

ORIGINAL PAPER

Bcl-2 expression in Hodgkin's lymphoma progression

CORINA FLANGEA¹⁾, ELENA POTENZ²⁾, RODICA MIHĂESCU³⁾,
S. GÎJU⁴⁾, A. ANGHEL¹⁾

¹⁾Biochemistry Department

²⁾Pathology Department

³⁾Hematology Department

"Victor Babeș" University of Medicine and Pharmacy, Timisoara

⁴⁾Central Laboratory,
County Hospital No. 1, Timisoara

Abstract

Introduction: Hodgkin's lymphoma study by immunohistochemical expression of Bcl-2 in Hodgkin and Reed–Sternberg cells can precise these cases evolutive way. **Material and methods:** Sixty-three cases of classical Hodgkin's disease, hospitalized into the Hematology Department of the County Hospital No. 1 Timisoara, were studied. Histopathological diagnostic was performed using common staining methods, and for revealing the tumoral developments immunohistochemical staining was performed Bcl-2. **Results and discussion:** In our study, the results were noticed a direct relation between the rise of tumoral proliferation index expressions of Bcl-2 and progression of the disease ($p \leq 0.001$). For I and II stages Bcl-2 expression does not overcome (-+) category while the III and IV stages, all the cases are situated in (+/-) and (+) categories. No connection we can be noticed between the histological type and Bcl-2 expression although the classic Hodgkin's lymphoma with lymphocyte depletion is considered the most aggressive histological type ($p \leq 1$). In our study, we found this correlation very important because the main cause of relapses is inadequate staging. In some cases, this staging is difficult; some little lymph nodes could be overlooked because they can be placed in less accessible areas and cannot be evidenced by the most imagistic methods. **Conclusions:** All the cases were Bcl-2 expression higher than (+/-) and are staged as I and II stages should be reinvestigated and restaged. This immunohistochemical reaction, although less used in Romania, is very accurate. That is very important because the therapeutically attitude is different in advances stages compared to earlier stages.

Keywords: Bcl-2, Hodgkin's lymphoma, clinical stage.

□ Introduction

Bcl-2 oncprotein is involved in apoptosis regulation as inhibitor. It is localized at mitochondrial cytoplasmatic internal side, endoplasmic reticulum and nuclear membrane level [1–4].

Increased expression of Bcl-2 causes resistance to chemotherapeutic drugs and radiation therapy, while decreasing Bcl-2 expression may promote apoptotic responses to anticancer drugs. In addition, overexpression of Bcl-2 may result in accumulation of cells in the G0 phase of cell cycle division and contribute to chemoresistance [5].

In addition, Bcl-2 protects cells from a wide range of cytotoxic insults, including cytokine deprivation, UV- and γ -irradiation, and chemotherapeutic drugs. Hence, Bcl-2 is a critical cellular factor contributing to the pathogenesis and progression of cancer [6, 7].

Bcl-2 expression is related to relapses and non-responders patients' category in lymphomas [1]. Overexpression of Bcl-2, which is frequently found in both high- and low-grade lymphomas, was reported to inhibit cytolysis mediated by cytotoxic T-lymphocyte [8, 9].

Even if Hodgkin's lymphoma is a malignant tumor, in most cases has a good response to treatment, 20–30%

patients relapse or die because of complications and the progressive disease, which is not influenced by therapy [10, 11]. Due to this fact, we have investigated this expression in Hodgkin's disease for identification a connection between clinical stage, proliferation and Bcl-2 expression.

□ Material and methods

We had 63 patients suffering from classical Hodgkin's lymphoma hospitalized into the Hematology Department of the County Hospital No. 1 Timișoara between January 2000 and June 2004. All the lymph nodes were formalin fixed (10%) and paraffin embedded, sectioned at 4 μ m. Prof. dr. Elena Lazar offered them kindly. Usual staining was performed with Hematoxylin–Eosin and PAS reaction. We also performed the CD30 and CD15 immunohistochemical reaction for diagnostic confirmation.

We used Dako reagents for immunohistochemical reactions. Monoclonal antibody Bcl-2 (clone 124, subclass IgG1 kappa) dilution was 1:50 in Antibody diluent. Bcl-2 reaction was revealed by APAAP technique using New Fuchsin as chromogen. Counterstaining was performed with Hematoxylin. Final reaction product has a cytoplasmatic localization

red color. Negative components are blue. Bcl-2 samples were appreciated semi quantitative in Hodgkin and Reed-Sternberg cells as follows:

- expression (+) – all the malignant cells positive;
- (+/-) – positive malignant cells 50–95%;
- (-/+) – positive cells under 50%;
- (-) – all the malignant cells negative.

Statistical analysis was done by non-parametric test chi square (χ^2). Statistical significance was appreciated in confidence degrees, which will lead to accepting or rejecting a hypothesis. For significance, p should be less than or equal to 0.05 ($p \leq 0.05$) and χ^2 must fit in critical values function to degrees of freedom of particularly situation.

Results

The majority of the patients elected for our study are young persons, 43% of them being under 30 years:

- 11–20 years, 15 cases (23%);
- 21–30 years, 13 cases (20%);

Table 1 – Bcl-2 expression function to the disease stage and histological type

Clinical stage	Bcl-2 expression											
	No. of cases (+)			No. of cases (+/-)			No. of cases (-/+)			No. of cases (-)		
	MC-CHL	LD-CHL	NS-CHL	MC-CHL	LD-CHL	NS-CHL	MC-CHL	LD-CHL	NS-CHL	MC-CHL	LD-CHL	NS-CHL
Stage I	–	–	–	–	–	–	1	–	–	–	–	1
Stage II	–	–	–	5	2	3	2	1	–	–	–	–
Stage III	3	2	2	16	9	4	–	–	–	–	–	–
Stage IV	3	3	1	2	2	1	–	–	–	–	–	–

We performed histopathological diagnostic using REAL/WHO classification. Using usual staining the lymph node architecture, we observed also the malignant cells morphology and characteristic of the reactive cellular background. From the whole patients, 32 cases (50%) were mixed cellularity (MC-CHL), 12 cases (19%) were nodular sclerosis (NS-CHL), 19 cases (29%) were lymphocyte depletion (LD-CHL); we not met lymphocyte rich classic Hodgkin's lymphoma (Table 1).

NS-CHL cases were characterized by nodular aspect with fibrosis, many lacunar and Reed-Sternberg cells (Figure 1).

Some of the neoplastic cells have shown a mummified aspect probably due to apoptotic process. A diffuse aspect and a large number of Hodgkin and Reed-Sternberg cells, with necrosis areas, characterized MC-CHL cases. Reactive background was composed by plasma cells, eosinophils, histiocytes and lymphocytes surrounded tumoral cells (Figures 2 and 3).

LD-CHL cases were characterized by a relative or absolute abundance of neoplastic cells with a variable fibrosis reaction. Cellular background has low content composed by small lymphocytes, plasma cells and eosinophils (Figure 4).

Fibrosis has evidenced by PAS reaction and revealed lymph node structure. The fibrosis bands were broad or small, branched, delimiting groups of cells or shown a complete disorganized structure (Figures 5 and 6).

- 31–40 years, nine cases (14%);
- 41–50 years, 11 cases (19%);
- 51–60 years, seven cases (11%);
- over 60 years, eight cases (13%).

From clinical point of view, the staging was performed according to Costwolds classification: 36 cases (56%) were diagnosed in the stage III, 13 cases (20%) in the stage II, 12 cases (19%) in the stage IV, and only two cases (5%) in the stage I (Table 1).

Systemic symptoms – fever, night sweats, and body weight loss – are detected in 41 patients (65%).

From all cases, 16 patients had a biopsy from a single lymph node and 47 had more lymph node biopsy (2–3 lymph nodes from the same patient). They usually present latero-cervical lymph nodes (34 lymph nodes) but other regions were involved, too (six submandibular, two gastropiploic, two from spleen hilum, 12 retroperitoneal, six mediastinal, 12 subclavicular, 16 axillary). Nine cases presented bulky disease (six with stage IV and three with stage III).

In our study, Bcl-2 expression increases according to disease progression. The patients with stages II and III Bcl-2 expression was quantified as (+/-) in majority of the situations: 10 patients had stages II (83%) and 29 patients had stage III (80%). In the case of those with stage IV, the quantified Bcl-2 expression (+/-) was met in five patients (42%) and the quantified 1 (+) was met in seven patients (58%). Those with stage I showed a Bcl-2 expression (-) and (-/+) in 50% each of them.

For stages I and II, Bcl-2 expression does not overcome (-/+) category while into stages III and IV all the cases are situated into (+/-) and (+) categories (Table 1). This distribution was statistically significant: degrees of freedom = 9; chi-square = 57.11363636364; $p \leq 0.001$.

The majority histological types we met in category (+/-): 23 cases (72%) had MC-CHL, eight cases (66%) had NS-CHL and 13 cases (68%) had LD-CHL histological types. These are followed by (+) category and then by (-/+) category: six cases (19%), three cases (9%) in MC-CHL respectively, three cases (26%), no cases in NS-CHL respectively and five cases (26%) and one case (6%) in LD-CHL respectively.

No association is observed between Bcl-2 expression and the histological type even though DL-CHL is considered the most aggressive histological type. Distribution is not statistical significant: degrees of freedom = 6; chi-square =

5.90894138755981 (for significance at the 0.05 level, chi-square should be greater than or equal to 12.59); $p \leq 1$.

Bcl-2 expression was intense in majority of cases in cytoplasm of lymphocytes and Hodgkin and Reed-Sternberg cells in (+) interval (Figures 7 and 8).

We also found mummified neoplastic cells Bcl-2 positive (Figure 9).

Some of malignant cells shown a weak staining in (+/-) and (-/+) intervals (Figures 10 and 11).

Few NS-CHL cases presented a zonal positivity of Bcl-2 expression (Figure 12).

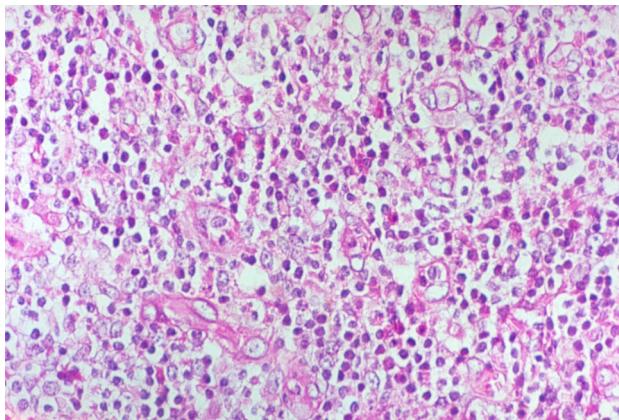


Figure 1 – Nodular sclerosis classical Hodgkin's lymphoma with many lacunar cells.
HE staining, $\times 400$
(private collection)

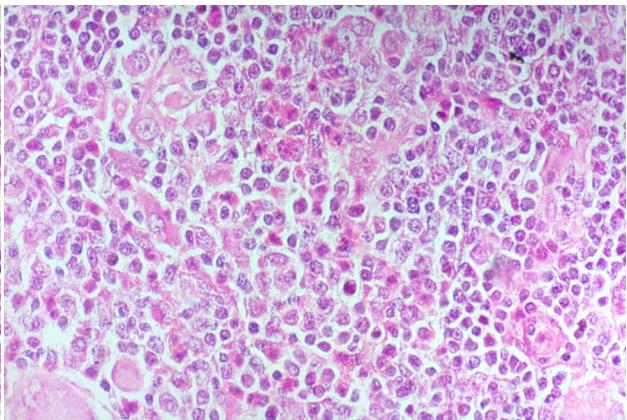


Figure 2 – Plasma cells, histiocytes and Reed-Sternberg cells in mixed cellularity classical Hodgkin's lymphoma. HE staining, $\times 400$
(private collection)

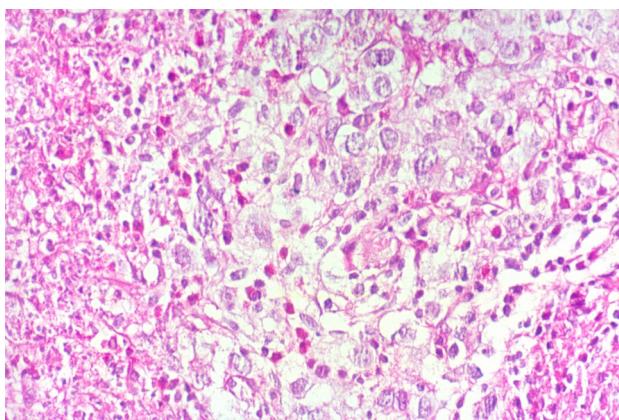


Figure 3 – Necrosis in mixed cellularity classical Hodgkin's lymphoma. HE staining, $\times 400$
(private collection)

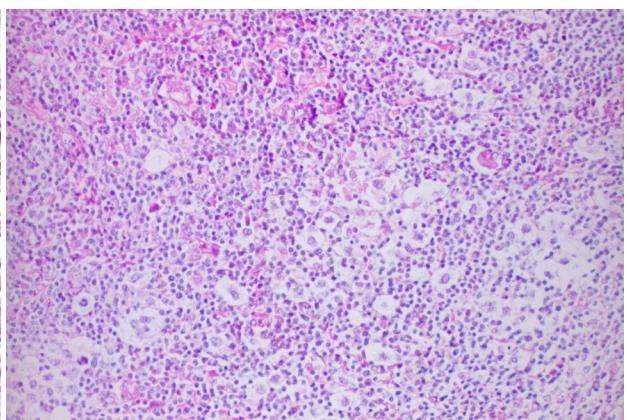


Figure 4 – Uniform aspect of a lymph node with lymphocyte depletion classical Hodgkin's lymphoma. HE staining, $\times 400$
(private collection)

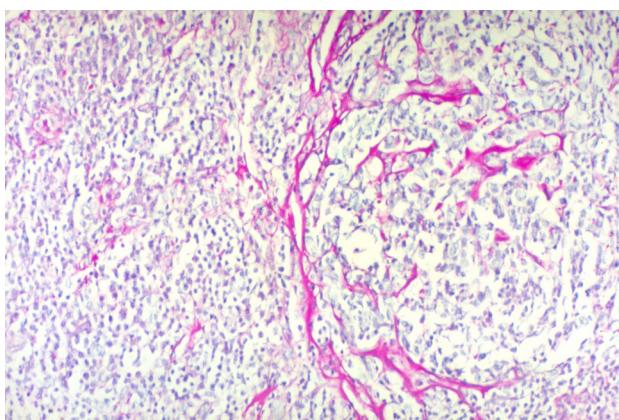


Figure 5 – Branched bands PAS-positive in mixed cellularity classical Hodgkin's lymphoma. PAS staining, $\times 200$
(private collection)

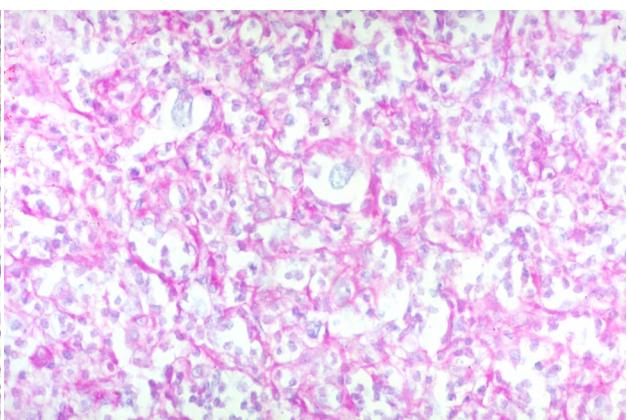


Figure 6 – Small bands delimiting the Reed-Sternberg cells in lymphocyte depletion classical Hodgkin's lymphoma. PAS staining, $\times 400$
(private collection)

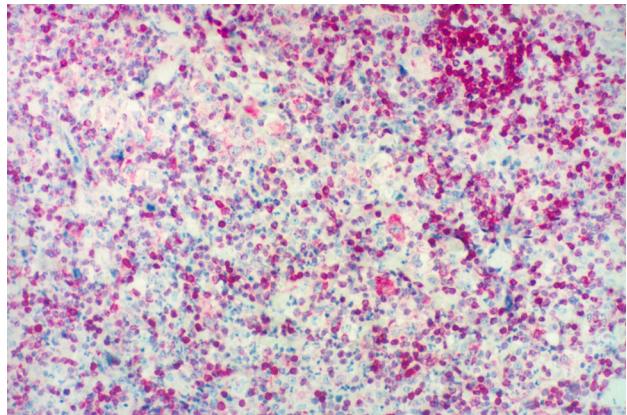


Figure 7 – Lymphocyte depletion classical Hodgkin's lymphoma with a *Bcl-2* (+) expression.
APAAP–New Fuchsin technique,
×200 (private collection)

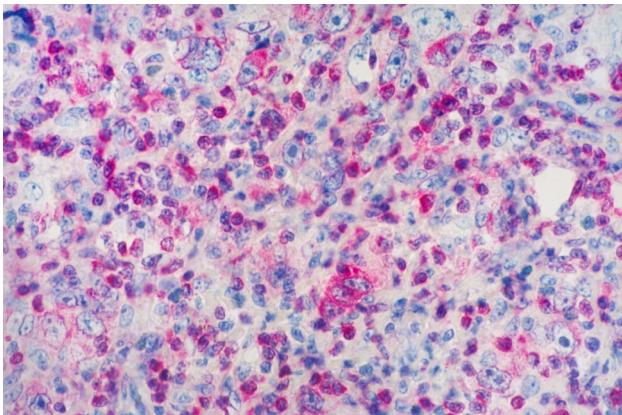


Figure 8 – Lymphocyte depletion classical Hodgkin's lymphoma with a *Bcl-2* (+) expression.
APAAP–New Fuchsin technique,
×400 (private collection)

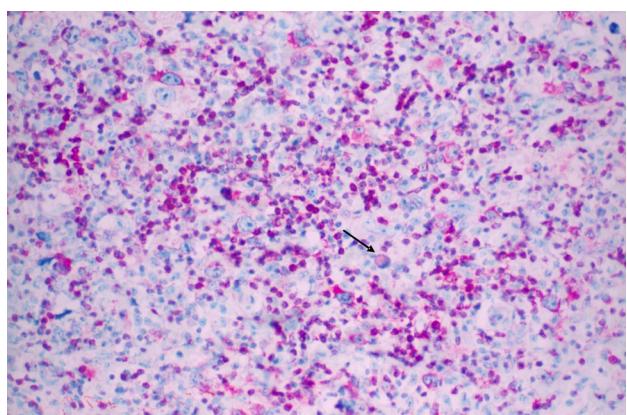


Figure 9 – Mixed cellularity classical Hodgkin's lymphoma with *Bcl-2* (+) expression; a mummified Hodgkin cell *Bcl-2* positive (arrow). AAPA–New Fuchsin technique, ×200 (private collection)

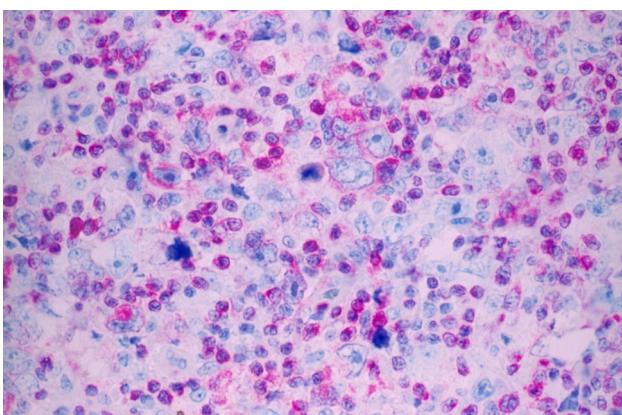


Figure 10 – Mixed cellularity classical Hodgkin's lymphoma with *Bcl-2* (+/-) expression.
APAAP–New Fuchsin technique,
×400 (private collection)

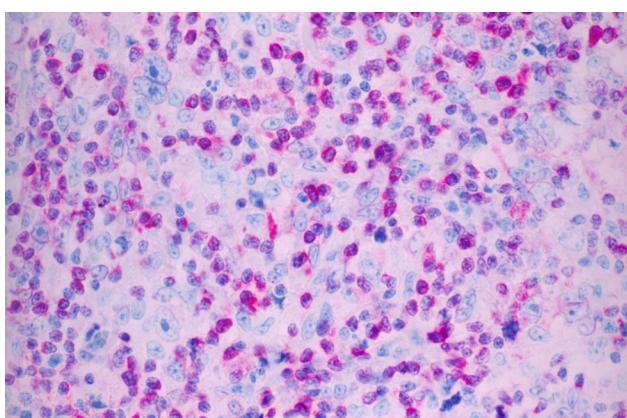


Figure 11 – Mixed cellularity classical Hodgkin's lymphoma with *Bcl-2* (-/+) expression.
APAAP–New Fuchsin technique,
×400 (private collection)

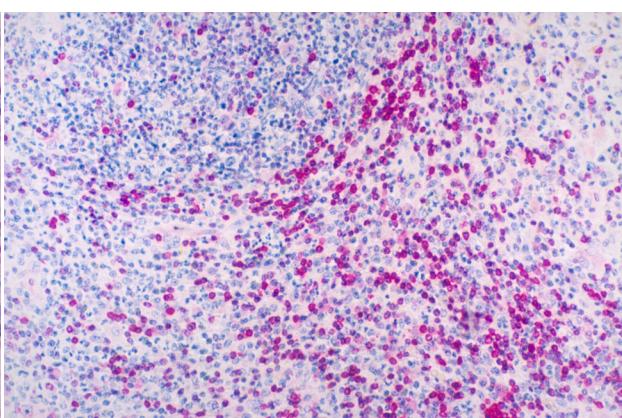


Figure 12 – Nodular sclerosis classical Hodgkin's lymphoma with (+/-) *Bcl-2* expression and a zonal positivity of staining. AAPA–New Fuchsin technique,
×200 (private collection)

Discussion

The *Bcl-2* expression in Hodgkin's lymphoma is frequent observed. Almost 54% from classical Hodgkin's lymphoma cases revealed a variable proportion of HRS cells *Bcl-2* positive without any connection with Epstein–Barr virus [12–14].

The *Bcl-2* expression was identified in 44–45% from aggressive lymphomas [15], being associated with clinical stage III and IV and with a higher rate of relapses [15, 16]. The genetic mechanism, which leads to *Bcl-2* supraexpression in Hodgkin and Reed–Sternberg cells, is unknown. In that way occur different

opinions regarding the role of t(14;18) chromosomal translocation in Hodgkin's lymphoma [17].

Because the presence of t translocation in Hodgkin's lymphoma is controversy, Lorenzen *J et al.* have done a non-isotopic study using *in situ* hybridization and they have determined RNA of Bcl-2. They observed an intact post-transcriptional regulation of Bcl-2 protein. Mummified cells presence was independent to Bcl-2 expression suggesting that its action could be inhibited by other members of Bcl-2 protein family, like Bcl-X [18].

In follicular lymphoma, Bcl-2 supraexpression play an important role in lymphoma-genesis process having as consequence an extension of survival of malignant cells due to apoptosis process blocking or slowing down [19–23].

Some authors sustain that neoplastic cells from follicular lymphoma and those from Hodgkin's lymphoma have a common precursor and the differentiation happens at germinal centers of B-cells [24, 25].

Montalban *C et al.* [26] correlated supraexpression of Bcl-2 in Hodgkin's lymphoma patients with a low complete or partial remission period. In addition, the other members of Bcl-2 family were identified in Hodgkin's lymphoma [17].

Bcl-2 and Bcl-XL supraexpression is present at patients with short periods of complete remission [27].

Kim LH *et al.* found a statistic correlation between Bcl-2 expression, Bcl-XL expression and apoptosis. They sustain that Bcl-XL play an important role in Hodgkin and Reed-Sternberg cells survival and Bax expression could be neutralized by other antiapoptotic members as Bcl-2 and/or Bcl-XL [17].

Zander T *et al.* [28] studied Bcl-2 expression in HRS cell and they have calculated an index. The rise of this index in their study has a bad prognostic.

A higher percent of HRS cells Bcl-2 positive and a weak Bcl-2 expression in surrounding small lymphocyte is associated with bad prognostic and relapses at young patients with classical Hodgkin's lymphoma, NS-CHL especially [29–31] due to antiapoptotic action [17].

Bcl-2 expression in Hodgkin and Reed-Sternberg cells inhibit the apoptotic process determined by the absence of B cells functional receptor, inducing tumor genesis in that case. Therefore, Bcl-2 expression can explain resistance of treatment by apoptosis repression of Hodgkin and Reed-Sternberg cells [13, 21, 30, 32].

In Sup SJ *et al.* study, Bcl-2 is overexpressed in a significant proportion of classical Hodgkin's lymphoma patients and is an independent adverse prognostic factor. Expression of Bcl-2 in classical Hodgkin's lymphoma is a useful, independent prognostic marker and can be used in association with clinical parameters to identify newly diagnosed patients with a good, intermediate, or poor prognosis. Their findings support the use of this biologic marker in conjunction with clinical parameters such as age and stage to identify patients at diagnosis who are at a low, intermediate, or high risk for primary treatment failure [33]. These risk groups might then be used to guide standard therapy and

develop risk-stratified protocols. These data provide a rationale for novel therapies targeting Bcl-2 expression in CHL [33, 34].

From this reason, ADASP (*Association of Directors of Anatomic and Surgical Pathology*) [35] recommend using the Bcl-2 expression as tumoral proliferation marker, which must accompanied any morphological examination.

The *International Prognostic Score*, which incorporates seven clinical and laboratory parameters, was developed for patients with advanced-stage Hodgkin's lymphoma but has also been applied to patients with early-stage disease [36]. Nevertheless, the most prognostic systems used to date, including the International Prognostic Score, fail to identify with sufficient accuracy the proportions of patients with favorable or unfavorable responses to treatment [26].

In our study, we found this correlation very important because the main cause of relapses is inadequate staging. In some cases, this staging is difficult; some little lymph nodes could be overlooked because they can be placed in less accessible areas and cannot be evidenced by the most imagistic methods. For this reason, they cannot reveal in the most of situations. This correlation between Bcl-2 expression and advanced disease occurs in reference data mentioned above.

Our contribution in accuracy staging is using this expression for overview the patients' clinical and aggressive disease status by immunohistochemical Bcl-2 examination in Hodgkin and Reed-Sternberg cells. For this reason, we recommended reinvestigation and restaging all the cases with Bcl-2 expression higher than (+/-) which are staged as stages I or II. We say that because we met (+/-) and (+) expression only in advanced stages.

Conclusions

In our study, we conclude that using Bcl-2 immunohistochemical reaction helps to precision staging, with avoid errors in most of the cases. This is very important because the main cause of relapses is inadequate staging.

Immunohistochemical reaction for Bcl-2 expression although less used in Romania, is very accurate. In addition, in Romania, the diagnostic process is based on old methods in majority of cases.

Investigation Bcl-2 expression is a prominent part of diagnostic because an early accurate staging improve the patients' prognostic, therapeutically attitude being different in advances stages compared to earlier stages.

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Corresponding author

Corina Flangea, Assistant Professor, MD, PhD, Biochemistry Department, "Victor Babeș" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300 041 Timișoara, Romania; Phone +40256–204 476, E-mail: flangeacorina@yahoo.com

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