

Immunohistochemistry in the therapeutic decision for Hodgkin's lymphoma

Mihaela LEBEDENCO^{1,3}, Nicoleta BERBEC¹, Oana STANCA¹, Andrei COLITA¹, Andrei TURBATU¹, Stefan MARIN³, Camelia DOBREA², Anca Roxana LUPU¹

¹Department of Hematology, Coltea Clinical Hospital, Bucharest, Romania

²Department of Hematology, Fundeni Clinical Institute, Bucharest, Romania

³Ambulatory of Hematology, "Sf. Ioan" Emergency Clinical Hospital, Bucharest, Romania

ABSTRACT

Hodgkin's lymphoma is a highly curable malignant hemopathy in both adults and children with therapy adapted to risk factors, including both chemotherapy and radiotherapy. Through effective and curable therapeutic schemes there is a huge potential to improve the vital prognosis and the quality of life in the long term. 15-20% of patients in early stages (stages I, II) and 35-40% of those in advanced stages (III and IV) have relapses or resistance to first-line therapy.

Molecular analysis of the cells facilitated the discovery that classical Hodgkin lymphoma, in the vast majority of cases, and predominantly lymphocytic lymphoma present clonal abnormalities derived from the germinal center of B cells.

RS cells and their mononuclear variants – Hodgkin cells (HRS) demonstrate an antigenic expression without lineage specificity: CD 15(Leu-M1) and CD 30 (Ki-1). Immunohistochemistry helps to identify the two major categories of Hodgkin's lymphomas.

The diagnosis of Hodgkin's lymphoma requires a histopathological examination on a lymph node biopsy or, more rarely, a biopsy for extranodal determination of the disease.

The identification of Reed-Sternberg cells and their variants is essential for establishing the histological diagnosis of Hodgkin lymphoma.

Comparative study (retrospective and prospective) of two groups of patients with Hodgkin's Lymphoma: Lot 1 – with favorable evolution; Lot 2 – refractory/relapsed/partial remission cases.

Study groups – 80 patients diagnosed with classical Hodgkin's lymphoma over a 10-year period, between 2007-2017:

- Lot 1-53 patients with a favorable evolution – in complete remission after the first line of treatment;*
- Lot 2-27 patients with unfavorable evolution (relapsed/refractory/partial remission cases).*

Keywords: Hodgkin lymphoma, immunohistochemistry

INTRODUCTION

The current work aims to evaluate the characteristics of the tumor microenvironment and malignant cells using techniques that are already available or that can be successfully implemented in the laboratories associated with hematology clinics in the country. The realiza-

tion of the project aims at histopathology, immunohistochemistry (IHC) techniques for the phenotypic characterization of the microenvironmental and malignant cells, to highlight the association of EBV and to study the expression of some genes characteristic of malignant and non-malignant cells in the tumor mass [1-4].

Corresponding author:

Mihaela Lebedenco

E-mail: mihaela.lebedenco@yahoo.com

Article History:

Received: 6 February 2024

Accepted: 28 March 2024

The present study is a retrospective/prospective analytical observational type and includes patients diagnosed with classical Hodgkin's lymphoma (LHC) between 2007 and 2017, in the records of the Hematology Clinic of the Coltea Clinical Hospital, Bucharest, followed up until December 2019 (or until the occurrence of death), in which we wanted the selection of clinical, biological and therapeutic response parameters, with proven prognostic value.

CASE PRESENTATION

The objectives pursued were:

- Follow-up of clinical evolution and response to treatment in Hodgkin's lymphomas, correlated with possible prognostic, clinical and biological factors.
- Comparison of patient evolution according to histological subtype, disease stage and treatment applied, correlated with prognostic factors present at the onset.
- Comparative studies with data from the literature to identify particularities regarding incidence, evolution and response to treatment.

The most realistic assessment of a patient's prognosis at the time of diagnosis is a prerequisite for an optimal therapeutic approach with as few long-term side effects as possible.

We analyzed certain risk factors (clinical, paraclinical) and evaluated the survival of patients with LHc depending on them, on the evolutionary stage of the disease and on the therapeutic means used. The different evolution of patients with LH, in the same clinical stage, is explained not only by the different therapies used, but also by characteristics of the host organism and tumor proliferation.

The aim is to describe a prognostic model based on the biological characteristics of LHc with predictive value on the therapeutic response and which allows a therapy adapted to the degree of risk of each patient [5,6]

Highlighting EBV expression in tumor cells to establish prognostic implications and a differentiated therapeutic approach in LH are not common in current pathological investigations. The identification of the markers associated with the tumor process is extremely important because these molecules can constitute targets for effective biological therapies [7,8].

Therefore, the complex approach, with the totality of clinical, biological and therapeutic factors with possible influence on the patient's prognosis and the individualization of each individual case guides in the choice of the most effective therapy.

The study group was made up of 80 patients diagnosed with classical Hodgkin's lymphoma (HCL) over a period of 14 years, between 2007-2017, in the Hema-

tology Clinic of the Coltea Clinical Hospital, followed up until December 2019 (or until upon the occurrence of death).

In the analysis, patients diagnosed with LHc with all four histological subtypes (according to the WHO classification) previously, but also during the course of the study, were randomly included, based on the inclusion criteria defined before their selection:

Correct and complete diagnosis of the type of lymphoma through the histopathological and immunohistochemical examination of the tumor biopsy material.

- The presence in the observation sheets of all evaluated parameters.
- In order to achieve clinical-histological correlations in patients with LHc, the group was subdivided, depending on the response to treatment at the end of the study, into two subgroups, namely:
 - Group 1 made up of 53 patients with favorable disease evolution - in complete remission after the first line of treatment;
 - Group 2 made up of 27 patients with unfavorable disease evolution or refractory cases - in which we introduced patients in partial remission, patients with progressive disease, as well as patients with stable disease after the first line of treatment.

The study was based on the analysis of patient-related factors (age, sex, clinical performance status, significant personal pathological antecedents), parameters that evaluate the tumor mass (biopsy location, presence of B symptoms, Ann Arbor stage, number of affected lymph node areas, presence Bulky tumor masses, the type and number of extranodal determinations, the presence of medullary determination, the presence of hepatomegaly/splenomegaly, the serum level of LDH, the serum level of beta2-microglobulin), of the prognostic factors related to the stage, histological subtype, biological parameters (hemogram, sideremia, the level of ESR, the level of serum albumin, the presence of the Epstein-Barr virus), prognostic factors related to the treatment (type of chemotherapy used in induction, reduction of the tumor mass by more than 50% after the first treatment, use of radiotherapy, monoclonal antibodies, transplantation autologous hematopoietic stem cells, the need for intermediate PET-CT) and the new prognostic factors that assess the evolution of the disease (BCL2 overexpression in malignant cells, the presence of PD1 in lymphocytes and the antigen CD68 in macrophages in the tumor microenvironment, as well as the presence of EGFR).

The diagnosis of Hodgkin's lymphoma was established after going through several stages:

- Histopathological and immunohistochemical diagnosis
- Evaluation of the clinical stage

The histopathological diagnosis was established based on the analysis of samples from the lymph node or other tissue, collected by incisional or excisional biopsy. The first step in establishing the histopathological diagnosis was the anatomical-pathological examination of the collected representative tumor fragments, through which, on the one hand, non-hematological proliferations and benign lymphoproliferations were excluded, on the other hand, an attempt was made to define the histological type. Later, the immunophenotypic profile of lymphoproliferation was established by performing immunohistochemical tests on the paraffin-embedded tumor preparations. The analysis of the gene expression profile in malignant cells and the cytogenetic study could not be carried out due to the lack of accessibility of these methods, although it would have considerably improved the accuracy of prognosis prediction and would have been useful in customizing the therapeutic approach, depending on the characteristics of the tumor microenvironment.

The immunophenotypic study by immunohistochemistry on the paraffin-embedded tissue was carried out over time in several laboratories: in the Pathological Anatomy Laboratory of the “Victor Babes” Institute, Bucharest, then in the OncoTeam Diagnostic Laboratory at the Monza Hospital, later in the Clinics Royal Hospital Bucharest. Very rarely results were also obtained from other private laboratories.

Immunohistochemistry on paraffin-embedded tissue allows the use of the usual prepared tissue and the study of archival material. Hodgkin-Reed-Sternberg cells represent only a low proportion of the total cell population in biopsies. Since most cells have a malignant substrate, it is essential to establish the cytological features of cells that react with different antibodies. Therefore, it is preferable to use paraffin sections for the immunophenotypic study of LH, which allow the appreciation of both the architecture of the lesion and the cytological details of the cells that react with a certain antibody. Most subtypes of LHc have a distinct phenotype consisting of a unique combination of a lymphoid activation antigen (CD30) and a myelomonocytic antigen (CD15), with the absence of the common leukocyte antigen (LCA, CD45). This characteristic phenotype, encountered in 85% of cases, does not have a normal counterpart among the cells of the lymph node. CD15 and CD30 characteristically localize to both the Golgi complex and cell membranes of RS cells and their variants. The combination of reactivity for the Golgi complex and the surface gives the characteristic “on target” appearance. Apart from this unique phenotype and cellular localization, LHc can have a distinct histological appearance, especially in the nodular sclerosis subtype. In this subtype, lacunar cells form clusters, suggesting cellular syncytium. Thus, a syncytial array of

CD15+CD30+CD45 “targets” is characteristic of LH with nodular sclerosis. Finally, even if a marker is present or absent, the best criterion for the diagnosis of LHc is the histological context or immunoarchitecture, which is pathognomonic.

IHC staining is a two-stage indirect phenotyping technique performed on paraffin-embedded tissue sections. IHC stainings were performed using two panels of antibodies (Ab) – panel 1: CD30, CD15, CD20, PAX5, CD3; extended panel: EBV/LMP1, BC:2, PD1, EGFR, CD68.

IHC stainings were performed by the automatic and manual staining technique, respectively:

- a) The automated IHC staining technique (performed according to the manufacturer's instructions) was performed using two types of immunostaining:
 - Group Mark Ultrastainer Module immunostainer (Ventana, Roche Diagnostics) for antiCD20, CD3, PAX5, bcl2 antibodies (highlighting of the Ag-Ab reaction was done using the Ultra View Universal DAB detection kit) and for antiCD30 antibodies (highlighting of the Ag reaction -Ac was done using the Opti View DAB IHC Detection Kit and the Opti View Amplification Kit);
 - Group-III immunostainer (LeicaBiosystems) for antiCD15 (the reaction was highlighted using the Bond Polymer Refine Detection Kit)
- b) Manual staining technique was used for EBV/LMP1, PD1, EGFR and CD68 antibodies. Manual IHC staining technique: [9,11]
 - The included histological sections were deparaffinized, rehydrated and washed in phosphate-saline buffer solution (PBS), pH=7.4;
 - Unmasking antigenic sites by heat treatment (boiling in a microwave oven in EDTA pH-9 buffer solutions);
 - Incubation with specific needle at room temperature, 1 hour;
 - Incubation with Ultra Vision Quanto Detection System HRP DAB polymer for 30 minutes at room temperature;
 - Development in 3-3'diaminobenzidine (DAB) for 5-10 minutes;
 - Counterstaining with Mayer's Hematoxylin for 2-3 minutes;
 - Dehydration, clarification and mounting of slides.

Reaction assessment for additional Ac panel:

- the reaction for EBV/LMP1 in RS tumor cells was marked with “+”, respectively “–”, without the percentage quantification of the number of positive tumor cells (qualitative reaction);
- the reaction for BCL2 in RS tumor cells was marked with “+”, respectively “–”; for “+” cases, a

qualitative assessment was made of the intensity of the reaction (on a scale from + to +++, related to the BCL2 reaction from reactive small lymphocytes) and a quantitative assessment of the percentage of BCL2 positive tumor cells;

- the reaction for PD1 was noted in the peritumoral microenvironment, assessing, quantitatively, the percentage of PD1+ small lymphocytes and their disposition relative to the RS tumor cells (possibly peritumoral rosette);
- the reaction for CD68 was noted in the peritumoral microenvironment, assessing, quantitatively, the percentage of CD68+ cells and their disposition relative to RS tumor cells (possibly peritumoral rosette);
- reaction for EGFR: five fields with the highest vascular density were selected for each section (selected with a 10x objective, 20x eyepiece with a Leica DMC 2900 microscope); these were captured with a digital camera with Leica Application Suite (LAS) software; vessels labeled by the EGFR reaction in each of the fields were counted and averaged;

DISCUSSIONS

The processing and statistical interpretation of the obtained data was carried out with the help of Microsoft Excel 2021 professional, SPSS Statistics 15.0.0 (SPSS Inc – 2006) – for ANOVA analysis, Crosstabulation- Chi square, survival time (Kaplan-Meyer), Odd Ratio (OR) and MedCalc Version 14.2.1., 1993-2014)-for ROC analysis and Cronbach's Alpha. A p value <0.05 was considered statistically significant.

The studied sample was composed of 80 patients with a confirmed diagnosis of classic Hodgkin's lymphoma, according to international WHO criteria, in which a series of clinical and paraclinical parameters with a

proven prognostic role, but also potential prognostic factors correlated with the evolution of the disease, were analyzed, which was noted in dynamics. The response to the therapy was followed during a relatively long period in which the study took place, between 2007 and 2017, within the Coltea Hematology Clinic.

The entire lot was divided into two subgroups: Group 1 = Cases with favorable evolution (53 cases; 66.25%) and Group 2 = Refractory cases (27 cases; 33.75%)

All data in the database, starting with age and ending with survival/death, were analyzed by various methods.

CONCLUSIONS

1. Hodgkin's lymphoma has become one of the most curable hematologic malignancies, both in adult patients and especially in pediatric patients with a current high overall cure rate.

2. In the studied group, there was an increasing incidence in the last half of the study period (2012-2017) of patients diagnosed with classical LH – 68.75%, the equivalent of 57 patients, results consistent with the data from the specialized literature.

3. Predominant histological subtypes SN (nodular sclerosis) = 50.00% and CM (mixed cellularity) = 45.00%, BL (lymphocyte rich) + DL (lymphocyte depletion) = 5.00%. SN is majority between 18-40 years, and CM between 18-30 years, SN was located especially in stages II and IV and CM in stages III and IV.

4. Although the prognosis is relatively good and the current overall cure rate of LH is high, it is important to emphasize that clinico-biological factors still remain the main pillars in guiding therapeutic strategies.

Conflict of interest: none declared

Financial support: none declared

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