5. Adamson PC, Balis FM, Miser J, *et al.* Pediatric phase I trial, pharmacokinetic study and limited sampling strategy for Piritrexim administered on a low-dose, intermittent schedule. *Cancer Res* 1992, 52, 521–524.
6. Vokes EE, Dimery IW, Jacobs Ch D, *et al.* A phase II of Piritrexim in combination with Methotrexate in recurrent and metastatic head and neck cancer. *Cancer* 1991, 67, 2253–2257.
7. Uen WC, Huang AT, Mennel R, *et al.* A phase II study of Piritrexim in patients with advanced squamous head and neck cancer. *Cancer* 1992, 69, 1008–1011.
8. Miller AB, Hoogstraten B, Staquet M, *et al.* Reporting results of cancer treatment. *Cancer* 1981, 47, 207–214.

*European Journal of Cancer* Vol. 30A, No. 7, pp. 1045–1046, 1994.
Elsevier Science Ltd
Printed in Great Britain
0959–8049/94 $7.00 + 0.00

0959-8059(94)E0152-T

# Prognostic Significance of Epstein-Barr Virus Association in Hodgkin's Disease

**A.A. Armstrong, A. Lennard, F.E. Alexander, B. Angus, S.J. Proctor, D.E. Onions and R.F. Jarrett**

THERE IS now strong evidence to suggest that Epstein-Barr virus (EBV) is involved in a proportion of cases of Hodgkin's disease (HD) [1–4]. Mixed cellularity HD (HDMC) is more likely to be EBV-positive compared with nodular sclerosis HD (HDNS), and, in addition, our data suggest that paediatric and older cases are more likely to be EBV-positive than young adult cases [2–4].

Despite improvements in the treatment of HD, there are still a number of cases with poor clinical outcome. In order to identify these cases and initiate alternative treatment regimes early in disease, we have previously devised a prognostic index. Since older age and HDMC as compared to HDNS have been associated with poor prognosis [6], we assessed the relationship between EBV-positivity and clinical outcome. The use of EBV-positivity in the prognostic index was also evaluated.

Paraffin-embedded sections from 59 HD cases (35 males, 24 females) diagnosed over a 13-year period (1976–1989) were investigated for the presence of EBV using immunohistochemical and *in situ* methods, as described previously [7, 8]. Cases of HD were considered EBV-associated if Reed-Sternberg (RS) cells expressed the EBV LMP-1 protein or EBER-1 RNA. We have found EBER-1 RNA *in situ* hybridisation to be the most reliable method of detecting EBV in HD tumours [7]. The series included 27 HDNS, 28 HDMC, 3 lymphocyte-predominant HD cases and 1 case of lymphocyte-depleted HD. 16 patients had stage I, 13 stage II, 19 stage III and 11 stage IV disease. The prognostic indices were calculated on prospectively collected data, but do not include additional weighting for bulk disease, as this information was not available. Early stage disease was treated with radiotherapy alone. Later stage or bulky disease was treated with a four-drug regimen (chlorambucil, vinblastine, procarbazine and prednisolone), plus or minus radiotherapy. Minimum follow-up is 48 months.

21 cases were categorised as EBV-associated. The age distribution and histological subtype of the EBV-associated cases were in agreement with those of previous studies [2–4]. Using standard survival analysis techniques, there was no evidence of an association between EBV status and survival (hazard ratio = 0.56, 95% confidence limits 0.11–2.68) (Figure 1a, b).

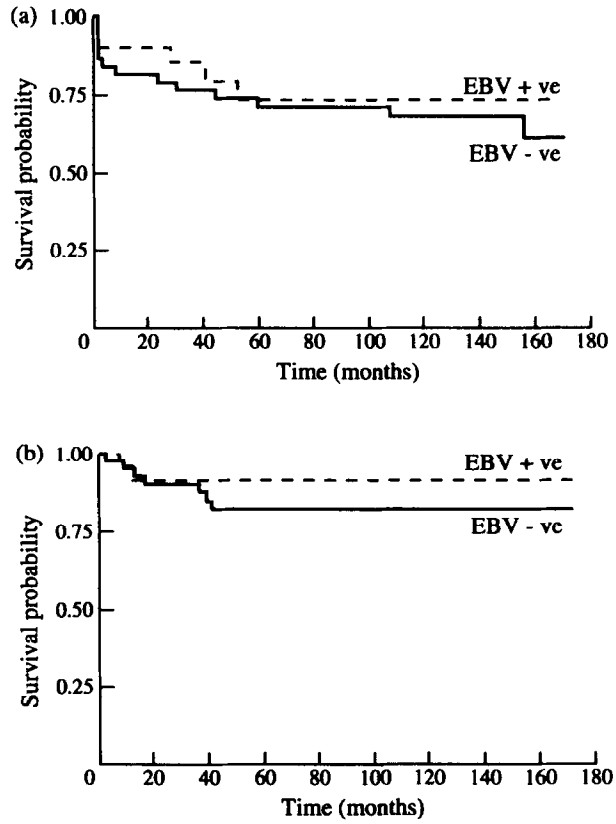


**Figure 1. (a) Disease-free survival by EBV status. (b) Total survival by EBV status.**

This observation applies to unadjusted analyses of the entire data set and was confirmed by analyses stratified by age and by age plus histological subtype. In addition, EBV status did not provide an improvement to the prognostic index in explaining survival. Our results are consistent with two recent studies which investigated the association between EBV status and clinical outcome, using immunohistochemical techniques and the polymerase chain reaction, respectively [9, 10].

This study provides no evidence to support the hypothesis that EBV-associated cases of HD have a less favourable clinical outcome than EBV-negative cases. We can conclude that the detection of EBV within RS cells in individual HD cases is not a clinically useful prognostic marker.

Correspondence to A.A. Armstrong
A.A. Armstrong , D.E. Onions and R.F. Jarrett are at the LRF Virus Centre, Department of Veterinary Pathology, University of Glasgow, Bearsden Road, Glasgow G61 1QH; A. Lennard and S. J. Proctor are at the Department of Haematology, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4PL; F.E. Alexander is at the Department of Public Health Sciences, University of Edinburgh Medical School, Tewiot Place, Edinburgh EH8 9AG; and B. Angus is at the Department of Pathology, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP, U.K.
Revised 28 Feb. 1994; accepted 28 Mar. 1994.

1. Weiss LM, Strickler JG, Warnke RA, Purtillo DT, Sklar J. Epstein–Barr viral DNA in tissues of Hodgkin's disease. *Am J Pathol* 1987, **129**, 86–91.
2. Pallesen G, Hamilton-Dutoit SJ, Rowe M, Young LS. Expression of Epstein–Barr virus latent gene products in tumour cells of Hodgkin's disease. *Lancet* 1991, **337**, 320–322.
3. Jarrett RF, Gallagher A, Jones DB, *et al.* Detection of Epstein–Barr virus genomes in Hodgkin's disease: relation to age. *J Clin Pathol* 1991, **44**, 844–848.
4. Delsol G, Brousset P, Chittal S, Rigal-Huguet F. Correlation of the expression of Epstein–Barr virus latent membrane protein and *in situ* hybridisation with biotinylated BamH1-W probes in Hodgkin's disease. *Am J Pathol* 1992, **140**, 247–253.
5. Proctor SJ, Taylor P, Donnan P, Boys R, Lennard A, Prescott RJ. A numerical prognostic index for clinical use in identification of poor-risk patients with Hodgkin's disease at diagnosis. *Eur J Cancer* 1991, **27**, 624–629.
6. Walker A, Schoenfeld ER, Lowan JT, Mettlin CJ, MacMillan J, Grufferman S. Survival of the older patient compared to the younger patient with Hodgkin's disease. *Cancer* 1990, **65**, 1635–1640.
7. Armstrong AA, Weiss LM, Gallagher A, *et al.* Criteria for the definition of Epstein–Barr virus association in Hodgkin's disease. *Leukemia* 1992, **6**, 869–874.
8. Armstrong AA, Gallagher A, Krajewski AS, *et al.* The expression of the EBV latent membrane protein (LMP-1) is independent of CD23 and *bcl-2* in Reed-Sternberg cells in Hodgkin's disease. *Histopathology* 1992, **21**, 72–73.
9. Vestlev PM, Pallesen G, Sandvej K, Hamilton-Dutoit SJ, Bendtzen SM. Prognosis of Hodgkin's disease is not influenced by Epstein–Barr virus latent membrane protein. *Int J Cancer* 1992, **50**, 670–671.
10. Fellbaum C, Hansmann M-L, Niedermeyer H, *et al.* Influence of Epstein–Barr virus genomes on patient survival in Hodgkin's disease. *Am J Pathol* 1992, **98**, 319–323.