EBV-mediated immune evasion, is robustly expressed in nasopharyngeal tumour cells and has emerged as a promising serological marker for NPC, given its potential involvement in immune evasion and viral pathogenesis.[10 11] Preliminary investigations indicate that anti-BNLF2b antibodies (P85-Ab) exhibit high sensitivity for detecting NPC across different stages, potentially outperforming conventional serological markers such as EBNA1-IgA and VCA-IgA.[10 11] By enabling more reliable identification of early-stage NPC, BNLF2b detection could facilitate timely treatment and improve long-term patient outcomes. However, current evidence regarding its diagnostic accuracy and clinical utility remains limited, emphasising the need for well-designed prospective studies to validate its role in high-incidence regions.[6 12]

Accordingly, the primary objective of this study is to compare the diagnostic performance of BNLF2b antibody, EBV-DNA and the dual-antibody method (VCA-IgA/EBNA1-IgA) in accurately detecting NPC. Conducted in a high-incidence region, our investigation also seeks to determine whether BNLF2b antibody enhances early-stage identification, a critical factor for timely treatment initiation and favourable outcomes. Ultimately, the findings may help refine current screening protocols in high-risk settings and mitigate the considerable clinical and economic burden imposed by this disease.

METHODS

Patient and public involvement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

Study population

Patients with suspected NPC who presented between January 2024 and June 2024 at our hospital were prospectively enrolled. Inclusion criteria were as follows: (1) residency in Hunan Province, a region with high incidence of NPC; (2) age≥18 years; (3) clinical suspicion of NPC based on the presence of metastatic cervical lymph nodes or a nasopharyngeal abnormality accompanied by non-specific symptoms (such as nasal obstruction and epistaxis); and (4) provision of written informed consent. The exclusion criteria were as follows: a prior history of NPC or other head and neck malignancies, presence of severe immunological or systemic diseases and inability to complete all required diagnostic evaluations.

The study was approved by the Medical Ethics Committee of Hunan Cancer Hospital (approval number: KYJJ-2023–342). All participants provided written informed consent prior to enrolment. The study was conducted in compliance with the Declaration of Helsinki.

Data collection

Demographic and clinical data were collected, including age, sex, body mass index (BMI), smoking and alcohol consumption status, dietary habits (eg, salted food intake), educational attainment (≥9 years of formal education), family history of NPC and comorbidities such as hypertension, diabetes and chronic bronchitis. The frequency of salted food intake was categorised as rarely (monthly or less), sometimes (2–3 times per month) and often (weekly or more). The clinical staging of patients diagnosed with NPC was also included.

All patients provided blood samples for biomarker analysis. Serological testing included measurement of BNLF2b antibody, VCA-IgA, EBNA1-IgA and EBV-DNA levels. BNLF2b antibody levels were determined using the Wan200+system and EBV BNLF2b antibody detection kit (chemiluminescent microparticle immunoassay; Xiamen Innodx Biotechnology Co., Ltd., Xiamen, China), with reactivity defined as a signal-to-cutoff ratio (S/CO) ≥1.0 and non-reactivity defined as S/CO<1.0. EBV-DNA was quantified via the EBV nucleic acid detection kit (fluorescence probe-based real-time PCR; Sansure Biotech Inc., Changsha, China), with a positivity threshold of >400 copies/mL. VCA-IgA and EBNA1-IgA were measured using the EBNA1-IgA antibody detection kit and VCA-IgA antibody detection kit (ELISA; Tracine BioMed Inc., Beijing, China), respectively. Risk probability for NPC based on VCA-IgA and EBNA1-IgA was calculated using the following formula: logit P = −3.934+4.797 × EBNA1-IgA+2.203 × VCA-IgA. Patients were categorised as high (p≥0.98), moderate (0.65≤P < 0.98), or low (p<0.65) risk based on these calculations, and positives were defined as the serological high risk. Furthermore, positivity based on biomarker combinations was defined as follows: EBV-DNA+BNLF2 b positivity required both EBV-DNA and BNLF2b antibody to be positive. Dual-antibody+BNLF2 b positivity also required

both the dual-antibody panel (VCA-IgA and EBNA1-IgA) and BNLF2b antibody to be positive.

Diagnostic confirmation

All participants underwent nasopharyngeal fibre-optic endoscopy and biopsies. Histopathological examination confirmed the presence of NPC, serving as the gold standard diagnosis.

Follow-up

Patients not diagnosed with NPC underwent follow-up assessments 6 months after the initial visit. Follow-up evaluations included repeated serological testing and nasopharyngoscopy, with biopsies performed if necessary.

Statistical analysis

The sample size was determined based on the primary objective of evaluating biomarker diagnostic performance. Assuming a sensitivity of approximately 80%, a specificity of approximately 90% and an NPC prevalence of around 20% among clinically suspected cases in our high-incidence setting and allowing for a precision of ±10% with a two-sided alpha of 0.05, we estimated a required sample size of approximately 320 participants. Considering a potential dropout rate of around 10%, a final sample size of 350 participants was targeted.

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarised as mean±SD or median (IQR) and compared using independent t-tests or Mann–Whitney U tests, depending on the data distribution. Categorical variables were presented as frequencies and percentages and compared using χ^2 or Fisher's exact tests. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were computed for individual biomarkers and their combinations. Sensitivity and specificity were calculated using the true positive (TP) rate, true negative (TN) rate, false negative (FN) and false positive (FP) according to the formula: sensitivity = TP/(TP + FN); specificity = TN/(TN + FP). PPV was calculated as the proportion of TP ases among all individuals who tested positive: PPV = TP/(TP + FP). NPV was calculated as the proportion of TN cases among all individuals who tested negative: NPV = TN/(TN + FP). All calculations were based on baseline testing results compared against the gold standard diagnosis (histopathological confirmation). The Wilson score interval was used to confirm the 95% CI. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

Baseline characteristics

A total of 350 participants were included in this study (figure 1). Among them, 50.86% were male, and 49.14% were female, with a median age of 52 (IQR, 40–61) years. The median BMI was 24.80 (IQR, 21.66–27.49). Smoking and alcohol consumption were reported by 50.86% and 44.86% of the participants, respectively. Approximately

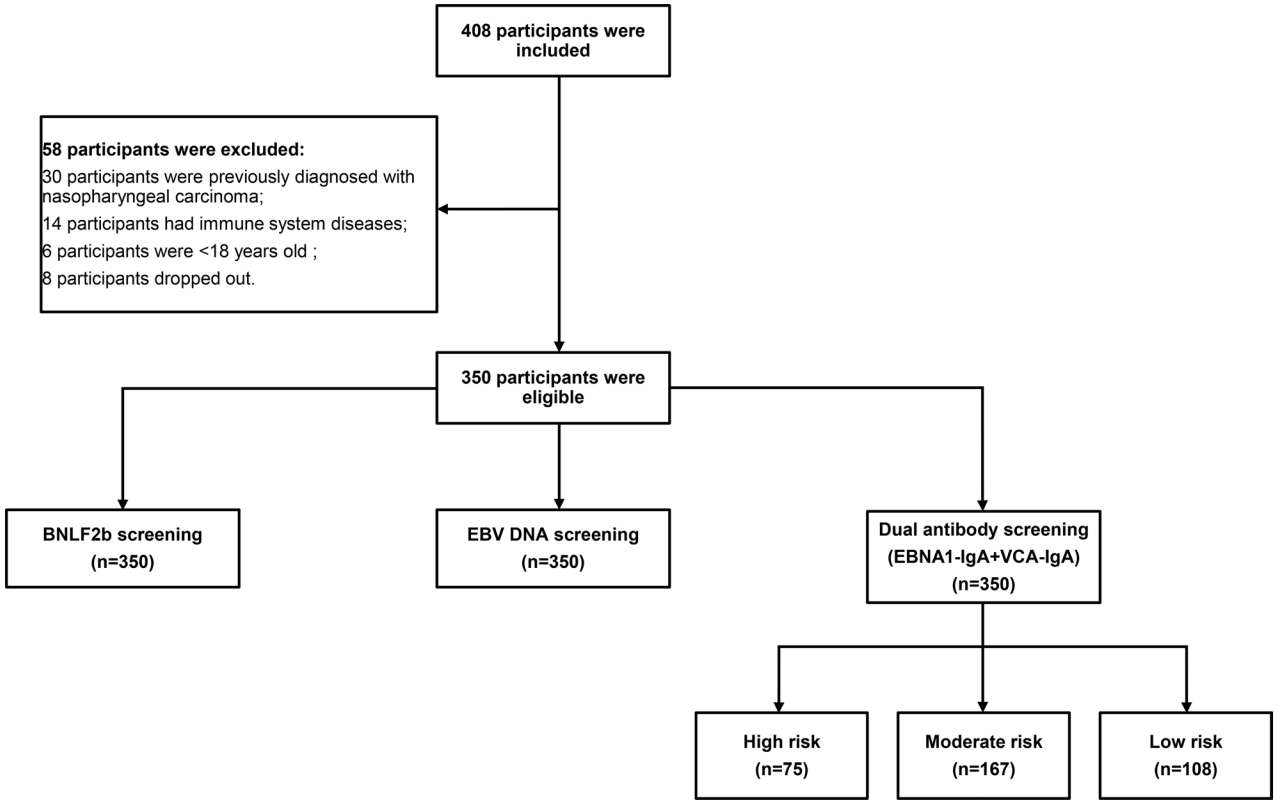

Figure 1 Screening flow chart for the included patients. EBNA1-IgA, Epstein–Barr virus nuclear antigen; VCA-IgA, viral capsid antigen.

Table 1 Baseline characteristics of the enrolled participants

Variables	N (%)
Age, n (%)	
Median (IQR), year	52.00 (40.00, 61.00)
<40	83 (23.71%)
40–49	71 (20.29%)
50–59	99 (28.29%)
60–69	97 (27.71%)
Sex, n (%)	
Male	178 (50.86%)
Female	172 (49.14%)
BMI, median (IQR)	24.80 (21.66, 27.49)
Smoking, n (%)	
Yes	178 (50.86%)
No	172 (49.14%)
Alcohol, n (%)	
Yes	157 (44.86%)
No	193 (55.14%)
Salted food, n (%)	
Rarely	178 (50.86%)
Sometimes	109 (31.14%)
Often	63 (18.00%)
Family history of NPC, n (%)	
Yes	106 (30.29%)
No	244 (69.71%)
Education, year	
≤9	151 (43.14%)
>9	199 (56.86%)
Hypertension, n (%)	
Yes	164 (46.86%)
No	186 (53.14%)
Chronic bronchitis, n (%)	
Yes	178 (50.86%)
No	172 (49.14%)
Diabetes, n (%)	
Yes	157 (44.86%)
No	193 (55.14%)

30.29% had a family history of NPC. Detailed baseline characteristics are summarised in table 1.

Comparison of demographic factors between different biomarker status

Among the 350 suspected NPC participants, 74 tested positive for BNLF2b antibody, 70 tested positive for EBV-DNA, 75 tested positive by the dual-antibody method, and ultimately 74 were diagnosed with NPC through biopsy (table 2). Participants with positive BNLF2b or EBV DNA results had a significantly higher prevalence of family history of NPC than those with negative results ($p<0.05$). These trends were consistent with the pathological biopsy results, suggesting a potential association between family history and NPC. In addition, alcohol consumption was significantly more common among dual antibody-positive participants than among antibody-negative participants ($p<0.05$). No statistically significant differences were identified in age, sex distribution, BMI, salted food intake, educational attainment or the prevalence of hypertension, diabetes and chronic bronchitis between the positive and negative groups for any biomarkers or pathological diagnoses.

Clinical characteristics of NPC patients

Of the 74 NPC patients, 62 (83.78%) were positive for the BNLF2b antibody, 39 (52.70%) tested positive for EBV-DNA and 58 (78.38%) were positive using the dual-antibody method (figure 2). Tumour staging revealed that 47.30% had early-stage primary tumours (tumour stage T1), and lymph node involvement (nodal stages N1–N3) was present in 64.86%. Distant metastatic disease (metastatic stage M1) was observed in 12 patients (16.22%). BNLF2b antibody was identified in 31 out of 35 (88.57%) stage T1 tumours, indicating strong sensitivity for detecting early-stage disease, which was superior to the dual-antibody method (28/35, 80.00%) and EBV-DNA (22/35, 62.85%). Similarly, BNLF2b identified 22 out of 26 N0 cases (84.62%) in nodal stage detection and 9 out of 12 M1 cases (75.00%) for metastatic disease, demonstrating better or comparable performance than the dual-antibody method and EBV-DNA across these classifications (table 3).

Biomarker performance

Subsequently, the diagnostic performance of individual and combined biomarkers was evaluated (table 4). BNLF2b antibody showed the highest sensitivity (83.78%; 95% CI 74.12 to 91.02) and specificity (95.65%; 95% CI 92.34 to 97.68) among single biomarkers. Combining BNLF2b with the dual-antibody method improved the specificity to 99.64% (95% CI 97.96 to 99.98) and the PPV to 98.04% (95% CI 89.13 to 99.73), but the sensitivity decreased to 67.57% (95% CI 56.36 to 77.20).

Follow-up

Participants not diagnosed with NPC (n=276) were followed up. During the follow-up period, a participant was diagnosed with NPC, and the remaining 275 participants had no clinically confirmed NPC. This NPC patient tested positive for BNLF2b antibody and in the dual-antibody method at baseline and follow-up, highlighting the importance of these biomarkers in identifying at-risk individuals. In addition, 20 cases were positive for BNLF2b antibody and in the dual-antibody method during follow-up but remained histopathologically negative. The diagnostic results and performance of the different biomarker tests after follow-up are shown in online supplemental table S1.

Table 2 Demographic characteristics of participants who underwent NPC screening

Variable	BNLF2b		Dual antibody		EBV-DNA		Pathological biopsy	
	Positive (n=74)	Negative (n=276)	Positive (n=75)	Negative (n=275)	Positive (n=70)	Negative (n=280)	Diagnosed (n=74)	Undiagnosed (n=276)
Age, years	51.00 (38.00, 59.75)	52.00 (41.00, 61.00)	49.00 (36.00, 59.00)	52.00 (42.00, 61.00)	49.00 (38.00, 57.75)	53.00 (40.00, 61.00)	50.00 (38.00, 58.75)	52.00 (40.75, 61.00)
Male, n (%)	37 (50.00%)	141 (51.09%)	38 (50.67%)	140 (50.91%)	39 (55.71%)	139 (49.64%)	36 (48.65%)	142 (51.45%)
BMI	25.53 (21.65, 27.79)	24.73 (21.67, 27.41)	24.55 (21.83, 27.69)	24.80 (21.56, 27.38)	24.69 (21.65, 27.43)	24.79 (21.67, 27.45)	25.32 (21.65, 27.66)	24.78 (21.67, 27.45)
Family history of NPC, n (%)	30 (40.54%)	76 (27.54%)*	26 (34.67%)	80 (29.09%)	29 (41.43%)	77 (27.50%)*	30 (40.54%)	76 (27.54%)*
Smoking, n (%)	38 (51.35%)	140 (50.72%)	37 (49.33%)	141 (51.27%)	34 (48.57%)	144 (51.43%)	35 (47.30%)	143 (51.81%)
Alcohol, n (%)	34 (45.95%)	123 (44.57%)	42 (56.00%)	115 (41.82%)*	32 (45.71%)	125 (44.64%)	36 (48.65%)	121 (43.84%)
Education, year								
≤9	41 (55.41%)	158 (57.25%)	37 (49.33%)	162 (58.91%)	43 (61.43%)	156 (55.71%)	43 (58.11%)	156 (56.52%)
>9	33 (44.59%)	118 (42.75%)	38 (50.67%)	113 (41.09%)	27 (38.57%)	124 (44.29%)	31 (41.89%)	120 (43.48%)
Salted food, n (%)								
Rarely	39 (52.70%)	139 (50.36%)	36 (48.00%)	142 (51.64%)	30 (42.86%)	148 (52.86%)	34 (45.95%)	144 (52.17%)
Sometimes	22 (29.73%)	87 (31.52%)	21 (28.00%)	88 (32.00%)	25 (35.71%)	84 (30.00%)	23 (31.08%)	86 (31.16%)
Often	13 (17.57%)	50 (18.12%)	18 (24.00%)	45 (16.36%)	15 (21.43%)	48 (17.14%)	17 (22.97%)	46 (16.67%)
Hypertension, n (%)	37 (50.00%)	127 (46.01%)	32 (42.67%)	132 (48.00%)	33 (47.14%)	131 (46.79%)	35 (47.3%)	129 (46.74%)
Chronic bronchitis, n (%)	35 (47.30%)	143 (51.81%)	32 (42.67%)	146 (53.09%)	32 (45.71%)	146 (52.14%)	32 (43.24%)	146, (52.9%)
Diabetes, n (%)	34 (45.95%)	123 (44.57%)	40 (53.33%)	117 (42.55%)	33 (47.14%)	124 (44.29%)	36 (48.65%)	121 (43.84%)

*p<0.05, statistically comparing the differences between the positive group and the negative group in different groups.
EBV-DNA, Epstein–Barr virus DNA; NPC, nasopharyngeal carcinoma.

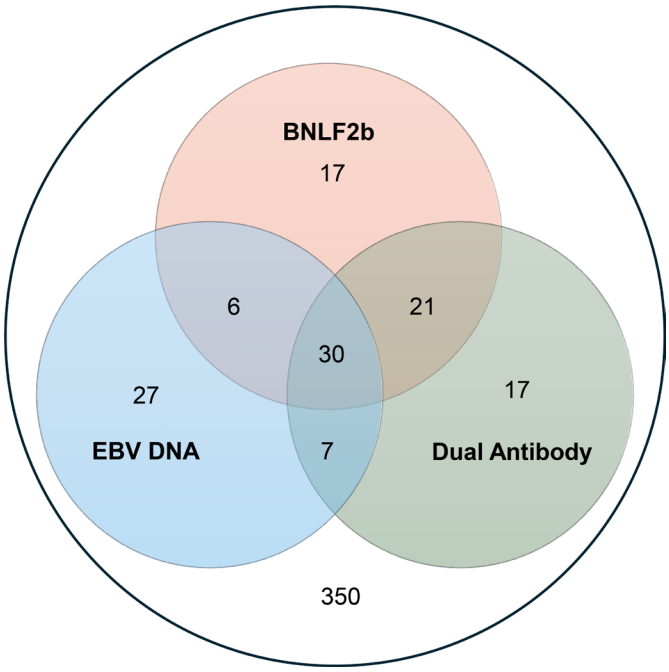

Figure 2 Venn diagram of different antibody test results.

Table 3 Clinical characteristics of NPC patients

Variable	NPC	BNLF2b-positive group	Dual antibody-positive group	EBV-DNA-positive group
n	74	62/74	58/74	39/74
AJCC stage				
I	10	8	7	4
II	19	17	16	9
III	18	14	13	8
IVA	7	7	6	5
IVB	8	7	8	5
IVC	12	9	8	8
Tumour stage				
T1	35	31	28	22
T2	19	13	15	7
T3	12	10	9	4
T4	8	8	6	6
Nodal stage				
N0	26	22	22	12
N1	24	21	17	12
N2	15	11	11	9
N3	9	8	8	6
Metastatic stage				
M0	62	53	50	31
M1	12	9	8	8

AJCC, American Joint Committee on Cancer; EBV-DNA, Epstein–Barr virus DNA; NPC, nasopharyngeal carcinoma.

DISCUSSION

In China, Hunan Province, a region with a high incidence of NPC, provides an ideal setting for evaluating diagnostic biomarkers for NPC. The significant disease burden and pronounced familial clustering in this area underscore the importance of refining diagnostic approaches and screening strategies tailored to NPC high-risk populations.[13 14] In this prospective study, we evaluated the diagnostic value of the BNLF2b antibody, EBV-DNA, and dual-antibody method (VCA-IgA+EBNA1 IgA) for NPC detection.

In this study, over 20% of the enrolled patients were diagnosed with NPC, further confirming the substantial diagnostic burden of NPC in endemic regions like Hunan. This high diagnostic rate is likely attributable to two key factors: the inherent high incidence of NPC in the region and the study's focus on individuals already suspected of having the disease. These patients were selected based on the presence of NPC-related symptoms—although non-specific, such as nasal obstruction or epistaxis—which significantly increased the pre-test probability of NPC. Additionally, over half of the confirmed cases were diagnosed at the T1 stage, highlighting the potential for early detection in a high-incidence setting when leveraging effective diagnostic biomarkers.

The BNLF2b antibody demonstrated the highest sensitivity and specificity among individual biomarkers tested, aligning with emerging evidence on its role in enhancing early detection of NPC. For instance, Li et al[10] demonstrated its superior diagnostic performance in mass screening for NPC, highlighting its potential clinical utility. Lam and Chan further reported that the anti-BNLF2b antibody is a promising biomarker for the early detection of NPC, particularly in high-risk populations.[11] Additionally, Ma et al[12] validated that incorporating the BNLF2b antibody in multiplex assays improved the diagnostic accuracy for NPC, supporting its role as a critical component in serological screening.

Plasma EBV-DNA measurement has been extensively studied as a non-invasive marker for detecting and monitoring NPC. Lee et al[15] demonstrated its clinical utility in routine screening, noting its value in identifying NPC cases, particularly in endemic regions. However, while EBV-DNA showed relatively high specificity, its sensitivity was notably lower than that of the BNLF2b antibody. This finding corroborates earlier research indicating that EBV-DNA alone may miss a significant proportion of early-stage NPC cases.[16 17] He et al[18] emphasised the variability of EBV-DNA detection due to fluctuating viral loads in different disease stages, advocating for multi-marker approaches to improve early detection rates. These findings collectively suggest that while EBV-DNA remains a valuable diagnostic tool, it benefits significantly from complementary biomarkers like BNLF2b for enhanced sensitivity in early-stage NPC screening.

Liu et al[19] confirmed the dual-antibody method's diagnostic value with pooled sensitivity exceeding 92.8%, underscoring its reliability as a screening tool. In our

Table 4 Diagnostic performance of different biomarker tests

Biomarker	NPC, n		Non-NPC, n		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
	Positive	Negative	Positive	Negative				
BNLF2b	62	12	12	264	83.78% (74.12 to 91.02)	95.65% (92.34 to 97.68)	83.78% (74.12 to 90.37)	95.65% (92.34 to 97.68)
Dual-antibody method	58	16	17	259	78.38% (68.13 to 86.35)	93.85% (90.18 to 96.25)	77.33% (67.26 to 85.14)	94.18% (91.01 to 96.29)
EBV-DNA	39	35	31	245	52.70% (41.52 to 63.70)	88.78% (84.39 to 92.09)	55.66% (45.30 to 65.54)	87.50% (82.54 to 91.02)
BNLF2b+ dual-antibody method	50	24	1	275	67.57% (56.36 to 77.20)	99.64% (97.96 to 99.98)	98.04% (89.13 to 99.73)	91.95% (88.26 to 94.67)
BNLF2b+EBV DNA	35	39	1	275	47.30% (36.17 to 58.65)	99.64% (97.96 to 99.98)	97.22% (85.55 to 99.30)	87.66% (83.13 to 91.03)

EBV-DNA, Epstein–Barr virus DNA; NPC, nasopharyngeal carcinoma; NPV, negative predictive value; PPV, positive predictive value.

study, the dual-antibody method (VCA-IgA+EBNA1 IgA) demonstrated relatively high sensitivity (78.38%) and robust specificity (93.85%). Li et al[10] similarly reported a sensitivity of 72.3% and a specificity of 97.0% for the dual-antibody method, showing comparable diagnostic performance. Both our results and those of Li et al indicate that although the dual-antibody method is less sensitive than anti-BNLF2b, its specificity is enhanced when combined with BNLF2b, ultimately improving PPV. Notably, one participant who tested positive for both biomarkers was diagnosed with NPC during follow-up, highlighting the clinical significance of biomarker co-positivity in identifying individuals at high risk of harbouring early lesions. Conversely, 20 participants who showed positive results for both BNLF2b and in the dual-antibody method remained histopathologically negative by the study's conclusion. While this outcome might initially be interpreted as indicative of FP, it is equally plausible that these individuals harboured latent or subclinical forms of NPC, undetectable through conventional biopsy techniques. Such cases emphasise the need for extended follow-up and repeated endoscopic evaluations, especially in high-incidence settings, to determine whether these individuals progress to histopathologically confirmed NPC. This observation underlines the potential role of biomarkers in identifying patients at an earlier stage of disease development, necessitating further investigation into their prognostic value.

Interestingly, this study revealed that patients with dual-antibody positivity were more likely to report alcohol consumption. Conversely, previous studies have not identified a direct association between alcohol consumption and EBV reactivation.[20] Further investigation into how behavioural and environmental factors interact with EBV reactivation is warranted to refine biomarker-based NPC screening strategies and integrate lifestyle interventions as part of risk management and possible lifestyle factors that could influence EBV serology or host immune status. Our data also confirm a significant association between family history of NPC and positivity for both BNLF2b and EBV-DNA. Previous studies have demonstrated that individuals with a family history of NPC exhibit higher risks of harbouring EBV-related biomarkers, highlighting the role of genetic susceptibility and shared environmental exposures.[15 21] In contrast, although salted food intake has long been implicated in NPC risk, particularly in high-incidence regions, our findings did not reveal meaningful differences. This observation aligns with the results of recent studies suggesting that while salted fish consumption was historically a major risk factor, its impact may be diminishing due to shifting dietary habits and the multi-factorial nature of NPC.[22]

Despite its strengths, this study has several limitations. First, this investigation was conducted at a single centre, potentially restricting generalisability to other geographic locations or ethnic populations. Second, the follow-up period, with a median of 7.15 months, may be insufficient to capture late-emerging cases or further changes in biomarker status. Third, only patients with suspected NPC were enrolled, increasing the baseline probability of NPC and possibly inflating the apparent sensitivity and specificity of the biomarkers. In addition, the sample size estimation was based on a relatively wide CI, which may limit the precision of the findings. Finally, unmeasured confounding factors, such as additional dietary variables or viral co-infections, may influence EBV reactivation and NPC onset. Larger multi-centre, prospective trials are warranted to validate these results across diverse populations.

Conclusion

This prospective study demonstrates that BNLF2b antibody is a highly sensitive and specific biomarker for

identifying early-stage NPC in high-risk populations. Although incorporating EBV-DNA and the dual-antibody method enhances specificity, careful attention should be paid to the resulting decline in sensitivity. Additional research is essential to optimise the balance between sensitivity and specificity, clarify the implications of persistent biomarker positivity with negative biopsies, and improve long-term patient outcomes. By refining these screening algorithms, especially in high-incidence settings, we can bolster early detection efforts and reduce the overall burden of NPC.

Contributors BQ conceptualised the study, designed the research methodology, supervised the data collection and contributed to manuscript drafting. LS led the statistical analysis, interpreted the data and assisted in manuscript revision. HD assisted in patient recruitment, clinical assessments, data acquisition and manuscript revision. QL conducted laboratory testing and biomarker analysis. HS reviewed the study protocol, contributed to data validation and assisted in manuscript revision. PX provided overall supervision, critically revised the manuscript for important intellectual content and approved the final version for submission. PX is responsible for the overall content as the guarantor.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study was approved by the Medical Ethics Committee of Hunan Cancer Hospital (approval number: KYJJ-2023-342). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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