# Clinical and demographic characteristics of Epstein-Barr virus-associated childhood Burkitt's lymphoma in Southeastern Brazil: epidemiological insights from an intermediate risk region

Rocio Hassan,[1] Claudete Esteves Klumb,[2] Fabricio E. Felisbino,[1] Deisy M. Guiretti,[1] Lídia R. White,[1] Claudio Gustavo Stefanoff,[1] Mario Henrique M. Barros,[1] Héctor N. Seuánez,[3,4] and Ilana R. Zalcberg[1]

[1]Bone Marrow Transplantation Centre (CEMO); [2]Hematology Service, [3]Genetics Division, Instituto Nacional de Câncer (INCA); [4]Genetics Department, Universidade Federal do Rio de Janeiro (HNS), Rio de Janeiro, RJ, Brazil

## ABSTRACT

We studied a group of 54 children with Burkitt's lymphoma from Southeastern Brazil, where epidemiological status of Burkitt's lymphoma is poorly understood. Epstein-Barr virus association showed an intermediate frequency (~60%) between endemic and sporadic subtypes. Median age was five years. Epstein-Barr virus infection was significantly associated to low age (Epstein-Barr virus$^+$ four years *vs.* Epstein-Barr virus$^-$ eight years). Sex ratio (M:F) was 2:1, with a significantly higher number of males in old age classes. Young age at diagnosis and excess of males at older ages, as well as a causal relationship between low age, epstein-barr virus and Burkitt's lymphoma risk, may characterize Burkitt's lymphoma in Brazil.

Key words: Burkitt's lymphoma, Epstein-Barr virus, clinico-demographic, Brazil.

*Citation: Hassan R, Klumb CE, Felisbino FE, Guiretti DM, White LR, Stefanoff CG, Barros MHM, Seuánez HN, and Zalcberg IR. Clinical and demographic characteristics of Epstein-barr virus-associated childhood Burkitt's lymphoma in Southeastern Brazil: epidemiological insights from an intermediate risk region. Haematologica 2008 May;93(5):780-783. doi: 10.3324/haematol.12424*



## Introduction

Burkitt's lymphoma (BL) shows marked variation across different geographical regions with respect to age-specific incidence, primary tumor site and association with Epstein-Barr virus (EBV) infection,[1] which characterize the endemic (eBL) and sporadic (sBL) subtypes.[2] eBL is almost always EBV-associated while sBL shows only 10%-30% EBV association.[1] Descriptions of BL outside endemic and sporadic regions allow for a progressive identification of world areas where BL exhibits specific characteristics and intermediate incidence.[3]

An alternative classification to the standard types, based on three different epidemiological conditions, considers: (i) BL in areas of high risk or incidence (endemic), (ii) BL in areas of low risk or incidence (sporadic cases in USA, Northern and Eastern Europe, and Japan), and (iii) BL in areas of intermediate risk or incidence (Northern Africa, the Middle East, and northern and central regions of South America).[3] BL in Brazil might be included in this third subtype,[4,5] although the epidemiological status of BL, in a geographically extensive and heterogeneous country like Brazil, remains poorly understood. We report a study of 54 children with BL in Southeastern Brazil and analyse their clinicopathological characteristics and EBV association.

## Design and Methods

### Patients and pathology samples

Fifty-four children (up to 16 years old), diagnosed with BL at the Instituto Nacional de Câncer (INCA), Brazil, between 1992 and 2004, were included in this study based on availability of pathology specimens and clinical records, following approval by INCA´s Ethics Committee. The clinical characteristics of 29 patients have been previously described.[5]

Histopathological diagnosis was performed according to the WHO classification.[2] Statistical analyses were based on non-parametric methods and a $p$ value $<0.05$ was considered significant.

### EBV diagnosis and genotyping

Infection was diagnosed by EBERs *in situ* hybridization.[6] DNA was isolated from biopsies and from paraffin-embedded

*Funding: this work was supported by the Conselho Nacional de Pesquisa (CNPq) Grant 478264–2003-8 (Brazil) and Swissbridge Foundation (Switzerland). Manuscript received October 23, 2007. Manuscript accepted November 22, 2007.*
*Correspondence: Rocio Hassan, PhD, Instituto Nacional de Câncer, INCA-Bone Marrow Transplantation Centre (CEMO). Praça da Cruz Vermelha 23, 6th floor, 20230-130, Rio de Janeiro, RJ, Brazil. E-mail: biomol@inca.gov.br*

tissue. Genotyping was carried out by nested-PCR for the distinctive EBV-1 and -2 *EBNA2* regions.[6] Detection of the polymorphic 30 or 69 bp deletion in the carboxy-terminus of the *LMP1* gene was carried out by specific nested-PCR assays.[7]

## Results and Discussion

### Demographic characteristics

BL was diagnosed in 50 immunocompetent patients and in 4 patients with AIDS (AIDS-BL) (Table 1). Age distribution (median 5 years, range 2-14) showed 42.5% of the patients in the 0-4 year age class, 42.5% in the 5-9 class, and 15% in the 10-14 class, indicating predominance of young children, with 54% equal to or greater than the median age. A similar age distribution was observed in 52 children from Bahia (Northeastern Brazil),[8] suggesting that it might be characteristic of pediatric BL in Brazil.

In high risk areas, BL is a pediatric disease, with most cases in Equatorial Africa occurring in the 5-9 year age-class although in Europe, childhood cases are equally distributed among different age classes.[9,10] A compilation of studies comparing BL median age from different regions showed an estimate of 6.1 years in Africa against 19.2 years in North America.[9] In the Middle East and Brazil, median ages were similar to those observed in Africa,[4,8-13] indicating that factors associated with early morbidity in these regions require investigation.

The sex ratio of the entire group was 2:1 (36M:18F) and a significant sex ratio variation in respect to age class was observed, with an increase of affected males in older age classes ($\chi^2$= 10.96; $p$=0.004). The age of males was significantly higher than in females, with a median of 6 years (range 3-14) *vs.* 4 years (2-9) (Mann-Whitney test, $p$=0.001). A positive correlation between median age per class and sex ratio per class was observed (Rho Spearman = ~1, $p$<0.01). Distortion of sex ratio in childhood cancer is a known finding, representing a classical risk factor; childhood cancers showing an adjusted-by-age sex ratio of 1.2, with non-Hodgkin's lymphomas showing the highest sex ratio (3:1).[14] BL is more frequent in males, mainly in low risk areas like Europe, contrary to early African reports where males and females were almost equally affected while, in intermediate areas, sex ratio was found to be variable.[3,9] In our patients, the positive correlation between sex ratio and age class was the same as the lower proportion of young males in high risk (endemic) areas. Interestingly, the opposite is not the case in the USA or the Middle East,[9,15] indicating that global sex ratio estimates in endemic and sporadic areas do not represent the extremes of a single distribution. This reinforces the need to carry out genetic and environmental studies per age class to search for biological factors involved in age-specific risk in BL.

### Clinical presentation

In 53 children with available clinical records, the abdomen was the most frequently involved region, accounting for the primary presentation in 38 cases (72%) (Table 2). Analysis of 22 cases with available data on abdominal involvement showed 7 tumors (32%) localized in the intestinal wall while 15 (68%) occurred at other sites (meso/epiplon 5 cases; retroperitoneum 4; ovary 2; liver 1; stomach 1; and others sites 2 cases). One patient (2%) showed a mandibular primary involvement. Nodal presentation (11%) was observed mainly in older children. Twelve (23%) patients showed bone marrow involvement (6 cases with FAB L3-ALL). The central nervous system (CNS) was involved in 5 patients, 2 (4%) of whom were diagnosed as primary CNS-BL. Patients at advanced (III/IV) stages (75%) were younger than stage I/II patients (median 4.5 *vs.* 7 years; Mann-Whitney test, $p$=0.03). This seems to be a characteristic of Brazilian BL,[4,5,8] contrary to that observed in a large series in Kenya, where stages I/II were predominant in childhood BL.[16] Abdominal involvement was unrelated to age class, while in older patients, a trend of nodal involvement was evident ($\chi^2$=5.48; $p$=0.064). There was no association between clinical characteristics and sex.

Like sBL, our patients showed predominance of abdominal tumors in all age classes, intestinal involvement characteristic of sBL was observed only in approximately 32% of cases. This pattern of intra-abdominal involvement is in agreement with another study from Brazil.[8] The low frequency (2%) of head and neck tumors in our patients also agreed with other reports, showing the lowest world frequency of head and neck involvement despite the young age of affected children.

### EBV association

EBV was detected in 61% of BL cases, and in 58% of the HIV$^-$ group. Remarkably, all HIV$^+$ children were also EBV$^+$. The frequency of EBV association was intermediate between eBL (95%) and sBL (10-30%),[1,4] predominantly of type 1 (Table 1), as previously reported in Brazil.[5,8,17] In immunocompetent children, a significant association between EBV infection and lower age was observed ($\chi^2$=10.24; $p$=0.006) (Figure 1 A-B). EBV$^+$ BL showed a median age of 4 years, against a median of 8 years in EBV-negative cases (Mann-Whitney test;

**Table 1.** Characteristics of patients with Burkitt's lymphoma, according to HIV and EBV association, age distribution and sex ratio.

| *Pathology* | *N* | *Median age (years)* | *Range (years)* | *(M:F)1* | *Sex ratio* | *EBV (+)* | *EBV-1 (% of positive)* | *EBV-2 (% of positive)* |
|---|---|---|---|---|---|---|---|---|
| BL HIV-negative | 50 | 5 | 2-14 | (35:15) | 2.33:1 | 29 (58%) | 25 (86%) | 4 (14%) |
| BL HIV-positive | 4 | 4 | 3-4 | (1:3) | 0.33:1 | 4 (100%) | 2 | 2 |
| Total | 54 | 5 | 2-14 | (36:18) | 2:1 | 33 (61%) | 27 (81%) | 6 (19%) |

[1]*(M:F): males:females.*

$p$=0.002). A high frequency of EBV$^+$ BL in younger children was also observed in Northeastern Brazil,[8] reinforcing the need for further investigation of the relationship between low age of EBV seroconversion and BL risk in Brazil. In eBL, an early and severe EBV infection during the first months of life was shown to be a key event for subsequent BL development.[18] We could not assess the serological status of our patients. However, a study on EBV seroprevalence in healthy individuals from the same Brazilian region showed an intermediate curve of seroconversion age with respect to developed and underdeveloped countries. It also found an earlier EBV seroconversion in low-income groups and, interestingly, associated to low maternal literacy.[19]

We also investigated LMP1 C-terminal deletion polymorphisms, the pathogenic role of which in EBV-associated lymphomas is controversial.[7] LMP1 nested-PCR resulted in a 175 bp, a 145 bp or a 106 bp amplified fragment for wild type, del30 or del69 variants respectively. Deletion variants were more frequent in BL (17/33, 52%) than in non-neoplastic controls (4/11, 36%) although the difference was not statistically significant ($p$=0.157). The del69 variant was detected in only one BL case. Our results in a large group of BL children do not support a pathogenic role of del30/del69 LMP1 variants in BL, which had been proposed for this region.[17] However, in a group of Hodgkin's lymphomas (HL) from our institution, a significant association of del30 variants with lymphoma cases was observed.[7] These differences may reflect the fact that *LMP1* is expressed and therefore subjected to selection in HL but not in BL, in which EBV expresses a restricted pattern of gene expression (latency I). Children with AIDS-BL were younger than immunocompetent BL children, as expected from perinatal HIV infection, confirmed in 3 patients. All 4 cases were EBV$^+$ compared with 58% of HIV$^-$ cases, in agreement with EBV$^+$ AIDS-related pediatric lymphomas *vs.* 10% EBV$^+$ cases in immunocompetent BL from Argentina.[20] This differs from the 6/11 EBV$^+$, AIDS-BL from the USA.[21] The scarcity of pediatric AIDS-BL reported to date does not allow us to determine whether these differences reflect characteristics of populations at risk or socio-geographically determined pathogenic factors, mirroring epidemiological patterns of AIDS-non-related BL. However, the high frequency of EBV association in AIDS-related and -unrelated young children with BL in this study suggested that EBV might play a pathogenic role in the development of BL in Brazil.

**Table 2. Clinical characteristics of children with Burkitt's lymphoma.**

| *Primary presentation* | *N (%)* |
|---|---|
| Extranodal | 47 (89%) |
| Abdomen | 38 (72%) |
| L3-ALL | 6 (11%) |
| Head and neck | 1 ( 2% ) |
| CNS | 2 ( 4% ) |
| Bone | 1 ( 2% ) |
| Nodal | 6 (11%) |
| Stage | |
| I/II | 12 (25%) |
| III/IV | 36 (75%) |
| Site[2] | |
| Abdomen | 41 (77%) |
| BM | 12 (23%) |
| Lymph node | 8 (15%) |
| CNS | 5 (9%) |
| Bone | 2 (4%) |
| Orbit | 2 (4%) |
| Jaws | 1 (2%) |
| Subcutaneous soft tissues | 1 (2%) |

*[2]Twelve out of 53 patients (23%) showed more than one involved site. CNS: central nervous system; BM: bone marrow. Clinical staging was based on St. Jude's staging system.*

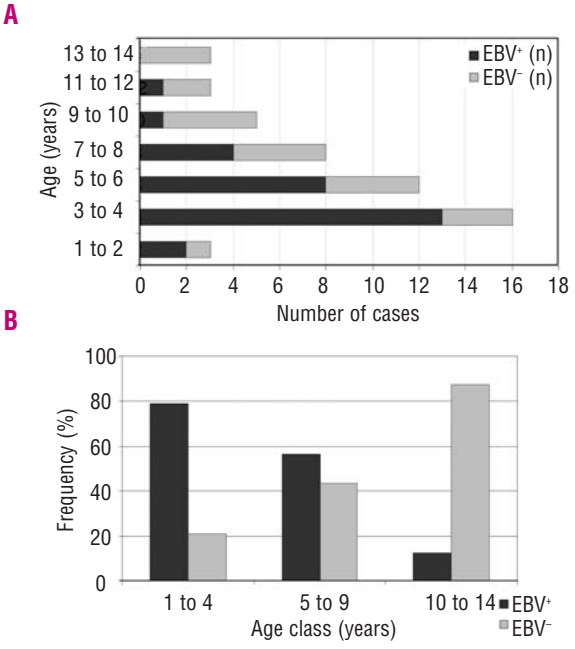


**Figure 1. Association of EBV with childhood Burkitt's lymphoma in Southeastern Brazil. (A) Age distribution of EBV$^+$ and EBV$^-$ children (number of cases). (B) Frequency of EBV association per age class. Cases include 50 non-AIDS-related Burkitt's lymphoma children.**

## Authorship and Disclosures

RH designed the study, performed ISH and developed molecular assays, analyzed data and wrote the manuscript. CEK took care of the patients, performed clinical review of the cases, and contributed to the interpretation of data and the final version of the manuscript. FEF, DMG and LRW performed ISH and molecular assays, interpreted data and contributed to the final version of the manuscript. MHMB was involved in histopathology and immunohistochemistry analyses. HNS and IZR contributed to the interpretation of data and reviewed the final version of the manuscript. All authors made a substantial contribution, reviewed and approved the final version of the manuscript. The other authors reported no potential conflicts of interest.

## References


1. Magrath IT, Jain V, Bathia K. Epstein-Barr virus and Burkitt's lymphoma. Semin Cancer Biol 1992;3:285-95.
2. Diebold J, Raphael M, Jaffe ES, Warnke RA. Burkitt lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization classification of tumours, pathology and genetics of haemopoietic and lymphoid tissues. Lyon: IARC Press, 2001.p. 1181-4.
3. Cardy AH, Sharp L, Little J. Burkitt's lymphoma: A review of the epidemiology. Kuwait Med J 2001;33:293-306.
4. Sandlund JT, Fonseca T, Leiming T, Verissimo L, Ribeiro R, Lira V, et al. Predominance and characteristics of Burkitt's lymphoma among children with non-Hodgkin lymphoma in northeastern Brazil. Leukemia 1997; 11:743-6.
5. Klumb CE, Hassan R, Elgui de Oliveira D, Magalhães de Resende LM, Carriço MK, Dobbin JA, et al. Geographic variation in EBV associated Burkitt's lymphoma in children from Brazil. Int J Cancer 2004; 108: 66-70.
6. Hassan R, White LR, Stefanoff CG, Elgui de Oliveira D, Felisbino FE, Klumb CE, et al. Epstein-Barr Virus (EBV) detection and typing by PCR: A contribution to diagnostic screening of EBV-positive Burkitt's lymphoma. Diagn Pathol 2006;1:17.
7. Guiretti DM, Chabay PA, Valva P, Stefanoff CG, Barros MHM, de Matteo E, et al. Structural variability in the carboxy-terminus of Epstein-Barr virus (EBV)- encoded latent membrane protein 1 gene in EBV-associated Hodgkin's lymphomas. J Med Virol 2007;79:1722-30.
8. Araujo I, Foss HD, Bittencourt A, Hummel M, Demel G, Mendonça N, et al. Expression of Epstein-Barr virus gene products in Burkitt's lymphoma in Northeast Brazil. Blood 1996;87: 5279-86.
9. Shapira J, Peylan-Ramu N. Burkitt's lymphoma. Oral Oncol 1998;34: 15-23.
10. Mwanda OW, Rochford R, Moormann AM, Macneil A, Whalen C, Wilson ML. Burkitt's lymphoma in Kenya: geographical, age, gender and ethnic distribution. East Afr Med J 2004;81:S68-77.
11. Çavdar AO, Gozdasoglu S, Yavuz G, Babacan E, Unal E, Uluoglu O, et al. Burkitt's lymphoma between African and American types in Turkish children: clinical, viral (EBV) and molecular studies. Med Pediatr Oncol 1993;21:36-42.
12. Anwar N, Kingma DW, Bloch AR, Mourad M, Raffeld M, Magrath I, et al. The investigation of Epstein-Barr viral sequences in 41 cases of Burkitt's lymphoma from Egypt. Epidemiologic considerations. Cancer 1995;76:1245-52.
13. Mansoor A, Stetler SM, Li RZ, Frekko K, Wiss W, Ahmed M, et al. Prevalence of Epstein-Barr sequences and EBV LMP1 oncogene deletions in Burkitt's lymphoma from Pakistan: Epidemiological correlations. Hum Pathol 1997;28:283-8.
14. Pearce MS, Parker L. Childhood cancer registrations in the developing world: still more boys than girls. Int J Cancer 2001;91:402-6.
15. Levine PH, Karamaju LS, Connelly RR, Berard CW, Dorfman RF, Magrath I, et al. The American Burkitt's lymphoma registry; eight years' experience. Cancer 1982;49:1016-22.
16. Mwanda OW. Clinical characteristics of Burkitt's lymphoma seen in Kenyan patients. East Afr Med J 2004;81:S78-88.
17. Cheng W-G, Chen Y-Y, Bacchi MM, Bacchi CE, Alvarenga M, Weiss LM. Genotyping of Epstein-Barr virus in Brazilian Burkitt's lymphoma and reactive lymphoid tissue. Type A with a high prevalence of deletions within the latent membrane protein gene. Am J Pathol 1996;148:17-23.
18. de Thé G, Geser A, Day NE, Tukei PM, Williams EH, Beri DP, et al. Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma for Ugandan prospective study. Nature 1978;274:756-61.
19. Figueira-Silva CM, Pereira FEL. Prevalence of Epstein-Barr virus antibodies in healthy children and adolescents in Vitória, State of Espírito Santo, Brazil. Rev Soc Bras Med Trop 2004;37:409-12.
20. Chabay P, De Matteo E, Maglio S, Grinstein S, Preciado MV. Assessment of Epstein-Barr virus association with pediatric Non-Hodgkin lymphoma in immunocompetent and immunocompromised patients in Argentina. Arch Pathol Lab Med 2002;126:331-5.
21. McClain KL, Leach CT, Jenson HB, Joshi VV, Pollock BH, Hutchison RE, et al. Molecular and virologic characteristics of lymphoid malignancies in children with AIDS. JAIDS J Acq Imm Def Synd 2000;23:152-9.