Published in final edited form as:

Int J Cancer. 2012 June 15; 130(12): 2886–2891. doi:10.1002/ijc.26334.

Antibody titers against EBNA1 and EBNA2 in relation to Hodgkin lymphoma and history of infectious mononucleosis

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Abstract

A role for Epstein Barr virus (EBV) in Hodgkin lymphoma (HL) pathogenesis is supported by the detection of EBV genome in about one-third of HL cases, but is not well defined. We previously reported that an elevated pre-diagnosis antibody titer against EBV nuclear antigens (EBNA) was the strongest serologic predictor of subsequent HL. For the present analysis, we measured antibody levels against EBNA components EBNA1 and EBNA2 and computed their titer ratio (anti-EBNA1:2) in serum samples from HL cases and healthy siblings. We undertook this analysis to examine whether titer patterns atypical of well-resolved EBV infection, such as an anti-EBNA1:2 ratio = 1.0, simply reflect history of infectious mononucleosis (IM), an HL risk factor, or independently predict HL risk. Participants were selected from a previous population-based case-control study according to their history of IM. We identified 55 EBV-seropositive persons with a history of IM (IM+; 33 HL cases, 22 siblings) and frequency-matched a comparison series of 173 IM history-negative, EBV-seropositive subjects on HL status, gender, age, and year of blood draw (IM–; 105 cases, 58 siblings). In multivariate logistic regression models, an anti-EBNA1:2 ratio = 1.0 was significantly more prevalent in HL cases than siblings (odds ratio, 95% confidence interval=2.43, 1.05–5.65); similar associations were apparent within the IM+ and IM– groups. EBNA antibodies were not significantly associated with IM history in HL cases or siblings. These associations suggest that chronic or more severe EBV infection is a risk factor for HL, independent of IM history.

Keywords

Hodgkin lymphoma; Epstein-Barr virus; infectious mononucleosis; EBNA1; EBNA2

Introduction

A substantial body of evidence links the Epstein-Barr virus (EBV) to the occurrence of Hodgkin lymphoma (HL) (1). EBV is a ubiquitous gamma herpes virus that establishes lifelong latent infection in host B lymphocytes. Several lines of molecular evidence support a role for EBV in HL pathogenesis. First, virally encoded proteins and gene products are detected in the Reed-Sternberg cells in tumor biopsies of about one-third of cases (2). Also, the latent EBV episome is monoclonal in EBV genome-positive cases (3). Serologic evidence supporting the association includes our early findings that HL cases have altered

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profiles of antibody to EBV, both prior to and following diagnosis (4). In addition, a history of infectious mononucleosis (IM) is a risk factor for HL among young adults (1, 4), and for EBV genome-positive young adult HL (5). Delayed infection with EBV often manifests as IM, and persons whose childhood social environment afforded protection from early infections, and thus susceptibility to delayed EBV infection, are also at greater risk of young adult HL (1). The synthesis of these seemingly consistent observations into a unifying hypothesis of oncogenesis remains elusive. For example, it is not known whether the altered profiles of antibody to EBV that predict HL risk are simply a reflection of a person's history of IM, or whether the altered EBV serologic profiles predict HL risk independently of a history of IM. The role of another oncogenic virus in the etiology of EBV genome-negative HL also remains unclear (6–8).

We previously reported that an aberrant pre-diagnosis pattern of antibodies to EBV-encoded antigens predicted a subsequent diagnosis of HL (4). This antibody pattern is not typically observed in persons with well-controlled latent EBV infection and involves elevated titers against antigens from the viral lytic and latent cycles. Among the individual antibodies in the at-risk profile, an elevated titer against the Epstein-Barr nuclear antigens (EBNA) was the strongest independent predictor of HL risk (4).

The EBNAs comprise six nuclear proteins, the full spectrum of which is expressed in the “latency III” pattern of EBV latent cycle gene transcription; the latency III pattern is observed in EBV-transformed lymphoblastoid cell lines (9). In contrast, no EBNA protein is expressed in EBV-infected B cells in latency 0, and only EBNA1 (among the EBNA antigens) is expressed in latency I and II infections. In vivo, EBV-infected populations of B lymphocytes with each of the four latency patterns have been described; down-regulation of EBNA gene expression (i.e., from the latency III pattern to the patterns that feature expression of fewer or no EBNA genes) occurs with expansion of host immune control and resolution of primary infection (10).

The EBNA2 antigen plays an essential role in the transformation of EBV-infected B lymphocytes to lymphoblastoid cells (10). This transformation is an early event following primary infection with EBV and an important mechanism by which the virus colonizes the host B lymphocyte system. The early expression of EBNA2 in primary infection is also reflected in the host serologic response. In primary EBV infection, antibodies against EBNA2 (anti-EBNA2) normally appear first. Resolution of acute infection is mediated by the action of EBV-specific cytotoxic T-lymphocytes (CTL), and following resolution, the anti-EBNA2 titers diminish in conjunction with the appearance of antibodies against EBNA1 (anti-EBNA1). Rickinson and Kieff have suggested that this change in antibody predominance could reflect the predominant expression of the corresponding EBNA protein genes at different times in the course of infection (10). This interpretation implies that relatively few EBV-infected cells in resolved (i.e., latent) infection express EBNA2, and that EBNA1 expression tends to predominate after resolution of primary infection.

In an early longitudinal study of EBV serology in IM patients, Henle et al. (11) reported that an abnormal antibody pattern detected two or more years after IM onset, specifically one in which the anti-EBNA1 titer was less than or equal to the anti-EBNA2 titer resulting in a ratio of anti-EBNA1:2 titers of ≤ 1.0 , characterized patients with a protracted course of IM. This “low” anti-EBNA1:2 ratio was also found in some cases of chronic EBV infection (11). A low anti-EBNA1:2 ratio was also more commonly observed among a subset of patients with HL or non-Hodgkin lymphoma who developed unusually high antibody titers to EBV viral capsid antigen (VCA) or early antigen (EA), which are lytic cycle proteins and indicative of lytic viral replication, as well as in patients with AIDS or congenital immune deficiency (11). Thus, the low anti-EBNA1:2 ratio appears to be an indicator of poor host

control of the EBV infection, which may reflect an underlying state of host immune dysfunction.

To further address the association of altered EBV serologic profiles with HL risk, and specifically whether EBV antibody titer-HL associations are independent of IM history, we conducted a serologic case-control study focused on the antibody response to EBNA1 and EBNA2.

Materials and Methods

Study participants

The subjects of this study were drawn from a previous population-based case-control study of HL patients diagnosed between July 1, 1973 and December 31, 1977 among residents of a defined population in eastern Massachusetts and their unaffected siblings who served as a control group (12). Essentially all of the HL cases had been treated prior to interview. Among 324 cases aged 15 or older, serum specimens were obtained from 304 (94%). For each case interviewed, we sought to interview the two siblings who were closest in age to the case. The 383 siblings interviewed for the study represented a majority (80%) of the potential sibling controls, and serum specimens were obtained from 285 (74%) of the interviewed siblings. In the original study, we reported an increased prevalence of elevated titers of immunoglobulin G (IgG) antibodies against the VCA and the EA from the EBV lytic cycle among HL cases compared to unaffected siblings (12). Antibodies to EBNA proteins were not measured in the original study.

For the present investigation, we identified 58 individuals who had self-reported a history of IM (IM+) at time of enrollment, including 35 HL cases and 23 siblings of cases, among the original 589 subjects with blood specimens. We obtained a comparison series of subjects who did not self-report a history of IM (IM-) by frequency matching three IM- persons for each IM+ person on HL status (case/sibling), gender, age (i.e., using age at diagnosis for HL cases or year of birth for siblings), and year of blood draw. When necessary to identify a suitable IM- match, the age-related or year of blood draw criterion was relaxed incrementally by ± 1 year.

Antibody testing

Archived serum samples from these subjects were tested by one of us (EL) for IgG antibodies to EBNA1 and EBNA2 expressed in stably transfected DG75 cells, a human Burkitt lymphoma-like cell line that normally does not harbor EBV. Each serum sample was tested separately but in parallel against EBNA1-transfected cells (DG75-EBNA1), DG75-EBNA2 cells, and non-transfected DG75 cells, using anti-complement immunofluorescence methods previously described (13). The antibody titer was defined as the greatest serial dilution at which a sample demonstrated reactivity to the given antigen. A positive titer was defined as reactivity at 1:5 dilution. The original antibody data against VCA and against diffuse (EA-D) and restricted (EA-R) forms of EA were abstracted for the present subjects. Those assays had been done in the laboratory of the late Dr. Alfred Evans (12) using standard assays. All laboratory assays were performed without knowledge of the case/sibling or IM status of individual specimens.

Statistical analysis

For consistency with our previous analysis (4), we defined "elevated" titers as the reciprocal of the upper 10–15% of the titer distribution among the siblings, which corresponded to anti-EBNA1 1280 and anti-EBNA2 80. Elevated cutoffs for the lytic cycle antibodies were previously defined as anti-VCA 320, anti-EA-D 10 and anti-EA-R 40 (4). We

categorized the anti-EBNA1:2 ratio according to the *a priori* criterion for an abnormal anti-EBNA response profile (i.e., 1.0 v. >1.0) (11). The calculation of geometric mean titer (GMT) included only subjects with a positive titer.

We used logistic regression to compute odds ratios (OR) and 95% confidence intervals (CI) to assess the association of elevated anti-EBNA1, elevated anti-EBNA2, and a low anti-EBNA1:2 ratio with IM history. We first constructed separate logistic regression models for each EBNA antibody variable, followed by a model which mutually adjusted for all the EBNA antibody variables. All the models were adjusted for elevated anti-VCA and -EA titers, gender, and age (modeled as a continuous variable: years of age at diagnosis for cases, or at the matched case's diagnosis for siblings) and stratified by HL status.

We next examined the association of HL occurrence with each EBNA antibody variable (separately first, then with mutual adjustment) in logistic regression models that controlled for the co-variables noted earlier, with stratification by IM status. In additional models we evaluated the association of the EBNA antibody variables with HL occurrence across all subjects, controlling for IM history as well as the previously noted co-variables. We assessed the statistical significance of a given OR according to whether or not the corresponding 95% CI included the null value of 1.0 (i.e., assuming a two-tailed α -error level of 0.05). All statistical analyses were performed with SASTM (Cary, NC).

Results

To insure that all subjects in this analysis were seropositive for EBV, we excluded three anti-VCA negative subjects (2 HL cases, 1 sibling; each was IM+). We also excluded one IM- sibling whose specimen had non-specific EBNA reactivity (12). Thus, the final analysis included 228 subjects: 55 IM+ (33 HL cases, 22 siblings) and 173 IM- (105 HL cases, 68 siblings). The subgroups defined by HL status and IM history were similar to one another in age and gender distributions (Table 1). Also, in IM+ persons, the interval from IM onset to blood collection was similar for HL cases and siblings (Table 1).

A comparison of the crude distributions of antibodies against EBNA antigens suggested some differences in the prevalence of abnormal titers by HL status and IM history (Table 2 and Supplementary Figures S1–S3). For antibodies against EBNA1, the IM+ HL cases had the lowest GMT, in comparison to IM- HL cases and to sibling controls with or without a history of IM. The prevalence of an elevated anti-EBNA2 titer was highest in IM+ HL cases, lower in IM- cases and IM+ siblings, and lowest in IM- siblings. An anti-EBNA1:2 ratio 1.0 was more frequent in IM+ than IM- persons of similar HL status and was higher in HL cases than unaffected siblings regardless of IM history.

Using covariate-adjusted logistic regression, we examined the association of atypical antibody responses to EBNA antigens with history of IM within strata defined by HL status (Table 3). Sibling controls with a history of IM had an approximately three-fold higher prevalence of an elevated anti-EBNA2 titer than IM- siblings (OR, 95% CI=3.10, 0.93–10.33; $p=0.07$). Among HL cases, IM history was not associated with any indicator of a response to EBNA antigens (Table 3). Mutual adjustment for all the EBNA antibody variables yielded similar results (data not shown).

We then evaluated whether the EBNA antibody variables were associated with the occurrence of HL itself among all subjects combined, and within strata defined by IM history (Table 4). In IM- persons, a low anti-EBNA1:2 ratio (OR, 95% CI=2.71, 1.00–7.39; $p=0.05$), an elevated anti-EBNA1 titer (2.03, 0.75–5.54), and an elevated anti-EBNA2 titer (2.33, 0.96–6.63; $p=0.06$) were more prevalent among HL cases than among unaffected

siblings (Table 4). Among the IM+ participants, an elevated anti-EBNA1 or anti-EBNA2 titer was less prevalent among the HL cases than the sibling controls; however, a low anti-EBNA1:2 ratio was two times more prevalent in IM+ cases compared with IM+ siblings (2.07, 0.38–11.41). When all subjects were combined, a low anti-EBNA1:2 ratio occurred at a significantly higher prevalence among HL cases than siblings (2.43, 1.05–5.65) with control for IM history and other co-variables (Table 4). Mutual adjustment for all of the EBNA antibody variables did not alter these findings (data not shown).

Discussion

In this cross-sectional study, we examined the prevalence of elevated antibodies against the two major components of the EBV-encoded EBNA antigens, EBNA1 and EBNA2, in relation to IM history and HL occurrence. We undertook these analyses to evaluate whether antibody responses to these EBV latent cycle antigens were altered in HL cases and if so, whether such altered antibody responses simply reflected the higher prevalence of IM history in HL cases or instead represented a primary risk factor for HL independent of IM history.

The prevalence of abnormal antibody responses to EBNA antigens did not vary significantly by IM history among HL cases. Instead, we observed a higher prevalence of an anti-EBNA1:2 ratio > 1.0 among HL cases compared to siblings. This atypical antibody profile was associated with HL in both IM+ and IM– subgroups, and significantly so when subjects were combined across IM history. These findings collectively support the view that an atypical response to EBNA antigens independently predicts HL risk (4) and does not simply reflect the association of HL with IM.

Infection with EBV is highly prevalent, and most people acquire their infection relatively early in life. Transmission occurs primarily by oral contact with a carrier who is shedding infectious virions in saliva. A herpes virus, the EBV establishes latency (predominantly types I/II) in a subset of B lymphocytes. Most childhood primary infections do not manifest as clinically recognized IM. However, prospective cohort studies of college students in the 1960s and 1970s demonstrated that primary infection in adolescence could result in clinical IM in up to half of the EBV seroconverters (14). A history of IM is therefore indicative of delayed acquisition of the EBV and of a more severe primary infection. Viral load is high during acute IM, and lytic replication of the virus occurs in the peripheral blood. Viral load then diminishes as the infection is cleared (15, 16). During acute infection, the expression of the EBNA antigens in latently infected lymphocytes is heterogeneous with a small proportion of cells expressing EBNA2, whereas EBNA2 is not normally expressed following resolution of the primary response to EBV in healthy carriers (15, 17).

EBV infection is essentially controlled by a “type 1” (or T_H1) immune response featuring EBV-specific CTLs (18), which are primarily directed against EBNA2, 3a, 3b, and 3c (19). Antibody responses to EBV-encoded antigens are unlikely to afford control of the virus but may be correlated with the relative level of *in vivo* expression of the corresponding viral proteins. The antibody profile may also reflect an imbalanced T_H1/T_H2 immune response to the infection; altered secretion of both type 1 and type 2 cytokines has been observed in young adult HL patients (20, 21). In either case, EBV antibody profiles have served as empiric indicators of viral/host interaction in a variety of EBV-associated diseases (10).

In 1987, Henle and colleagues (11) described the evolution of antibody responses to EBNA1 and EBNA2 in IM and chronic EBV infection, including the early predominance of anti-EBNA2 and the subsequent reversal of the anti-EBNA1:2 ratio. In their study, persistence of a low anti-EBNA1:2 ratio was indicative of chronic EBV infection. The Henle group (11)

also reported that a low anti-EBNA1:2 ratio was more prevalent in a subset of HL patients who developed unusually high anti-VCA or anti-EA titers. Similarly, Seigneurin et al. (22) reported detection of anti-EBNA2 in a subset of HL and documented the inverted anti-EBNA1:2 ratio in a renal transplant patient, an AIDS patient, and a persistent IM patient. Others have documented the low anti-EBNA1:2 ratio in progressive HIV infection (23) and in HIV-infected children (24). Collectively, these data further corroborate the view that the low anti-EBNA1:2 ratio occurs primarily in persons with compromised immune control of EBV. In the present analysis a low anti-EBNA1:2 ratio was more prevalent among HL cases than among sibling controls. This association was independent of the associations with HL that we previously reported for elevated anti-VCA and anti-EA in this study population (12) and suggests that poorly controlled EBV and/or an underlying status of diminished type 1 immunity contributes to the etiology of HL.

We also observed a marginally significant increase in the prevalence of elevated anti-EBNA2 in IM+ compared to IM- siblings, consistent with our previous finding that the IM+ siblings had a higher prevalence of elevated anti-VCA and anti-EA (4). Given that the blood samples collected from the IM+ siblings were drawn at least two years after onset of IM (range, 2 to 24 years), the observed abnormalities in EBV serology in IM+ siblings cannot be attributed simply to recent onset of IM. Rather, these findings suggest that a subset of the IM+ siblings had poorly resolved EBV infection. The reasons for and health implications of the poor control of EBV infection in these siblings are unclear but warrant further investigation. Insights gleaned from such studies would improve the interpretation of EBV serologic markers with regard to host immune status and/or risk for complications of chronic or severe EBV infection.

Strengths of the present analysis include the population-based design and relatively high participation rates among HL cases and siblings included in the original case-control study from which the present study subjects were sampled. These characteristics minimize the influence of selection and recall bias on the findings and enhance their generalizability. Also, we were able to jointly examine antibody responses to lytic and latent antigens encoded by EBV to identify serologic markers of HL risk that were independent of one another and of IM history.

A few potential limitations to this study should also be noted. These include the cross-sectional design since the effect of HL and/or its treatment may affect antibody levels against EBNA antigens. However, the present findings do not differ markedly from those we reported in two previous studies of pre-diagnosis EBV antibody profiles, an observation which argues against a treatment bias (4, 25). Of note, in one of those studies we prospectively evaluated a low anti-EBNA1:2 ratio in relation to risk of EBV+ HL and observed an increased risk, but found no association of a low anti-EBNA1:2 ratio with EBV- HL (25). Also, a treatment bias cannot explain apparent differences in antibody profiles among HL cases by IM status, unless the treatment effect on antibody levels varied by (often quite distant) IM history. Another limitation is our inability to classify HL patients with regard to tumor EBV status; the diagnostic tumor specimens are no longer available because the original case-control study was performed in the 1970's at multiple hospitals. Hjalgrim and colleagues (5) reported that a history of IM was primarily associated with young adult EBV+ HL, and not with EBV- or older adult HL. In a recent case-case comparison by Chang et al. (26) that included both younger and older adult HL patients from a different population-based case-control study, we observed that elevated anti-VCA and anti-EA-D, as well as an anti-EBNA1:2 ratio ≥ 1.0 , were significantly more prevalent among tumor EBV+ than tumor EBV- HL patients. The latter observations were not restricted to younger or older adult HL subgroups. The present observation of an association of an anti-EBNA1:2 ratio ≥ 1.0 with HL risk in general, obtained from an analysis using

sibling controls, is consistent with the case-case and prospective findings of an association of a low anti-EBNA1:2 ratio with EBV+ HL (25, 26). However, explicit confirmation of the association of altered EBV serology with EBV+ HL in additional populations is warranted.

In summary, our findings are consistent with the notion that having had a more clinically severe EBV infection – as indicated by a history of IM – is associated with some abnormality in anti-EBNA1 and anti-EBNA2 responses. Furthermore, we found that the atypical antibody profile captured by a low anti-EBNA1:2 ratio was a more robust indicator of poor host control of the EBV than the individual markers for elevated anti-EBNA1 or anti-EBNA2 and was independent of previously reported HL and IM associations with elevated anti-VCA or anti-EA titers (4). Of particular interest, the association of HL with atypical EBV serologic profiles appears to be independent of a history of IM, suggesting that a more severe or chronic EBV infection is a risk factor for HL itself. Whether this is true for all HL, or is restricted to EBV genome-positive cases, remains to be confirmed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by Public Health Service grant K07 CA115687 (BMB). The authors thank Ms. Catherine Suppan for technical and editorial assistance. We also gratefully acknowledge the HL patients and siblings who participated in this study and thereby made the research possible.

Abbreviations used

CI	confidence interval
EA	Epstein-Barr virus early antigen
EBNA (EBNA1, EBNA2)	Epstein-Barr nuclear antigen (type 1, 2)
EBV	Epstein-Barr virus
HL	Hodgkin lymphoma
IM	infectious mononucleosis
OR	odds ratio
VCA	Epstein-Barr virus capsid antigen

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Novelty

The present study is the first to report the association of altered antibody responses to Epstein Barr virus (EBV) nuclear antigen components 1 and 2 (EBNA1, EBNA2), including an anti-EBNA1:2 ratio ≥ 1.0 —which has been shown empirically to be indicative of severe or chronic EBV infection—with young adult HL risk within strata defined by IM history.

Impact

Altered EBV serologic patterns were associated with risk of HL, including young adult HL, in previous reports, but it was not clear whether those associations simply reflected a greater prevalence of a history of infectious mononucleosis (IM) among HL patients. The present findings support the view that those associations do not simply reflect the prevalence of IM history, and that poorly controlled EBV infection (as characterized by altered antibody patterns) is a primary risk factor for young adult HL independent of IM history. This observation invites further investigation into the role of subclinical host immune dysfunction and poor control of EBV in the pathogenesis of HL, and whether chronic or severe EBV infection specifically predicts risk of tumor EBV+ HL.

Table 1

Selected characteristics of Hodgkin lymphoma (HL) cases and unaffected siblings by infectious mononucleosis (IM) history.

IM Status	HL Cases	Siblings
IM+	33	22
% male	49	41
Mean age, years [†]	27	25
Mean (range) interval, IM onset to blood draw, years	8.9 (4–28)	8.9 (2–22)
IM–	105	68
% male	51	44
Mean age (years)	27	25

Abbreviations: HL, Hodgkin lymphoma; IM, infectious mononucleosis.

[†] at blood draw.

Table 2

Prevalence of elevated titers against EBNA1 and EBNA2 and of the anti-EBNA1:2 titer ratio 1.0, and geometric mean titer (GMT) of antibodies, by HL status and IM history.

EBV antibody	HL Cases		Siblings	
	IM+ (33) [†]	IM– (105)	IM+ (22)	IM– (68)
EBNA1 (1:1280)	0.12	0.18	0.09	0.10
GMT	122	325	226	261
EBNA2 (1:80)	0.42	0.32	0.36	0.13
GMT	54	52	45	26
EBNA1:2 1.0	0.33	0.23	0.14	0.09

Abbreviations: EBV, Epstein-Barr virus; EBNA, EBV nuclear antigen; GMT, geometric mean titer; HL, Hodgkin lymphoma; IM, infectious mononucleosis.

[†] Number of subjects in analyses.

[‡] Among those with detectable titer.

Table 3

The association of history of IM with elevated antibody titers against EBNA antigens and with a low anti-EBNA1:2 ratio by HL status.

EBV Antibody	HL Cases [†]			Siblings [†]		
	IM+ (%)	IM- (%)	OR (95% CI)	IM+ (%)	IM- (%)	OR (95% CI)
N [‡]	33	105		22	68	
EBNA1	12.1	18.1	0.58 (0.17–1.99)	9.1	10.3	1.06 (0.18–6.38)
EBNA2	42.4	32.4	1.15 (0.48–2.77)	36.4	13.2	3.10 (0.93–10.33)
EBNA1:2 1.0	33.3	22.9	1.52 (0.61–3.82)	13.6	8.8	1.28 (0.26–6.35)

Abbreviations: EBV, Epstein-Barr virus; EBNA, Epstein-Barr nuclear antigen; HL, Hodgkin lymphoma; IM, infectious mononucleosis; OR, odds ratio; CI, confidence intervals; VCA, viral capsid antigen; EA, early antigen.

[†] ORs and 95% CIs, adjusted for age, gender, anti-VCA and anti-EA.

[‡] Number of subjects.

Table 4

The association of HL with elevated antibody titers against EBNA antigens and with a low anti-EBNA1:2 ratio in all participants combined and with stratification by history of IM.

EBV Antibody	IM+ Subjects [†]			IM– Subjects [†]			Subjects Combined [‡]
	Cases (%)	Siblings (%)	OR (95% CI)	Cases (%)	Siblings (%)	OR (95% CI)	OR (95% CI)
N [§]	33	22		105	68		
EBNA1	12.1	9.1	0.52 (0.06–4.72)	18.1	10.3	2.03 (0.75–5.54)	1.64 (0.67–4.05)
EBNA2	42.4	36.4	0.78 (0.18–3.32)	32.4	13.2	2.33 (0.96–6.63)	1.72 (0.84–3.54)
EBNA1:2 1.0	33.3	13.6	2.07 (0.38–11.41)	22.9	8.8	2.71 (1.00–7.39)	2.43 (1.05–5.65)

Abbreviations: HL, Hodgkin lymphoma; EBV, Epstein-Barr virus; OR, odds ratio; CI, confidence interval; EBNA, Epstein-Barr nuclear antigen; IM, infectious mononucleosis; VCA, viral capsid antigen; EA, early antigen.

[†] ORs and 95% CIs, adjusted for age, gender, anti-VCA and anti-EA

[‡] ORs and 95% CIs, adjusted for age, gender, anti-VCA, anti-EA, and IM history

[§] Number of subjects