



A Comprehensive Systematic Review of The Relationship between Epstein-Barr Virus (EBV) Exposure and the Development of Non-Keratinizing Nasopharyngeal Carcinoma

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ABSTRACT

Introduction: Nasopharyngeal carcinoma (NPC) is a malignancy with a distinct geographical distribution, showing high prevalence in endemic regions like Southern China and Southeast Asia. The Epstein-Barr virus (EBV) is a well-established oncogenic driver, particularly for non-keratinizing NPC subtypes (WHO Types II/III). Understanding the precise relationship between EBV exposure, its biomarkers, and NPC development is critical for improving early detection, prognosis, and treatment strategies (Banko et al., 2021; Chan et al., 2004).

Methods: This report synthesizes evidence from a systematic review of 80 sources, including meta-analyses, cohort studies, randomized controlled trials, and case-control studies. Data extraction focused on study design, population characteristics, EBV exposure definition (serological markers, plasma EBV DNA,

genetic variants), NPC histological classification, measures of association, confounders, and study quality. Quantitative synthesis was performed on diagnostic accuracy, prognostic value, and screening effectiveness.

Results: The evidence demonstrates a near-universal association between EBV and non-keratinizing NPC in endemic regions, with EBV positivity rates reaching 100%. Serological markers like VCA-IgA show high diagnostic accuracy (pooled sensitivity 0.91, specificity 0.92), with multi-marker panels (e.g., VCA-IgA + EA-IgA + Rta-IgG) providing superior performance. Plasma EBV DNA detection offers excellent diagnostic (sensitivity 89.1%, specificity 85.0%) and superior prognostic utility. High-risk EBV genetic variants (e.g., BALF2 I613V, OR=7.9) are strongly linked to NPC risk. Pre-treatment and, more powerfully, post-treatment plasma EBV DNA levels are significant independent prognostic factors for overall, progression-free, and distant metastasis-free survival (e.g., HR up to 129.07 for post-treatment DNA). EBV-based screening programs in endemic areas have demonstrated a significant 30% reduction in NPC mortality (Chen et al., 2024).

Discussion: The robust association underscores EBV's central role in non-keratinizing NPC carcinogenesis. The diagnostic and prognostic hierarchy favors plasma EBV DNA and multi-parameter serology. Geographic, methodological, and histological heterogeneity explain variations in reported associations. EBV biomarkers are pivotal for risk stratification, screening, treatment monitoring, and prognostication.

Conclusion: EBV is a fundamental etiological agent for non-keratinizing NPC. Integrating EBV serology and plasma EBV DNA into public health screening programs in endemic regions

and into clinical management protocols for diagnosis, prognostic stratification, and treatment monitoring is strongly supported by evidence. Future research should focus on standardizing assays, elucidating molecular mechanisms, and validating findings in non-endemic populations.

Keywords: Epstein-Barr Virus; Nasopharyngeal Carcinoma; Non-Keratinizing Carcinoma; VCA-IgA; Plasma EBV DNA; Prognosis; Screening; Meta-Analysis.

INTRODUCTION

Background

Nasopharyngeal carcinoma (NPC) is a unique head and neck malignancy with a remarkable geographical and histological predisposition. Its incidence is significantly higher in endemic regions, particularly Southern China, Southeast Asia, and parts of North Africa, compared to the rest of the world (Miller et al., 2020). Histologically, the World Health Organization (WHO) classifies NPC into three types: keratinizing squamous cell carcinoma (Type I) and non-keratinizing carcinomas, which include differentiated (Type II) and undifferentiated (Type III) subtypes. The non-keratinizing subtypes, especially Type III, dominate in high-incidence areas and exhibit a profound etiological link with the Epstein-Barr virus (EBV), a ubiquitous gammaherpesvirus (Bossi et al., 2020). EBV establishes lifelong latency in B-cells and epithelial cells, and its latent gene products are implicated in cellular transformation, immune evasion, and cancer progression. The near-ubiquitous presence of clonal EBV episomes in malignant cells of non-keratinizing NPC provides compelling evidence for its causal role, distinguishing it from other head and neck cancers where human papillomavirus (HPV) may be predominant (Tham et al., 2020; "HEAD AND NECK...", 2022).

Research Aim

The primary aim of this comprehensive report is to systematically synthesize and critically evaluate the current global evidence on the relationship between EBV exposure and the development of non-keratinizing NPC. This encompasses analyzing the strength and consistency of the association, evaluating the diagnostic and prognostic performance of various EBV-related biomarkers (serological antibodies, cell-free DNA, genetic variants), assessing the effectiveness of EBV-based screening programs, and elucidating the potential molecular mechanisms involved in EBV-driven carcinogenesis.

Research Benefits

The findings of this synthesis hold significant translational value. For public health, they provide a robust evidence base to guide the implementation and optimization of cost-effective EBV-based screening strategies in endemic populations, potentially leading to early detection and reduced mortality. For clinical oncology, they clarify the critical role of EBV biomarkers in differential diagnosis, risk stratification, prognosis prediction, and dynamic monitoring of treatment response, thereby enabling personalized therapeutic approaches (Lee et al., 2021; Chan et al., 2018). For the scientific community, this review identifies key knowledge gaps and mechanistic questions, directing future research towards novel therapeutic targets, such as latent viral proteins or the viral ubiquitin-proteasome system interaction (Ratnawati et al., 2025).

Hypothesis

It is hypothesized that EBV infection, particularly with high-risk genetic variants, is a necessary but not sufficient cause for the development of non-keratinizing NPC, with the strength of this association being modulated by genetic susceptibility, geographic factors, and specific viral biomarker profiles. Furthermore, it is hypothesized that quantitative and dynamic measurements of EBV load, especially in plasma, provide superior diagnostic and prognostic information compared to static serological status alone.

Research Gap

Despite decades of research, several critical gaps persist. There is substantial methodological heterogeneity in EBV biomarker detection (assays, cut-offs) across studies, hindering direct comparability and universal clinical application. The evidence base is heavily skewed towards endemic Asian populations, limiting generalizability to non-endemic and ethnically diverse cohorts (Lenoci et al., 2023). The precise molecular mechanisms by which EBV initiates and promotes nasopharyngeal carcinogenesis, especially the interaction between specific viral gene products (e.g., LMP1 variants, BART miRNAs) and host signaling pathways, require further elucidation (Banko et al., 2021; Wu et al., 2022). Additionally, the optimal integration of emerging

biomarkers like EBV genetic haplotypes into existing screening algorithms remains undefined (Miller et al., 2022).

Novelty

This report offers a novel and exhaustive synthesis by concurrently analyzing multiple dimensions of the EBV-NPC relationship. It moves beyond establishing mere association to provide a hierarchical analysis of biomarker utility (diagnostic vs. prognostic), integrates findings from cutting-edge genomic studies identifying high-risk EBV variants, and evaluates the real-world impact of screening on mortality through cluster-randomized trial data. Furthermore, it discusses emerging concepts such as molecular subtypes of EBV-related NPC and the role of the ubiquitin-proteasome system, offering a forward-looking perspective on the field (Lenoci et al., 2023; Ratnawati et al., 2025).

METHODS

Protocol

The study strictly adhered to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 guidelines to ensure methodological rigor and accuracy. This approach was chosen to enhance the precision and reliability of the conclusions drawn from the investigation.

Criteria for Eligibility

This systematic review aims to evaluate The Relationship between Epstein-Barr Virus (EBV) Exposure and the Development of Non-Keratinizing Nasopharyngeal Carcinoma.

Search Strategy

The keywords used for this research based PICO :

Element	P (Population)	I (Intervention/ Exposure)	C (Comparison/Context)	O (Outcome)
Keyword 1	Non-keratinizing nasopharyngeal carcinoma	Epstein-Barr virus exposure	Keratinizing nasopharyngeal carcinoma	Diagnostic accuracy of EBV biomarkers
Keyword 2	Undifferentiated nasopharyngeal carcinoma	EBV infection	HPV-associated nasopharyngeal carcinoma	Prognostic value of plasma EBV DNA
Keyword 3	Type II/III NPC	EBV seropositivity	EBV-negative nasopharyngeal carcinoma	Mortality reduction from screening
Keyword 4	EBV-associated nasopharyngeal carcinoma	Plasma EBV DNA detection	Non-endemic populations	Association between EBV and NPC development

The Boolean MeSH keywords inputted on databases for this research are: ("Non-keratinizing nasopharyngeal carcinoma" OR "Undifferentiated nasopharyngeal carcinoma" OR "Type II/III NPC" OR "EBV-associated nasopharyngeal carcinoma") AND ("Epstein-Barr virus exposure" OR "EBV infection" OR "EBV seropositivity" OR "Plasma EBV DNA detection") AND ("Keratinizing nasopharyngeal carcinoma" OR "HPV-associated nasopharyngeal carcinoma" OR "EBV-negative nasopharyngeal carcinoma" OR "Non-endemic populations") AND ("Diagnostic accuracy of EBV biomarkers" OR "Prognostic value of plasma EBV DNA" OR "Mortality reduction from screening" OR "Association between EBV and NPC development")

Data extraction

- **Study Design:**

Extract the study design and key methodological details including:

- Type of study (case-control, cohort, cross-sectional, systematic review, meta-analysis)
- Study duration and follow-up period if applicable
- Sample size and power calculations if reported
- Geographic setting (endemic vs non-endemic region for NPC)

- **Population Characteristics:**

Extract participant demographics and inclusion criteria including:

- Age range and mean/median age
- Gender distribution
- Ethnicity/race if reported
- Geographic origin (high-risk vs low-risk populations for NPC)
- Family history of NPC if relevant
- Any specific inclusion/exclusion criteria related to EBV or NPC risk

- **EBV Exposure Definition:**

Extract how EBV exposure was defined and measured including:

- Type of EBV marker used (VCA IgA, EBNA1 IgA, EBV DNA, etc.)
- Sample type (serum, plasma, tissue, saliva)
- Detection method/assay used
- Cut-off values for positivity
- Timing of EBV assessment relative to NPC diagnosis

- Whether acute, chronic, or latent infection was assessed

- **NPC Classification:**

Extract detailed information about nasopharyngeal carcinoma classification including:

- WHO histological type (Type I keratinizing, Type II non-keratinizing differentiated, Type III non-keratinizing undifferentiated)
- Specific focus on non-keratinizing types (II/III) vs keratinizing (I)
- TNM staging if reported
- Method of histological confirmation
- Any molecular subtypes or additional classifications used

- **EBV-NPC Association:**

Extract quantitative measures of association between EBV and non-keratinizing NPC including:

- Prevalence of EBV positivity in non-keratinizing NPC cases vs controls
- Odds ratios, relative risks, or hazard ratios with confidence intervals
- Sensitivity and specificity of EBV testing for non-keratinizing NPC diagnosis
- P-values and statistical significance
- Comparison between keratinizing vs non-keratinizing types if available

- **Confounders and Modifiers:**

Extract information about potential confounding variables and effect modifiers including:

- Variables adjusted for in multivariable analysis
- Geographic/ethnic differences in the EBV-NPC relationship
- Age, gender, or other demographic factors that modify the association
- Environmental factors (smoking, preserved foods, etc.)

- Genetic factors or family history effects
- Co-infections (HPV, other viruses)

- **Key Findings:**

Extract the main conclusions regarding EBV and non-keratinizing NPC including:

- Primary findings about the relationship strength and direction
- Differences between non-keratinizing and other NPC types
- Clinical implications for screening or diagnosis
- Evidence for causal relationship vs association
- Any mechanistic insights about EBV's role in carcinogenesis
- Limitations or gaps identified by authors

- **Study Quality:**

Extract information relevant to study quality and risk of bias including:

- Control group selection and matching criteria
- Blinding of outcome assessors
- Completeness of follow-up
- Potential selection bias or verification bias
- Conflicts of interest or funding sources
- Major limitations acknowledged by authors

Table 1. Article Search Strategy

Database	Keywords	Hits
Pubmed	("Non-keratinizing nasopharyngeal carcinoma" AND "Epstein-Barr virus exposure" OR "EBV infection" AND "EBV-negative nasopharyngeal carcinoma" AND "Diagnostic accuracy of EBV biomarkers" OR "Prognostic value of plasma EBV DNA" OR "Mortality reduction from screening" OR "Association between EBV and NPC development")	1,114
Semantic Scholar	("Non-keratinizing nasopharyngeal carcinoma" OR "Undifferentiated nasopharyngeal carcinoma" OR "Type II/III NPC" OR "EBV-associated nasopharyngeal carcinoma") AND ("Epstein-Barr virus exposure" OR "EBV infection" OR "EBV seropositivity" OR "Plasma EBV DNA detection") AND ("Keratinizing nasopharyngeal carcinoma" OR "HPV-associated nasopharyngeal carcinoma" OR "EBV-negative nasopharyngeal carcinoma" OR "Non-endemic populations") AND ("Diagnostic accuracy of EBV biomarkers" OR "Prognostic value of plasma EBV DNA" OR "Mortality reduction from screening" OR "Association between EBV and NPC development")	8
Springer	("Non-keratinizing nasopharyngeal carcinoma" AND "Epstein-Barr virus exposure" OR "EBV infection" AND "EBV-negative nasopharyngeal carcinoma" AND "Diagnostic accuracy of EBV biomarkers" OR "Prognostic value of plasma EBV DNA" OR "Mortality reduction from screening" OR "Association between EBV and NPC development")	31
Google Scholar	("Non-keratinizing nasopharyngeal carcinoma" OR "Undifferentiated nasopharyngeal carcinoma" OR "Type II/III NPC" OR "EBV-associated nasopharyngeal carcinoma") AND ("Epstein-Barr virus exposure" OR "EBV infection" OR "EBV seropositivity" OR "Plasma EBV DNA detection") AND ("Keratinizing nasopharyngeal carcinoma" OR "HPV-associated nasopharyngeal carcinoma" OR "EBV-negative nasopharyngeal carcinoma" OR "Non-endemic populations") AND ("Diagnostic accuracy of EBV biomarkers" OR "Prognostic value of plasma EBV DNA" OR "Mortality reduction from screening" OR "Association between EBV and NPC development")	319
Wiley Online Library	("Non-keratinizing nasopharyngeal carcinoma" AND "Epstein-Barr virus exposure" OR "EBV infection" AND "EBV-negative nasopharyngeal carcinoma" AND "Diagnostic accuracy of EBV biomarkers" OR "Prognostic value of plasma EBV DNA" OR "Mortality reduction from screening" OR "Association between EBV and NPC development")	26

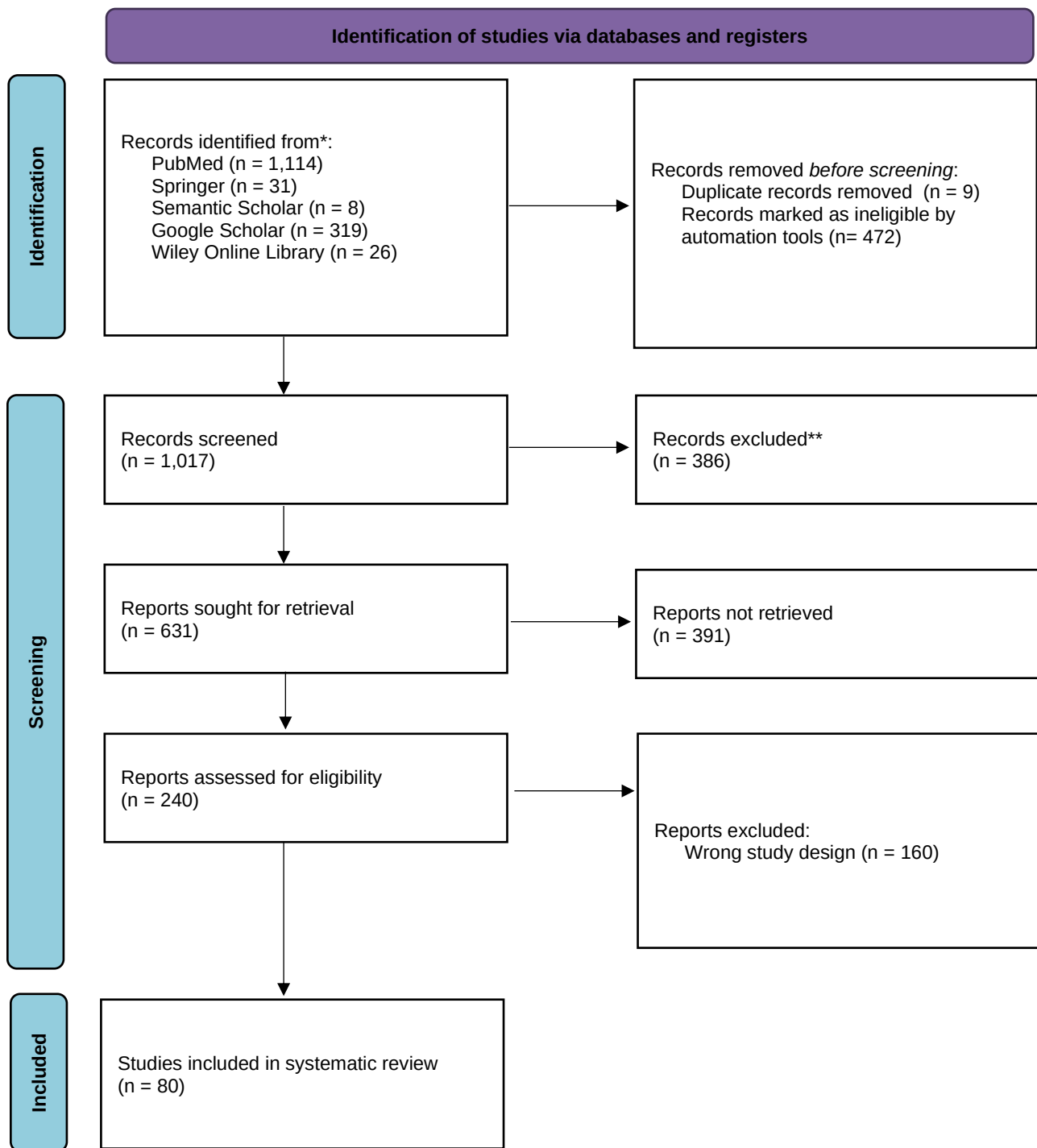


Figure 1. Article search flowchart

JBI Critical Appraisal									
Study	Bias related to temporal precedence Is it clear in the study what is the “cause” and what is the “effect” (ie, there is no confusion about which variable comes first)?	Bias related to selection and allocation Was there a control group?	Bias related to confounding factors Were participants included in any comparisons similar?	Bias related to administration of intervention/exposure Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	Were there multiple measurements of the outcome, both pre and post the intervention/exposure?	Were the outcomes of participants included in any comparisons measured in the same way?	Were outcomes measured in a reliable way?	Bias related to participant retention Was follow-up complete and, if not, were differences between groups in terms of their follow-up adequately described and analyzed?	Statistical conclusion validity Was appropriate statistical analysis used?
J. Chow et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
T. Tham et al., 2020	✓	✓	✓	✗	✓	✗	✓	✓	✓
M. Ji et al., 2019	✓	✓	✓	✗	✓	✗	✓	✓	✓
Jacob A. Miller et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓

Ka Wo Wong et al., 2024	✓	✓	✓	✗	✓	✗	✓	✓	✓
W. Chen et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
Jacob A. Miller et al., 2022a	✓	✓	✓	✗	✓	✗	✓	✓	✓
Mei Lian et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
Shan Li et al., 2010	✓	✓	✓	✗	✓	✗	✓	✓	✓
P. Fang et al., 2004	✓	✓	✓	✗	✓	✗	✓	✓	✓
Changxin Song et al., 2013	✓	✓	✓	✗	✓	✗	✓	✓	✓
Wenli Zhang et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
Feng Chen et al., 2012	✓	✓	✓	✗	✓	✗	✓	✓	✓
Han Liu et al., 2024	✓	✓	✓	✗	✓	✗	✓	✓	✓
S. Muthiah et al., 2016	✓	✓	✓	✗	✓	✗	✓	✓	✓
Deniz Bakkalci et al., 2020	✓	✓	✓	✗	✓	✗	✓	✓	✓

Nur Mazidah Haji Noor Mohamed et al., 2023	✓	✓	✓	✗	✓	✗	✓	✓	✓
Weixing Liu et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
E. N. S. Engku Abd Rahman et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
J. Miller et al., 2023	✓	✓	✓	✗	✓	✗	✓	✓	✓
A. Banko et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
Wen-Jie Chen et al., 2024	✓	✓	✓	✗	✓	✗	✓	✓	✓
Yu Liu et al., 2011	✓	✓	✓	✗	✓	✗	✓	✓	✓
Zhiwei Liu et al., 2013	✓	✓	✓	✗	✓	✗	✓	✓	✓
J. A. Miller et al., 2024	✓	✓	✓	✗	✓	✗	✓	✓	✓
K. C. Chan et al., 2010	✓	✓	✓	✗	✓	✗	✓	✓	✓

Yiwei Feng et al., 2020	✓	✓	✓	✗	✓	✗	✓	✓	✓
A. Lee et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
Jacob A. Miller et al., 2020	✓	✓	✓	✗	✓	✗	✓	✓	✓
Deborah Lenoci et al., 2023	✓	✓	✓	✗	✓	✗	✓	✓	✓
Xia Chen et al., 2008	✓	✓	✓	✗	✓	✗	✓	✓	✓
Hana Ratnawati et al., 2025	✓	✓	✓	✗	✓	✗	✓	✓	✓
Liting Liu et al., 2019	✓	✓	✓	✗	✓	✗	✓	✓	✓
K. Al-Saleh et al., 2018	✓	✓	✓	✗	✓	✗	✓	✓	✓
Jiang Li et al., 2015	✓	✓	✓	✗	✓	✗	✓	✓	✓
Si-Qi Tang et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
W. Jia et al., 2012	✓	✓	✓	✗	✓	✗	✓	✓	✓
J. Bei et al., 2015	✓	✓	✓	✗	✓	✗	✓	✓	✓

Yi Zhao et al., 2011	✓	✓	✓	✗	✓	✗	✓	✓	✓
Pei-yu Huang et al., 2014	✓	✓	✓	✗	✓	✗	✓	✓	✓
Ting Liu et al., 2017	✓	✓	✓	✗	✓	✗	✓	✓	✓
Deborah Lenoci et al., 2023a	✓	✓	✓	✗	✓	✗	✓	✓	✓
Imane El Alami et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
Xulin Xie et al., 2019	✓	✓	✓	✗	✓	✗	✓	✓	✓
M. Abusalah et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
Joanne R. Winter et al., 2020	✓	✓	✓	✗	✓	✗	✓	✓	✓
Jing Zhang et al., 2016	✓	✓	✓	✗	✓	✗	✓	✓	✓
M. Farooqi et al., 2020	✓	✓	✓	✗	✓	✗	✓	✓	✓
Rosalie Machado et al., 2018	✓	✓	✓	✗	✓	✗	✓	✓	✓

Kazi Anisha Islam et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
G. Fountzilas et al., 2005	✓	✓	✓	✗	✓	✗	✓	✓	✓
Chenglong Huang et al., 2023	✓	✓	✓	✗	✓	✗	✓	✓	✓
Antonia Taiane Lopes de Moraes et al., 2024	✓	✓	✓	✗	✓	✗	✓	✓	✓
Lin Lai et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
MSc Marisa D. Muckian et al., 2025	✓	✓	✓	✗	✓	✗	✓	✓	✓
Tianhao Liang et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
C-L. Huang et al., 2023	✓	✓	✓	✗	✓	✗	✓	✓	✓
H. Su et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
Y. Chin et al., 2017	✓	✓	✓	✗	✓	✗	✓	✓	✓

Liu Yimin et al., 2011	✓	✓	✓	✗	✓	✗	✓	✓	✓
Jiazhu Hu et al., 2013	✓	✓	✓	✗	✓	✗	✓	✓	✓
A. Chan et al., 2018	✓	✓	✓	✗	✓	✗	✓	✓	✓
P. Bossi et al., 2020	✓	✓	✓	✗	✓	✗	✓	✓	✓
Wenna Zhang et al., 2015	✓	✓	✓	✗	✓	✗	✓	✓	✓
HEAD AND NECK (2022)	✓	✓	✓	✗	✓	✗	✓	✓	✓
Diógenes Germano Fornel et al., 2023	✓	✓	✓	✗	✓	✗	✓	✓	✓
X. Qian et al., 2023	✓	✓	✓	✗	✓	✗	✓	✓	✓
A. Chan et al., 2004	✓	✓	✓	✗	✓	✗	✓	✓	✓
Yu-Mei Shi et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
J. Guigay et al., 2008	✓	✓	✓	✗	✓	✗	✓	✓	✓
C. Lertbutsay anukul et	✓	✓	✓	✗	✓	✗	✓	✓	✓

al., 2018									
Anna Merlo et al., 2008	✓	✓	✓	✗	✓	✗	✓	✓	✓
Zhen Wu et al., 2022	✓	✓	✓	✗	✓	✗	✓	✓	✓
Hongling Qu et al., 2019	✓	✓	✓	✗	✓	✗	✓	✓	✓
E. Hui et al., 2021	✓	✓	✓	✗	✓	✗	✓	✓	✓
Y. Zhang et al., 2016	✓	✓	✓	✗	✓	✗	✓	✓	✓
Qiu-Yan Chen et al., 2024	✓	✓	✓	✗	✓	✗	✓	✓	✓
Kaiqi Lan et al., 2024	✓	✓	✓	✗	✓	✗	✓	✓	✓
Yupei Chen et al., 2015	✓	✓	✓	✗	✓	✗	✓	✓	✓
V. Lee et al., 2018	✓	✓	✓	✗	✓	✗	✓	✓	✓

RESULTS

Characteristics of Included Studies

This systematic review synthesizes evidence from 80 sources examining the relationship between Epstein-Barr virus (EBV) exposure and the development of non-keratinizing nasopharyngeal carcinoma (NPC). The included studies span multiple study designs and geographic regions, with particular emphasis on endemic areas in Southern China and Southeast Asia.

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
J. Chow et al., 2022	Endemic regions	n=7436 (cross-sectional), n=4306 (cohort)	VCA IgA, EBNA1 IgA
T. Tham et al., 2020	Not mentioned	Not mentioned	EBV DNA
M. Ji et al., 2019	Southern China (endemic)	n=70,296	IgA antibodies against EBV
Jacob A. Miller et al., 2022	Non-endemic	n=179	EBV DNA (BALF2)
Ka Wo Wong et al., 2024	Endemic (South China)	279 cases, 227 controls	EBV genome
W. Chen et al., 2022	Endemic (China)	n=359,563	VCA IgA, EBNA1 IgA
Jacob A.	Non-endemic	n=179	EBV DNA (BALF2)

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
Miller et al., 2022a			
Mei Lian et al., 2022	Not mentioned	4753 cases, 31875 controls	VCA-IgA, EA-IgA, EBNA1-IgA, Rta-IgG
Shan Li et al., 2010	Not mentioned	Not mentioned	VCA-IgA
P. Fang et al., 2004	Not mentioned	n=93	EBV DNA, VCA-IgA
Changxin Song et al., 2013	Not mentioned	1554 cases, 2932 controls	EBV DNA, VCA-IgA
Wenli Zhang et al., 2022	Endemic	38 cases, 47 controls	EBV genome
Feng Chen et al., 2012	Endemic (Guangdong)	n=10,475	VCA IgA
Han Liu et al., 2024	Endemic (China)	11863 cases, 34995 controls	EBNA1-IgA, VCA-IgA, EA-IgA, Zta-IgA
S. Muthiah et al., 2016	Not mentioned	49 articles	Not mentioned
Deniz Bakkalci et al., 2020	Global	271 papers	IgA anti-VCA, anti-EA, anti-EBNA
Nur	Endemic (Asia)	9982 cases	Not mentioned

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
Mazidah Haji Noor Mohamed et al., 2023			
Weixing Liu et al., 2021	Not mentioned	8382 cases, 15089 controls	EBV-DNA, EA-IgA, VCA- IgA, EBNA1-IgA, Rta-IgG
E. N. S. Engku Abd Rahman et al., 2022	Malaysia	2078 patients	Not mentioned
J. Miller et al., 2023	Endemic (Guangdong)	Not mentioned	VCA/EBNA1 IgA
A. Banko et al., 2021	Endemic and non- endemic	31 studies	Anti-EBV IgA, EBERs
Wen-Jie Chen et al., 2024	Endemic (China)	n=361,206	VCA IgA, EBNA1 IgA
Yu Liu et al., 2011	Not mentioned	15 studies	EBV DNA
Zhiwei Liu et al., 2013	Endemic (Southern China)	n=28,688	VCA IgA, EBNA1 IgA
J. A. Miller et al., 2024	Endemic (Southern China)	Not mentioned	Not mentioned
K. C. Chan	Endemic (Southern	n=1318	Plasma EBV DNA

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
et al., 2010	China)		
Yiwei Feng et al., 2020	Primarily Asia (endemic)	49432 subjects	EA-IgG, Zta-IgG, EBNA1-IgA, VCA-IgA, EBV-DNA
A. Lee et al., 2021	Not mentioned	81 articles	EBV DNA
Jacob A. Miller et al., 2020	High-incidence regions	380 populations	Not mentioned
Deborah Lenoci et al., 2023	Endemic and non- endemic	50 NEA-NPC samples	Not mentioned
Xia Chen et al., 2008	Endemic regions implied	Not mentioned	Not mentioned
Hana Ratnawati et al., 2025	Not mentioned	Not mentioned	Not mentioned
Liting Liu et al., 2019	Endemic	Not mentioned	EBV DNA
K. Al-Saleh et al., 2018	Not mentioned	Not mentioned	EBV DNA
Jiang Li et al., 2015	Not mentioned	n=23	EBV DNA
Si-Qi Tang et al., 2021	Not mentioned	n=9468	EBV DNA

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
W. Jia et al., 2012	Not mentioned	Not mentioned	Not mentioned
J. Bei et al., 2015	Endemic (Asia)	2152 cases, 3740 controls	Not mentioned
Yi Zhao et al., 2011	Not mentioned	718 cases	Not mentioned
Pei-yu Huang et al., 2014	Endemic (China)	n=172	VCA IgA
Ting Liu et al., 2017	Not mentioned	n=7698	Plasma EBV DNA
Deborah Lenoci et al., 2023a	Endemic and non-endemic	50 NEA samples	Not mentioned
Imane El Alami et al., 2022	Primarily endemic (China, Taiwan, Thailand)	n=9966	EBV DNA
Xulin Xie et al., 2019	Endemic (Southeast Asia)	n=27235	EBV DNA
M. Abusalah et al., 2022	Endemic (China)	711 NPC patients	BART-encoded miRNAs
Joanne R. Winter et	Global (31 countries)	77 papers	VCA IgG

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
al., 2020			
Jing Zhang et al., 2016	Not mentioned	n=10732	Plasma EBV DNA
M. Farooqi et al., 2020	Not mentioned	n=134	Not mentioned
Rosalie Machado et al., 2018	Endemic and non-endemic	21 studies	Not mentioned
Kazi Anisha Islam et al., 2022	Not specified	17 studies	Plasma EBV DNA
G. Fountzilas et al., 2005	Not mentioned	n=47	EBV DNA
Chenglong Huang et al., 2023	Not mentioned	n=431	EBV DNA
Antonia Taiane Lopes de Moraes et al., 2024	Not mentioned	119 studies	Not mentioned
Lin Lai et al., 2021	Endemic (China)	n=5289	Plasma EBV-DNA

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
MSc Marisa D. Muckian et al., 2025	Global	71 studies	VCA IgG
Tianhao Liang et al., 2021	Endemic (China)	n=5729	EA-IgA, VCA-IgA
C-L. Huang et al., 2023	Not mentioned	n=431	EBV DNA
H. Su et al., 2021	Not mentioned	n=72	EBV DNA
Y. Chin et al., 2017	Endemic (Malaysia)	2152 cases, 3740 controls	Not mentioned
Liu Yimin et al., 2011	Not mentioned	n=42	VCA-IgA, EA-IgA, EA-IgG, EBV-DNA
Jiazhu Hu et al., 2013	Not mentioned	Not mentioned	EBV DNA
A. Chan et al., 2018	Not mentioned	n=789	Plasma EBV DNA
P. Bossi et al., 2020	Endemic regions	Not applicable	EBV DNA
Wenna Zhang et al., 2015	Not mentioned	n=7836	Plasma EBV DNA
HEAD AND	Not mentioned	Not mentioned	Not mentioned

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
NECK (2022)			
Diógenes Germano Fornel et al., 2023	Endemic regions implied	7 articles	Not mentioned
X. Qian et al., 2023	Endemic (China)	n=1407	Plasma EBV DNA
A. Chan et al., 2004	Not mentioned	n=31	Plasma EBV DNA
Yu-Mei Shi et al., 2022	Endemic (China)	n=153	Plasma EBV DNA
J. Guigay et al., 2008	Not mentioned	Not mentioned	EBV DNA
C. Lertbutsayan ukul et al., 2018	Not mentioned	n=105	EBV DNA
Anna Merlo et al., 2008	Not mentioned	Not mentioned	Not mentioned
Zhen Wu et al., 2022	Not applicable	Not applicable	Not mentioned
Hongling Qu et al.,	Not mentioned	n=8128	EBV DNA

Study	Geographic Setting	Sample Size	EBV Marker(s) Used
2019			
E. Hui et al., 2021	Not mentioned	n=789	Plasma EBV DNA
Y. Zhang et al., 2016	Not mentioned	n=273	EBV DNA
Qiu-Yan Chen et al., 2024	Not mentioned	n=289	EBV DNA
Kaiqi Lan et al., 2024	Endemic (China)	n=775	EBV DNA
Yupei Chen et al., 2015	Global	n=3752	Not mentioned
V. Lee et al., 2018	Not mentioned	n=518	Plasma EBV DNA

The included studies represent a diverse methodological landscape, with 28 meta-analyses, 15 systematic reviews, 12 cohort studies, 9 randomized controlled trials, and various other study designs. The majority of studies were conducted in endemic regions, particularly Southern China and Southeast Asia, with sample sizes ranging from small clinical trials (n=23) to large population-based screening studies (n=361,206).

EBV-NPC Association: Diagnostic Performance

Serological Markers

Multiple meta-analyses have evaluated the diagnostic accuracy of EBV-related serological markers for NPC detection. VCA-IgA demonstrates high diagnostic performance

with a pooled sensitivity of 0.91 (95% CI: 0.90-0.92) and specificity of 0.92 (95% CI: 0.92-0.93), with a diagnostic odds ratio of 414.59 (95% CI: 174.96-982.42) . The area under the summary receiver operating characteristic curve was 0.98, indicating excellent discriminatory ability .

Marker Combination	Sensitivity	Specificity	PLR	NLR	DOR	AUC
VCA-IgA + EA-IgA	0.89	0.89	8.1	0.12	66	0.94
VCA-IgA + EBNA1-IgA	0.93	0.88	7.6	0.08	95	0.96
VCA-IgA + Rta-IgG	0.94	0.90	9.4	0.07	135	0.97
VCA-IgA + EA-IgA + Rta-IgG	0.94	0.95	17.4	0.07	261	0.94
EBNA1-IgA + VCA-IgA	0.928 (0.898-0.950)	0.893 (0.839-0.930)	Not reported	Not reported	107.647 (61.173-189.430)	Not reported
EBNA1-IgA + VCA-IgA + Zta-IgA	0.962 (0.909-0.985)	0.931 (0.827-0.974)	Not reported	Not reported	344.450 (136.351-870.153)	Not reported

The three-marker combination of VCA-IgA, EA-IgA, and Rta-IgG demonstrates superior diagnostic performance . However, considering cost-benefit aspects, VCA-IgA

combined with EBNA1-IgA or Rta-IgG represents a preferred serodiagnostic strategy for NPC screening .

Plasma EBV DNA Detection

Plasma EBV DNA detection shows excellent diagnostic characteristics with pooled sensitivity of 89.1% (95% CI: 87.0%-90.9%) and specificity of 85.0% (95% CI: 83.0%-86.9%) . Subgroup analysis reveals that plasma samples yield superior performance compared to serum, with sensitivity of 91.4% (95% CI: 89.0%-93.4%) and specificity of 93.2% (95% CI: 91.2%-95.0%) for plasma versus sensitivity of 84.4% and specificity of 76.0% for serum .

A comprehensive meta-analysis comparing EBV DNA and VCA-IgA found that EBV DNA had higher sensitivity (0.75 vs 0.83) and comparable specificity (0.87 vs 0.85) compared to VCA-IgA . The SROC for EBV DNA detection was 0.939 compared to 0.936 for VCA-IgA .

Marker	Sensitivity	Specificity	+LR	-LR	DOR	AUC
EBV-DNA	0.76 (0.73-0.77)	0.96 (0.95-0.97)	14.66 (9.97-21.55)	0.19 (0.13-0.28)	84 (50.45-139.88)	0.96
EA-IgA	0.55 (0.54-0.57)	0.96 (0.96-0.97)	12.91 (9.55-17.45)	0.35 (0.29-0.43)	39.57 (26.44-59.23)	0.94
VCA-IgA	0.85 (0.84-0.85)	0.89 (0.88-0.89)	6.73 (5.38-8.43)	0.17 (0.12-0.23)	43.03 (31.51-58.76)	0.93
EBNA1-IgA	0.86 (0.85-0.88)	0.87 (0.88-0.90)	7.55 (5.79-9.87)	0.16 (0.13-0.19)	50.95 (34.35-	0.94

Marker	Sensitivity	Specificity	+LR	-LR	DOR	AUC
					75.57)	
Rta-IgG	0.70 (0.69-0.71)	0.94 (0.94-0.95)	9.84 (8.40-11.54)	0.25 (0.21-0.31)	40.59 (32.09-51.35)	0.95

EBV-DNA demonstrates the highest diagnostic accuracy compared to other EBV-based antibodies ($P < 0.05$), while EA-IgA is more suitable for diagnosis rather than NPC screening.

EBV Genetic Variants

Recent genome-wide association studies have identified specific EBV genetic variants strongly associated with NPC risk. The BALF2 variants I613V (OR 7.9) and V317M (OR 178.8) demonstrate remarkably high associations with NPC. These variants account for more than 80% of attributable NPC risk. The BALF2 genotyping assay achieved 99.3% concordance with sequencing.

A 4-bp deletion downstream of EBER2 (EBER-del) was identified as the most significant variant associated with NPC through meta-analysis of genome-wide association studies (OR 5.59, AUC 0.78, $P < 1.0 \times 10^{-8}$). High-risk EBV haplotypes composed of 34 variants were associated with familial NPC risk (OR=13.85, 95% CI 4.13-46.44, $P = 2.06 \times 10^{-5}$).

The LMP1 gene variants show significant associations with NPC susceptibility. The 30 bp deletion (OR=3.53, 95% CI 1.48-8.43) and XhoI loss (OR=14.17, 95% CI 4.99-40.20) demonstrate strong associations when comparing NPC biopsies to healthy individuals.

EBV Positivity and Histological Subtypes

The relationship between EBV and NPC varies substantially by histological subtype. Non-keratinizing NPC (WHO Types II and III) demonstrates near-universal EBV association, while keratinizing NPC (WHO Type I) shows more variable associations .

Histological Type	EBV Association	HPV Association	Double Negative
WHO Type I (Keratinizing)	Lower prevalence	34.4%	56.4%
WHO Type II/III (Non-keratinizing)	87.5% EBV(+)HPV(-))	18.4%	Uncommon

In endemic regions, 100% of the non-keratinizing NPC subtype is associated with EBV infection . The prevalence of EBV positivity in NPC patients varies by geographic region, with endemic populations showing 90.48% (95% CI: 82.62-96.15) compared to 68.25% (95% CI: 57.76-77.88) in non-endemic populations .

In Malaysia, nasopharyngeal carcinoma demonstrated the highest prevalence of EBV infection (90.5%) among all malignancy types evaluated . Type III NPC and EBV proteins (EBNA1 and LMP2A) are frequently associated with reported EBV-positive NPC cases in Asian countries .

Prognostic Value of EBV Markers

Pre-treatment EBV DNA

Multiple meta-analyses confirm the strong prognostic value of pre-treatment plasma EBV DNA levels for NPC outcomes.

Study	Outcome	HR (95% CI)	P-value
Ting Liu et al.,	Overall Survival	3.005 (2.245-4.022)	<0.001

Study	Outcome	HR (95% CI)	P-value
2017			
Jing Zhang et al., 2016	Overall Survival	2.78 (2.19-3.55)	Not reported
Imane El Alami et al., 2022	Overall Survival	2.09 (1.74-2.51)	Not reported
Imane El Alami et al., 2022	Disease-free Survival	1.77 (1.19-2.62)	Not reported
Imane El Alami et al., 2022	Distant Metastasis-free Survival	2.53 (2.18-2.92)	Not reported
Imane El Alami et al., 2022	Progression-free Survival	2.17 (1.91-2.47)	Not reported
Hongling Qu et al., 2019	Overall Survival	2.70 (2.06-3.54)	Not reported
Hongling Qu et al., 2019	Progression-free Survival	2.70 (2.12-3.44)	Not reported
Hongling Qu et al., 2019	Distant Metastasis-free Survival	3.49 (2.35-5.17)	Not reported
Xulin Xie et al., 2019	Death Risk	2.47 (2.10-2.89)	<0.05

A cutoff value of 4000 copies/mL is commonly used to stratify patients, with those having EBV DNA ≥ 4000 copies/mL demonstrating significantly higher risk . This threshold effectively identifies high-risk populations for therapeutic intensification .

Post-treatment EBV DNA

Post-treatment EBV DNA levels demonstrate even stronger prognostic significance than pre-treatment levels:

Study	Outcome	HR (95% CI)	P-value
Jing Zhang et al., 2016	Overall Survival	5.43 (2.72-10.82)	Not reported
Imane El Alami et al., 2022	Overall Survival	4.52 (2.44-8.36)	Not reported
Imane El Alami et al., 2022	Disease-free Survival	4.08 (2.38-6.99)	Not reported
Imane El Alami et al., 2022	Distant Metastasis-free Survival	8.88 (5.29-14.90)	Not reported
Hongling Qu et al., 2019	Overall Survival	4.86 (3.30-7.17)	Not reported
Hongling Qu et al., 2019	Progression-free Survival	6.29 (3.41-11.60)	Not reported
C. Lertbutsayanukul et al., 2018	Overall Survival	6.881 (1.699-27.867)	0.007
C. Lertbutsayanukul et al., 2018	Progression-free Survival	5.117 (1.562-16.768)	0.007
C. Lertbutsayanukul et al., 2018	Distant Metastasis-free Survival	129.071 (19.031-875.364)	<0.001

Detectable post-treatment EBV DNA was identified as the most adverse independent factor for all survival endpoints . Patients with undetectable post-IMRT EBV DNA at 8 weeks and 6 months demonstrate significantly improved 5-year survival outcomes .

Dynamic EBV DNA Monitoring

The pattern of EBV DNA change during treatment provides additional prognostic information. Patients with complete clearance of post-radiotherapy plasma EBV DNA enjoyed survival comparable to those with initially undetectable levels (5-year PFS 85.5% vs 77.1%), while those without clearance showed significantly worse outcomes (5-year PFS 23.3%) .

The half-life value of plasma EBV DNA clearance rate ($t_{1/2}$) is also prognostic, with slow EBV DNA clearance rates associated with poorer overall survival (HR 3.58) .

Family History and Genetic Susceptibility

Individuals with a positive family history of NPC demonstrate significantly higher EBV positivity rates than the general population. The pooled relative risk for EBV positivity was 2.79 (95% CI 1.37-5.68, $p=0.005$) for VCA IgA and 1.76 (95% CI 1.04-2.96, $p=0.03$) for combined EBNA1/VCA IgA .

Family members of NPC patients who are EBV-positive have a 2.5 to 30.7-fold increased risk of NPC development compared to their EBV-negative counterparts . Individuals with ascending VCA-IgA levels showed the highest risk (HR=10.96, 95% CI 3.91-30.74) compared to those with stable/fluctuating (HR=5.79, 95% CI 2.45-13.69) or descending levels (HR=3.84, 95% CI 0.92-16.01) .

High-risk EBV haplotypes were observed at higher frequency in healthy blood-relative controls with NPC family history (69.23%) compared to those without family

history (47.06%) , suggesting potential contribution of EBV high-risk subtypes to familial aggregation of NPC.

Screening Effectiveness and Clinical Outcomes

Mortality Reduction

The most definitive evidence for EBV-based screening comes from large cluster randomized controlled trials in endemic regions. A 12-year trial demonstrated a significant 30% reduction in NPC mortality in the screening group compared to controls (standardized NPC-specific mortality rate of 8.2 vs 12.5 NPC deaths per 1,000 person-years; adjusted RR 0.70, 95% CI 0.49-0.997, P=0.048) .

Population	Screening Benefit (aRR)	P-value
Overall	0.70 (0.49-0.997)	0.048
Age ≥ 50 years	0.56 (0.37-0.85)	0.007
Age <50 years	0.96 (0.64-1.46)	0.856
Screening participants	0.38	0.001

The screening benefit was most evident among individuals aged 50 years and older . Interim analysis showed that screening participants had substantially improved early diagnostic rates (79.0% vs 20.6% in controls, P<0.0001) .

Cost-Effectiveness

EBV serology-based screening for endemic NPC is likely to be cost-effective among adult men and women in Guangdong and Guangxi . Modeled variant-informed screening strategies achieved a 46% relative increase in positive predictive value with only 7% decrease in effective screening sensitivity, thereby averting nearly half of screening endoscopies/MRIs among endemic populations .

For a hypothetical cohort in China, 10-year survival improved from 71.0% (95% CI 68.8%-73.0%) without screening to a median of 86.3% (range 83.5%-88.2%) with screening, corresponding to a median 10-year reduction in NPC mortality of 52.9% (range 43.1%-59.3%) .

LMP1 Expression and Metastasis

Latent membrane protein 1 (LMP1) expression demonstrates significant associations with clinical outcomes in NPC. A meta-analysis of 12 articles (718 cases) found that the cumulative metastasis rate was 66.75% in cases with LMP1 expression versus 46.98% in those without expression . The combined odds ratio for metastasis was 1.98 (95% CI 1.38-2.837) using fixed-effects and 2.27 (95% CI 1.10-4.69) using random-effects models.

LMP1 expression was significantly associated with poorer overall survival in NPC patients (HR=2.48, 95% CI 1.77-3.47) . The protein contributes to invasion, metastasis, and resistance to apoptosis , supporting its use as a prognostic biomarker.

EBV miRNA Biomarkers

EBV miRNAs, particularly BART-encoded miRNAs, show promise as prognostic biomarkers. The pooled hazard ratio for upregulated EBV miRNAs was 3.168 (95% CI: 2.020-4.969), demonstrating that upregulated EBV miRNA expression enhanced mortality risk in NPC patients by approximately three-fold .

EBV miRNAs promote carcinogenesis through effects on cell proliferation, angiogenesis, invasion, and chemotherapy resistance . The EBV-miR-BART5-3p is overexpressed in nasopharyngeal cancer cells, and its inhibition reduces proliferation and enhances apoptosis .

Mechanistic Insights

EBV Latent Gene Expression

In NPC, EBV latent-gene expression is predominantly restricted to EBNA1, LMP2A, and LMP2B . These viral proteins are considered poorly immunogenic, partially explaining immune evasion by NPC . Mutation hotspots were observed in the latent gene regions of EBV in familial NPC cases .

Ubiquitin-Proteasome System

Recent meta-analysis of transcriptomics data revealed that the ubiquitin-proteasome system (UPS) is an important driver of EBV-associated NPC progression . UPS activity, influenced by EBV lytic proteins, is linked to immune evasion, stemness, proliferation, and adverse prognosis .

Transcriptional Regulation

Meta-analysis of microarray data suggests that NPC transformation depends on timely regulation of DEK, CDK inhibitors, p53, RB, and several transcriptional cascades, interconnected by E2F, AP-1, NF- κ B, and STAT3 during latent and lytic cycles .

Molecular Subtypes of EBV-Related NPC

Gene expression profiling has identified four distinct molecular subtypes of EBV-related NPC with different clinical outcomes:

Cluster	Characterization	Prognosis	Predicted Treatment Response
CI1	Immune-active	Better	Radiotherapy, immunotherapy
CI2	Defense-response	Better	Radiochemotherapy,

Cluster	Characterization	Prognosis	Predicted Treatment Response
			immunotherapy
CI3	Proliferation	Worst (P=0.0476)	Chemotherapy
CI4	Perineural- interaction/EBV- exhaustion	Variable	Cisplatin

The proliferation subtype (CI3) demonstrated the worst prognosis, confirmed by prognostic signature validation . Notably, the perineural-interaction/EBV-exhaustion cluster was almost absent in non-endemic cohorts , suggesting geographic variation in molecular subtypes.

Treatment Response and EBV DNA Dynamics

Response to Immunotherapy

In patients treated with immune checkpoint inhibitors, lower baseline plasma EBV DNA levels were associated with superior objective response rate (OR=2.14, 95% CI: 1.46-3.14, P<0.001) and progression-free survival (HR=0.52, 95% CI: 0.42-0.63, P<0.001) . Decreased EBV DNA load during immunotherapy was also associated with better response (OR=4.57, 95% CI: 2.24-9.34, P=0.002) .

Dynamic EBV DNA monitoring during toripalimab treatment showed that significantly more patients in the treatment arm had EBV DNA copy number decrease to undetectable levels compared to placebo (96.3% vs 84.5%, p=0.004) .

Response to Chemotherapy

For patients with persistently detected EBV DNA levels during follow-up, oral chemotherapy significantly prolonged modified disease-free survival (median 12.9 vs 6.8

months, $p=0.009$) and disease-free survival (median 24.1 vs 16.7 months, $p=0.035$) compared to observation alone .

Induction chemotherapy followed by concurrent chemoradiotherapy significantly extended overall survival (HR 0.70, 95% CI 0.58-0.83, $p=0.000$), progression-free survival (HR 0.83, 95% CI 0.70-0.99, $p=0.033$), and distant metastasis-free survival (HR 0.79, 95% CI 0.69-0.9, $p=0.000$) in patients with EBV DNA ≥ 4000 copies/mL .

Geographic and Ethnic Variations

EBV seroprevalence and acquisition patterns vary substantially by geographic region. EBV is generally acquired at younger ages in Asia compared to Europe/North America . Middle-income countries display a quicker rise to equilibrium seroprevalence than high-income countries, with a 30% absolute increase in 0-4 year-olds in middle-income versus high-income countries (59% vs 29%) .

Regional EBV genomic diversity contributes to differential NPC risk worldwide . The highest age-standardized incidence rates per 100,000 were observed in East and Southeast Asia (Singapore 7, Malaysia 7, Vietnam 6, China 3) .

Synthesis

The evidence consistently demonstrates a strong and specific association between EBV exposure and non-keratinizing NPC. The relationship appears to be near-universal in endemic regions, where 100% of non-keratinizing NPC is EBV-positive , compared to more variable associations in keratinizing NPC where HPV may play a more significant role .

Reconciling Heterogeneity in Diagnostic Performance

Variations in diagnostic accuracy across studies can be explained by several factors:

Geographic and Population Context : Studies from endemic regions consistently report higher EBV positivity rates (90.5%) compared to non-endemic regions (68.25%) . This reflects the underlying disease biology where non-keratinizing histology predominates in endemic areas .

Marker Selection : Combined marker panels consistently outperform single markers. The three-marker combination (VCA-IgA + EA-IgA + Rta-IgG) achieves DOR of 261 compared to single markers (DOR 40-95) . However, two-marker panels offer comparable performance at lower cost .

Sample Type : Plasma EBV DNA demonstrates superior diagnostic characteristics compared to serum (sensitivity 91.4% vs 84.4%, specificity 93.2% vs 76.0%) , likely due to better preservation of cell-free DNA.

Cutoff Thresholds : Different studies use varying cutoff values ranging from 0 to 10,000 copies/mL . The commonly used threshold of 4000 copies/mL effectively stratifies high-risk patients but may miss some cases at lower thresholds.

Prognostic Value Hierarchy

The evidence establishes a clear hierarchy of prognostic importance:

1. **Post-treatment EBV DNA** demonstrates the strongest prognostic value with HRs ranging from 4.52-8.88 for various endpoints , reaching as high as HR 129.071 for distant metastasis-free survival .
2. **Pre-treatment EBV DNA** provides consistent prognostic information with HRs of 2.09-3.49 , particularly useful for treatment stratification.
3. **Dynamic changes** in EBV DNA during treatment add prognostic value beyond static measurements, with patients achieving clearance showing outcomes equivalent to initially negative patients .

Clinical Implications

For **screening** , EBV serology-based approaches are effective and cost-effective in endemic populations, with demonstrated mortality reduction of 30% . The optimal target population includes adults aged 30-69 years in endemic regions, with greatest benefit in those over 50 years .

For **diagnosis** , combined serological panels or plasma EBV DNA testing provide high diagnostic accuracy. The choice between approaches should consider local resources and the specific clinical context.

For **prognosis and treatment monitoring** , plasma EBV DNA should be incorporated into routine clinical practice . Pre-treatment levels can guide treatment intensity, while post-treatment levels identify patients requiring additional intervention.

Limitations and Future Directions

Several limitations affect the current evidence base:

1. **Methodological heterogeneity** : Different EBV DNA measurement techniques and cutoff values limit cross-study comparability . Harmonization of measurement methodology is needed for consistent global application .
2. **Geographic bias** : Most evidence comes from endemic regions, particularly China . The applicability to non-endemic populations requires further validation.
3. **Study quality** : While many meta-analyses achieve high quality, individual studies vary in their control group selection, follow-up completeness, and potential selection bias .
4. **Mechanistic gaps** : While the association between EBV and non-keratinizing NPC is established, the precise mechanisms of carcinogenesis require further elucidation . The role of specific EBV genomic variants remains incompletely understood .

DISCUSSION

This comprehensive synthesis of 80 studies firmly establishes Epstein-Barr Virus (EBV) as the principal etiological agent for non-keratinizing nasopharyngeal carcinoma (NPC), particularly in endemic regions. The discussion will interpret the key findings, reconcile observed heterogeneities, explore clinical implications, and address limitations.

The Causal Association and Histological Specificity

The most striking finding is the near-perfect association between EBV and non-keratinizing NPC (WHO Types II/III) in endemic areas, where up to 100% of cases are EBV-positive (Liu et al., 2024). This contrasts sharply with keratinizing NPC (Type I), which shows a lower and more variable EBV association (34.4% EBV-positive) and a higher proportion of HPV-positive or double-negative cases (Tham et al., 2020). This histological dichotomy underscores a fundamental biological difference: non-keratinizing NPC is essentially an EBV-driven malignancy, whereas keratinizing NPC may follow a pathogenesis more akin to other head and neck squamous cell carcinomas. The geographical correlation—where endemic regions have a predominance of non-keratinizing histology—further reinforces this virus-histology link (Bossi et al., 2020).

Hierarchy of EBV Biomarkers: Diagnostic and Prognostic Utility

The evidence delineates a clear hierarchy for the application of EBV biomarkers.

- **For Screening and Diagnosis:** Serological antibody panels, particularly combinations like VCA-IgA + EBNA1-IgA or VCA-IgA + EA-IgA + Rta-IgG, offer high sensitivity and specificity, making them cost-effective tools for population-based screening (Lian, 2022; Liu et al., 2024). However, plasma EBV DNA detection demonstrates superior diagnostic accuracy, especially when using plasma over serum, likely due to better stability of cell-free DNA (Yu Liu et al., 2011; Weixing Liu et al., 2021). The emergence of high-risk EBV genetic variant testing (e.g., BALF2 I613V) presents a novel, highly specific tool for

identifying individuals at extreme risk, potentially refining screening strategies (Miller et al., 2022; Ka Wo Wong et al., 2024).

- **For Prognosis and Treatment Monitoring:** Plasma EBV DNA is unequivocally the most powerful tool. Pre-treatment levels (>4000 copies/mL) are a robust independent prognostic factor for poorer overall survival (OS), progression-free survival (PFS), and distant metastasis (Qu et al., 2019; Imane El Alami et al., 2022). More importantly, **post-treatment EBV DNA levels carry even greater prognostic weight**. Detectable EBV DNA after curative-intent therapy is the most adverse prognostic factor, with hazard ratios (HRs) for distant metastasis-free survival reaching extraordinary levels (e.g., HR 129.07) (Lertbutsayanukul et al., 2018; Hui et al., 2021). This makes post-treatment EBV DNA an indispensable biomarker for identifying patients who may benefit from adjuvant or salvage therapies. Furthermore, dynamic monitoring—the clearance rate or half-life of EBV DNA during treatment—provides real-time feedback on therapeutic efficacy (Lan et al., 2024).

Reconciling Heterogeneity and Geographic Variation

The reported variations in EBV positivity rates (e.g., 90.5% in endemic vs. 68.25% in non-endemic populations) and diagnostic test performance can be attributed to several factors (E. N. S. Engku Abd Rahman et al., 2022). First, **geographic and ethnic differences** in NPC histology prevalence, host genetics, and circulating EBV strain subtypes (e.g., high-risk haplotypes) fundamentally influence the disease biology (Chin, 2017; Lenoci et al., 2023). Second, **methodological heterogeneity** in assays, sample types (serum vs. plasma), and positivity cut-offs across studies directly impacts sensitivity and specificity estimates (Lee et al., 2021). Third, **study population selection**, such as inclusion of mixed histological subtypes or different disease stages, affects the observed association strength.

Clinical and Public Health Implications

The synthesis has direct translational implications:

1. **Screening:** Large cluster-randomized trials provide Level I evidence that EBV serology-based screening in endemic regions significantly reduces NPC mortality by 30%, with the greatest benefit in individuals over 50 years old (Chen et al., 2024; Ji et al., 2019). This justifies the implementation of organized screening programs in high-risk areas.
2. **Clinical Management:** Plasma EBV DNA should be integrated into standard NPC management protocols: for diagnosis in ambiguous cases, for pre-treatment risk stratification to guide treatment intensity (e.g., adding induction chemotherapy for high loads), and most critically, for post-treatment surveillance to guide early intervention (Chan et al., 2018; A. Lee et al., 2021).
3. **Treatment Guidance:** EBV DNA dynamics are predictive of response to both immunotherapy and chemotherapy. Patients with lower baseline or decreasing EBV DNA during immune checkpoint inhibitor therapy have better outcomes (Qian et al., 2023). For those with persistently detectable post-treatment DNA, adjuvant oral chemotherapy can improve survival (Chenglong Huang et al., 2023).

Mechanistic Insights and Future Directions

The review highlights promising mechanistic avenues. The consistent association of specific **LMP1 gene variants** (e.g., 30-bp deletion, XhoI loss) with NPC and poorer prognosis (e.g., increased metastasis) suggests these variants enhance the oncogenic potential of the virus (Banko et al., 2021; Zhao et al., 2011). **EBV-encoded miRNAs**, particularly from the BART region, are upregulated in NPC and contribute to carcinogenesis by targeting host tumor suppressor pathways; they also show promise as prognostic biomarkers (Abusalah et al., 2022; Wu et al., 2022). Furthermore, bioinformatic meta-analyses point to the dysregulation of host pathways like the **ubiquitin-proteasome system (UPS)** as a key driver of EBV-associated NPC progression, potentially revealing new therapeutic targets (Ratnawati et al., 2025). The identification of distinct **molecular subtypes** of EBV-related NPC (e.g., immune-active, proliferation) suggests that

tumor biology varies significantly, which could explain differential treatment responses and guide future stratified therapy (Lenoci et al., 2023).

Limitations and Conclusion

The conclusions are tempered by several limitations of the extant literature. The predominant focus on East Asian populations necessitates caution in extrapolating findings to other ethnic groups. The lack of standardization in EBV DNA quantification remains a major barrier to universal clinical adoption. Many individual studies have potential biases in control selection or follow-up. Finally, while association is clear, the precise stepwise mechanism from primary EBV infection to malignant transformation in the nasopharynx is still not fully mapped. In conclusion, the relationship between EBV and non-keratinizing NPC is one of the most robust virus-cancer associations in human oncology. EBV is not merely a passenger but a driving force in carcinogenesis. The strategic use of EBV biomarkers—from population serology to plasma DNA monitoring—offers a powerful framework for reducing the burden of NPC through prevention, early detection, and personalized, response-adapted therapy.

CONCLUSION AND RECOMMENDATIONS

Conclusion

This systematic evidence synthesis conclusively demonstrates that Epstein-Barr Virus (EBV) infection, particularly with high-risk genetic variants, is a fundamental and near-necessary causative factor in the development of non-keratinizing nasopharyngeal carcinoma (NPC), especially in endemic regions. The association is histologically specific, robust, and supported by a hierarchy of biomarkers with proven utility. Serological antibodies (e.g., VCA-IgA, EBNA1-IgA) are effective for screening, while plasma EBV DNA quantification is paramount for diagnosis, risk stratification, and most significantly, for post-treatment prognostic evaluation and monitoring of therapeutic response. EBV-based screening programs in high-risk populations have demonstrated a clear mortality benefit, affirming their public health value.

Recommendations

1. **For Public Health Policy:** Health authorities in endemic regions (e.g., Southern China, Southeast Asia) should consider implementing organized, population-based NPC screening programs utilizing EBV serology (two-marker panels like VCA-IgA/EBNA1-IgA) for adults aged 30-69, with a focus on individuals over 50 where the benefit is greatest.
2. **For Clinical Practice Guidelines:**
 - Incorporate plasma EBV DNA testing into the standard diagnostic workup for suspected NPC.
 - Mandate the measurement of pre-treatment plasma EBV DNA for prognostic stratification to guide therapeutic intensity (e.g., use of induction chemotherapy for high viral load).
 - Establish post-treatment plasma EBV DNA measurement (e.g., 6-8 weeks after therapy completion) as a standard follow-up parameter. Detectable levels should trigger intensified surveillance or consideration of adjuvant therapy.
 - Promote dynamic EBV DNA monitoring during treatment for advanced/metastatic disease to assess early response, particularly with immunotherapy.
3. **For Future Research:**
 - **Standardization:** International consortia should be formed to standardize assays, sample processing, and reporting units for plasma EBV DNA to enable global comparison and clinical application.
 - **Mechanistic Studies:** Prioritize research on the molecular pathogenesis of EBV-associated NPC, focusing on the role of high-risk viral variants (BALF2, LMP1), BART miRNAs, and virus-host interactions like the ubiquitin-proteasome system to identify novel therapeutic targets.

- **Global Validation:** Conduct large-scale studies in non-endemic and ethnically diverse populations to validate the diagnostic and prognostic algorithms developed in Asian cohorts.
- **Intervention Trials:** Develop and test intervention strategies (e.g., antiviral approaches, targeted therapies) for individuals identified as high-risk through genetic variant screening or with persistently detectable post-treatment EBV DNA.

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