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Prognostic Utility of Anti-EBV Antibody Testing for Defining NPC Risk among Individuals from High-Risk NPC Families

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Abstract

Purpose—Epstein–Barr virus (EBV) infection and a family history of nasopharyngeal carcinoma (NPC) are associated with NPC risk. We examined the risk associated with EBV markers and their clinical utility to identify NPC susceptibles within high-risk NPC families.

Experimental Design—We evaluated antibody titers against viral capsid antigen (VCA) IgA, EBV nuclear antigen-1 (EBNA1) IgA, and DNase among unaffected relatives of NPC cases from 358 multiplex families in Taiwan. Incident NPC cases were identified via linkage to the National Cancer Registry. Clinical examinations of 924 individuals were also done to identify occult, asymptomatic NPC. Baseline EBV serology was used to estimate NPC risk using rate ratios with 95% CI. Associated sensitivity/specificity and receiver operating characteristic (ROC) curves were calculated.

Results—A total of 2,444 unaffected individuals with 15,519 person-years (6.5 years median follow-up) yielded 14 incident NPC cases (nearly 11 times the general population rate). The absolute rate of NPC among anti-EBV EBNA1 IgA seropositives using a standard positivity cutoff versus an optimized cutoff point defined by ROC analyses was 265/100,000 person-years with a 4.7-fold increased risk of NPC (95% CI: 1.4–16) and 166/100,000 person-years with a 6.6-fold increase (95% CI: 1.5–61), respectively. Sensitivity and specificity using the optimized positivity cutoff points were 85.7% and 51.2%, respectively. It is estimated that active evaluation of 49% of individuals from high-risk NPC families seropositive for this marker could lead to earlier detection of up to 86% of NPC cases. Risks associated with the other three EBV markers were weaker.

Conclusions—Future efforts are needed to identify susceptibility markers among high-risk NPC families that maximize both sensitivity and specificity.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Introduction

Epstein–Barr virus (EBV) is strongly associated with the development of nasopharyngeal carcinoma (NPC). EBV is ubiquitous worldwide, with more than 90% infected and usually with lifelong persistence (1, 2). Most EBV infections are asymptomatic, but the virus can be associated with rare malignant transformations in lymphoid cells or epithelial tissue including NPC (3).

It is known that the vast majority of NPC cases present alterations in levels of antibodies directed against various EBV antigens (4–7). Numerous studies have shown both diagnostic and prognostic utility of EBV antibody testing for EBV-associated malignancies (4–7), in particular IgA antibodies indicating lytic viral replication. Studies in China and Taiwan have observed, for example, that individuals with detectable levels of anti-EBV VCA IgA and EBV DNase are at an elevated risk of developing NPC in subsequent years (8, 9). In one large prospective study from Taiwan, serum was tested for 2 anti-EBV antibodies (VCA IgA and anti-DNase) known to be associated with elevated risk of prevalent NPC in general population studies (8). Chien and colleagues found individuals that tested positive for 1 of the 2 markers examined (VCA IgA or anti-DNase) had a prospective relative risk of 4.0 compared with seronegative individuals, and those positive for both markers had a relative risk of 33 in the 16 years follow-up period (8).

Although studies have indicated that EBV serologic profile is predictive of sporadic NPC risk, less is known about the utility of these same markers to predict risk and thereby be used as a clinical screening tool among individuals from families with a predisposition for NPC development. In a previously published paper from our group, Pickard and colleagues showed that healthy individuals from high-risk NPC multiplex families have elevated levels of anti-EBV VCA IgA, anti-EBV EBNA1 IgA, and anti-EBV DNase Ig compared with healthy individuals from the general population (10). In that study, nearly 50% of unaffected members of high-risk families were found to be positive for 1 or more of the 3 EBV markers evaluated compared with only 18% of the population controls. Although it is tempting to speculate based on these findings that individuals from high-risk NPC families with demonstrable elevations in anti-EBV antibody levels are at increased risk of NPC and that anti-EBV antibody levels can be used to screen for NPC in these families, it is also possible that elevations observed reflect shared environmental exposure to EBV that is unrelated to disease risk.

To formally evaluate whether EBV serologic responses measured among healthy individuals from high-risk multiplex NPC families are predictive of subsequent NPC risk and whether EBV serology can be used clinically as a screening tool, we report herein the results of our efforts to follow individuals from our high-risk multiplex families in Taiwan with and without altered EBV serologic profiles for incident NPC.

Materials and Methods

Study population

Participants in the present study were selected from among those included in an ongoing NPC multiplex family study in Taiwan. Details of this parent study have been described elsewhere (10–12). In brief, 358 NPC multiplex families were identified and recruited into our study. From these families, a total of 659 NPC cases (404 NPC cases alive at time of recruitment) and 2,557 unaffected family members were enrolled. Unaffected family members included parents of NPC cases, up to 5 siblings, and for deceased NPC cases, their spouse and up to 3 children. When necessary to link affected individuals in our families, additional relatives were recruited.

Families were administered a questionnaire by a trained nurse interviewer to obtain complete family history information. As part of a questionnaire that was self-administered to participants or proxies for deceased individuals, age, gender, date of birth, name, and national identification number were also obtained. Informed consent was obtained from live participants or from their relatives in the case of deceased individuals. Institutional Review Boards at both the NIH and the National Taiwan University approved the study protocol and informed consent.

For this follow-up effort, we sought to determine NPC status for all 2,557 unaffected family members from the 358 NPC multiplex families included in the parent study. Both passive (linkage to the national tumor registry) and active (clinical evaluation) methods were used to identify incident NPC diagnosed since original recruitment into the parent study. Each is described, in turn, in the following text.

Record linkage

We used the national identification number to link participants in our study to the National Cancer Registry. For those subjects without a reported national identification number, we used their sex and date of birth to link. Linkage was done for each unaffected subject to identify incident cancers diagnosed after enrollment into our study (which started in 1996) through 2005. Using this information, we were able to link more than 90% of our subjects to the registry. Additional linkage to the National Death Registries in Taiwan was done to confirm that all cancer diagnoses were identified via linkage to the tumor registry. There were no additional cases identified through linkage to the National Death Registry.

Active follow-up and clinical evaluation

Eligible participants were recontacted by invitation letter and then by phone to describe the follow-up study and to invite them to participate by making an appointment to visit one of the 4 study ENT (ear, nose, and throat) clinics for a clinical evaluation. A total of 2,461 individuals were recontacted, of which 949 (38.6%) agreed to visit one of our study clinics, 90 (3.7%) were found to be deceased, 247 (10%) refused, 13 (0.5%) were too ill, 1 (0.04%) was not mentally competent, and the remainder were lost to follow-up or were still pending response at the time our study ended.

Clinical evaluation was done by 1 of 2 otolaryngology specialists on clinic days specifically set up for the study. The clinical evaluation consisted of an examination of the ears to check for the presence of middle ear effusion; an examination of the nose to check for the presence of any tumorous lesion in the nasal cavity; palpation of the neck to check for the presence of any pathologic lymph nodes; and an examination of the nasopharynx by a fiberscope. Biopsy specimens were collected, when needed, for histologic diagnoses of suspected NPC.

In addition, NPC diagnoses made between the time of initial participation in the parent study and participation in this follow-up phase were ascertained through a questionnaire administered during this follow-up study. In total, there were 21 biopsies done out of the 924 screened subjects of which 1 was histologically confirmed as NPC, 13 were reactive lymphoid hyperplasia, 4 were chronic inflammation, 1 was keratinous cyst, 1 was neurilemmoma, and 1 was insufficient for diagnosis. No additional incident NPC cases were identified based on the self-reported questionnaire.

EBV biomarkers

Plasma obtained from participants during the initial (baseline) phase of our study was tested for EBV markers of interest—viral capsid antigen (VCA) IgA by the immunofluorescent (IF) test, EBV nuclear antigen-1 (EBNA1) IgA, and EBV anti-DNAse antibodies as

previously described (10, 13). The reproducibility of these markers were also evaluated and reported previously (10). In addition, we included a commercially available VCA IgA by the ELISA test as a fourth assay following manufacture protocol using 1 dilution factor of 1:101 [ELISA kit Cat-No RE56271; Immuno-Biological Laboratories (IBL)].

A positive test for anti-VCA IgA was defined as optical density at 405 nm (OD_{405}) > 0.50. Initial cutoff points used to establish positive versus negative EBV antibody titers for each of the individual markers were based on cutoffs previously defined in the literature based on studies of sporadic NPC (10). For the present evaluation, we included the 2,444 unaffected individuals with at least one EBV biomarker result available. A total of 113 (4.4%) unaffected individuals with missing EBV results were excluded. None of the excluded individuals developed NPC during follow-up.

Statistical analyses

We estimated the risk of NPC based on baseline EBV serologic results by calculating the rate ratio (RR) with 95% CI of developing NPC in those individuals who were unaffected in the original family study. Multiple RR estimates were calculated as follows: (i) including all subjects regardless of the time of NPC diagnosis, (ii) excluding those with NPC diagnosis made within 1 year after their initial enrollment into the original family study to account for the possible missed prevalent NPC cases, and (iii) restricting to those NPC cases diagnosed within 5 years of study recruitment. We also evaluated the risk based on the number of EBV biomarker positivity, using the following categories: none, 1, 2 or more, and none versus any. Adjusting for age-standardized rates did not affect the estimates and therefore, we chose to present the crude rate ratios for ease of comparison to the absolute rates. Age-standardized RRs are provided in the Supplementary Tables 1 and 2. All statistical tests were 2-sided.

Positivity was initially defined using cutoff points based on studies of sporadic NPC (as defined previously). Receiver operating characteristic (ROC) curves were drawn to assess the appropriateness of these cutoff points for screening of individuals from high risk families. When combination ROC curves were drawn, individuals missing any of the 4 EBV biomarkers of interest were deleted from analysis. Results for individual EBV biomarkers are presented, including all individuals for whom results were available. Analysis restricted to individuals with data for the complete EBV marker set resulted in comparable findings (data not shown). The Youden index statistic was calculated, conditional on a sensitivity of 80% or more to account for the requirement for high sensitivity of a test used to screen high-risk subpopulations such as ours. Results from the ROC evaluation were used to redefine cutoff points for individual assays and the RRs were reestimated using these new cutoff points.

Results

For the present evaluation, 2,444 unaffected individuals from the NPC Multiplex Family Study in Taiwan contributed a median follow-up time of 6.5 years (range: 0.36–11 years). A total of 15,519 person-years were accrued, within which 14 incident NPC cases were diagnosed. Thirteen NPC cases were identified via linkage to the Taiwan cancer registry; 1 additional case was identified through clinical examination in our follow-up effort. Table 1 shows the time between recruitment and development of NPC, sex, age at NPC diagnoses, TNM staging, and WHO type for each of the 14 incident NPC cases. As shown, cases with earlier stage diagnoses had overall shorter follow-up period and were younger than later stage diagnoses (stages 1–2: median follow-up = 3 and median age at diagnoses = 47 years vs. stages 3–4: median follow-up = 6 and median age at diagnoses = 59 years). In addition, there was no distinct pattern in EBV seropositivity and staging (data not shown). The rate of

NPC observed in our study was 90 per 100,000 person-years, nearly 11 times higher than general population rates for comparably aged individuals in Taiwan (8.4 per 100,000; Table 2). When we excluded subjects with NPC diagnosed within 1 year of study recruitment ($n = 2$), the incidence was 77 per 100,000 person-years.

The RRs of NPC are presented in Table 2, stratified by each of the 4 EBV seromarkers evaluated and combinations of markers. Standard cutoff points reported in the literature (based on studies of sporadic NPC) were used to define positivity for each of the markers. We observed that anti-EBV EBNA1 IgA was the marker with the strongest association with NPC; individuals positive for anti-EBV 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]. Rates for the other 3 markers were also elevated [anti-EBV VCA IgA (immunofluorescent) = 110.6/100,000; RR = 1.6 (0.34–6.3); anti-EBV VCA IgA (ELISA) = 175/100,000; RR = 2.0 (0.046–13); anti-EBV DNase = 168.2/100,000; RR = 2.2 (0.39–8.5)], although none reached significant levels. Positivity to any biomarker versus none yielded a RR of 2.3 (95% CI: 0.71–8.9). When risk stratification was examined by number of markers positive, a dose response was observed, with individuals who tested positive for a single marker having an RR = 1.7 (95% CI: 0.39–7.4) and those who tested positive for 2+ markers having an RR = 4.4 (95% CI: 0.87–20) compared with individuals negative for all markers ($P_{\text{trend}} = 0.01$). Individuals negative for all measured EBV markers had NPC rates (58.3/100,000) that were nearly 7 times higher than general population rates (8.4/100,000). Exclusion of the 2 incident NPC cases that were diagnosed within a year of study enrollment did not materially alter our findings (data not shown).

We calculated RRs stratified by time of NPC diagnosis relative to study enrollment. There were 10 NPC cases diagnosed within the first 5 years and 4 cases after more than 5 years since study enrollment. The overall incidence was 93.6 per 100,000 person years and 32.1 per 100,000 person-years for NPC diagnosis within the first 5 years and beyond 5 years, respectively. Risk patterns by EBV sero-markers were similar to the overall pattern in analyses restricted to the first 5 years of follow-up, with the exception of anti-EBV EBNA1 IgA which yielded a risk ratio estimate of 12 in analysis restricted to the first 5 years of follow-up (RR = 12; 95% CI: 2.7–71; Table 2). In our study population, there were only 4 NPC cases diagnosed beyond 5 years of follow-up, limiting our ability to formally evaluate risk in this subgroup stratified by EBV seromarkers. It should be noted, however, that of the 4 NPC cases diagnosed beyond 5 years, all were negative for both anti-EBV VCA IgA by ELISA assay and anti-EBV EBNA1 IgA based on the standard cutoff points suggesting that these 2 particular EBV markers may have limited long-term predictability for NPC.

The standard cutoff points used in Table 2 to define positivity for each of the 4 seromarkers evaluated were defined based on published studies of sporadic NPC. To assess the appropriateness of these cutoff points for individuals from high-risk NPC families, ROC curves were evaluated. Figure 1 displays the ROC curve for each of the EBV biomarkers along with their area under the curve (AUC; anti-EBV VCA IgA IF, AUC = 0.55; anti-EBV VCA IgA ELISA, AUC=0.54; anti-EBV DNase, AUC=0.53; anti-EBV EBNA1 IgA, AUC = 0.73). Use of combinations of markers did not markedly improve AUC estimates (data not shown). The best 2-way EBV marker combination was observed for anti-EBV EBNA1 and anti-EBV DNase (AUC = 0.70), best 3-way EBV marker combination was observed for anti-EBV EBNA1, anti-EBV DNase, and anti-EBV VCA IgA (IF; AUC = 0.72). The 4-way EBV marker combination yielded an AUC of 0.73. When we restricted the data to the first 5 years, AUC scores for the best 2-way, 3-way, and 4-way combinations reported previously were 0.71, 0.90, and 0.91, respectively.

Using these ROC curves, we derived new cutoff points for each of the EBV biomarkers. Because our study population is known to be at high risk of NPC development, maximizing sensitivity over specificity was deemed desirable. Therefore, we defined the ideal cutoff points for our high-risk population by maximizing the Youden score conditional on a sensitivity estimate of more than 80%. By using this criterion, we were unable to identify a cutoff point that yielded a sensitivity of more than 80% for the anti-EBV VCA IgA by IF, so this marker was not evaluated further. For the remaining 3 EBV seromarkers examined, the following positivity cutoff points were identified: $OD_{405} \geq 0.10$ for anti-EBV VCA IgA by ELISA, $OD_{405} \geq 0.10$ for anti-EBV EBNA1 IgA, and 160 or more neutralizing units of DNase neutralizing activity for EBV anti-DNase antibody. The RR estimates that were observed using these new cutoff points for positivity are summarized in Table 3 and are as follows: anti-EBV VCA IgA RR = 2.6 (95% CI: 0.39–109), anti-EBV EBNA1 IgA RR = 6.6 (95% CI: 1.5–61), and anti-EBV DNase RR = 1.4 (95% CI: 0.40–5.2). Patterns observed in analysis restricted to the first 5 years of follow-up were similar to those observed overall (Table 3).

Sensitivity ranged from 7.1% for VCA IgA (ELISA) to 50.0% for EBNA1 IgA using standard assay cutoff values compared with 85.7% for EBNA1 IgA, 92.9% for VCA IgA (ELISA), and 84.6% for DNase using the optimized cutoff values (Table 4). Specificity ranged from 73.8% for VCA IgA (IF) to 95.7% for VCA IgA (ELISA) and was 84% for EBNA1 IgA using standard cutoff values and were 16.0% for VCA IgA (ELISA), 51.2% for EBNA1 IgA, and 92.3% DNase using the optimized cutoff values (Table 4). The negative predictive values were greater than 99% in contrast to the less than 6% positive predictive value for all markers for the entire follow-up period irrespective of the cutoff values (Table 4).

Discussion

We sought to determine (i) whether EBV seromarkers, specifically anti-EBV VCA IgA by IF and ELISA assays, anti-EBV EBNA1 IgA, and anti-EBV DNase were associated with risk of incident NPC in unaffected individuals from high-risk multiplex families and (ii) the clinical utility of EBV biomarkers in our high-risk families.

All 4 markers evaluated were associated with risk and risk increased with the number of positive markers. Our study shows that individually anti-EBV EBNA1 IgA seropositivity was the marker most strongly associated with NPC risk. Individuals seropositive for this marker using the standard cutoff value for positivity ($OD_{405} > 0.20$) were 4.7 times more likely to develop NPC during follow-up than seronegative individuals. The rate of NPC among those positive by the EBNA1 IgA test was 265 per 100,000 person-years, which was nearly 32 times greater than the general population of similar age.

The findings that EBV antibodies are predictive of NPC risk in high-risk NPC families are consistent with findings in general population studies that have found EBV markers to be predictive of sporadic NPC. In contrast to previous studies, however, we found that anti-EBV EBNA1 IgA and not anti-EBV VCA IgA to be the most predictive marker. Whether these findings reflect true differences between individuals from high-risk families and the general population, difficulties in reproducibly measuring anti-EBV VCA IgA by the IF test (10), or a chance finding remains to be determined. In addition, most previously published studies have focused screening efforts using only anti-VCA IgA (IF and/or ELISA assay) in both general population and high-risk populations (9, 14–18). Other efforts have proposed the use of additional EBV markers including anti-Zebra IgG, EBV DNase, and thymidine kinase early antigen, but there has been no consensus on the use of these EBV proteins in serologic testing and the focus has been for diagnostic and prognostic purposes (19–29).

It is unknown whether standard positivity cutoff values reported in the literature (8, 10) are optimal to define risk associated with EBV serologic markers among individuals from high-risk NPC multiplex families. We therefore conducted ROC analyses to identify optimal positivity cutoff values to define NPC risk within high-risk families. Because unaffected individuals from NPC multiplex families are known to be at considerably elevated risk of NPC development themselves, we defined optimal cutoff points as those that achieved a minimum of 80% sensitivity and within that constraint maximized the sensitivity and specificity of the test (i.e., maximum Youden score). Using this approach, we again found that anti-EBV EBNA1 IgA seropositivity was the marker most strongly associated with NPC risk. Individuals seropositive for this marker using the optimized cutoff point ($OD_{405} \geq 0.10$) were 6.6 times more likely to develop NPC during follow-up than seronegative individuals. The rate of NPC amongst those positive by the EBNA1 IgA test was 166 per 100,000 person-years. We did not observe appreciable improvement in risk prediction when other EBV markers or combinations of EBV markers were considered.

We noted that unaffected family members from high-risk multiplex families have rates of NPC that are nearly 11 times higher than those observed in the general population in Taiwan. However, even among this high-risk population, only a small subset of individuals will ultimately develop NPC. Markers capable of distinguishing individuals predisposed to NPC within high-risk families could be of use clinically. We therefore evaluated whether EBV seromarkers could serve as a good clinical indicator of risk in our population. For this analysis, we evaluated the clinical utility of 4 EBV markers by assessing their sensitivity, specificity, and predictive values. An ideal screening tool should have both high sensitivity (ability to identify those who will develop disease) and specificity (ability to identify those who will not develop disease). Of the 4 markers we evaluated, we observed that anti-EBV EBNA1 IgA had the best clinical performance. Using the optimized assay cutoff points defined by ROC analyses, the anti-EBV EBNA1 IgA test was able to identify close to 90% of individuals in our families who developed NPC during our follow-up period (median = 6.5 years). Approximately 50% of individuals in our families who did not develop NPC during the study period also tested positive; therefore it is estimated that close to half of our unaffected family members would have required active follow-up to ensure detection of close to 90% of NPC cases. Although it is reassuring that the anti-EBV EBNA1 IgA test can rule out NPC development over the subsequent 5 to 10 years in about 50% of individuals from high-risk families, identification of EBV seromarkers with higher assay specificity (i.e., a test that would screen out a larger fraction of individuals who are not at risk of NPC development) is important to optimize clinical care.

A main limitation of our study is the small number of cases accrued during the median 6.5 years of follow-up. Although Taiwan has a higher incidence of NPC compared with the world average, the estimated incidence rate of NPC is still low as for most cancers, at 11/100,000 in the general population 30 years or older and 13/100,000 in those 50 years or older. Another limitation is the lack of repeated anti-EBV serology markers, which precluded an evaluation of whether EBV serology patterns over time are predictive of NPC risk. Nonetheless, our study is the largest study of NPC multiplex families to date, covering about 50% of all high-risk population in Taiwan. Although our results suggest that anti-EBV EBNA1 IgA is a predictive marker of NPC, the EBV markers we evaluated have been validated for research purposes only. Before EBV markers can be used clinically, several points need to be considered carefully: (i) our results need to be independently reproduced and (ii) if reproduced, efforts will be needed to further characterize assay performance to ensure robustness and reproducibility over time and across laboratories.

We have in this study provided proof-of-concept that EBV seromarkers have the potential to differentiate individuals from high-risk NPC families who are at risk of developing NPC

from those who are not. This raises the possibility that targeted clinical follow-up of a subset of individuals from high-risk families at highest risk of NPC could lead to earlier diagnosis and therefore improved prognosis and/or higher survival rates.

Translational Relevance

Individuals with a family history of nasopharyngeal carcinoma (NPC) are at increased risk for developing NPC. Individuals with altered Epstein–Barr virus (EBV) serologic profiles are at increased risk for sporadic NPC. There are currently no clinical tests that can identify susceptible individuals within high-risk NPC families. We tested antibody titers from blood collected between 1996 and 2004 against viral capsid antigen IgA, EBV nuclear antigen-1 (EBNA1) IgA, and DNase from 2,557 unaffected relatives of NPC cases from 358 multiplex families. All markers were associated with increased risk of NPC and risk increased with the number of positive markers. Of the four tests evaluated, the EBNA1-IgA antibody test provided the best predictive ability. However, cutoff points required to achieve high clinical sensitivity result in low specificity, limiting the clinical utility of current tests. Additional efforts are warranted to identify markers of susceptibility among high-risk NPC families that preserve high sensitivity while improving specificity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
ROCs curve for each of the EBV biomarkers.

Characteristics of 14 subjects with nasopharyngeal carcinoma diagnosed after recruitment

Table 1

Person-time, y ^a	Sex	Age at diagnosis, y	T	N	M	WHO
0.4	F	43	T3	N2	0	III
0.6	M	46	T1	N0	0	II
1.1	M	50	T1	N0	0	II
1.7	M	59	T3	N2	0	II
2.9	M	47	T2	N1	0	II
3.1	M	41	T1	N0	0	II
3.2	F	53	NA	NA	NA	NA
3.3	M	47	T1	N1	0	II
3.7	M	42	T2	N3	0	II
4.9	M	45	T2a	N1	M1	III
6.4	F	62	T4	N3	0	III
6.8	M	62	T4	N0	0	III
7.2	F	34	T3	N3	0	II
8.1	M	66	T2	N1	0	III

Abbreviation: NA, not available.

^a Person-time accrued defined by enrollment year (vary by individuals) until 2005.

Table 2

Rate ratio of NPC for specific EBV biomarker profiles in high-risk families

Variable ^a	No. of subjects	Person-years FU	No. of NPC cases ^b	Rate of NPC/100,000 person-years	Rate ratio (95% CI) overall (n = 14)	Rate ratio (95% CI) first 5 y of follow-up (n = 10)
Entire follow-up period	2,444	15,519	2	14	90.2	
VCA IgA (IF)						
Negative	1,372	10,122.2	7	69.2		
Positive (>1:10 dilution of serum)	488	3,616	4	110.6	1.6 (0.34–6.3)	2 (0.30–12)
VCA IgA (ELISA)						
Negative	2,285	14,591.4	13	89.1		
Positive (OD ₄₀₅ > 0.50)	103	570.9	1	175.2	2 (0.046–13)	2.6 (0.060–19)
EBNA1 IgA						
Negative	2,008	12,518.3	7	55.9		
Positive (OD ₄₀₅ > 0.20)	380	2,644	7	264.8	4.7 (1.4–16)	11.9 (2.7–71)
DNase						
Negative	2,023	12,974.2	10	77.1		
Positive (>400 neutralizing units)	275	1,783.7	3	168.2	2.2 (0.39–8.5)	2.1 (0.21–11)
Number of marker positivity						
0	1,431	8,574	5	58.3		
Any	957	6,588.30	9	136.6	2.3 (0.71–8.9)	3.2 (0.73–19)
1	735	5,024.30	5	99.5	1.7 (0.39–7.4)	1.8 (0.24–13)
2+	222	1,563.90	4	255.8	4.4 (0.87–20)	7.7 (1.3–52)

^aThere were 3 cases with missing VCA IgA by IF and 1 case with missing DNase biomarker values.

Rate ratio of NPC (based on optimized cutoff points) for specific EBV biomarker profiles for which sensitivity levels of more than 80% could be achieved in high-risk families

Table 3

Variable ^a	No. of subjects	Person-years FU	No. of NPC cases ^b	Rate of NPC/100,000 person-years	Rate ratio (95% CI) overall (n = 14)	Rate ratio (95% CI) first 5-y of follow-up (n = 10)
Entire follow-up period	2,444	15,519.2	14	90.2		
VCA IgA (ELISA)						
Negative	382	1,218	1	40		
Positive (OD ₄₀₅ > 0.10)	2,006	1,170	13	102.7	2.6 (0.39–109)	1.9 (0.26–81)
EBNA1 IgA						
Negative	1,218	7,941	2	25.2		
Positive (OD ₄₀₅ > 0.10)	1,170	7,221.2	12	166.2	6.6 (1.5–61)	4.3 (0.85–41)
DNase						
Negative	1,904	12,114.2	10	82.5		
Positive (> 160 neutralizing units)	394	2,643.7	3	113.5	1.4 (0.40–5.2)	1.4 (0.29–6.6)

^aThere were 3 cases with missing VCA IgA by IF and 1 case with missing DNase biomarker values.

Sensitivity, specificity, and negative and positive predictive values at different cutoff points for each of the EBV biomarkers

Table 4

	Sensitivity (%)	Specificity (%)	% Family member positive	Negative predictive value (%)	Positive predictive value (%)
VCA IgA (IF) ^a					
Positive (>1:10 dilution of serum)	36.4	73.8	26.2	99.5	0.8
VCA IgA (ELISA)					
Positive (OD ₄₀₅ > 0.50)	7.1	95.7	4.3	99.4	1
Positive (OD ₄₀₅ > 0.10)	92.9	16	84	99.7	0.6
EBNA1 IgA					
Positive (OD ₄₀₅ > 0.20)	50	84.3	15.9	99.7	1.8
Positive (OD ₄₀₅ > 0.10)	85.7	51.2	49	99.8	1
DNase					
Positive (>400 neutralizing units)	23.1	88.1	12	99.5	1.1
Positive (>160 neutralizing units)	84.6	92.3	8.1	99.9	5.6

^aROC yielded no change in cutoff point for this particular EBV biomarker assay.