The prognostic role of EBER in pediatric cancer

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**Abstract**

The Epstein–Barr virus (EBV) infection is an endemic disease, over 90% of the population being exposed to it by adulthood. EBV is implicated in the etiology of a significant number of neoplasms, which acquire particular features in terms of course and prognosis. Incidence rates are much higher in children. To establish the link between EBV and neoplasms, EBER (Epstein–Barr virus non-encoded RNAs) needs to be highlighted in tumor tissue. The role of EBV in patient response to oncological treatment remains controversial.

**Keywords**: EBER, cancer, children, chemotherapy.

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**Introduction**

The incidence of pediatric cancer is continuously growing and contributes, from an epidemiological standpoint, to the high incidence of the disease worldwide. In 2012, GLOBOCAN estimated to 14.1 millions the number of annual new cases, to 8.2 millions the number of annual cancer-related deaths and to 32.6 millions the number of cancer patients for whom less than five years have passed since diagnosis/treatment [1].

The growing incidence of pediatric cancer depends on the age of onset of the disease, some studies estimating the growth rate to be much lower. However, an extremely important difference between pediatric and adult cancer is made by prognosis. An Italian study assessing data since diagnosis/treatment [1].

The multifactorial etiology of cancer poses significant problems in terms of screening, early diagnosis or effective treatment. The continuous phenotypic change of cancer cells in their effort to adapt to the aggression of their host’s immune system or of the oncological treatments administered represents an extremely important cause of treatment failure.

The Epstein–Barr virus (EBV) is representative for a virus group that is extremely widespread in the general population, over 90% of the individuals developing antibodies before reaching adulthood. The initial infection is asymptomatic in most of the cases, which allows for a lifelong persistent latent infection to settle in the host’s body. Expression of a true arsenal of proteins with extremely diverse roles, it ensures cell proliferation and the survival of the virus. In the latency period, expressed proteins ensure the proliferation of cells responsible for preserving the viral reservoir, keeping it “invisible” to the immune system and infecting new cells or hosts [3].

The factors associated with EBV reactivation or dramatic clinical presentations, which may culminate in patient death, remain unknown. EBV has the versatile capacity of infecting a wide variety of cell types: T-cells, B-cells, natural killer (NK), epithelial cells and muscle cells [4].

EBV is considered by World Health Organization (WHO) to be a class I carcinogenic agent and it is associated in terms of etiologic factors with nasopharyngeal cancer, Hodgkin lymphoma, non-Hodgkin lymphoma – Burkitt lymphoma. The tumor spectrum in which its involvement is identifiable is constantly changing, the latest sites referring to stomach and breast cancer [5, 6]. It is one of the largest viruses with double-stranded linear DNA genomes, encoding over 100 genes, most of whom are dormant during the latency period. During the latent infection stage, some of them encode important proteins such as EBV nuclear antigen (EBNA) 1, 2, 3A, 3B, 3C and EBNA-leader protein (EBNA-LP), latent membrane proteins (LMP) 1, 2A, 2B [7]. The EBV genome also contains two small EBER (Epstein–Barr virus non-encoded RNAs), EBER-1 and EBER-2 [7]. EBER is the sole gene expressed in all types or latency stages [8].

EBV may be considered the causative agent of a malignancy provided that the presence of viral genes or gene products in the tumor tissue is demonstrable. Given its constant presence, EBER is an ideal candidate in proving the viral etiology of EBV for some malignancies. Demonstrating the viral etiology of a malignancy may have a practical relevance through the potential screening of the oncological treatment administered: the viremia level or other proteins expressed by the viral infection may become therapeutic targets. The presence of EBER in tumor tissue is determined through in situ hybridization, the gold standard in place. Among all EBV genes, EBER is the most intensely expressed in EBV-induced tumors [9].

The pro-tumor role of EBER has not been fully clarified...
yet. It appears that, in gastric cancer, EBV stimulates the insulin-like growth factor (IGF-1), an autocrine factor, which accelerates tumor growth [10]. On the other hand, the presence of EBV though LMP-1 increases cancer cell resistance to Fluorouracil (5FU)- and Taxotere-based chemotherapy [10]. Another mechanism consists in stimulating IL-10 secretion, thus favoring cell multiplication [11]. It appears that EBER inhibits apoptosis by blocking the protein kinase phosphorylation and it confers resistance to the pro-apoptotic effects of IFN-γ [11, 12]. Moreover, the detachment of EBV-infected cells in the EBER interstitial environment stimulates the production of type 1 IFN and of proinflammatory cytokines, responsible for the presence of B-type clinical symptoms in lymphoproliferative disorders [11].

**Working hypothesis. Goals**

In patients with EBV-induced neoplasms, the natural history and the prognosis of the disease are different. The immunosuppression induced by the tumor itself or by the treatment administered may be accountable for the viral reactivation of EBV. This can be objectified by determining the anti-viral capsid antigen immunoglobulin G titers (anti-VCA IgG) and viral DNA in the blood. The pre-therapeutic anti-VCA IgG titer may be considered an independent tumor prognostic factor, as in the case of nasopharyngeal carcinoma [13]. The sensitivity and specificity values of EBV DNA appear to be higher than those of anti-VCA Ig in cancer diagnosis [14].

EBV DNA serum is a marker of viral replication correlated with viral load. Published studies also revealed that the EBV DNA level in cancer patients and their response to treatment are directly proportional [15]. Still, EBV DNA cannot replace EBER in identifying the viral etiology of a neoplasm [16]. The goals of this study were to assess possible correlations between the anti-VCA IgG titer (serological marker for EBV infection intensity) and the presence of EBER in tumor tissue and also to evaluate the prognostic role of EBER in the response treatment of pediatric neoplasms.

**Materials and Methods**

A retrospective study including 20 children monitored either by the “Prof. Dr. Ion Chiricuță” Oncological Institute of Cluj-Napoca or by the Pediatric Emergency Hospital of Cluj-Napoca (Romania) was conducted. Inclusion criteria: age <18 years, histopathologically confirmed malignancy, chemotherapeutic treatment, available paraffin block, positive serous status with known anti-VCA IgG serum level. Exclusion criteria: absence of histopathological confirmation of the tumor type, biological or clinical status not allowing for the administration of oncological treatment, non-compliant patient, incomplete clinical or paraclinical data and chemotherapy in a medical center other than those indicated above. The study group included 12 boys and eight girls. Paraffin blocks were retrieved from the archives of the “Prof. Dr. Ion Chiricuță” Oncological Institute or of the Pediatric Emergency Hospital (Cluj-Napoca). Glass slides were prepared from these blocks. They were first read to confirm initial diagnostic and they subsequently followed the preparation stages imposed by immunohistochemistry (IHC).

Clinical and paraclinical data from the patients’ records were also assessed — following classic prognostic factors in connection with the EBER status: treatment response, tumor dimension, “bulky” stage, changes in hematological and biochemical parameters during chemotherapy.

**Detection of EBV by in situ hybridization**

**Section selection**

Four to 6 μm sections were cut from the paraffin blocks and stained with Hematoxylin and Eosin (HE), in compliance with standard protocol. The glass slides of interest were selected among the examined sections (criteria: maximal density of cancer cells or of malignant lymphoid cells).

**Section preparation for hybridization**

New 5 μm sections were cut from the paraffin blocks from which the sections examined previously had been obtained. The sections were spread on glass slides in compliance with the usual procedure. The deparaffination of the sections was performed by successively immersing them in three Xylene baths of three minutes each, followed by two minute-baths in 99% and 95% ethyl alcohol respectively. The glass slides were subsequently immersed in double-distilled water for one minute.

**Pre-treatment and protease digestion**

Proteinase K was applied on the sections – 100 mL of 15 μg/mL solution (500 μg of lyophilized Proteinase K to which 1 mL of 50 mM Tris/HCl buffer with a pH 7.6 was added). The glass slides were incubated for 30 minutes at a temperature of 37°C. After digestion, the glass slides were immersed in a double-distilled water bath for three minutes.

**Section dehydration**

Section dehydration was achieved by three minute-immersions in two ethyl alcohol baths (95% and 99%, respectively).

**Hybridization**

An oligonucleotide probe was used for the detection of Epstein–Barr viral RNA, conjugated with Fluorescein (Novocastra Fluorescein-conjugated probes for in situ hybridization Epstein–Barr Virus Probe ISH Kit), out of which 20 μL of probe were added per section. The process lasted for 20 minutes and unfolded at a temperature of 37°C. Digestion was blocked by incubating the glass slides for 10 minutes with 100 μL of blocking solution.

**Detection**

The anti-FITC/AP antibody (Anti-Fluorescein Isothiocyanate/Alkaline Phosphatase) was added to the sections, for binding purposes, for 30 minutes. The glass slides were placed in a Tris-buffered saline (TBS) solution for three minutes and in Alkaline Phosphatase substrate buffer for five minutes. In order to prove Alkaline Phosphatase activity, the glass slides were incubated in the dark, overnight, at room temperature, with the mixture obtained from 5-Bromo-4-chloro-3-indolyl phosphate and Nitro blue...
tetrazolium, in Dimethylformamide, to which Levamisole was added in compliance with the proportions indicated by the producer. The following day, the glass slides were washed for five minutes, counterstained with Mayer’s Hematoxylin and mounted (aqueous mounting medium).

**Statistical analysis**

Chi-square or Fisher Exact Test was used to evaluate correlations between quantitative variables, following standard application criteria for each test. Normality of continuous data was tested using the Shapiro–Wilks test. Based on normality testing results, between-group differences in continuous data were assessed using either the Student t-test or the Mann–Whitney U-test. Receiver operating characteristic (ROC) analysis, including curve construction, area under curve (AUC) with a 95% confidence interval (95% CI) and cut-off determination was performed. Sensitivity and specificity were also determined for the identified cut-off value. A significance threshold of \( p \leq 0.05 \) was selected for all tests. SPSS 13.0 (Chicago, IL, USA) and MedCalc 8.3.1.1 software applications were used for data analysis.

**Results**

The mean age of the patients enrolled in the study group was of 12.4 years, the youngest subject being two years and the oldest being 17 years.

The types of neoplasms developed by these patients were: Hodgkin lymphoma – eight patients, non-Hodgkin lymphoma – eight patients, nasopharyngeal carcinomas – four patients.

Several aspects related to the neoplastic tumor the patients had developed were assessed in correlation with the EBER status.

**Histological type**

The classification according to tumor type and EBER status, as well as the microscopic details of the neoplasms studied are presented in Table 1 and Figures 1–6.

**EBV infection intensity**

In a previous cohort study (previous published), which included 35 patients, the regression curve revealed an anti-VCA IgG cut-off value of 213.44 IU/mL, predictive of whether or not complete remission will be achieved in children with EBV-induced neoplasm at the end of treatment with classic chemotherapy agents.

Comparing the EBER status against the EBV infection intensity – highlighted using the anti-VCA IgG titer level – results in the following contingency table (Table 2).

**Treatment response**

In the study group, complete remission was achieved for 70% of the EBER-negative patients and for 60% of the EBER-positive patients (Table 3), the difference being statistically insignificant \( (p=0.639) \).

**Initial tumor dimension**

The initial dimension of the tumor does not appear to have influenced the EBER-positive status in any way, the differences obtained being statistically insignificant \( (p=0.497) \).

**Variations in hematological and biochemical parameters during treatment, contingent on the EBER status**

We assessed the connection between the EBER status (positive or negative) and the initial values, as well as their variations (nadir) during chemotherapy, so as to verify whether or not the presence of EBV induces additional toxicity. Blood count was used to assess the level of leukocytes, neutrophils, lymphocytes, monocytes, thrombocytes and hemoglobin level. The following parameters were taken into account from biochemical analyses: aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and lactate dehydrogenase (LDH) (Table 4).
Out of the elements taken into account in the study, the EBER-positive status only had a statistically significant impact on the ASAT level, during the second and third chemotherapy cycles. A tendency towards statistical significance was noted for the forth cycle, also suggesting that EBER presence significantly increases cholestasis in the context of chemotherapy.

**Disease-free survival rate versus EBER status**

Disease-free survival (lack of relapse) does not seem to be influenced by the EBER status, the differences being statistically insignificant (Tables 5 and 6; Figure 7).

![Figure 1](image1.jpg)  
**Figure 1** – Lateral cervical lymph node metastasis of nasopharyngeal carcinoma. HE staining, ×400.

![Figure 2](image2.jpg)  
**Figure 2** – EBER positive for the case presented on previous figure: nasopharyngeal carcinoma. IHC for EBER, ×200.

![Figure 3](image3.jpg)  
**Figure 3** – Nodular sclerosis Hodgkin lymphoma. HE staining, ×400.

![Figure 4](image4.jpg)  
**Figure 4** – Nodular sclerosis Hodgkin lymphoma, EBER-positive. IHC for EBER, ×1000.

![Figure 5](image5.jpg)  
**Figure 5** – Burkitt lymphoma. HE staining, ×100.

![Figure 6](image6.jpg)  
**Figure 6** – EBER positive Burkitt lymphoma. IHC for EBER, ×100.

<table>
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<tr>
<th>Table 5 – Disease free survival rate versus EBER status</th>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>EBER-negative</td>
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<td>EBER-positive</td>
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<td>Total</td>
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<th>Table 6 – Disease free survival versus EBER status: statistical significance</th>
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<td><strong>Chi-square</strong></td>
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cases in low-incidence areas (limited available data) [20]. EBV DNA can be identified in geographic areas with high incidence of nasopharyngeal carcinoma. In virtually all cases, the authors reported an increasing trend in the number of cases [21]. The number of new cancer cases that can be attributed to EBV infection varies according to the regional socioeconomic level, with a high number of cases in areas with neoplasms significantly vary. On the one hand, this discrepancy reflects the geographical differences recorded in connection with the incidence of the EBV infection. A Chinese study reveals an incidence rate of 73.5% whereas an Iraqi study reports an EBER-positive rate of less than 5% in children with lymphoma [17, 18]. It appears that EBV is most frequently associated with the mixed cellularity subtype (71%) and with nodular sclerosis (54.2%) [19].

The number of new cancer cases that can be attributed to EBV infection varies according to the regional socioeconomic level, with a high number of cases in areas with limited development [20]. Khan & Hashim estimated that the global death burden, for the period of 1990–2010 attributed to EBV-related neoplasm worldwide was of 142 979, which is translated into 1.8% of all cancer deaths. More concerning is that for the mentioned period the authors reported an increasing trend in the number of cases [21].

The EBV infection is strongly linked to the development of nasopharyngeal carcinoma. In virtually all cases, EBV DNA can be identified in geographic areas with high and intermediate-incidence and in approximately 80% of cases in low-incidence areas (limited available data) [20]. The virus infects epithelial cells from the oropharynx and the associated B-cells, in the mucosa, by using the CD21 complement receptor [22].

Evidence regarding the implication of EBV in carcinomaogenesis of nasopharyngeal carcinoma is brought by the identification of a clonal EBV genome in the tumor cells [23, 24]. These facts argue in favor of the presence of the infection prior to the development of the neoplastic process [22]. However, the exact role of the EBV infection in the development of nasopharyngeal carcinoma is still debated. In most cases, the virus establishes a non-lithic or minimally lithic relationship with the host cell, favored by a low level of viral gene expression [21]. In the case of nasopharyngeal carcinoma, the virus assumes a type II latency behavior [characterized by expression of EBV-encoded RNAs (EBERs), BamHI-A rightward transcripts (BARTs), Qp promoter-induced EBV nuclear antigen-1 (EBNA-1) and a variable expression of LMP-1, LMP2A and LMP2B] [21, 23]. In undifferentiated nasopharyngeal carcinomas, the most common early chromosomal abnormalities involve losses of genetic material in 3p and 9p. This is translated into inactivation of RASSF1A, p16, p14ARF, CDKN2A [23, 25]. Evidence suggests that p16 hypermethylation significantly increases the risk of development of nasopharyngeal carcinomas [26, 27]. The silencing of p16 and RASSF1A creates a favorable context for the progression of the EBV infection, which in turn will promote tumor progression [23].

In the case of Hodgkin’s lymphoma, EBV shows a similar latency state [23]. The detection of EBV gene products in tumor cells is highly variable and dependent of several factors (age, area and histological subtype) [20]. Although the data is limited, it estimated that the overall prevalence of EBV infection in HL is around 40% [20].

The tumor cells of classic Hodgkin’s lymphoma [Hodgkin and Reed–Sternberg (HRS) cells] are considered transformed B-cell with a germinal center or post germinal center origin [23, 27]. The infection does not adversely affect the integrity and viability of the cell, but favors viral replication. LMP-1 mimics the presence of the CD40 receptor in its active state, which leads to the stimulation of cell proliferation [22]. LMP-2 shows a homologous function to the B-cell receptor and LMP-2 signaling plays a key role in the immortalization HRS cells [28]. EBV uses the cellular signaling pathways (NF-kB, JAK/STAT and phosphatidylinositol 3-kinase/AKT) through LMP-1, which adds a survival and proliferation advantage to HRS cells [22, 29]. Evidence suggests that EBV impede cytotoxic T-cell response [30].

The mean age of the patients enrolled in the study was of 12 years. Literature suggests an early onset of the EBV infection in developing countries, where population density is higher [31].

Patient age is a determining factor in EBV-positive classic Hodgkin lymphoma (cHL) prognosis. Studies have shown that EBV+cHL have a worse prognosis especially in the elderly [32–36]. For young adults (16 to 34 years of age), some studies have shown a survival advantage in EBV+ cases [37]. For EBV-associated Burkitt’s lymphoma, it seems that age does not influence the presentation type abdominal versus extra abdominal [38]. Overall survival is statistical influenced by age only in univariate analysis [39]. In contrast, in other studies, the presence of EBV itself represents a negative prognostic factor for overall and progression free survival of diffuse large cell lymphoma [40]. Moreover, the presence of EBV in strong enough as prognostic factor for both groups of patients: young and aged. Non-Hodgkin’s lymphoma represents only 6–10% of pediatric neoplasia [40]. In pediatric population the EBV-associated lymphoma has a significantly higher incidence among patients <10 years and immunosuppressed [41]. EBV could be implicated in
approximately 30% of sporadic Burkitt’s lymphoma compared to 25–40% in immunocompromised hosts [42]. In Indian population like other developing countries, Epstein–Barr virus (EBV) association of malignant hemopathies confirmed by Epstein–Barr virus encoded RNA (EBER) RISH or EBV-LMP-1 IHC revealed an EBV association of 93% [43]. In Western countries, the EBV etiology of Hodgin’s disease is met more lately and with a lower frequency – 20–40% [16].

There was no statistically significant correlation between the EBV infection intensity and the EBER status for the group under study, although there are studies that establish a connection between EBV positivity and high antibody levels [44].

Nowadays is little known regarding the correlation, if any, between EBV-negative DNA load and EBER status. In a retrospective study of 140 patients [45], EBV DNA load was significantly higher in EBER positive than in negative lymphomas. Despite this fact, a significant DNA load (more than median number of copies) was present also in EBER-negative patients (>70% of patients). DNA intense load in EBER-negative patients altered the overall and progression free survival, underlying that DNA load is not a surrogate for EBER status, on the contrary, it could represent an independent prognostic factor [45]. In Hodgkin’s lymphoma patients, the serum level of EBV DNA seems to be correlated with response to chemotherapy [15]. Even in immunocompromised hosts, Epstein--Barr DNA is not detected in malignant cells of all lymphoma subtypes. In AIDS-related lymphoma, EBV viral load had a significant decrease after chemotherapy treatment [46], but this viral load remains significantly higher in EBER-positive patients than in EBER-negative. EBV viral load could be predictive for oncological response; meanwhile, EBER expression was associated to advanced stages of disease and worse immune status. EBV DNA in plasma has a high specificity (90%) but low sensitivity (65%) in order to certify the EBV association in HL. [16]. EBV viral load correlate with high infiltration of the tumor with macrophage and low serum level of EBNA1 and lymphocytes, which may suggest a reduction of immunosurveillance needed for expansion of Reed–Sternberg cells.

The standard method applied to identify EBER is in situ hybridization (ISH). Automated analysis is more effective, with a sensitivity of 94%, a specificity of 94% and a global accuracy of 83% [47]. The positivity rate for Hodgkin lymphomas varies significantly, ranging from 20 to 70% [48]. The comparison between immunohistochemistry (IHC) for LMP-1, PCR for EBER-1 and PCR for BamHI W fragment revealed the superiority of the PCR-based method [48].

In cytoplasm, EBER is sometimes represented by over one million copies/cell, while LMP-1 is found in cytosol and on the surface of the cytoplasmic membrane [24]. Their expression on the Reed–Sternberg (RS) cells abides by the “all or nothing” rule. If equivocal results are obtained, the material is likely to have been degraded during the preparation stage [49]. The major advantage of EBER technique is represented by a particularity of these particles, which consist in being amplified and present at high levels in all latency forms of EBV infection. By this, they are ideal targets for ISH, which is widely considered the gold standard for the detection of EBV latent infection, more sensitive than the immunohistochemical evaluation of LMP-1 expression [42].

Plasma EBV-DNA could serve as a surrogate for EBER-ISH and need further studies to explore its prognostic utility in HL. In a published study, Cancer Cooperative Intergroup Trial E2496, a cut-off value of 60 copies/100 μL plasma yielded 96% concordance with positivity of EBER on ISH [50].

No correlation was established between EBER status and response to chemotherapy in our study. The prognostic role of the EBV infection remains controversial despite the description of distinct entities of EBV-positive lymphoproliferations. A possible explanation lies in the role played by EBV in maintaining the RS cells in the cycle, which renders them highly sensitive to chemotherapy and radiotherapy, thus increasing the chances of achieving complete remission [51]. Other studies reports that EBER-positive status of lymphoma is correlated with poor survival [45]. The positivity of EBER is associated with male gender, poor performance status and lower response to the first line of chemotherapy [40]. EBV positive status is linked to more advanced stage, more than one extranodal involvement, higher International Prognostic Index (IPI) risk group, presence of B-symptom [52].

Classical Hodgkin’s lymphoma (cHL) is characterized by a small number of neoplastic cells in a background of reactive cells. In a population of 100 children with HL, a morphological analysis revealed that children <10 years and EBV-positive cases have an intense T-cell infiltrate, exhibiting a cytotoxic/Th1 profile, characterized by higher numbers of CD3+, CD8+, TIA1+ and TEBET+ lymphocytes [53]. That could be explained by physiological changes of the immunity depending on time of developing of the disease and by interactions with EBV.

The survival rate of the patients included in the study was not influenced by the EBER status. Other studies on pediatric patients confirm these results [54]. However, EBER seems to be correlated with the tumor proliferative index [54]. EBV appears to be absent in lymphomas of low malignancy [55]. In nasopharyngeal carcinomas, where EBV has a very strong causative role, the survival rate of EBV-positive patients being statically higher than the survival rate of EBV-negative patients. By contrast, in aggressive non-Hodgkin’s lymphomas, the presence of EBV may be a negative prognostic factor, especially in elderly patients [56].

The presence of EBV in hematological hemopathies could represent a factor, which aggravates the clinical behavior of a tumor. This influence can affect recipients with normal or altered immunity. EBV favors a window in immunological surveillance, which permits proliferation of EBV-transformed B-cells. Existing published report present infast prognostic for patients with coinfection of EBV after hematopoietic stem cell transplantation by the developing of post-transplant lymphoproliferative disorder [57]. Chemotherapy used in such cases include a target therapy consisted in Rituximab an anti-CD20 antibody. Despite aggressive treatment, the clinical evo-
lution could be aggressive with little or no response to chemotherapy regimen. The tumor suffers genetic and clonal changes induced by chemotherapy action, for example, CD20-negative could disappear and be replaced by CD19-negative CD20-negative EBER-positive, more aggressive and less responsive to treatment. In a larger analysis of bone marrow transplants recipients, negative prognostic factors linked with high rate of mortality were (amongst others): involvement of extralymphoid tissue, acute graft-versus-host disease (GVHD), and a lack of reduction of immunosuppression upon post-transplant lymphoproliferative disease (PTLD) diagnosis [58]. In immunocompetent patients, the appearance of an EBV-positive lymphoma (especially NK or T) is characterized by an aggressive evolution. In the vast majority of them, they will present B-symptoms (80%), an advanced Ann Arbor stage (III, IV) (87%) and an International Prognostic Index high or high/intermediate (87%) [59]. The association of EBV (quantified by positivity of EBER) with NK and peripheral T-cell lymphomas not otherwise specified (PTCL NOS) was underlined by a morphopathological analysis [60]. The mismatch EBV between donor and recipient it is thought to be a risk factor [61].

In order to minimize the risk of PTLD it is necessary to closely monitor the cytomegalovirus (CMV) reactivation. In multivariate analysis, the reactivation of CMV is strongly related with EBV reactivation [62]. A single administration of Rituximab in patients with EBV DNA more than 10,000 copies/100 μL seems to do the viral clearance and to prevent the risk of progression into EBV-related PTLD [62].

Closely monitoring the viral load of EBV could identify the patients at risk for lymphoproliferative disorders [63]. Nearly 75% of those with less than 1000 copies/100 μL will not have any EBV-associated lymphoproliferative disease. Monitoring the viral load of EBV could help physicians for an early intervention in EBV-associated lymphoproliferative disease and also for follow-up of the efficacy of the antiviral therapy.

Conclusions

The results of this study did not highlight the prognostic factor of EBER in the evolution of children neoplasms. There were no statistically significant correlations between infection intensity, measured using the anti-VCA IgG titer and EBER, between EBER and patient response to oncological treatment or between EBER and the disease-free survival rate. The results of this study must be interpreted cautiously, given the low number of patients included in the study, the histological heterogeneity of the neoplasms studied as well as the potential presence of false-negative reactions caused by the degradation of viral miRNA during the processing stage.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

This paper was published under the frame of European Social Fund, Human Resources Development Operational Program 2007–2013, Project No. POSDRU/159/1.5/138776.

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Received: April 6, 2015
Accepted: December 9, 2015