

Indolent Lymphoma: Diagnosis and Prognosis in Medical Practice

Anca CIOBANU^a; Oana STANCA^b; Irina TRIANTAFYLIDIS^b; Anca LUPU^b

^aDepartment of Hematology, Coltea Clinical Hospital, Bucharest, Romania

^b“Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

ABSTRACT

Introduction: Non-Hodgkin lymphomas represent malignant tumors of lymphoid cells. These chronic lymphoproliferative disorders stand for malignancies with varied histological aspects, clinical features, evolution, prognosis and aggressiveness. Follicular lymphomas are the most frequent form of indolent lymphomas and they represent around 25% of all malignant lymphomas in adults.

Material and method: Between 2011 and 2012, we have retrospectively observed, analyzed and described a group of 24 patients diagnosed with follicular lymphomas in the Department of Hematology from Coltea Hospital. The admittance criteria were: age, gender, hemoglobin and LDH levels, number lymph nodes affected and the Ann Arbor lymphoma staging system. Also used as patient study parameters were the following immunohistochemical criteria: CD20, UCHL1, CD79a, expression of Bcl 2 and Bcl 6, CD10 and the proliferative index (Ki-67).

Results: Multiple studies have shown that prognosis depends far more on clinical and histology parameters, including age, the presence of extra-node diseases and the performance status. In our study, regarding the ratio between the two genders, the male patients were more numerous than the female patients. The impairment of the male patients is associated with an unfavorable prognosis. From the age perspective, most of the diagnosed patients were part of the age group over 60. The age exceeding 60 is considered a negative prognosis factor. The serum lactate dehydrogenase (LDH) level is also considered an unfavorable prognosis factor. In our study, stage III and IV were frequently and this represents a poor prognosis factor.

Conclusions: Although it was a small number of patients, the results obtained correspond to the results existing in literature

Keywords: Follicular lymphoma, diagnosis, prognosis

INTRODUCTION

Follicular lymphomas are the most frequent indolent non-Hodgkin lymphoma, presenting as a painless, slowly progressive adenopathy. They are defined as malignant proliferations of the follicular centre cells: centrocytes (small

cleaved cells) and centroblasts (large noncleaved cells). The frequency of follicular lymphomas is around 20% in Western European countries. In Romania, the frequency is 5-7%, similar to other Eastern European and Asian countries (1).

Follicular lymphomas include clinical symptoms caused by node damage. They can be

Address for correspondence:

Anca Ciobanu, Coltea Clinical Hospital, Department of Hematology, I.C. Bratianu Avenue, 030171, Bucharest.

E-mail: ancaciobanu05@yahoo.com.

Article received on the 19th of November 2012. Article accepted on the 4th of November 2013.

evaluated by morphological and biological criteria. Follicular lymphoma patients most frequently present with a late advanced stage disease.

Although many of the cases of follicular lymphomas (of 1-2 degree) are considered indolent lymphomas, their clinical evolution is often unpredictable. We have patients who exceed the survival average considerably, by 8-10 years, and patients which turn into aggressive lymphomas shortly after the diagnosis. This aspect hinders the diagnosis, especially for follicular lymphomas with mainly centroblastic cells (2). The follicular lymphomas distinguish themselves through the clinical evolution with multiple relapses, disease free survival (DFS) and overall survival (OS) varying from one patient to another.

Studies of non-Hodgkin lymphomas at molecular and gene levels (through FISH/CISH, PCR, RT-PCR techniques, defining the gene profile with cDNA microarray, etc.) has allowed the identification of new entities, but has also opened up the possibility of new, customized, therapeutic approaches, with higher results compared to traditional treatments.

The translocation t (14;18) has been described as being specific and diagnostic for follicular lymphomas. In this translocation, the bcl2 gene approaches the Ig heavy chain gene. The bcl2 gene encodes a protein capable of inhibiting apoptosis, so that the cancer cells have a longer life resulting in their local accumulation (3).

Recently, immunophenotypic and genotypic analysis carried out with the help of monoclonal antibodies and the new techniques in molecular biology, have allowed the identification of malignant populations with monoclonal proliferations and the recognition of morphological and functional differentiation stages at a cell line level (4).

Modern cytogenetic methods demonstrated that all lymphomas have chromosomal changes such as translocations, deletions, rearrangements, inversions. Recent research has identified the factors which activate cell proliferation: c-myc 62 phosphoprotein, the proliferation of cell nuclear antigen (PCNA). Also the Ki 67 antigen is increased, also having a prognosis value (4).

The prognostic variables in follicular lymphomas may be heterogeneous.

The Follicular Lymphoma International

Prognostic Index (FLIPI) is used more frequently for follicular lymphomas.

The FLIPI index results in 3 risk groups reported to a 10 year survival rate (low - 71%, intermediate - 51% and high - 36%).

The immunohistochemical expression of the bcl 2 anti-apoptotic protein A is also considered to be a prognostic factor. The overexpression of this protein is a marker that suggests a poor prognosis, while the expression of bcl 6 or CD10 (germinal center markers) indicate a favorable prognosis (5).

The relatively recent discovery of the humanized monoclonal antibodies anti-CD20 has opened up a new era of treatment for follicular lymphomas. The combination of anthracyclines with alkylating agents and the administration of interferon or purine analogues, or the association with the autologous transplant with hematopoietic cells, treatments previously attempted for stage II/IV patients could not be standardized. The treatment with monoclonal antibodies of Rituximab type has gradually become more widespread and has the tendency of becoming a first-line therapy, with or without any association with chemotherapy. Another category of therapeutic agents is represented by radioimmunoconjugates associated or not with chemotherapy. □

MATERIAL AND METHOD

Starting from the existing patient data regarding prognostic factors, a group of 24 patients was selected from patients admitted in "Coltea" Hospital Hematology Clinic in 2011-2012. Some studies have shown that age, sex, number of extra-nodal determinations, disease B symptoms, and erythrocyte sedimentation rate (ESR) and serum lactate dehydrogenase (LDH) levels may be prognostic predictors (6).

FLIPI index (considered an important indicator for prognosis) comprises the following negative factors: age over 60, AnnArbor stage III or IV, hemoglobin <12g / dl, >4 lymph nodes affected areas, increased serum LDH (7).

The following parameters were analyzed for the stratification of patients included in the study: patient data (sex, age), clinical balance (ECOG performance status, disease B signs, syndrome tumor, stage of disease at diagnosis), laboratory findings balance (renal and hepatic function, eg. histopathology of lymph node and bone biopsy, molecular biology tests in select-

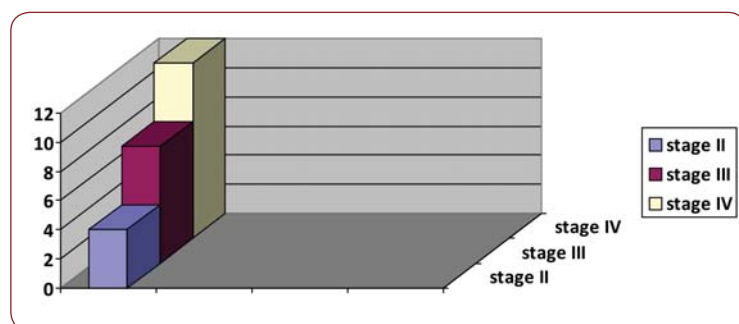


FIGURE 1. The distribution of the patients considering the stage of the disease.

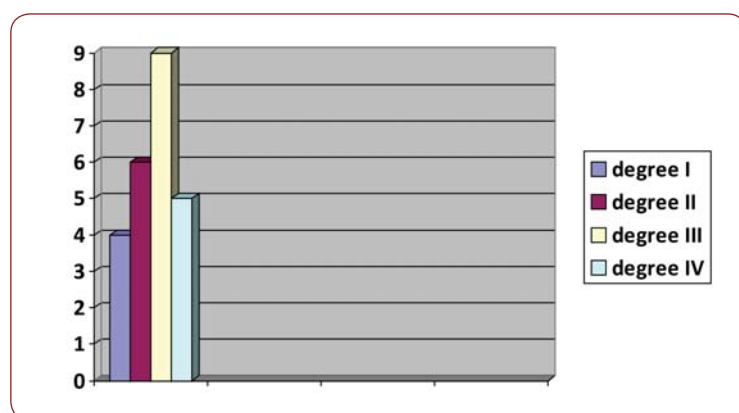


FIGURE 2. The distribution of patients considering the histological degree.

ed cases), imaging tests - chest X-ray, ultrasound and computer tomography. The immunohistochemical criteria used in the study of the patients were as follows: CD20, UCHL1, CD79a, bcl 2, CD10 and the proliferative index (Ki-67). It represents a nuclear antigen present during the cell cycle (G, G2, S, M) and absent in the resting cells (6,7). It is identified by monoclonal antibody Ki-67 MoAb as a proportion of Ki-67 + cells in the total cell. □

RESULTS

The average age in the patient group was 61, with thresholds between 34 and 83 years of age. The results showed that the average age at diagnosis of patients suffering from follicular lymphoma is 60.

Follicular lymphoma is an elderly lymphoma, with increasing incidence, thus the group older than 75 will report an increase of 1.8% per year.

In our study, the gender ratio was: men/women 16/8.

Smoking is considered an important factor in the etiology of lymphomas. In the patient group, 14 patients were smokers.

Half of the patients in our group have shown general signs of the disease.

The Ann-Arbor staging system is a necessary but not sufficient prognostic parameter (7). For 10% of the patients with follicular lymphoma, the disease was localized upon diagnosis.

Follicular lymphomas of degree 1 and 2 can be found at onset in stages III-IV, in 80% of the cases, with bone marrow involvement in 50% of the cases, but this will not radically impact the prognosis.

In our study, the distribution of patients by the stage of the disease at the onset was the following: in stage II – 4 patients; in stage III – 8 patients, and in stage IV – 12 patients (Figure 1).

The distribution of patients depending on the histological degree was as follows: Degree I – 4 patients; degree II – 6 patients; degree IIIA – 9 patients; degree IIIB – 5 patients (Figure 2). It was noticed that most of the patients had histology type III, which corresponds to an unfavorable prognosis.

Laboratory findings analysis: the complete blood count is normal in most of the cases at onset or during localized stages. Usually, there is no pathognomonic data.

The number and leucocyte count may be normal or sometimes there might be a decrease in the lymphocyte count. There is a small percentage of cases in which the peripheral discharge of lymphoid cells with atypical features is similar to the ones found in the lymph nodes (7).

In our study, 18 patients had normal values of hemoglobin at the disease onset, while 6 patients had low values.

In our group, 9 patients showed a normal value of the erythrocyte sedimentation rate at onset, while 15 patients had increased values.

The serum lactate dehydrogenase (LDH) level is an absolute bias parameter of overall survival in follicular lymphomas and is treated conventionally (5). 16 of our patients had increased values of serum lactate dehydrogenase (LDH) at onset.

All the cases in the study group have tested positive for bcl 2 and CD 10. From an immunohistochemical perspective, the reaction for bcl 2 was intense and diffuse (Figure 3).

In patients with small cell follicular lymphoma, the proliferative index was variable, both for the areas with nodular pattern and for the ones with diffuse pattern, but a little higher

than it is estimated in the existing data; only Ki67 had a values under 10% (7).

In our group, 10 patients had an increased Ki-67 >70% (Figure 4, 5).

Generally speaking, the analyzed cases were positive for L26, the positive nature being intense and diffuse. Yet in certain cases, L26 was negative, the only B line marker being CD79a. In all cases, there was also a population of small T cells, positive for UCHL1. These cells will be analyzed in a subsequent stage with the purpose of identifying the Treg cells and their receptors. □

DISCUSSIONS

According to the existing data, the age over 60 represents an unfavorable prognosis factor. In our study, most of the patients were over 60.

The specialized literature shows the fact that the evolution of follicular lymphomas is more severe in male patients than in female ones.

The existence of B symptoms at the onset of the disease represents an unfavorable prognostic factor. Regarding follicular lymphomas, the existence of the B symptoms is reduced at onset, being more frequent in advanced stages and influencing the survival considerably.

Multiple studies have shown that prognosis depends far more on clinical and histology parameters, including age, the presence of extra-node diseases and the performance status. Stage III and IV represent a poor prognosis factor (6).

The histological degree is used for therapeutic guidance concerning follicular lymphomas and is also correlated with the prognosis.

The erythrocyte sedimentation rate represents the biological parameter which is best correlated with the clinical stage of the disease, while the value of the C reactive protein is correlated best with the histological degree of malignancy (6). Serum lactate dehydrogenase (LDH) represents an indicator of the response to treatment, the higher values at onset being associated with the low rate of complete and partial remissions. It plays the role of a specific tumor marker.

The peripheral cytopenias (leukopenia and thrombocytopenia) are most frequently a secondary effect of the cytostatic therapy; but they can also be generated by a massive bone marrow infiltration or a hypersplenism (5).

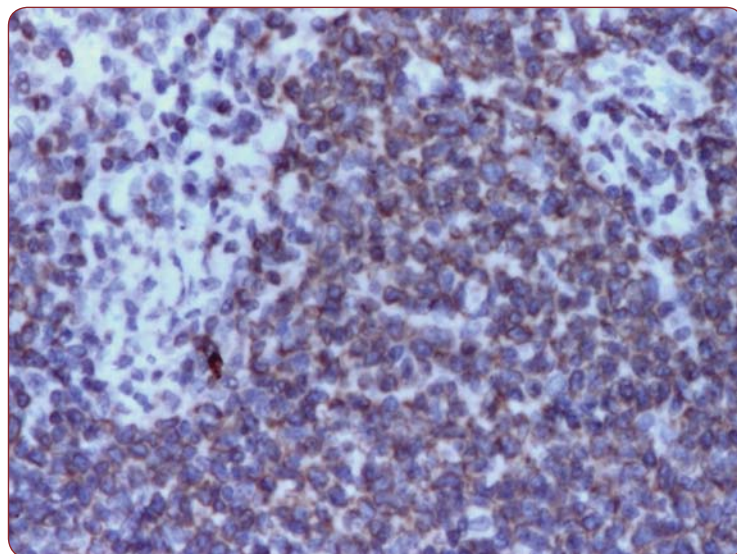


FIGURE 3. Malignant non-Hodgkin Follicular Lymphoma with nodular patterns, small B cells. Col. IHC for bcl 2 – positive diffuse reaction Ob. 20x.

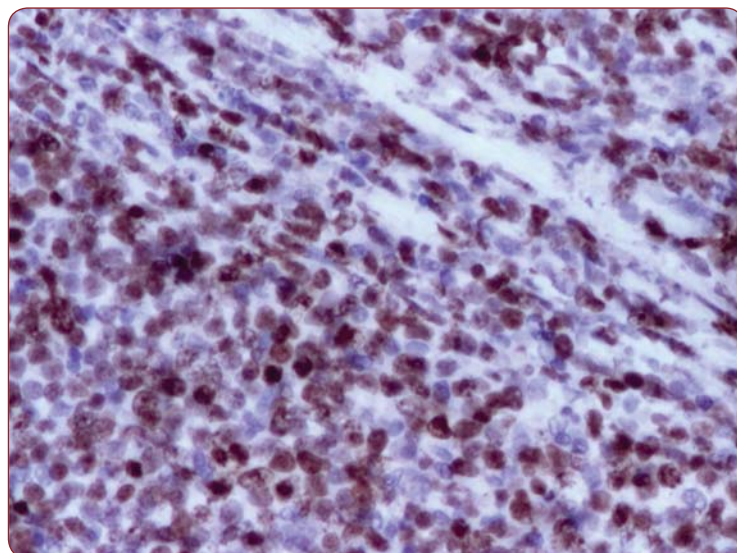


FIGURE 4. Follicular lymphoma with large B cells, degree 3b. Col. IHC for Ki67. Positive reaction in approximately 70% of the cells. Ob. 20x.

The following factors have a well-known importance: the immunohistochemical expression of the bcl 2 anti-apoptotic protein, usually resulted from t (14,18) with the juxtaposition of the BCL2 oncogene at the locus of the IGH heavy chains. The overexpression of this protein is a poor prognosis marker, while the expression of bcl 6 or CD10 (germinal center markers) indicates a favorable prognosis (5).

The relatively recent discovery of the humanized monoclonal antibodies anti-CD20 has opened up a new treatment era for follicular lymphomas. The treatments previously attempted in the patients with stage II/IV, such as

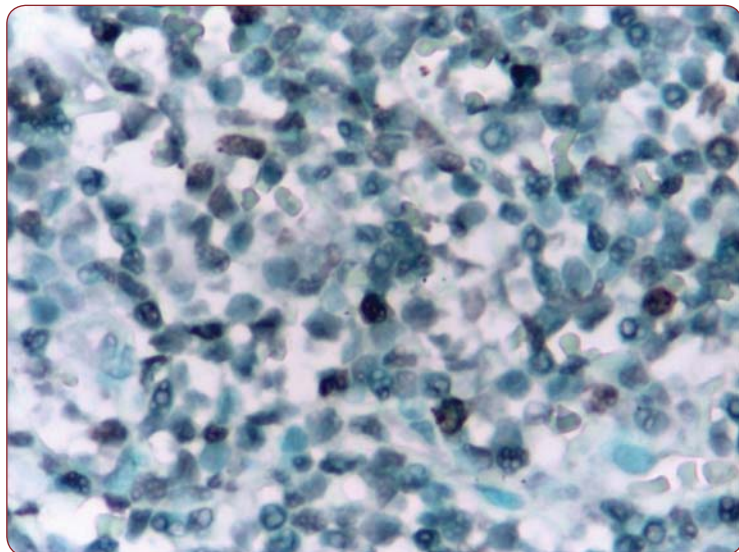


FIGURE 5. Follicular lymphoma with follicular and diffuse pattern. Col.IHC for Ki67. Proliferative index 7-8%. Ob. 40x.

the combination of anthracyclines with alkylating agents and the administration of interferon or purine analogues or the association with the autologous transplant with hematopoietic cells, could not be standardized. The treatment with monoclonal antibodies such as Rituximab has gradually become more widespread and has the tendency of becoming a first-line therapy (8), with or without any association with chemotherapy. Another category of therapeutic agents is represented by the radioimmunoconjugates associated or not with chemotherapy.

In our study, treatment response was defined as complete remission (CR), partial remission (PR), non-response (NON-R).

Chemotherapy was chosen according to age, comorbidity, stage of lymphoma at diag-

nosis, presence of extra-node determinations and their location.

The most common chemotherapy treatment used was R-CHOP followed by R-COP and R-COEP. Up to 57.7% reduction in tumor mass was obtained with >50% having a favorable prognosis. In the study group, the largest tumor mass reduction rate, after the initial therapy, was obtained with R-CHOP.

We achieved complete remission (CR) in 42.3% of patients in the study group and partial remission in 32.4% overall response rate being 74.7%. □

CONCLUSIONS

Although they refer to a small number of patients, the results obtained correspond to the results existing in literature. Regarding the ratio between the two genders, the male patients were more numerous than the female patients. The impairment of the male patients is associated with an unfavorable prognosis. From the age perspective, most of the diagnosed patients were part of the age group over 60. The age exceeding 60 is considered a negative prognosis factor. The serum lactate dehydrogenase (LDH) level is also considered an unfavorable prognosis factor. The molecular biology techniques allow not only the increase in the accuracy of the diagnosis, but also the monitoring of the therapeutic response by detecting the minimal residual disease.

Conflict of interests: none declared.

Financial support: none declared.

REFERENCES

1. Montoto S, Davies AJ, Matthews J, et al. – Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol* 2007; 25:2426-33
2. Montoto S, Davies AJ, Matthews J, et al. – Risk and clinical implications of transformation of follicular lymphoma to diffuse large B-cell lymphoma. *J Clin Oncol* 2007; 25:2426-2433
3. Ross CW, Ouillet PD, Saddler CM, et al. – Comprehensive analysis of copy number and allele status identifies multiple chromosome defects underlying follicular lymphoma pathogenesis. *Clin Cancer Res*. 2007; 13:4777-85
4. Yamamoto K, Ono K, Katayama Y, et al. – Derivative (3)t(3;18)(q27;q21)t(18;16)(q21;?) involving the BCL2 and BCL6 genes in follicular lymphoma with t(3;14;18)(q27;q32;q21). *Cancer Genet Cytogenet*. 2007; 179:69-75
5. Zha H, Raffeld M, Charboneau L, et al. – Similarities of prosurvival signals in bcl 2 positive and bcl 2 negative follicular lymphomas identified by reverse phase protein microarray. *Lab Invest* 2004; 84:235-44
6. Gordon LI, Anderson J, Colgan J, et al. – Advanced diffuse non Hodgkin's non lymphoma. Analysis of prognostic factors by the international index and by lactic dehydrogenase in an intergroup study. *Cancer* 1995; 75:856-73
7. Solal-Céligny P, Roy P, Colombat P, et al. – Follicular Lymphoma International Prognostic Index; *Blood*, 1 september 2004; 104:1258-1265
8. Salles GA – Clinical features, prognosis and treatment of follicular lymphoma. *Hematol* 2007; 216-25.