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Original article

Clinicopathologic features of adult EBV-associated B-cell lymphoproliferative disease

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ABSTRACT

In the present study, 21 cases of adult/late-onset EBV-associated lymphoproliferative disease (AELPD) with an uncertain malignant potential were investigated with regard to their histomorphology, immunophenotype, clonal rearrangement of the heavy chain (IgH) and T-cell receptor (TCR) genes and clinical course.

The cases were histomorphologically reevaluated and assigned to one of three morphological groups: mononucleosis-like, Hodgkin-like, or polymorphous. In addition, cases with or without detectable necrosis were investigated for differences in clinical outcome.

Overall survival was highest in the group with Hodgkin-like morphology (4/4 patients), followed by patients with mononucleosis-like phenotype (4/5 patients surviving). Cases with polymorphous morphology showed the poorest survival rates with 7/12 patients dead of disease (58%). 4/6 patients with histologically detectable necrosis died (66%), but only 4/15 patients without necrosis (27%). 11/21 cases with AELPD showed clonal rearrangement for IgH (n = 4), TCR (n = 5) or IgH + TCR (n = 2). 5/11 patients with clonal rearrangement died (45%), and this percentage was similar in all of the three subgroups.

In conclusion, the present study shows that polymorphous morphology and detection of necrosis in AELPD are frequently linked to a fatal clinical course, whereas Hodgkin-like morphology seems to be associated with a more favourable prognosis. Clonal rearrangement of IgH or TCR is frequent in AELPD, but prognosis is unpredictable from this feature.

1. Introduction

Epstein Barr Virus (EBV) is a ubiquitous DNA-virus with a high rate of endemic infection. It is involved in the development of a broad spectrum of lymphatic diseases, some of them benign, some malignant, and some with ill-defined malignant potential [1,2].

Primary infection is usually asymptomatic, especially in childhood, but sometimes progresses to acute infectious mononucleosis (IM), a benign and self-limiting disease. After primary infection, EBV shows long-term persistence.

In patients with impaired T cell-mediated immune regulation, primary infection can present as fatal infectious mononucleosis, characterized by an uncontrolled B-cell lymphoproliferation [3,4]. Similarly, T-cell immunodeficiency caused by immunosuppression of individuals with solid organ or stem cell transplantation results in post-transplantation lymphoproliferative disease (PTLD). Both conditions are associated with poor prognosis.

Patients with chronic active EBV infection suffer from EBV-related symptoms for more than 3 months and show increased EBV genome levels or EBV antibodies, with associated high morbidity and high mortality [5].

Malignant lymphatic diseases associated with EBV include Hodgkin lymphoma, Burkitt lymphoma, as well as T- and NK-cell lymphomas. However, there are still several problematic cases occurring in routine diagnostics showing EBV-association without completely meeting the criteria of any of these diseases. The biological behaviour of most of these cases is not definitely predictable.

A search in current literature for further information on such cases with emphasis on epidemiology, morphological or molecular patterns and clinical outcome found results spread over a wide range.

In 2009, Schrager et al. [6] described a spectrum of EBV-associated B-cell lymphoproliferative diseases (LPD) and classified 116 cases into five categories: (i) lymph node based reactive hyperplasia with increased EBV-positive B-cells, (ii) EBV-positive nodal B-cell lymphoproliferations resembling PTLD, (iii) EBV-positive extranodal B-cell proliferations resembling PTLD, (iv) EBV-positive diffuse large B-cell

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Table 1
Literature on EBV-infection and clonality.

Author	No. of cases	Age (y.)	mc IgH	mc TCR	outcome
Schrager 2009 [6]	88	30–101	~60%	~20%	1 progressed
	28 (i)	45–93	0	3	
Salama 2008 [9]	3	1–4	2	0	fatal
Kojima 2007 [8]	6	52–74			self-limiting
Wick 2002 [4]	6	2–17	2	3	fatal
Plumbley 2002 [11]	18	1–80	0	0	self-limiting
Quintanilla-Martinez 2000 [15]	6	2–37	0	5	fatal self-limiting
	8		0	0	
Setsuda 1999 [16]	7	6–37	0	–	self-limiting
Krafft 1999 [10]	3	–	–	2	self-limiting
Malik 1996 [17]	1	–	–	1	self-limiting
Gaillard 1992 [18]	1	16	–	1	fatal
Christensson 1987 [19]	1	30	0	0	fatal
Kornstein 1989 [20]	1	17	0	0	fatal

(i) = cases with lymph-node based reactive hyperplasia with increased EBV-positive B-cells.

lymphoma, and (v) EBV-positive B-cell proliferations resembling classical Hodgkińs lymphoma. Of 28 patients with EBV-associated reactive lymphoid hyperplasia, one showed progression to nodal EBV-positive B-cell lymphoma within one year. Most of the EBV-associated B-cell LPD in this study were found to be extranodal. The subgroups of this study, together with DLBCL of the elderly and lymphomatoid granulomatosis, were previously summed up by the term “adult – late-onset EBV-associated B-cell LPD” [7].

Histomorphological features of EBV-related reactive lymphoproliferative disorders were also described by Kojima et al. [8], the clinical course of all six patients being self-limited.

Other authors postulated a link between clonality and EBV-infection, the patients ranging from children to elderly patients, the outcomes from self-limiting to fatal, with variable clonal rearrangements (see Table 1). Some findings suggested that patients with a fatal outcome of EBV-infections are more likely to have monoclonal gene rearrangements [4,9,10], and that patients with infectious mononucleosis usually lack monoclonal rearrangement [11].

The aim of this study was to find common morphological and molecular features as well as possible correlations to similar clinical outcomes in cases with adult EBV-associated lymphoproliferative disorders to support predictions concerning the prognosis of these cases, and thus help with therapeutic decisions. The present study was planned within the context of the diagnostic problems described above to further explore the clonality and clinical outcome of cases with infectious mononucleosis (IM) for the purpose of comparison.

2. Materials and methods

2.1. Case selection

All cases were collected from the archives of the reference center of lymph node pathology Würzburg Germany (Institute of Pathology at the University of Würzburg, Director at the time of sampling and investigation Professor HK Müller-Hermelink).

Only completed cases were selected to rule out any interference with current or ongoing diagnostic investigations. To achieve this, a period of eight years was chosen (2000–2008), beginning at the introduction of the current diagnostic software until five years before the start of our investigations. A first computational research for the terms “EBV-associated” and “lymphoproliferation” provided 387 cases in this period (details see Fig. 1). After initial evaluation of these 387 reports, 101 cases were potentially includable (with all other reports containing the search terms in a different context). From these 101 cases, lesions with typical PTLD, known immunodeficiency or immunosuppression were excluded. Of the remaining 41 cases, 21 could be found with available paraffin blocks containing enough tissue for further investigations. After thorough examination, repetition of immunohistochemical stainings and completion of molecular analysis, all 21 cases with atypical EBV-associated alterations of lymphatic tissues were definitely identified (20 with manifestation in lymph nodes, 1 in a tonsil). The criteria for the definition of adult EBV-associated lymphoproliferation (AELPD) in this study are shown in Table 2.

Additionally, 65 tonsils with infectious mononucleosis were selected from the archives, received during the period 2000–2008.

Clinical information was obtained from the medical reports of treating physicians whenever possible. As a consequence of not being the primary diagnosticians we did not always have access to complete clinical data with most clinical reports only giving selected information without standardization. After compilation of data, all personal details of patients were blinded. This process was done in accordance with the ethical standards given by the ethics committee of University of Wuerzburg.

2.2. Immunostaining

Immunohistochemical studies were performed using formalin-fixed (4% buffered formalin), paraffin-embedded tissue sections on an automated immunostainer (Ventana Medical Systems, Tucson, Arizona) according to the companýs protocol. For all cases of AELPD, immunohistochemical studies included at least CD5, CD15, CD20, CD30, Kappa/Lambda, Ki67, LMP and Pax-5 (ImmunKit Advance Dako, Glostrup, Denmark), in most cases with several further stains being available.

2.3. In situ-hybridization for EBV

EBER-ISH was performed using an automated system (Ventana Medical Systems; Inform EBER, Roche, Grenzach-Whylen, Germany) according to the companýs protocol.

2.4. DNA extraction

Genomic DNA was extracted from paraffin blocks in all cases. Three to five sections, 5–10 µm thick, were cut from each paraffin-embedded tissue block. The sections were deparaffinized by rinsing twice with xylene, followed by ethanol. Dried samples were resuspended in lysis buffer containing Proteinase K and incubated overnight at 56 °C. Proteinase K was inactivated at 70 °C for 10 min and the tissue was pelleted by centrifugation. Purification of the DNA contained in the supernatand was carried out using QiaAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the companýs protocol.

Measurement of DNA concentrations was performed with Genequant (GE Healthcare, Freiburg, Germany).

2.5. Polymerase chain reaction

Our routine clinical IgH and TCR-γ rearrangement assay was used, performing PCR in a total volume of 20 µl. Each PCR was performed with four different dilutions of DNA from 1 to 1:1600, depending on concentrations of DNA, PCR performed, and PCR results.

The components of the PCR cocktail included the following components:

- JH/FR3A: DNA, magnesium chloride, dNTP, PCR Buffer, Primer FR3A (ACA CGG CYS TGT ATT ACT GT), Primer LJH (TGA GGA GAC GGT GAC C), AmpliTaq Gold Polymerase (Applied Biosystems, Brandburg, New Jersey).
- JH/FR2A: DNA, magnesium chloride, dNTP, PCR Buffer, Primer FR2A (TGG $R_{(A/G)}$TC $CGM_{(A/C)}$ GAC $S_{(G/C)}GY_{(C/T)}$ $Y_{(C/T)}Y_{(C/T)}$C $N_{(A/G/C/T)}$GG), Primer LJH, Primer VLJH (GTG ACC AGG $GTN_{(A/G/C/T)}$