Utility of Epstein-Barr virus (EBV) antibodies as screening markers for nasopharyngeal carcinoma: a narrative review

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Abstract

Background and Objective: Nasopharyngeal carcinoma (NPC) is a tumor of the head and neck that arises from the mucosal epithelium of the nasopharynx. Epstein-Barr virus (EBV) is a human herpes virus and the necessary cause for NPC. The 5-year survival rate for NPC patients is higher when diagnosed at an earlier stage of disease. Therefore, NPC screening should be prioritized for early detection. The objective of this narrative review is to synthesize the existing literature from the past decade describing evaluations of EBV-based serological markers for NPC screening.

Methods: We performed a literature search in PubMed for studies published from 2010 to 2020. Studies were required to be English-language articles. Twelve articles fulfilled all inclusion criteria, including eight studies conducted among the general population in southeastern China, three studies in genetically high-risk Taiwanese families, and one study comparing EBV serology versus circulating EBV DNA for NPC prediction.

Key Content and Findings: Studies suggest that EBV-based serology has the potential to be an effective tool to aid in early detection of NPC. The synthesized research also collectively

suggests that incorporation of antibody against multiple EBV targets, as well as efforts to optimize assay output, can improve the ability of EBV serological markers to detect NPC. Finally, recent data from the only randomized trial provide preliminary evidence that screening using anti-EBV immunoglobulin A (IgA) antibody may achieve the goal of reducing mortality from NPC.

Conclusions: Late diagnosis is one of the reasons for poor survival after an NPC diagnosis. In high-risk areas, early diagnosis aided by EBV antibody could therefore improve survival.

Keywords

Epstein-Barr virus (EBV); nasopharyngeal carcinoma (NPC); EBV serology; NPC screening

Background

Epstein-Barr virus (EBV) is a necessary cause of undifferentiated, non-keratinizing nasopharyngeal carcinoma (NPC) (1–3). Although EBV is generally acquired during early childhood, the virus remains latent in host B-cells for the duration of adulthood in >90% of individuals globally and can lead to epithelial and lymphoid tumors in a subset of infected persons (4–6). In the absence of vaccines to prevent long-term EBV infection and associated disease, efforts to identify viral biomarkers to aid in the identification of individuals most likely to develop NPC, including differential immune response to EBV, could facilitate detection at stages when treatment is most effective. NPC is highly treatable when diagnosed early, with 5-year disease-free survival of approximately 90%. This contrasts with 5-year survival rates of less than 50% for NPC diagnosed at later stages (7).

Historically, the majority of such screening biomarker research has focused on examining how circulating antibodies against EBV proteins perform as prospective metrics of NPC risk. This approach is based on the premise that increased immune response to EBV is evidence of poor viral control and thereby susceptibility to disease. This is particularly true for immunoglobulin A (IgA) antibody produced after exposure to virus in the oral cavity where EBV is transmitted and periodically reactivates. A pivotal study conducted among >9,500 men from Taiwan convincingly demonstrate that higher antibody levels, including IgA against the EBV viral capsid antigen (VCA), preceded the development of NPC (8). Specifically, men who tested positive for VCA IgA antibody at baseline were approximately 22-fold more likely to develop NPC during follow-up (8). Although the underlying principle of EBV-based biomarkers as a useful NPC risk stratification tool may be accepted, the details of which marker combinations are optimal has been the subject of much research in the past decade, which will be reviewed here. The objective of this narrative review is to summarize published findings from the past decade that represent evaluations of the utility of EBV-based serology (i.e., anti-EBV antibodies) for NPC screening. We present the following article in accordance with the Narrative Review reporting checklist (available at https://anpc.amegroups.com/article/view/10.21037/anpc-21-12/rc).

Methods

To identify articles for this review, the following Medical Subject Headings (MeSH) terms were entered into PubMed: ["Epstein-Barr Virus Infections", "Herpesvirus 4, Human",

"Nasopharyngeal Carcinoma" and "((Epstein-Barr Virus Infections) OR (Herpesvirus 4, Human)) AND (Nasopharyngeal Carcinoma)"] between June 21, 2021 and November 5, 2021. Criteria for inclusion were as follows: the study must (I) be published in the last decade (2010 to 2020); (II) be an English-language article; (III) include measurement of at least one IgG or IgA anti-EBV antibody, referred to here often as EBV serological markers; and (IV) the study had to be prospective in nature, with EBV serological markers measured prior to NPC diagnosis. This final requirement eliminated many studies focused on use of EBV serology testing to aid in NPC diagnosis or as a tool for monitoring NPC prognosis. However, this requirement was pivotal to ensure that the summarized data were relevant to the overarching objective of providing data to inform NPC screening and early detection. Altered EBV biomarkers must be present for an extended period prior to NPC diagnosis to be viable tools for population-based cancer screening of adults without disease. Exclusion criteria included studies that were: (I) not published in English; (II) study without measurement of at least one EBV serological marker; and (III) not a prospective study. Initial review was completed by SS, and articles were then independently reviewed by AEC. The final publication list was augmented by a review of the senior author's files to ensure that all relevant articles were included.

We identified twelve articles that met the above criteria for this narrative review. This included eight reports assessing the potential utility of EBV serology for NPC prediction in Guangzhou Province of southeastern China. This region has reported NPC rates (~50 cases per 100,000 individuals) that are up to 50 times higher than what is observed in the United States. We further identified three studies conducted among Taiwanese multiplex family members, defined as members of families with at least 2 first- or second-degree relatives affected by NPC. Such families have documented NPC rates as high as ~100 cases per 100,000 individuals. Finally, we discuss one study conducted in Singapore that provided a comparison of EBV serology versus circulating EBV DNA for NPC prediction. For each study, the following variables were extracted: author, study year and population, the number of NPC cases identified, a summary of the study objectives, and EBV serological markers evaluated. Given that many studies were conducted in the same high-risk region of southeastern China, effort is made to highlight unique contributions of each study to understanding EBV serology as an NPC screening tool. The search stratergy is summarized in Table 1.

Results

EBV serology for population-based NPC screening in China

Historically, evaluations of anti-EBV antibody, including IgA against the EBV VCA and early antigen (EA) proteins, have demonstrated strong positive associations with NPC onset in Guangdong Province in southeastern China (8,9). As noted above, southeastern China has one of the highest general population NPC rates globally. Findings from the eight studies we identified from Sihui County and Zhongshan City of Guangdong Province, southeastern China, are summarized in Table 2.

Sihui County—The first study reports on 20 years of follow-up data from a large, population-based study in four towns of Sihui County that originally recruited 18,986 individuals ages 30–59 years between 1987 and 1992 (10). The goal of this effort was to improve the early detection of NPC using VCA and EA IgA as blood-based screening tests. After >300,000 person-years of follow-up, 125 cases of newly diagnosed NPC were identified. NPC onset differed according to EBV serological status. The age-adjusted NPC incidence rate was 29.4 among subjects seronegative for VCA IgA, compared to 188.2 among those seropositive for VCA IgA but seronegative for EA IgA, and 617.4 among those seropositive for both EBV serological markers. This was a dose-dependent association for VCA IgA, with hazard ratios ranging from 6.7 to 41.9 based on increasing antibody titers measured at baseline. Among 962 individuals seropositive for VCA IgA with 3 blood collections during follow-up, 129 individuals were classified as ascending because antibody titers increased over time, 426 as stable, and the remainder as descending because antibody titers decreased. Corresponding hazard ratios for NPC risk in the first 5 years of follow-up were 21.3 (95% CI: 7.1–64.1), 6.2 (95% CI: 2.2–17.8), and 1.5 (not statistically significant), respectively.

In the same Sihui population, investigators later addressed the important question of how many NPC cases may be missed in a screening program based on EBV serological markers (11). The authors evaluated this by examining NPC detection rates in 17,106 persons who tested negative for VCA IgA at baseline. Different screening intervals (i.e., time between anti-EBV antibody testing) were employed, with two cities screened every 4–5 years classified as 'short interval' and two screened every 9–10 years as 'long interval'. Any NPC diagnosed 6 months after a screening visit and before re-screening was defined as an interval cancer (i.e., NPC case missed by screening). Seven interval cancers were diagnosed in the short interval group, (detection rate =0.07%). In contrast, 20 interval cancers were diagnosed (i.e., 20 NPC cases missed) in the long interval group (detection rate =0.28%). The authors posited that anti-EBV antibody is therefore best at detecting NPC during a shorter time frame (4–5 years).

Zhongshan City—Data are also available from a population-based study of 42,048 individuals ages 30–59 years originally recruited from Zhongshan City between 1986 and 1988 and followed 16 years, during which 171 NPC cases (51 Stage I and 120 Stages II–IV) were identified (12). The VCA IgA assay employed in this population used immunofluorescence (IF) technology to group individuals into seropositive and seronegative groups at baseline. VCA IgA seropositivity was associated with an NPC case detection rate (sensitivity) of 56%, although this was dependent upon time between antibody measurement and NPC detection. For cancers detected in the first 2 years of follow-up, VCA IgA was seropositive at baseline in 95%, compared to only being positive in 34% of NPC cases detected in the subsequent 16 years of follow-up.

Cluster-randomized clinical trial—The last section of Table 2 warrants special attention because it represents a cluster-randomized trial designed to formally evaluate whether a population-based VCA and EBV nuclear antigen (EBNA1) IgA screening program can effectively decrease NPC mortality (14). This mass screening trial was launched in 8

towns from Sihui (7 towns) and Zhongshan (1 town) and enrolled 28,688 individuals ages 30–59 years during the initial round of EBV serology screening between 2008 and 2010. Participants were offered fiberoptic endoscopy and/or biopsy if EBV-based serological testing reached a pre-defined threshold for high risk. The primary endpoint of this trial is whether residence in a screening town results in a lower NPC mortality rate compared to non-screening areas. However, multiple interim analyses of the link between EBV serology and NPC onset have been described. The first trial description published in 2012 noted that 41 NPC cases were detected during the first year of follow-up after initial screening, yielding an overall diagnosis rate of 0.14%. Illustrating the strong link between anti-EBV IgA antibody and future NPC risk, diagnosis rates were 0.008%, 0.05%, and 4.4% in the low-risk, medium-risk, and high-risk groups IgA groups, respectively.

Follow-up work conducted in subsets of the clinical trial participants examined distinct aspects of refining anti-EBV antibody algorithms, with the goal of maximizing NPC detection (i.e., increasing sensitivity) without falsely classifying individuals as high risk if they did not develop NPC (i.e., decreasing false positivity). The first study (N=5,481) conducted a head-to-head comparison of older VCA and EA IgA IF technology to more contemporaneous enzyme-linked immunosorbent assays (ELISAs) for those same target antigens (13). The ELISAs used in the referenced study, like many ELISAs, were commercialized (e.g., VCA IgA: EUROIMMUN AG, Germany) (18). The ability of a commercial lab to create a product that assays the target antibody in a sample consistently, with published standards and expected variation, is a major strength for scaling up to population-based cancer screening. In contrast, IF assays use a fluorescent tag for the target antibody, and difficulties in interpreting the intensity of the fluorescent output leads to difficulties in standardization and automation. Whereas sensitivity (case detection rate) and specificity (1-false positive rate) of early NPC detection for the IF method were 77.3% and 94.3.6% for VCA IgA, the corresponding test parameters were 91.6% and 82.7% for the ELISA IgA assays in those same participants. Importantly, the area under the ROC curve (AUC) that reflects overall predictive accuracy improved from 0.88 (95% CI: 0.85–0.91) with IF to 0.94 (95% CI: 0.92–0.97) with ELISA, demonstrating the potential of this more contemporaneous approach.

The second study (N=4,200) incorporated antibody responses to additional EBV proteins, Zebra (Zta) and thymidine kinase (TK) (15). Anti-TK IgG antibody was determined to be the most effective complementary marker for more traditional markers such as anti-EA IgA antibody. The overall sensitivity of the updated antibody algorithm including anti-TK and anti-EA IgA was 83.3%. Although thisdid not differ from the original screening approach, the specificity did significantly increase from 97% to 99.5%, again representing an opportunity to reduce false positive detection rates. A more recent effort utilized a larger number of participants (N=16,712) to illustrate that the choice of an alternate, optimized cutoff value for VCA and EBNA1 IgA ELISA output could improve NPC prediction (16). Ultimately, these interim analyses within the larger cluster-randomized trial scheme all point to the potential to improve NPC detection with incorporation of a broader spectrum of EBV targets and optimized interpretation of standard assay output.

The last study reports the first set of NPC mortality data from the cluster-randomized clinical trial, detailing the early case detection and mortality data for three towns in Zhongshan City, one of which was randomized to screening and two of which were randomized to the control arm (17). The early diagnosis rate for NPC was statistically significantly higher in the screening (45.9%) compared to the control (20.6%) arm. The all-cause mortality rates in the screening and control arms were 339.2 and 418.5 per 100,000, respectively. The intent-to-treat risk ratio did not demonstrate any difference in NPC-specific mortality between the trial arms (RR: 0.82; 95% CI: 0.37–1.79), whereas the risk ratio comparing NPC-specific mortality in the control arm to individuals who actually received at least one EBV serological screening was 0.22 (95% CI: 0.09–0.49), evidence of potential efficacy that warrants evaluation after longer follow-up in this high-risk region.

EBV serology for NPC screening in genetically high-risk Taiwanese families

Findings from studies of members of NPC multiplex families (i.e., families with at least 2 first- or second-degree relatives affected by NPC) are summarized in Table 3. The findings were generated from the Taiwan Family Study (TFS), a cohort of ~2,500 high-risk individuals recruited from 358 multiplex families in Taiwan starting in 1996 (22). The first study reviewed from this cohort evaluated IgA antibody titers against VCA and EBNA1 in relatives of multiplex NPC cases who were cancer-free at baseline (19). A total of 15,519 person-years of follow-up yielded 14 incident NPC cases. Individuals positive for anti-EBV EBNA1 IgA had nearly 5 times the rate of NPC compared to those who tested negative (risk ratio: 4.7; 95% CI: 1.4–16). In a subsequent analysis from the same population that identified 21 NPC cases after longer follow-up, the authors compared the baseline EBV serological profile in the 21 NPC cases to that of 84 multiplex family members who did not develop NPC during follow-up (20). Because earlier findings from this population were based on research-grade assays that may not be reproducible, the second study utilized chemically defined, peptide based ELISAs for IgA against VCA, EBNA1, and EA. The optimal threshold for a given IgA marker was defined as the value that successfully identified at least 80% of incident NPC cases. In this population, the threshold chosen for EBNA1 IgA resulted in the highest specificity (58%) while still identifying at least 80% of individuals who developed NPC.

The most recent publication from this genetically high-risk population evaluated antibody against 199 peptide sequences from 86 EBV proteins, a pronounced expansion beyond the conventional set of VCA, EBNA, and EA IgA (21). As those three antibodies represent a very small fraction of the immune response against EBV's nearly 100 proteins, targeting additional EBV proteins to improve risk stratification is an important effort. The study utilized a two-step approach to compare both IgG and IgA antibody against the 199-marker anti-EBV peptide panel between prevalent NPC cases and cancer-free controls. This identified which EBV serological markers were most likely to differ by NPC diagnosis. This filtering step resulted in a risk stratification signature that included VCA and EBNA IgA antibody as well as 12 additional anti-EBV antibodies: BXLF1 (IgG and IgA), LF2 (IgG and IgA), BRLF1 IgA, BZLF1 IgG, BGLF2 IgG, BPLF1 IgA, BFRF1 IgG, BORF1 IgG, and 2 distinct BMRF1 IgA. Including the 12 array-identified antibodies improved NPC prediction. The 14-antibody risk score predicted NPC onset with 89% accuracy (AUC =88.7%; 95%

CI: 82.0–95.5%), a ~10% improvement compared to VCA and EBNA1 IgA alone (AUC =78.1%; 95% CI: 66.2–89.9%).

EBV DNA versus serology for NPC prediction

Recently, circulating cell-free EBV DNA measured in the serum or plasma has been shown to be elevated in NPC patients at the time of their cancer diagnosis, raising the possibility that EBV DNA could be a viable tool to improve NPC screening (23). This would represent a direct measurement of EBV itself, rather than antibodies marking the adaptive immune system's response to EBV exposure. Between 2004 and 2013, a group in Singapore recruited a cohort of 524 adult first-degree family members of NPC patients (24). The mean duration of follow-up was 58 months. Baseline and annual blood investigations were performed for VCA and EA IgA, as well as serum cell-free EBV DNA. Nasopharyngeal biopsies were conducted if any of the following criteria were met: clinical suspicion of NPC or observation of any nasopharynx abnormality during regular evaluations, EBV serology titers elevated (VCA 1:160 or EBV-EA IgA 1:10), or EBV DNA 1,000 copies/mL.

In this cohort, five NPC cases were identified during the one decade of follow-up, four of which were diagnosed at early stages. EBV serology titers were consistently elevated in all five NPC cases identified and preceded the NPC diagnosis in three cases. In contrast, serum EBV DNA was not elevated. No EBV DNA threshold that could reliably identify high-risk family members with NPC onset was found. Furthermore, eight family members with EBV DNA >1,000 copies/mL during screening, the level that triggered subsequent biopsy, were all determined to be negative for NPC, pointing to a potentially high rate of false positivity. Despite a limited sample size of 5 NPC cases, these data from multiplex family members provide preliminary evidence for the continued utility of EBV serology. It is feasible that different parameterizations of circulating EBV DNA (e.g., fractionation size) may improve prospective NPC identification (25).

Additional considerations for NPC screening

A crucial step in the practical evaluation of any EBV biomarker-based NPC screening program is computation of two important metrics: (I) the estimated number of individuals that will need to be screened for each case of NPC that is successfully detected [1/ (sensitivity × 5-year NPC risk)] and (II) the number of individuals who will test positive, which may require further clinical intervention, per detected NPC [1/(positive predictive value)]. One of the studies conducted among multiplex family members from Taiwan nicely illustrates use of these metrics (20). Optimizing interpretation of EBNA1 IgA antibody output in this population improved the sensitivity (i.e., case detection rate) from 80% to 90%. This decreased the number of individuals needing to be screened to detect a case of NPC from 164 to 146 individuals. However, increasing sensitivity was accompanied by a decrease in specificity from 58% to 40% (i.e., elevated false positive rate). This increased the number of individuals testing positive per NPC case from 69 to 88. The higher number of positive tests may be problematic if a positive test triggers an invasive or costly clinical follow-up.

The underlying incidence of cancer also has a large impact. In that same article, it was illustrated that 164 individuals needed to be screened to detect a case of NPC for a population with an NPC incidence rate of 100 per 100,000, which markedly differed from the 1,250 persons that would need to be screened to detect a case of NPC for a population with an incidence of 10 per 100,000. This type of practical analysis is strongly recommended when evaluating the implementation of screening biomarkers on a population-level.

In addition to careful selection of the target population, researchers must be able to clearly articulate which EBV peptides are targeted by their serological assay. For example, the VCA protein of EBV includes multiple peptide sequences that comprise the viral capsid; each of these sequences could serve as reasonable antibody targets. However, the human immune system may not produce antibody to these distinct sequences at the same efficiency, or assays designed against varying sequences could have different binding efficiencies. This was nicely illustrated by Liu and colleagues in their testing of standardized blood samples from the same set of individuals for 26 EBV serological assays, including six distinct VCA IgA assays (26). The optimal outcome would be to get similar output for all VCA IgA assays measured on the same set of samples. However, the authors observed two clusters of antibody response, with a median Spearman coefficient of only 0.41 (95% CI: 0.20–0.66). It is therefore strongly recommended that reports of the potential utility of EBV serological assays for NPC screening include a reporting of the exact peptide sequences of the assays evaluated as part of their study.

Conclusions

This article series is dedicated to emerging issues in NPC epidemiology and clinical care. This specific review summarizes the role of EBV-directed serological markers in predicting NPC. One of the noted features of current NPC diagnoses in high-risk regions is the generally late stage at diagnosis, which translates into poor survival. Effective early detection, a possibility posited by many of the articles cited here, could shift this diagnosis trend to earlier stages and thereby improve survival. The studies reviewed here suggest that this could be facilitated through reproducible ELISA assays in both average-risk and genetically high-risk persons and that incorporation of multiple EBV targets could further improve prediction. Finally, recent data from the only randomized trial provide preliminary evidence that screening using anti-EBV IgA antibody may achieve the goal of reducing patient mortality from NPC.

Funding:

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Table 1

The search strategy summary

Items	Specification
Date of search (specified to date, month and year)	June 21, 2021 to November 5, 2021
Databases and other sources searched	PubMed
Search terms used (including MeSH and free text search terms and filters)	Search terms: "Epstein-Barr Virus Infections", "Herpesvirus 4, Human", "Nasopharyngeal Carcinoma" Search strategy of PubMed: ("Epstein-Barr Virus Infections[MeSH terms]" OR "Herpesvirus 4, Human[MeSH terms]" OR "Nasopharyngeal Carcinoma[MeSH terms]") AND ((Epstein-Barr Virus Infections) OR (Herpesvirus 4, Human)) AND (Nasopharyngeal Carcinoma)
Timeframe	2010 to 2020
Inclusion and exclusion criteria (study type, language restrictions, etc.)	Inclusion criteria (I) Published in the last decade (2010 to 2020) (II) English-language article (III) Measurement of at least one IgG or IgA anti-EBV antibody, referred to here often as EBV serological markers (IV) Prospective study, with EBV serological markers measured prior to NPC diagnosis Exclusion criteria (I) Study was written in non-English language (II) Study without measurement of at least one EBV serological marker (III) Not a prospective study
Selection process (who conducted the selection, whether it was conducted independently, how consensus was obtained, etc.)	Initial review was completed by SS, and articles were then independently reviewed by AEC. The final publication list was augmented by a review of the senior author's files to ensure that all relevant articles were included

IgG, immunoglobulin G; IgA, immunoglobulin A; EBV, Epstein-Barr virus; NPC, nasopharyngeal carcinoma.

Table 2

EBV serology for population-based nasopharyngeal carcinoma screening in SE China

Author	Year	Population	NPC cases	Study objective	EBV antibody	Key finding to highlight
Sihui County						
Su-Mei Cao *et al.* (10)	2011	18,986	125 cases	Investigate dose-response association between EBV and NPC	VCA IgA and EA IgA	For follow-up <5 years, HRs were 6.7 (95% CI: 2.7 to 16.3), 9.4 (95% CI: 4.0 to 21.9), 22.5 (95% CI: 8.6 to 59.1) and 41.9 (95% CI: 16.0 to 110.2) for subjects with VCA/IgA antibody titers 1:5, 1:10, 1:20 and 1:40 on IF testing, respectively
Feng Chen *et al.* (11)	2012	17,106	27 cases	Describe NPC that occurs in those with a negative VCA IgA antibody test	VCA IgA	Seven interval cancers were diagnosed in the short interval (4- or 5-year follow-up) centers, none of which were aggressive. Twenty interval cancers were diagnosed in the long interval (9- or 10-year follow-up) centers, including four aggressive cancers
Zhongshan City						
Ming-Fang Ji *et al.* (12)	2011	42,048	171 cases	Determine if EBV serology is associated with NPC onset	VCA IgA	VCA IgA predicted 56% of 171 NPC cases detected over 16 years (sensitivity =56%). Higher sensitivity for detecting Stage I tumors was observed in the first 2 years after EBV screening (95%) compared to years 2–16 after screening (34%)
Cluster-randomized clinical trial in Guangzhou						
Yue Liu *et al.* (13)	2012	5,481	8 cases	Compare performance of IF versus ELISA for assessing EBV serology to predict NPC	VCA IgA and EA IgA	VCA IgA AUC improved from 0.88 (95% CI: 0.85–0.91) with IF to 0.94 (95% CI: 0.92–0.97) with ELISA. The corresponding metrics for EA IgA were 0.66 (95% CI: 0.63–0.69) for IF and 0.83 (95% CI: 0.79–0.87) for ELISA
Zhiwei Liu *et al.* (14)	2012	28,688	41 cases	Determine if EBV serology is associated with NPC onset	VCA IgA and EBNA1 IgA	41 NPC cases were detected during the first year after initial screening. NPC diagnosis rates were 0.008% (2/25,642), 0.05% (1/2,184), and 4.4% (38/862) in the low-risk, medium-risk, and high-risk EBV serology groups, respectively
Tingdong Li *et al.* (15)	2018	4,200	46 cases	Evaluate impact of incorporating additional anti-EBV antibodies for predicting NPC	VCA IgA, EBNA1 IgA, EA IgA, and TK IgG	Combining TK and EA(D) antibody markers achieved sensitivity for NPC detection of 97.83% and 93.48% for TK/IgA + EAD/IgA, and EAD/IgA + TK/IgG, respectively. Corresponding specificity was 48.67% and 85.93%, with specificity of EAD/IgA + TK/IgG being significantly better (P<0.001)
Xia Yu *et al.* (16)	2018	16,712	47 cases	Optimize interpretation of ELISA-based EBV serology for predicting NPC onset	VCA IgA and EBNA1 IgA	The EBV seromarker combination with the highest AUC was an optimized version of both VCA and EBNA1 IgA positivity (AUC: 0.93, 95% CI: 0.89–0.97), versus either VCA or EBNA1 IgA (AUC: 0.88, 95% CI: 0.82–0.94) or EBNA1 IgA alone (AUC: 0.87, 95% CI: 0.79–0.94)
Mingfang Ji *et al.* (17)	2019	524	45 deaths	Present interim analyses linking EBV serology screening to NPC mortality	VCA IgA and EBNA1 IgA	The adjusted RRs of dying from NPC were 0.82 (95% CI: 0.37–1.79) in the screening group and 0.22 (95% CI: 0.09–0.49) in the participants who actually received at least one EBV screening test, respectively, compared with the control arm

EBV, Epstein-Barr virus; SE, southeastern; NPC, nasopharyngeal carcinoma; VCA, viral capsid antigen; IgA, immunoglobulin A; AUC, area under the ROC curve; EA, early antigen; EBNA1, EBV nuclear antigen 1; IF, immunofluorescence; ELISA, enzyme-linked immunosorbent assay; TK, thymidine kinase; IgG, immunoglobulin G.

Table 3

EBV serology for nasopharyngeal carcinoma screening the Taiwan Family Study

Author	Year	Population	NPC case	Study objective	EBV antibody	Key finding to highlight
Kelly J. Yu et al. (19)	2011	2,444	14 cases	Determine if EBV serology is associated with NPC onset in multiplex family members	VCA IgA and EBNA1 IgA	High-risk family members positive for EBNA1 IgA had nearly 5 times the rate of NPC (265 per 100,000) compared with individuals who tested negative (56 per 100,000; RR =4.7; 95% CI: 1.4–16)
Anna E. Coghill et al. (20)	2014	2,557	21 cases	Investigate the association of a standardized ELISA anti-EBV antibody panel with NPC	VCA IgA, EBNA1 IgA, EA IgA	The optimized threshold for EBNA1 IgA (cutoff =0.72) had specificity of 58% and 80% sensitivity. The alternative threshold (cutoff =0.61) had higher (90%) sensitivity but lower (40%) specificity
Anna E. Coghill et al. (21)	2018	2,557	26 cases	Evaluate the utility of an anti-EBV peptide microarray to improve prediction of NPC	BXLF1 IgG/A, LF2 IgG/ A, BRLF1 IgA, BZLF1 IgG, BGLF2 IgG, BPLF1 IgA, BFRF1 IgG, BORF1 IgG, EA IgA, VCA IgA, and EBNA1 IgA	The 14-antibody panel that included 12 markers from the EBV microarray predicted NPC with 89% accuracy (95% CI: 82–96%), a significant improvement (P<0.01) compared with VCA + EBNA1 IgA alone (AUC =78%; 95% CI: 66–90%)

EBV, Epstein-Barr virus; NPC, nasopharyngeal carcinoma; VCA, viral capsid antigen; IgA, immunoglobulin A; EBNA1, EBV nuclear antigen 1; ELISA, enzyme-linked immunosorbent assay; EA, early antigen; IgG, immunoglobulin G; AUC, area under the ROC curve.