Originals

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Transformation in Lymphomas - Morphological, Immunophenotypic and Molecular Features

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During the course of lymphoma, a clinically more aggressive process with different morphology may develop, referred to as lymphoma transformation. Clonal relationship and pathogenic mechanism of this process are widely debated. The aim of the study was to evaluate morphology, immunophenotype (including EBV status) and clonal relationship in nine cases of lymphoma transformation. Among the six patients with low grade B-cell lymphomas three transformed into high grade B-cell lymphomas (two into diffuse large B-cell lymphoma, one into Burkitt lymphoma) and three into Hodgkin lymphoma. Three other patients with Hodgkin lymphoma presented with transformation into diffuse large B-cell lymphoma in two patients and peripheral T-cell lymphoma in one patient. In all cases there was a sudden clinical change as well as change in morphology and phenotype. In five of the nine patients studied EBV-LMP1 was demonstrated by immunohistochemistry in large transformed lymphoma cells. In two cases molecular studies revealed a different pattern of immunoglobulin gene rearrangement in the large transformed cells as compared to the small cells of primary indolent lymphoma. Thus, they represented secondary, arising *de novo* neoplasm.

Introduction

During the course of lymphoma a clinically, morphologically and molecularly more aggressive process may develop, referred to as lymphoma transformation. Clinically lymphoma transformation is characterized by a sudden or gradual appearance of a tumor in primary or a different localization associated with general symptoms (B symptoms) and changes in results of laboratory tests. The process is resistant to previously administrated treatment. Morphologically lymphoma transformation manifests as different tumor morphology, blastoid appearance of lymphoma cells and more mitoses. Molecular transformation means that in polymerase chain reaction (PCR) and sequencing analyses the primary and the more aggressive lymphoma derive from the same cell clone and are characterized by the same immunoglobulin heavy and/or light chain gene rearrangement. When the clinical course and changes of lymphoma morphology indicate transformation, but there is no clonal relationship between the two diseases, then we are dealing with a secondary neoplasm. "Composite lymphoma" is a descriptive morphological term for different lymphomas occurring simultaneously within the same or in different organs, irrespective of their clonal relationship. Follicular lymphoma, B-cell chronic lymphocytic leukemia (CLL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) undergo transformation relatively frequently. Lymphoma transformation seems to be the results of secondary genetic alterations leading to inactivation of cell cycle regulatory genes e.g. *p53*, $p21^{waf1}$, $p15^{INK4b}$, $p16^{INK4a}$ as well as dysregulation of *c-myc* and mutation occurring in translocated *bcl-2* and *bcl-6* genes. EBV seems to play a role in the transformation of indolent B-cell lymphomas into Hodgkin lymphoma (HL). Prior immunosuppressive therapies and impaired host immune surveillance (especially in CLL) may be also implicated in the process of lymphoma transformation [12, 13].

Material and Methods

The study involved nine patients with lymphoma transformation diagnosed between 1995 and 2005 in Department of Pathomorphology, Institute of Hematology and Department of Pathology of The Memorial M. Skłodowska-Curie Cancer Centre and Institute in Warsaw. Table 1 summarizes basic clinical data. All patients had clinical symptoms of transformation. In three patients transformation developed in the same anatomic site (cases 2, 3, 4), the others presented with transformed lymphoma at sites different than the primary lymphoma. Time intervals between the two diseases were from 1 to 95 months. In all patients lymph nodes and trephine biopsies were histopathologically examined. Samples from surgically removed stomach (cases 2 and 4), spleen, liver (case 4) and skin (cases 5 and 9) were also available. Sections were fixed in 10% buffered formaldehyde, and trephine biopsies in Hanover fixative. Routinely processed and paraffin-embedded sections were hematoxylin-eosin stained. Immunohistochemical studies were done (EnVision method) using the following mono- and polyclonal antibodies: CD45, CD20, CD30, CD15, bcl-6, bcl-2, CD38, IgM, CD3, OPD4, EMA, kappa and lambda light chains (DAKO), CD5, CD23 (Novocastra). Proliferative index was defined with MIB1 (Ki-67, Immunotech). Epstein Barr virus status was assessed by immunostains for EBV latent membrane protein (LMP1) (CS.1-4, DAKO). All lymphomas were diagnosed according to the WHO classification [9].

Immunoglobulin gene rearrangement

Molecular studies were done on DNA isolated from paraffin embedded samples using xylene method. PCR reaction was performed twice with 5 ng and 50 ng DNA according to BIOMED-2 protocol for testing immunoglobulin (Ig) gene rearrangement (IGH, IGK, IGL) [23]. IGH rearrangement test consisted of three multiplex PCR tubes with 27 forward and 5 reverse primers, IGK test consisted of 2 multiplex PCR tubes with 13 forward and 3 reverse primers, IGL test consisted of 1 multiplex PCR tube with 6 forward and 2 reverse primers. PCR products underwent heteroduplex analysis (95°C – 5 min, 4°C – 60 min) and were separated on polyacrylamide gel electrophoresis and visualized by ethidium bromide.

Results

Among six patients with low grade B-cell lymphoma three transformed into Hodgkin lymphoma (HL). HL displayed features of mixed cellularity (MC) type in two cases. In the third case only scattered Hodgkin and Reed-Sternberg (HRS) cells were noted within low grade lymphoma infiltration. In case 1 right cervical lymph node biopsy revealed marginal zone lymphoma composed of small monocytoid cells with phenotype: CD20+, Ig kappa+, CD43+/-, MIB1 in 15% of the cells, CD5-, CD23-, bcl-2-, cyclin D1-. Following the treatment with 10 courses of chemotherapy partial remission (PR) was achieved. However, 58 months later the patient complained of the left cervical lymphadenopathy, fever and night sweats. Histopathological examination of the lymph node showed HL MC type with CD30 and CD15-positive HRS cells with co-expression of CD20, in the background of inflammatory cells. Case 2 represented CLL in the cervical lymph node and the bone marrow (CD20+, CD5+, CD23+, CD43+, Ig lambda+, MIB1 in 10% of the cells). Following 6 courses of chemotherapy PR was achieved. Two years later B symptoms appeared and two large ulcerating tumors were found on gastroscopy. On histopathological examination of subtotally removed stomach classic HRS cells were seen dispersed in the inflammatory background composed of histiocytes, plasma cells, eosinophils and granulocytes. Similarly to the case 1, HRS cells were CD30 and CD15-positive with co-expression of CD20. In this case HL was observed in gastric mucosa simultaneously with CLL. The adjacent gastric wall had features of chronic gastritis with no evidence of Helicobacter pylori (Hp) infection. Six perigastric and 7 mesenteric lymph nodes showed infiltration by CLL, without the presence of HL. Isolated transformation of indolent CLL into HL MC type developing in the stomach was diagnosed. In the case 3, CLL was the primary diagnosis in the cervical lymph node and bone marrow. Two years later during second hospitalization due to hemolytic anemia histopathological examination of right axillary lymph node showed early transformation of CLL into HL. Contrary to the two described above cases, only scattered HRS cells (Fig. 1A) strongly expressing CD30, CD15 (Fig. 1B) and CD20, CD45-negative were noted within the background of CLL infiltration (Fig. 1C). Clinical symptoms of transformation (B symptoms, peripheral and abdominal lymphadenopathy) appeared 3 years later. In the evaluated left cervical lymph node the number of classical HRS cells was increased. Moreover, a more polymorphic appearance of the CLL background was noted with the presence of scattered macrophages, eosinophils and CD3+ and OPD4+ T lymphocytes. At that stage the morphology of the lymph node showed HL.

In the three discussed cases a strong cytoplasmic expression of EBV-LMP1 was demonstrated in the large transformed HRS cells.

The other three cases represented transformation of low grade B-cell lymphoma (two lymphoplasmocytic lymphomas and one CLL) into high grade B-cell lymphoma (two

into diffuse large B-cell lymphoma and one into Burkitt lymphoma). In the case 4, the morphology and immunophenotype (CD19, CD20, CD5, CD23 and Ig kappa-positive, MIB1 in 10% of the cells) of original cervical lymph node and bone marrow were consistent with the diagnosis of CLL. One month later progressive epigastric pain appeared. Histopathological evaluation of gastric biopsy and surgically removed stomach showed infiltration by large lymphoma cells (DLBCL) (Fig. 1D) with phenotype CD45+, CD20+, bcl-6+, bcl-2+, Ig lambda+, EBV-LMP1+, CD5-, CD23- and MIB1 in 85% of the cells (Fig. 1E). DLBCL cells were intermingled with foci of small CLL cells. The uninvolved mucosa showed the features of chronic gastritis without evidence of Hp infection. Lymph nodes of the small curvature, spleen and liver revealed CLL involvement without the presence of large transformed cells.

In the case 5 lymphoplasmocytic lymphoma (LPL) was diagnosed based on interstitial infiltration of the bone marrow by small lymphocytes, lymphoplasmocytes and plasma cells expressing CD20, CD38, IgM kappa (30% of the cells). IgM kappa corresponded to the isotype identified in the serum. Eight months later clinical features of transformation appeared: peripheral and abdominal lymphadenopathy, hepatosplenomegaly and two tumors located in subcutaneous tissue near the sternum and spine. Histopathological evaluation of the tumor located near sternum showed infiltration composed of large cells, mainly centroblasts with the phenotype: CD45+,CD20+, bcl-2+, bcl-6+, IgM kappa+, EBV-LMP1-, MIB1 in 90% of the cells, CD5-, CD23-, CD43-. DLBCL was diagnosed. The case 6 also represented lymphoplasmocytic lymphoma (CD20+, CD38+, IgM kappa+) diagnosed in the bone marrow. Transformation into nodal Burkitt lymphoma (CD20+, c-myc+, MIB1 in 95% of the cells, IgM kappa+, EBV-LMP1-) appeared 31 months after primary LPL diagnosis.

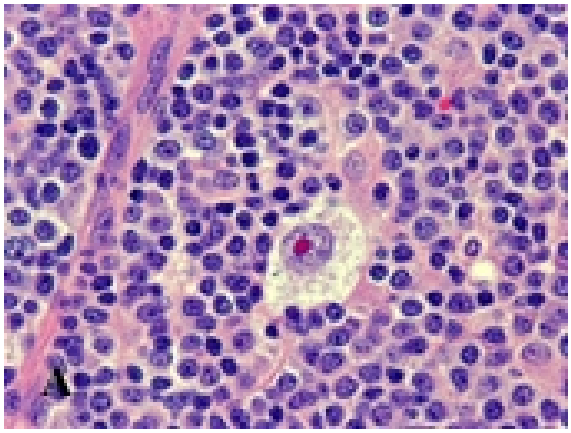
Among three patients with HL, two developed nodal DLCBL. The case 7 with the diagnosis of nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) transformed into T-cell rich B-cell lymphoma (TCRBCL). The original right cervical lymph node biopsy showed nodular architecture with scattered L&H cells (lymphocytic/histiocytic Reed-Sternberg cell variants) among small B lymphocytes. L&H cells were positive for CD20, CD45 and bcl-6 but lacked expression of CD30, CD15 and EMA. They were ringed by small CD3+ T cells. After 5 courses of chemotherapy PR was achieved. However, 95 months later in the right cervical lymph node large lymphoma cells were seen with centroblastic and immunoblastic appearance (some of them had multilobated nuclei) and HRS-like cells with abundant eosinophilic cytoplasm, polylobated nuclei and one to five prominent basophilic nucleoli (Fig. 1F). They were scattered between sheets of histiocytes and small T (CD3+) lymphocytes. Large B cells, which were relatively sparse expressed CD20 (Fig. 1G), bcl-6 and CD45 with cytoplasmic expression of EBV-LMP1 (Fig. 1H). In single cells CD30 was weakly positive, CD15 and EMA were negative. The large B cells of TCRBCL with morphology resembling HRS cells had the same phenotype as L&H cells in the previously diagnosed NLPHL. The case 8 presented with HL MC type. HRS cells were positive for CD30 and CD15 with co-expression of CD20. Sixty-three months after treatment peripheral T-cell lymphoma (PTLC) was diagnosed in the lymph node. Neither its morphology nor immunophenotype had any pathogenic relationship with the previously diagnosed HL. In the case 9, HL MC type with classical morphology and phenotype (CD30+, CD15+, CD20-, CD45-) transformed into DLBCL localized mainly in the mediastinum and the skin.

Molecular biology results

In the case 2 gene rearrangement analysis revealed a different clonal cell populations in the lymph node at the time of diagnosis of CLL and 2 years later in the gastric biopsy sample. Multiplex polymerase chain reaction (PCR) with heteroduplex analysis of PCR products revealed in the lymph node clonal rearrangement of IGH genes in VH-JH segments and IGL genes rearrangement (Vλ–Jλ segments). Additionally a germline rearrangement of IGH genes in segments DH7-JH1 was found. Analysis of IG clonality of gastric biopsy sample displayed different type of gene rearrangements. The above described rearrangements of IGH and IGL genes were not present. The only detected rearrangement was in IGK genes (Vk–Jk segments). In the case 4 there was a clonal rearrangement of IGH genes in VH–JH segments and in IGK genes (Vk–Jk segments) with no clonal rearrangements between Vk and intron segments and Kde segment in the lymph node. Molecular analysis of DNA from gastric biopsy sample obtained one month later showed lack of Ig genes rearrangements, visible previously in the lymph node, but the presence of IGL gene rearrangement in segments Vλ-Jλ. In the remaining patients we were unable to detect clonal rearrangement of Ig genes due to lack of signal of control gene. In three HL patients the material for molecular studies was not available.

Follow-up data

Six patients achieved response to therapy. Patient 4 diagnosed as transformation of CLL into DLBCL in the stomach, treated with gastrectomy and chemotherapy is still in complete remission (23 months). Three patients with HL transformation into DLBCL (cases 7 and 9) and into PTCL (case 8) achieved CR of 16, 4 and 4 months, respectively after chemotherapy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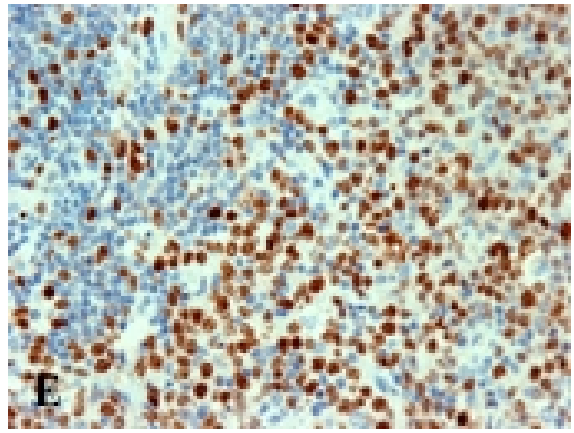
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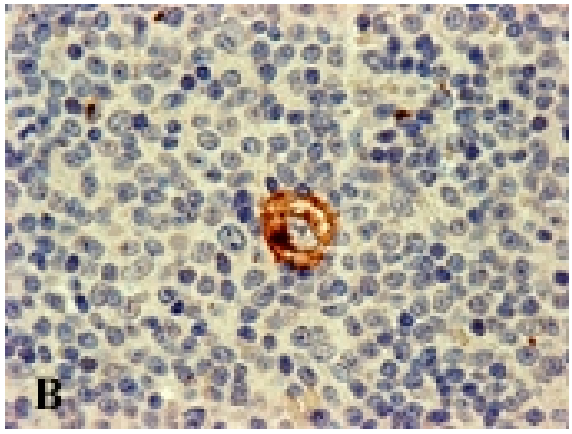
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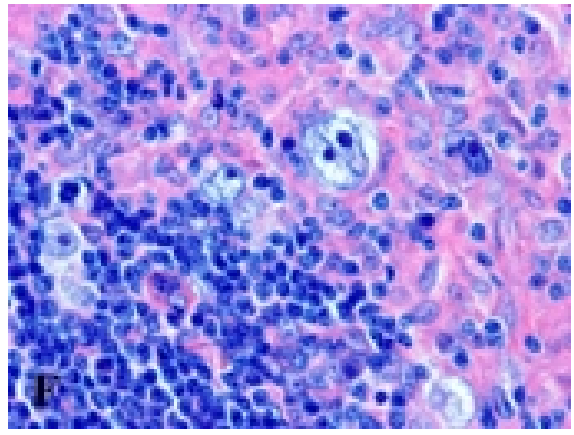
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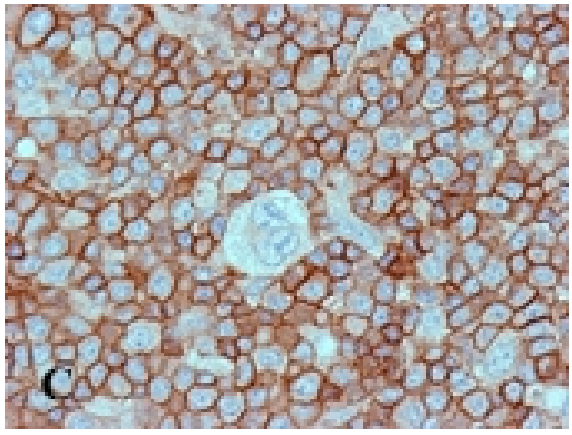
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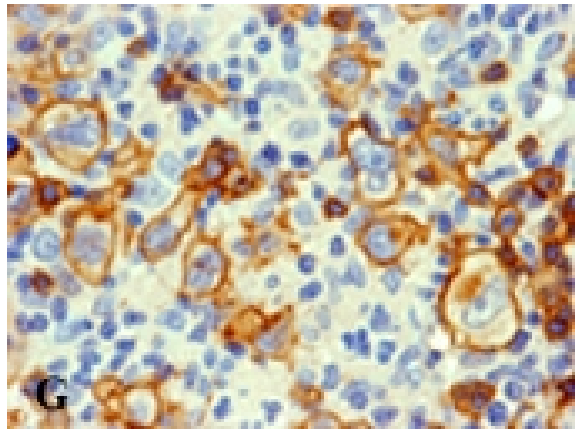
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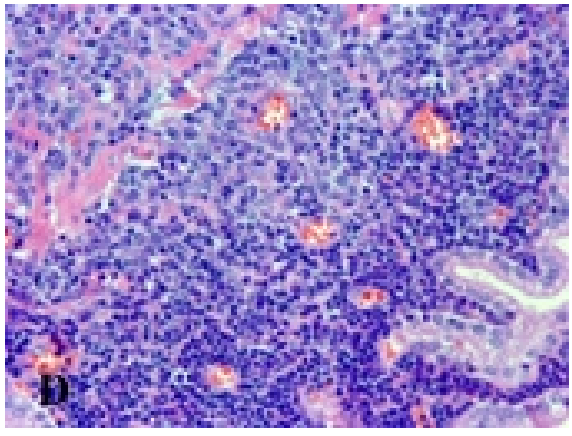
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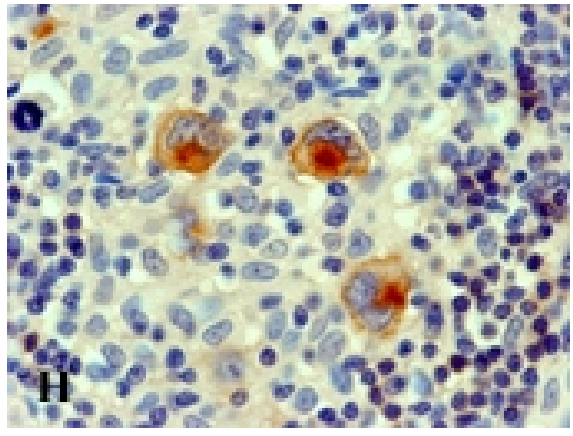
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and involved field radiotherapy. Two patients with transformation of MZL and CLL into HL (cases 1 and 3) had only PR to therapy (13 and 53 months). Three patients (cases 2, 5, 6) died. Patient 2 with transformation of CLL into isolated gastric HL died 15 days after gastrectomy (2 months after HL diagnosis) due to bronchopneumonia with respiratory and circulatory failures. Patient 5 died due to gastrointestinal hemorrhage two months after DLBCL diagnosis and two weeks after chemotherapy. Treatment was also unsuccessful in the patient 6 due to disseminated disease and NR.

TABLE 1
Basic clinical data of patients with lymphoma transformation

N	Age(y)/ sex(M/F)	Initial lymphoma (localization)	Transformed lymphoma (localization)	Interval between two diseases (m)	Treatment before transformation and response	Treatment after transformation and outcome (m)
1	47/M	Marginal zone lymphoma/ R cervical LN	Hodgkin lymphoma MC/L cervical LN	58	6xCHOP, 4x2-CdA/PR	3xABVD/ PR 13+
2	76/M	Chronic lymphocytic leukemia/cervical LN, BM	Hodgkin lymphoma MC/stomach	24	6x2-CdA, cyclophosphamide/PR	Gastrectomy/death
3	70/F	Chronic lymphocytic leukemia/L cervical LN	Hodgkin-Reed-Sternberg cells/ R axillary LN	24	Leukeran, Encorton/PR	4xMOPP, 2xCOP/PR 53+
4	67/M	Chronic lymphocytic leukemia/cervical LN, BM	Diffuse large B-cell lymphoma/stomach	1	No treatment	Gastrectomy, 6xCHOP/CR 23+
5	65/M	Lymphoplasmocytic lymphoma/BM	Diffuse large B-cell lymphoma/skin	8	2x2-CdA, cyclophosphamide/PR	1xCHOP NR/death
6	48/F	Lymphoplasmocytic lymphoma/BM	Burkitt lymphoma/ submandibular LN	31	VAD, VMCP/PR	3xCHOP NR/death
7	20/M	NLPHL, R cervical LN, CSII	T-cell rich B-cell lymphoma R cervical LN, CSII	95	5xMOPP/ PR	6xCHOP+ IFrth/CR 16+
8	35/F	Hodgkin lymphoma MC/cervical LN CSIII	Peripheral T-cell lymphoma inguinal LN, CSI	63	8xABVD/CR	6xCHOP+ IFrth/CR 4+
9	62/M	Hodgkin lymphoma MC/inguinal LN	Diffuse large B-cell lymphoma/ skin	4	6xABVD+ IF rth/CR	6xCHOP/CR 4+

y-years, M-male, F-female, m-months, LN-lymph node, BM-bone marrow, MC-mixed cellularity, NLPHL-nodular lymphocyte predominant Hodgkin lymphoma, L-left, R-right, CR-complete remission, PR-partial remission, NR-no response, CHOP-cyclophosphamide, adriamycin, vincristine, prednisolone, 2-CdA-cladribine, ABVD-doxorubicin, bleomycin, vincristine, decarbazine, MOPP-mechlorethamine, vincristine, procarbazine, prednisone, VAD-vincristine, doxorubicin, prednisolone, VMCP-vincristine, melphalan, cyclophosphamide, prednisolone, CS-clinical stage, IFrth-involved field radiotherapy

Fig. 1. Morphological and phenotypic spectrum of transformation in lymphomas.
A. Case 3. Typical Hodgkin cell in the background of small CLL cells. HE. Magn. 250×.
B. Case 3. CD15 expression in Hodgkin cell. EnVision. Magn. 250×.
C. Case 3. CD23 expression in CLL cells, the Reed- Sternberg cell is negative. EnVision. Magn. 250×.
D. Case 4. Gastric tumor. Large cells of DLBCL with morphology of centroblasts and immunoblasts (upper left) next to infiltration by small CLL cells (lower right). HE. Magn. 100×.
E. Case 4. MIB1 staining in 85% of the cells of DLBCL and in 10% of the cells of CLL. EnVision. Magn. 100×.
F. Case 7. TCRBCL. The presence of HRS-like cell among scattered large lymphoma cells. Histiocytes and small T lymphocytes in the background. HE. Magn. 250×.
G. Case 7. CD20 in large lymphoma cells. EnVision. Magn. 250×.
H. Case 7. TCRBCL with cytoplasmic staining of EBV-LMP1 in large atypical lymphoma cells. EnVision. Magn. 250×.

Discussion

Clonal relationship and pathogenic mechanisms of lymphoma transformation evoke great interest.

Transformation of chronic lymphocytic leukemia (CLL) into Hodgkin lymphoma (HL) is rare and occurs in about 0.5% of cases [5]. HL and CLL are clinically and morphologically distinct diseases, but recent molecular findings indicate that HRS-like cells in B-CLL represent the outgrowth of single germinal center B-cell-derived clones and may be potential precursors of Hodgkin and Reed Sternberg (HRS) cells in HL [10]. HRS-like cells in CLL, like HRS in HL derive from germinal center B cells, because they have somatic mutation in the variable region of Ig heavy chain genes (VH) [9].

There are two molecularly different types of CLL transformation into HL [18]. In one (case 4) of the two patients described above the immunohistochemical study showed the presence of small CLL cells CD20/CD5/CD23+ associated with only scattered in the background few CD30 and CD15-positive HRS cells. This case represents type 1 "CLL with Hodgkin's transformation". Ohno et al. [15] using single-cell PCR analysis and DNA sequencing of the hypervariable region of the IgH gene showed that HRS cells and CLL cells in this type derived from the same clone. The other patient (case 2) represents type 2 CLL transformation into HL, where HRS cells were situated in a typical background of non-neoplastic inflammatory cells. HRS cells in this case did not represent molecular transformation of underlining B-CLL, even though they expressed the B-cell specific antigen. Molecular analysis of DNA from gastric HL showed the presence of IGL gene rearrangement in segments Vλ–Jλ, while in the lymph node with CLL a different type of IGH and IGK gene rearrangement was found. Thus, the second case was not clonally related to CLL and represented *de novo* secondary neoplasm. Relatively few cases of nodal transformation of CLL into HL have been reported, but it seems that in patients with type 2 CLL transformations into HL with clinical features of Richter's syndrome the median survival time is usually shorter and response to chemotherapy is less effective than in *de novo* diagnosed nodal HL [4, 19]. Type 1 is characterized by indolent and stable course with better response to ABVD therapy and a longer survival [18], as in the discussed case 4.

Transformation into DLBCL occurs in 3–5% of the patients with CLL. Clinical symptoms are defined as Richter's syndrome (RS) [5]. Our case 4 represents a very rare transformation of CLL into DLBCL lymphoma localized in the stomach. Gastric DLBCL cells have a different immunophenotype than the original CLL cells, characterized by the lack of CD5 and CD23 antigens and show different surface expression of Ig light chains [16, 17]. This was observed in our case. Sometimes nodal transformed DLBCL cells exhibit expression of CD5, what indicates that similarly as in primary CLL they may derive from pre-germinal center B cells (naive cells) with germline hypermutation [14]. Gastric lymphoma cells in our patient were negative for CD5 and positive for bcl-6 indicating their origin from germinal center B-cells [7]. Moreover, gastric transformation of CLL into DLBCL usually requires morphological and immunohistochemical differential diagnosis with transformation of marginal zone B-cell lymphoma, MALT type. As in the presented case, in most patients with gastric DLBCL with pre-existing CLL, molecular analysis of paired samples from gastric tumors and lymph nodes reveals that they developed from different clones and represent *de novo* secondary neoplasms [16, 17]. On the other hand, molecular analysis of nodal progression of CLL revealed in about 50% of patients the same clonal origin of CLL and DLBCL representing a true molecular lymphoma transformation [2, 5]. Recent studies suggest some relationship between mutational status of the CLL immunoglobulin heavy chain genes variable region (VH genes) and clonal evolution in RS. Timar et al. analyzed 8 patients with RS and proved that clonal transformation of CLL into DLBCL occurs only in VH unmutated CLL, while clonally unrelated, secondary DLBCL originate mainly in CLL patients with mutated VH genes [20].

Similarly as in CLL, patients with lymphoplasmocytic lymphoma (LPL) may develop DLBCL in 5–13% of cases and these patients become resistant to therapy resulting in poor outcome [11]. Lin et al. [11] showed in 12 patients with LPL transformation that immunoglobulin light chains expression was identical in both LPL and DLBCL and suggested that the latter probably represented molecular transformation of LPL. That was true in our case 5. Unfortunately, in our case as well as in the above described patients DNA extracted from paraffin-embedded specimen was degraded and it was impossible to prove a clonal relationship between these two diseases.

While transformation into DLBCL does occur in extranodal marginal zone lymphomas (MZL), MALT type localized in the stomach or spleen, the transformation of nodal MZL into Hodgkin lymphoma presented in the case 1 is extremely rare and only few such cases has been reported [26]. Clonal relationship has not been investigated in these cases, so far.

During the course of HL in approximately 3–5% of cases transformation into DLBCL may appear and the lymphoma may arise simultaneously as well as subsequently to HL [6, 8]. Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is associated with higher incidence of

transformation into DLBCL than the other types of HL [6]. In our case 7 the L&H cells (lymphocytic/histiocytic variants of Reed-Sternberg cells) of NPLHL and large cells resembling HRS cells of T-cell rich B-cell lymphoma (TCRBCL) have a similar phenotype with expression of CD20 ,CD45 and bcl-6 indicating transformation from a common germinal center B cell. Some authors suggest that NLPHL is a polyclonal lymphoproliferation that arises in the germinal center and large B-cell lymphoma, especially TCRBCL represents a form of clonal, histological transformation of NLPHL [3]. Large B-cell lymphomas which develop following other types of HL (like in our case 9) have the immunophenotype (CD20+, bcl6+, CD30-, CD15-) that is distinctly different from classic HL (CD20-, CD30+, CD15+) [25]. Among the cases published with concomitant DLBCL and HL, immunoglobulin gene rearrangement and sequencing analysis revealed that both lymphomas might derive from separate as well as common precursors [1, 21, 24]. Most of the reported cases with clonal relationship between HL and DLBCL represented NLPHL [6, 24]. DLBCL may develop as a consequence of HL therapy (chemotherapy-induced lymphoma), especially when the two diseases occur with a long interval between the diagnoses. Some authors suggest that the large B-cell lymphomas associated with NLPHL, if localized, generally have a good prognosis [6]. In larger series of cases, Huang et al. found that in cases of NLPHL transformation into disseminated DLBCL prognosis is similar to that in *de novo* DLBCL and these patients should be treated aggressively [8].

In five of nine cases of lymphoma transformation (two CLL and one MZL into HL as well as CLL into DLBCL and NLPHL into TCRBCL), EBV-LMP1 staining by immunohistochemistry was positive in large transformed lymphoma cells and negative in pre-transformation indolent lymphomas. The role of EBV in lymphoma transformation is controversial. EBV infection and prior therapies (mainly fludarabine followed by immunosuppression) play a major pathogenic role in the transformation of CLL into HL [22]. However, according to some authors EBV is not implicated in pathogenesis of HL transformation into large cell lymphomas and into secondary DLBCL following CLL, LPL, MZL and FL [6, 11, 13].

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