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Comparison of Epstein-Barr virus copy number in white blood cells of chronic lymphocytic leukemia patients with laboratory prognostic biomarker

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Abstract

Background and objective The DNA load of EBV may play a part in CLL pathogenesis and prognosis. The objective of this cross-sectional study was to examine the prognostic value of EBV viral load in CLL patients in comparison with other common laboratory prognostic factors.

Materials and methods Whole blood and sera from forty untreated CLL patients were collected. Next, DNA was extracted from total white blood cells (WBC), and TaqMan real-time PCR was performed to determine the EBV-DNA load by amplifying a specific fragment in the *BNRF1* gene. In addition, parameters such as complete blood counts (CBC) and lactate dehydrogenase (LDH) were determined using an automated clinical laboratory analyzer.

Results Twenty-one patients (52.5%) were positive for EBV by real-time PCR analysis (ranged 20 to 30000 copies/μL). The difference in LDH mean levels between EBV positive and negative patients was marginally significant ($P = 0.05$). Furthermore, platelet (PLT) count ($P = 0.03$) and $CD5^{+}/CD19^{+}$ count ($P = 0.04$), between EBV positive and negative subgroups, were substantially different. In addition, individuals with a severe form of illness, as defined by an increase in LDH, a decrease in PLT, and an 11q deletion, had considerably higher EBV-DNA copy numbers (the ranges of viral loads were 9966.66 ± 20033 in the severe form vs. 137.13 ± 245.41 in the mild form).

Conclusion The EBV-DNA load could be used as a prognostic factor in the initial examination of CLL patients to better characterize the disease outcome and prognosis.

Keywords Chronic lymphocytic leukemia, Epstein-Barr virus, Viral load, Prognostic factor

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Introduction

Leukemia is the world's fifth-highest, and Iran's second-highest mortality rate among malignant tumors [1]. In 2018, more than 437,000 people worldwide were diagnosed with leukemia, with 309,000 fatalities [2]. Furthermore, in 2017, there were 114,000 cases of chronic lymphocytic leukemia (CLL) worldwide [3].

CLL is the most prevalent type of adult leukemia, characterized by clonal proliferation and an accumulation of abnormal mature B-cells in the blood, bone marrow, and secondary lymphatic organs [4, 5]. The appearance of monoclonal long-lived mature B-cells expressing CD5, CD19, and CD23 markers characterizes it [6].

CLL mostly affects the elderly, and the incidence rate rises rapidly beyond 50 years of age [4, 7]. Because the older population is continually growing, the total CLL incidence trend will continue to rise [4, 8]. The variable clinical course of CLL is one of its most notable features [4, 5]. As a result, rising incidence rates, a heterogeneous presentation of the disease, as well as side effects associated with existing treatments and the financial burden of treatment expenditures on the health system of the country, highlight the importance of looking for accurate prognostic criteria [9–11]. Prognostic factors will be highly valuable in CLL patients to distinguish those with a poor prognosis from those with a good prognosis [12, 13]. In this regard, several CLL prognostic markers have been discovered over the last two decades. In addition to the Binet and Rai staging systems, age, gender, hemoglobin level, absolute lymphocyte count, lactate dehydrogenase (LDH) serum level, Beta-2 microglobulin, thymidine kinase, Zeta-chain-associated protein kinase 70 (ZAP70), CD38, un-mutated immunoglobulin heavy chain variable region (IGHV), and specific chromosomal abnormalities were all used to determine the prognosis of CLL patients [7, 12–17].

Attempts to find the relationship between CLL and EBV, the first human oncovirus discovered, have accelerated in recent years [18–23]. EBV is the most common persistent viral infection in humans, infecting over 95% of the world population asymptomatically throughout the rest of their lives [24, 25]. This form of persistence is due to the particular adaption of EBV to B cells, as any disruption in this delicate process would result in cancers like Burkitt's lymphoma, Hodgkin's and non-Hodgkin's lymphoma, and lymphoproliferative disorders after transplantation [26–29].

In EBV-related malignancies such nasopharyngeal carcinoma and Hodgkin's lymphoma, EBV viral load is crucial in diagnosis, prognosis, and monitoring [30–33]. Furthermore, new studies have highlighted the significance of EBV-DNA load in the prognosis and outcome of some CLL patients [12, 18, 21, 23, 34]. The use of peripheral blood mononuclear cells (PBMC) rather than plasma or serum has been found to increase the sensitivity and accuracy of EBV-DNA detection. Also, EBV infection in a different type of white blood cell prompted us to use buffy coat, "a mixture of all WBC", for EBV copy number measurement [20, 23].

Therefore, the aim of this study was to compare the value of EBV viral load in CLL patients' WBCs for predicting prognosis in comparison to other laboratory tests that are commonly used prognostic markers.

Methods

Patients

Forty untreated CLL patients were recruited for this cross-sectional investigation, from April 2019 to May 2020. Clinical records, cell morphological assessment, and immunophenotypic assays were all used to confirm a definitive diagnosis of CLL by expert hemato-oncologists based on National Comprehensive Cancer Network Guideline, Version 2.2019. Before the study, all patients gave their written informed consent. Shiraz University of Medical Sciences Ethics Committee approved the project (IR.SUMS.REC.1398.405).

Blood sampling

Seven milliliters of peripheral blood was collected from each participant and split into EDTA-treated tubes (2 mL), as well as a tube containing a clot activator and a gel vacuum blood collecting tube (5 mL). An anti-coagulated sample was used for the CBC test. The buffy coat was also isolated by centrifugation at 1500 g for 15 min, aliquoted 30×10^6 cells, and kept at −20 °C until DNA extraction. In addition, the serum was extracted to evaluate the level of LDH.

Complete blood count assessment

CBC indexes were measured for all subjects immediately after sample collection using a Sysmex KX-21 N automated hematology analyzer (Sysmex Corporation, Kobe, Japan) to estimate hematological parameters that had already been accepted as prognostic factors. The device reports about 19 parameters, but we selected only those parameters that have been previously reported to be related to patient outcomes.

LDH measurement

All samples were analyzed twice on the Hitachi 917 automated analyzers for serum LDH levels using the Pars Azmoon kit (Pars Azmoon Co., Tehran, Iran) (Hitachi, Tokyo, Japan). In the method of this kit, lactate dehydrogenase catalyzes the conversion of pyruvate to lactate, and in this process NADH is oxidized to NAD^+. The rate of reduction of NADH cofactor is proportional to the activity of lactate dehydrogenase enzyme.

DNA extraction

The nucleic acids from a fixed number of WBCs (30×10^6) were extracted using a Roche High Pure viral nucleic acid kit (Manheim, Germany) according to the manufacturer's procedure. The NanoDropTM 2000 spectrophotometer was used to evaluate the concentration and purity of the isolated viral DNA (Thermo Scientific Inc., Waltham, MA, USA).

Determination of the EBV copy number by real-time PCR

To measure the EBV viral load, the TaqManreal-time PCR test was used with the EBV Primer DesignTM genesing standard Kit (PrimerDesign TM, London, UK). Based on comprehensive bioinformatics analysis, the primers and FAM-labeled probe sequences which targeted the *BNRF1* gene had 100% homology with a broad range of clinically relevant sequences.

The final volume of each reaction was 20 µL, which included 10 µL of 2× one-step master mixes (Applied Biosystem, USA), 1 µL of EBV primer/probe mix (FAM-labeled, BHQ quenched), 4 µL of RNase/DNase free water, and 5 µL of DNA template (nucleic acids extracted from a fixed number of WBCs from each sample). The cycling condition consisted of a 2-minute early denaturation stage at 95 °C, followed by 35 cycles of 95 °C for 5 s and 60 °C for both annealing and elongation, each lasting 20 s. The Rotor-Gene 6000 was used to do real-time PCR (Rotorgene-Q, Hilden, Germany). EBV-DNA copy numbers were calculated using the provided standard curve. The positive (total DNA from NC-37 cell line, with a certain average of EBV copy numbers) and negative controls were included in each run. As the sensitivity of the system was 10 copies/µL, all samples with the EBV-DNA copy number lower than the threshold were considered negative (EBV (-)).

Statistical analysis

Statistical analysis was performed using SPSS 23. The normal distribution of continuous variables was tested using the Shapiro-Wilk test. The student t-test and the Mann-Whitney U-test were used for intergroup comparisons of independent variables. The association between EBV-DNA loads and discrete variables was analyzed using Chi-square or Fisher exact test. The power and direction of the relationships between pairs of variables were determined based on the values of Spearman's coefficient of rank correlation (R). P-values less than 0.05 were considered statistically significant.

Table 1 Comparison between clinical and biological variables in CLL EBV (+) patients, CLL EBV (-)

Clinical and biological variables	EBV-DNA (+) patients Mean ± SD	EBV-DNA(-) patients Mean ± SD	*P*-value
Age	62.67 ± 2.25	61.79 ± 2.25	0.79
White blood cell count($\times 10^9$/L)	46.21 ± 10.92	45.90 ± 11.34	0.98
Absolute lymphocyte count($\times 10^9$/L)	38.39 ± 10.29	32.55 ± 8.04	0.96
Hemoglobin (g/dL)	14.15 ± 1.91	13.25 ± 2.49	0.20
Platelets count($\times 10^9$/L)	150.37 ± 15.83	193.38 ± 8.04	**0.03**
Lactate dehydrogenaseserum level (U/L)	347.38 ± 25.84	284.47 ± 16.95	**0.05**
$CD5^+/CD19^+$	65.17 ± 18.06	82.64 ± 9.18	**0.04**

Results

Demographic and clinico-pathological characteristics of CLL patients

Among 40 CLL patients, 12 (30%) were female and 28 (70%) were male. The mean age of the patients was 62.25 ± 10.49 years old, with a range of 42 to 77 years.

CBC

The analysis of CBC indexes (Table 1) indicated that WBCs count ranged from 3.8 to 215.92×10^9/L (mean $46.06\pm49.1\times10^9$/L). The absolute lymphocyte count (ALC) of the patients ranged from 0.9 to 198.64×10^9/L (mean $35.62\pm41.44\times10^9$/L). The platelet counts of the patients ranged from 44 to 327×10^9/L, with a mean value of $172.95\pm65.07\times10^9$/L. Hemoglobin concentration of the patients ranged from 7.2 g/dL to 17.10 g/dL with a mean value of 13.72 ± 2.22 g/dL.

LDH

The LDH serum level of the patients ranged from 161 to 599 U/L with a mean value of 317.5 ± 103.56 U/L.

CD38, FISH CLL panel and CD5+/CD19 + cells percentage

The results of CD38 expression, FISH CLL panel, and the percentage of malignant cells ($CD5^+/CD19^+$) were obtained from the patients' medical records. CD38 expression was determined only in 22 patients. Among these patients, 7 (31.82%) were positive and 15 (68.18%) were negative for this marker. Deletion of 11q and 17p was detected in 3 (7.5%) and 4 (10%) patients, respectively. The percentage of $CD5^+/CD19^+$ malignant cells was determined in 31 patients, ranging from 30 to 96.35% (mean = 73.06% ±16.99).

EBV-DNA copy number in patients with CLL

The results of real-time PCR showed that 21 patients (52.5%) were positive for EBV DNA with a median level of 3294.29 ± 1951.42 copies /µL (ranged 20 to 30000 copies/µL). The EBV- positive subgroup included 7(33.3%) females and 14(66.7%) males and the EBV-negative subgroup consisted of 5 (26.3%) females and 14 (73.7%) males, which was not significantly different (*P* = 0.62). The mean age of the EBV (+) and EBV (-) patients subgroup was respectively 62.67 ± 10.35 (ranged 42 to 77

years) and 61.79±10.91 (ranged 42 to 76 years) years old, with no significant difference ($P=0.79$). In comparison of LDH, PLT count, malignant cells ($CD5^+/CD19^+$), deletion of 11q in EBV (+) and EBV(-) subgroups, the EBV (+) subgroup had higher LDH mean level ($P=0.05$) but lower PLT count ($P=0.03$)(Table 1). Also, the percentage of malignant cells ($CD5^+/CD19^+$) was significantly lower in the EBV (+) subgroup ($P=0.04$), which showed a negative correlation with EBV copy numbers (P: 0.001, R: 0.58) (Table 2). Furthermore, the EBV (+) subgroup had a higher frequency of 11q deletion than EBV (-) subgroup insignificantly ($P=0.23$) (Table 3). In addition, individuals with a severe form of illness, as defined by an increase in LDH, a decrease in PLT, and an 11q deletion, had considerably higher EBV-DNA copy numbers (the ranges of viral loads were 9966.66±20033 in the severe form vs.137.13±245.41 in the mild form).

Table 2 Correlation between EBV copy numbers and prognostic factor

Clinical and biological variables	P-value	R
Age	0.64	0.07
White blood cell count(× 10^9/L)	0.72	0.05
Absolute lymphocyte count (× 10^9/L)	0.58	0.09
$CD5^+/CD19^+$	**0.001**	**-0.58**
Hemoglobin (g/dL)	0.46	0.11

Table 3 Association between EBV load and prognostic factor

Clinical and biological variables	EBV-DNA (+) patients	EBV-DNA (-) patients	P-value
Gender Female **OR** Male	7 (33.3%)/14 (66.7%)	5(26.3%)/14 (73.7%)	0.62
CD38status Positive **OR** negative	3 (30.0%)/7 (70.0%)	4(33.3%)/8(66.7%)	1.0
Platelets count $<150\times10^9$/L **OR** $>150\times10^9$/L	14 (66.7%)/7 (33.3%)	6 (31.6%)/13 (68.4%)	**0.02**
Deletion of 11q Positive **OR** Negative	3 (14.3%)/18 (85.7%)	0 (0%)/19 (100%)	0.23

Discussion

Because of the rising incidence rate, the heterogenic character of CLL, the adverse side effects of available medicines, and the burden on the health system of the country, developing effective prognostic markers are more essential than ever [12, 13]. Identification of patients with a poor prognosis, as well as an early diagnosis at the time of treatment, could be particularly valuable.

Our findings revealed that 30% of CLL patients were females and 70% were males, which was compatible with the global frequency (male/female ratio ~ 2). Our participants' mean age was 61.45 years (range 42–77 years), which was lower than the 72 years reported in the literature [4].

Analysis of the National Cancer Institute Surveillance and Epidemiology End Results (SEER) database indicated that 11% of CLL patients diagnosed in 2009 were younger than 55 years old [35]. Studies conducted in Europe have also shown that between 7% and 20% of CLL patients are in this age range at the time of diagnosis [36]. Young patients diagnosed with chronic lymphocytic leukemia exhibit a longer survival rate compared to individuals aged 55 years and above [37].

Based on the current findings, 21 CLL patients (52.5%) tested positive for EBV. In this regard, Visco et al. reported that 59% of Italian CLL patients were EBV positive [18]. Furthermore, according to Grywalska et al., 53.91% of Polish CLL patients were positive for EBV [23]. In a related study from Iran, it was found that 41.6% of CLL patients had detectable EBV-DNA in their PBMCs [38]. Furthermore, an investigation from the United States revealed that 38% of CLL patients had EBV-encoded small RNA1 (EBV-EBER1) by using in- situ hybridization [20]. The reported results may be influenced by the type of selected sample (PBMCs, buffy coat, etc.) and the method (in-situ hybridization, real-time PCR, etc.) used to detect the presence of EBV.

On the other hand, Liang et al. reported that only 10% of Chinese CLL patients had EBV-DNA positive [34]. It seems that in this study the whole samples were used for DNA extraction. Furthermore, a study from the United States found that 14% of CLL patients were positive for EBV-encoded latent membrane protein 1 (LMP-1) mRNA transcript [22]. This discrepancy is most likely due to differences in techniques, specimen type, reporting unit, lack of an agreed-upon calibrator, and an EBV detection cut-off [18]. Furthermore, since EBV has lytic and latent replication cycles with distinct gene expression patterns, these controversies could be caused by differing phase and protein detection [34]. Although latent infection plays an important role in EBV carcinogenesis, various findings clarify the role of lytic infection in EBV-associated cancers [39]. In this regard, the significance of lytic infection, which is associated with high viral load, has been reported in EBV-induced cancers [39]. Also, a study conducted in Uganda revealed that children who later developed Burkitt lymphoma (BL) exhibited elevated antibody levels against EBV viral capsid antigen (VCA), indicating a higher level of lytic infection during cancer development [40, 41]. Thus, different results reported by various studies might be related to the methods used.

Furthermore, we found that a positive EBV-DNA test was significantly associated with some poor prognostic clinical and biological biomarkers. Our findings revealed a marginally significant difference in the mean serum LDH concentration between EBV- positive and negative

subgroups; the EBV-positive patients had a higher LDH mean level. Additionally, some patients in the EBV-positive subgroup in comparison with the EBV-negative subgroup had insignificantly abnormal serum LDH concentration. Grywalska et al. and Tsimberidou et al. reported that positive patients for EBV tended to have higher LDH levels [23, 42]. As elevated serum LDH level was consistently identified as a poor prognostic factor for CLL patients [43], according to our results, EBV(+) patients might have a poorer prognosis than EBV(-) ones. On the other hand, Liang et al. found no correlation between the LDH concentration and EBV load [34].

As to the PLT count, our results showed that EBV DNA load was associated with a decreased PLT count, suggesting that EBV-positive individuals had thrombocytopenia (PLT > 150). Additionally, some patients in the EBV-positive subgroup in comparison with the EBV-negative subgroup had a significantly abnormal PLT count. In the same line, Grywalska et al. reported that EBV load was significantly higher in patients with thrombocytopenia. Moreover, slight to severe platelet decrease was reported in EBV-associated infectious mononucleosis and chronic active EBV disease (CAEBV) [44, 45].

It was also observed that EBV-positive patients had a lower percentage of malignant B-cells (CD5+ / CD19+) than EBV-negative patients. The amount of EBV load in malignant B-cells was negatively correlated with the percentage of malignant B-cells. This data showed that only a small proportion of malignant cells had EBV-DNA, supporting Tsimberidou et al.'s findings [20].

Based on our finding, in comparison with the EBV (-) subgroup, some patients in the EBV (+) subgroup had insignificant 11q deletion. Furthermore, as noted in earlier studies conducted by Visco et al. and Grywalska et al., a notable correlation was found between elevated EBV load (>2000copy/L) and the existence of 11q deletion encompassing the ATM locus, which is considered a negative prognostic chromosomal anomaly [18, 23]. However, similar to other studies, there was no significant association between older age, gender, leukocytosis, and EBV-DNA positivity [18, 23]. In addition, in our study, individuals with a severe form of illness, as defined by an increase in LDH, a decrease in PLT, and an 11q deletion, had considerably higher EBV-DNA copy numbers (the ranges of viral loads were 9966.66 ± 20033 in the severe form vs.137.13 ± 245.41 in the mild form).

Although no research has considered an association between the WBC count and EBV load, an equal count of WBC for all extractions was used to normalize the process.

Based on our current understanding, none of the few studies that have examined the EBV levels in CLL patients has utilized the buffy coat for assessing EBV load. The convenience and cost-effectiveness of utilizing the buffy coat could increase the satisfaction of new prognostic markers like EBV-DNA load. The small sample size and lack of follow-up of patients due to the short period of the investigation could be considered as limitations of the study. Also, small sample size as well as use of buffy coat as the sample could be the limitation of this study that should be overcome in future studies by working on B cells of many samples. This study has several limitations, including a small sample size, lake of long-term follow-up of patients as well as lack of diversity in the sample population that would be overcome in future studies.

Conclusion

The results showed that the EBV-related CLL subtype was associated with unfavorable prognostic characteristics, thrombocytopenia. Moreover, EBV DNA load might be included in the first assessment of patients to accurately represent and predict their prognosis and outcome. More research is necessary to confirm these results.

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Author contributions

The aim of this study was to Evaluation of white blood cells based on Epstein-Barr virus copy numbers in chronic lymphocytic leukemia patients as a new prognostic biomarker. Conceptand design: Azhdari F and Sarvari J; patients' selection: Haghighat S; Benchwork: Azhdari Fand Jamalidoust M; Data analysis; Azhdari F, Jamalidoust M and Hashemi SMA. Manuscriptdrafting: Azhdari F; Critical revision of the manuscript: Sarvari J, Hosseini SY, Zahra Faghih andHashemi SMA, Jamalidoust M.

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Data availability

The analyzed data sets generated during the study are available as supplementary information file.

Declarations

Ethics approval

The study was approved by the local Ethics Committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1398.405). Written informed consent was obtained from participants.

Competing interests

The authors declare no competing interests.

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References

1. Saffar A, Rahgozar M, Shahi F, Biglarian A. Survival analysis of acute myeloid leukemia. Razi J Med Sci. 2015;22(134):41–8.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Cancer J Clin. 2018;68(6):394–424.
3. Collaboration GBoDC. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 29 cancer groups, 1990 to 2017: a systematic analysis for the global burden of disease study. JAMA Oncol. 2019;5(12):1749–68.
4. Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. Am J Hematol. 2019;94(11):1266–87.
5. Milne K, Sturrock B, Chevassut T. Chronic lymphocytic leukaemia in 2020: the future has arrived. Curr Oncol Rep. 2020;22:1–9.
6. Falay M, Özet G. Immunophenotyping of chronic lymphocytic leukemia. Clin Lab. 2017;63(10):1621–6.
7. Kipps TJ, Stevenson FK, Wu CJ, Croce CM, Packham G, Wierda WG, et al. Chronic lymphocytic leukaemia. Nat Reviews Disease Primers. 2017;3(1):1–22.
8. Hao T, Li-Talley M, Buck A, Chen W. An emerging trend of rapid increase of leukemia but not all cancers in the aging population in the United States. Sci Rep. 2019;9(1):12070.
9. Chen Q, Jain N, Ayer T, Wierda WG, Flowers CR, O'Brien SM, et al. Economic burden of chronic lymphocytic leukemia in the era of oral targeted therapies in the United States. J Clin Oncol. 2017;35(2):166.
10. Allan JN. The clinical importance of prognostic markers in CLL. Clin Adv Hematol Oncology: H&O. 2022;20(10):606–8.
11. Ivanescu A, Oprea M, Momanu R, Coles E, Colita A, Lupu A. Prognosis factors in chronic lymphocytic leukemia. J Med Life. 2012;5(Spec Issue):48.
12. Furman RR. Prognostic markers and stratification of chronic lymphocytic leukemia. Hematology 2010. Am Soc Hematol Educ Program Book. 2010;2010(1):77–81.
13. Chen C, Puvvada S. Prognostic factors for chronic lymphocytic leukemia. Curr Hematol Malig Rep. 2016;11(1):37–42.
14. Hus I, Podhorecka M, Bojarska-Junak A, Roliński J, Schmitt M, Sieklucka M, et al. The clinical significance of ZAP-70 and CD38 expression in B-cell chronic lymphocytic leukaemia. Ann Oncol. 2006;17(4):683–90.
15. Zenz T, Döhner H, Stilgenbauer S. Genetics and risk-stratified approach to therapy in chronic lymphocytic leukemia. Best Pract Res Clin Haematol. 2007;20(3):439–53.
16. Dürig J, Nückel H, Cremer M, Führer A, Halfmeyer K, Fandrey J, et al. ZAP-70 expression is a prognostic factor in chronic lymphocytic leukemia. Leukemia. 2003;17(12):2426–34.
17. Rassenti LZ, Jain S, Keating MJ, Wierda WG, Grever MR, Byrd JC, et al. Relative value of ZAP-70, CD38, and immunoglobulin mutation status in predicting aggressive disease in chronic lymphocytic leukemia. Blood J Am Soc Hematol. 2008;112(5):1923–30.
18. Visco C, Falisi E, Young KH, Pascarella M, Perbellini O, Carli G, et al. Epstein-Barr virus DNA load in chronic lymphocytic leukemia is an independent predictor of clinical course and survival. Oncotarget. 2015;6(21):18653.
19. Rolinski J, Grywalska E, Bojarska-Junak A, Grabarczyk P. EBV infection leads to activation of chronic lymphocytic leukemia (CLL) cells and worsen clinical prognosis in CLL patients. Front Immunol. 2013. Conference Abstract: 15th International Congress of Immunology (ICI). https://doi.org/10.3389/conf.fimmu.2013.02.01073.
20. Tsimberidou A-M, Keating MJ, Bueso-Ramos CE, Kurzrock R. Epstein-Barr virus in patients with chronic lymphocytic leukemia: a pilot study. Leuk Lymphoma. 2006;47(5):827–36.
21. Dolcetti R, Carbone A. Epstein-Barr virus infection and chronic lymphocytic leukemia: a possible progression factor? Infect Agents cancer. 2010;5(1):1–5.
22. Tarrand JJ, Keating MJ, Tsimberidou AM, O'Brien S, LaSala RP, Han XY, Bueso-Ramos CE. Epstein-Barr virus latent membrane protein 1 mRNA is expressed in a significant proportion of patients with chronic lymphocytic leukemia. Cancer: Interdisciplinary Int J Am Cancer Soc. 2010;116(4):880–7.
23. Grywalska E, Roliński J, Pasiarski M, Korona-Glowniak I, Maj M, Surdacka A, et al. High viral loads of Epstein-Barr virus DNA in peripheral blood of patients with chronic lymphocytic leukemia associated with unfavorable prognosis. PLoS ONE. 2015;10(10):e0140178.
24. Cohen JI. Epstein–Barr virus infection. N Engl J Med. 2000;343(7):481–92.
25. Hardie DR. Human γ-herpesviruses: a review of 2 divergent paths to oncogenesis. Transfus Apheres Sci. 2010;42(2):177–83.
26. Saha A, Robertson ES. Mechanisms of B-cell oncogenesis induced by Epstein-Barr virus. J Virol. 2019;93(13):e00238–19.
27. Young LS, Rickinson AB. Epstein–Barr virus: 40 years on. Nat Rev Cancer. 2004;4(10):757–68.
28. Casabonne D, Benavente Y, Robles C, Costas L, Alonso E, Gonzalez-Barca E, et al. Aberrant Epstein-Barr virus antibody patterns and chronic lymphocytic leukemia in a Spanish multicentric case-control study. Infect Agents Cancer. 2015;10(1):1–7.
29. Young LS, Yap LF, Murray PG. Epstein–Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer. 2016;16(12):789–802.
30. Baumforth K, Young L, Flavell K, Constandinou C, Murray P. The Epstein-Barr virus and its association with human cancers. Mol Pathol. 1999;52(6):307.
31. Hohaus S, Santangelo R, Giachelia M, Vannata B, Massini G, Cuccaro A, et al. The viral load of Epstein–Barr virus (EBV) DNA in peripheral blood predicts for biological and clinical characteristics in Hodgkin lymphoma. Clin Cancer Res. 2011;17(9):2885–92.
32. Li W, Chen J, Liang B, Li Z, Li J, Yuan X, et al. Long-term monitoring of dynamic changes in plasma EBV DNA for improved prognosis prediction of nasopharyngeal carcinoma. Cancer Med. 2021;10(3):883–94.
33. Li W-F, Zhang Y, Huang X-B, Du X-J, Tang L-L, Chen L, et al. Prognostic value of plasma Epstein–Barr virus DNA level during posttreatment follow-up in the patients with nasopharyngeal carcinoma having undergone intensity-modulated radiotherapy. Chin J cancer. 2017;36:1–9.
34. Liang J-H, Gao R, Xia Y, Gale RP, Chen R-Z, Yang Y-Q, et al. Prognostic impact of Epstein-Barr virus (EBV)-DNA copy number at diagnosis in chronic lymphocytic leukemia. Oncotarget. 2016;7(2):2135.
35. Ferrajoli A. Treatment of younger patients with chronic lymphocytic leukemia. Hematology 2010. Am Soc Hematol Educ Program Book. 2010;2010(1):82–9.
36. Abrisqueta P, Pereira A, Rozman C, Aymerich M, Giné E, Moreno C, et al. Improving survival in patients with chronic lymphocytic leukemia (1980–2008): the Hospital Clinic of Barcelona experience. Blood. J Am Soc Hematol. 2009;114(10):2044–50.
37. Parikh SA, Rabe KG, Kay NE, Call TG, Ding W, Schwager SM, et al. Chronic lymphocytic leukemia in young (≤ 55 years) patients: a comprehensive analysis of prognostic factors and outcomes. Haematologica. 2014;99(1):140.
38. Tabibzadeh A, Niya MHK, Esghaei M, Bokharaei-Salim F, Ataei-Pirkooh A, Kiani SJ, Monavari SHR. Molecular epidemiology of epstein-barr virus (ebv) in patients with hematologic malignancies. Asian Pac J Cancer Prevention: APJCP. 2020;21(3):693.
39. Frappier L. Epstein-Barr virus: current questions and challenges. Tumour Virus Res. 2021;12:200218.
40. De-Thé G, Geser A, Day N, Tukei P, Williams E, Beri D, et al. Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan prospective study. Nature. 1978;274(5673):756–61.
41. Geser A, De Thé G, Lenoir G, Day N, Williams E. Final case reporting from the Ugandan prospective study of the relationship between Ebv and Burktit's lymphoma. Int J Cancer. 1982;29(4):397–400.
42. Tsimberidou A-M, Keating MJ, Bueso-Ramos C, Kurzrock R. Epstein-Barr Virus in patients with chronic lymphocytic leukemia. American Society of Hematology; 2005.
43. Alayvaz Aslan N, BÜYÜKAŞIK Y, SAYINALP N. The effect of prognostic features of patients with chronic lymphocytic leukemia on overall survival and treatment-free survival: a single-center experience. J Experimental Clin Med (Turkey). 2024;41(1).
44. Kimura H, Morita M, Yabuta Y, Kuzushima K, Kato K, Kojima S, et al. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. J Clin Microbiol. 1999;37(1):132–6.
45. Stevens SJ, Verkuijlen SA, Brule AJvd, Middeldorp JM. Comparison of quantitative competitive PCR with LightCycler-based PCR for measuring Epstein-Barr virus DNA load in clinical specimens. J Clin Microbiol. 2002;40(11):3986–92.

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