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CLINICAL – BIOLOGICAL AND PROGRESSIVE CORRELATIONS IN THE CHRONIC LYMPHOCYTIC LEUKEMIA

ABSTRACT OF PhD THESIS

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TABLE OF CONTENTS

I. GENERAL PART

II. SPECIAL PART

Keywords: CLL, clinical correlations, cholesterol, evolution, treatment

SYNTHESIS OF THE GENERAL PART

The chronic lymphocytic leukemia is characterized by the progressive accumulation of monoclonal lymphocytes, with specific phenotype ($CD5^+$, $CD19^+$, $CD23^+$, FMC7 ^{dim}, $CD20^{dim}$, $SmIg^{dim}$) in the peripheral blood, bone marrow and lymphoid tissues.

The classification systems of the chronic lymphoproliferative syndromes simultaneously developed with the evolution of the diagnosis techniques. REAL classification from 1990 is based on morphologic, immunologic and clinical criteria. Since 2009, most of the hematologists adopted the WHO 2008 system, where the molecular aspects appear for the first time.

CLL is the most common form of leukemia in adults from the western countries, representing around 30% of the total leukemias and an incidence of 3.7 for 100,000 individuals in the United States each year. The mean age for diagnosis is 72 years, but in recent years, a third of the new cases are diagnosed before the age of 55 years. Taking into consideration the long survival average rate (around 10 years), CLL prevalence is between 0.03% and 0.05%. The ratio between the incidence of the disease for men and women is around 1.5-2:1. The male gender represents an important risk factor for the malign hematological conditions, but the causal factors underlying this association are unknown. However, the occupational exposures can be incriminated.

CLL etiology is mostly unknown, despite the fact that the old age, Caucasian race and the family history of malign disorders or other lymphoproliferative diseases were constantly recognized as risk factors for CLL. The first-degree relatives of CLL patients present a risk to develop the same disease which is 2-4 times higher than for the general population. The farmers and other agricultural professions, as well as the individuals exposed to organic solvents and other chemical substances (i.e., benzene and butadiene) have an increased risk for developing the CLL.

The most frequent clinical manifestations are the lymph nodes hypertrophy (87%), splenomegaly (54%) and hepatomegaly (14%). For a reduced number of patients, "B"-type symptoms are reported (asthenia, fever, weigh loss or night sweats) and 25% of the patients are asymptomatic. The tonsillar hypertrophies are sometimes associated with the hypertrophies of the salivary and lacrimal glands (Mikulicz syndrome). If the number of white cells exceeds $500.000/mm^3$, the hyperviscosity syndrome appears (headache, vertigo, nystagmus, visual impairments).

If the CLL diagnosis is relatively simple to establish, the evolution of the disease is extremely variable. Some patients do not have symptoms or the symptomatology is minimal and they can have a normal life span, while for other patients the progression is fast and it can lead to death.

The biological markers associated with a more aggressive form of CLL are: fast dedublation rate of white cells, the cytogenetic modifications, beta-2 microglobulin, the CD38 and ZAP70 expression, the gene without mutation for the heavy immunoglobulin chains, the chromosomal anomalies, the length and mutational status of telomeres.

The most frequent complication specific to the disease are the infections and autoimmune phenomena. The transformations in aggressive forms or the occurrence of the second neoplasia are rarely encountered.

When establishing the diagnosis, most of the CLL patients do not require chemotherapy until they become symptomatic or there is evidence of fast progression of the disease. The CLL treatment becomes increasingly customized and it requires a detailed knowledge of the current treatment options: chemotherapy, immunotherapy, hematopoietic cell tranplantation, gene therapy, vaccination and immunomodulation or surgical therapy (spenectomy).

Excepting the case when the death occurs due to other disease, the untreated CLL inexorably progresses to exitus, regardless if it occurs within 12 months or within 20 years.

SYNTHESIS OF THE SPECIAL PART

OBJECTIVE OF THE THESIS

The objective of the thesis is to identify the clinical – biological parameters with prognosis role in the evolution of the patients with chronic lymphocytic leukemia. I will also analyze the epidemiologic data, the clinical and biological parameters in dynamics, the response manner to the treatment according to the various therapeutic schemes applied and the stage of the disease. Based on this analysis, I want to identify the therapeutic schemes with beneficial effects on the quality of life and with an optimal effect concerning the survival of the patients. I will also verify the extent in which the data obtained comply with the data from the specialized literature.

MATERIAL AND METHOD

This is an analytical, observational, cross-sectional study – the patients were taken over and evaluated when diagnosed, longitudinal prospective study – the patients were monitored up to the conclusion of the study and with a retroactive component. The study covered a

period of 7 years and it includes 74 patients admitted to the Hematology Department of Sibiu County Clinical Emergency Hospital between 2008-2014, diagnosed with CLL.

The patients included in the study were the patients for whom the CLL diagnosis conditions were fulfilled: clinical, laboratory, imagistic examination, immunophenotyping of peripheral blood or of bone marrow and sometimes, biopsy with immunohistochemistry.

The cases of B cell monoclonal lymphocytosis or lymphocytic lymphoma were excluded from the study.

For each patient, a monitoring chart was elaborated, starting from the identification data, the investigation of the observation chart, the analyses and immunophenotyping records, the medical file of the patient from the admission in the hematology department and up to the completion of the study, death or loss of the patients from the records. The cytogenic examination was conducted on a single individual.

The blood samples for hemoleucogram and the immunophenotyping examination were sampled in tubes emptied with EDTA K_3 anticoagulant and they were analyzed through automated counting with Sysmex XT 2000i analyzer, respectively Cytomic FC 500 Beckman-Coulter. For the biochemistry samples, the blood was sampled in tube without anticoagulant, with separating gel and analyzed after centrifugation, through the Arhitect analyzer.

The clinical and paraclinical data of each patient were organized in the database and statistically processed using Microsoft Office Excel for Windows XP, SPSS (Statistical Program for Social Sciences) version 20.0. For graphics, I used the software Excel and the SPSS was used for the descriptive analysis (frequencies, crosstabulation, etc.), Pearson correlation (for two variables of the same group), T test for independent variables (comparison between two groups), ANOVA test (for three or more groups) and Kaplan-Mayer test, T test, Fischer and Chi-Squared test for the analysis of the survival. It was considered that there are statistically significant differences for a value of the parameter lower than 0.05. For Kaplan-Mayer, the significance is given by Log Rank Test.

Study 1 - Clinical – biological characteristics of the batch of CLL patients studied throughout a period of 7 years

Material and method

From the monitoring charts of the 74 CLL patients I extracted the data related to age, gender, environment of origin, county of origin, stage of the disease when diagnosed, metabolic status (obesity, diabetes mellitus), arterial hypertension, existence of associated comorbidities, symptoms and signs when diagnosed, biochemical status (cholesterol,

triglycerides, transaminases, GGT, uric acid, creatinine, alkaline phosphatasis, LDH) and hematological status (absolute number of white cells, absolute number of lymphocytes, hemoglobin, number of platelets, medullar infiltration with lymphocytes) when diagnosed.

I statistically processed the data obtained using the descriptive analysis for frequencies, means and medians, maximum and minimal values, standard deviation, T test, Chi-Squared test, Pearson test.

Results and conclusions

Demographic data

A number of 74 patients who fulfilled the CLL diagnosis criteria were included in the study. All the patients were classified from the immunophenotyping or immunohistochemical point of view in B cell type.

The mean age of the patients was 67.15±10.37 years (with a minimum age of 40 years and a maximum age of 89 years), without significant differences between men and women (*p=0.400*). The highest rate of patients (65 patients, 88%) are over 55 years old and only a minor rate (9 patients 12%) have the diagnosis age under 55 years (*p<0.0001*).

Women predominate in the studied batch - 54% (40 women), compare to men - 46% (34 male patients), and the men/women ratio is 0.85. There are no statistically significant differences between the CLL incidences in women and men in the studied batch (*p=0.560*).

A number of 50 patients came from the urban environment and 24 patients from the rural environment (*p=0.004*), most of them (88%) from Sibiu county and only 12% from the neighboring counties.

I analyzed the relation between age or gender and the stage of the disease and I did not find statistically significant differences (*p=0.491*, respectively *p=0.427*).

Clinical data

From the batch studied, a number of 40 patients (54.1%) went to the doctor in the incipient stage of the disease (0, I or A), 27 patients (36.5%) in intermediary stages (II or B) and a reduced number, 7 patients (9.5%) in the advanced stage of the disease (III, IV or C) (*p=0.011*).

The incipient stages of the disease were associated more often with the female gender (28 women compared to 12 men), the ratio is reversed in the intermediary stages (9 women and 18 men) and in the advanced stages the ratio becomes almost unitary (3 women and 4 men) (p=0.01).

In the early stage of the disease, the mean diagnosis ages of women and men are very close (67.68 years for women and 68.42 years for men), in the intermediary stages the mean

age of women is slightly increased (66.89 years for women compared to 65.33 years for men) and in the advanced stage, the age of the women is significantly below the diagnosis age of men (65.5 years for women and 71 years for men) (*p=0.004*).

When diagnosed, more than 50% of the patients were in incipient stages of the disease (0, I or A), around 1/3 presented systemic symptoms of the disease (asthenia, fever, weigh loss or night sweats) and 36.8% were asymptomatic. The ganglion hypertrophy (52.7%) remains the most stable manifestation for the CLL patients (52.7%), compared to hepatomegaly (27%) or splenomegaly (29.7%).

An important percentage of patients showed no adenopathies when diagnosed (47.3%) or a single affected ganglion group (16.2%) and only a single patient had 6 hypertrophied ganglion territories.

According to the frequency of affectation, the ganglion areas are classified in the following manner: the most frequently affected in the axillary area (41.56%), then the lateral – cervical area (23.38%), the submandibular ganglions (10.39%) and the supraclavicular lymph nodes (7.79%).

The number of ganglion groups affected when diagnosed is correlated with the stage of the disease (*p=0.030*), but not also with the dimensions of these groups (*p= 0.170*).

The high dimension of adenopathies is situated at 2.10±1.09 cm, with a maximum value of 5 cm and a minimum value of 0.5 cm when diagnosed. One of the patients showed large adenopathic blocks when diagnosed. The dimensions of the hypertrophied ganglions are not correlated with the stage of the disease (*p= 0.060*).

Most of the patients studied (77.0%) show associated diseases: chronic ischemic cardiopathy, valvular cardiac failures, chronic cor pulmonale, chronic obstructive pulmonary disease, chronic hepatopathies, chronic pyelonephritis, post myocardial infarction sequella, prostate adenoma, chronic kidney failure (*p< 0.001*).

A number of 5 patients (6.8%) showed history of other neoplasia, namely: 2 surged and chemo-treated breast cancers, 1 surged skin cancer, 1 surged and radio-treated urinary bladder cancer and a chemotherapy treated chronic myeloproliferative disease.

The lymphocytic infiltration of the bone marrow when diagnosed is statistically significantly correlated with the number of adenopathies (*p=0.004*), presence of splenomegaly (*p=0.003*), presence of symptoms – other than the B-type syndromes (*p=0.017*), hemoglobin (*p< 0.001*), number of platelets (*p=0.009*) and stage of the disease when diagnosed (*p=0.016*).

Unfortunately, we have few data regarding the type of medullar infiltration with lymphocytes. The description of the infiltration type is available only for 5 patients: 2 cases of nodular and interstitial infiltration, for patients in early and respectively advanced stages of the disease and 3 cases with diffuse infiltration, in early and intermediary stages of the disease.

When diagnosed, most of the patients show normal serum levels for LDH (67.3%) and cholesterol (64.7%), but in terms of the triglycerides, half of the patients have normal values (50.0%) and the other half pathologic values (50.0%).

The stage of the disease when diagnosed is statistically significantly correlated with the presence of hepatomegaly, splenomegaly, hemoglobin value, number of platelets and cholesterol level. The correlations between the presence of splenomegaly, hemoglobin value, cholesterol and hepatomegaly are also statistically significant. The cholesterol is correlated with the level of triglycerides and the LDH is correlated with the hemoglobin value.

In the peripheral blood, the reduced percentage of white cells when diagnosed was 57.19% with the lymphocyte mean of 49.94% and a mean medullar lymphocytic infiltration of 73.02%. The mean values of hemoglobin and platelets (13.03g/dl and respectively 178.89 $x10^{3}$ /µl), as well as the means of cholesterol and triglycerides (192.96 mg/dl and respectively 154.75 mg/dl) were normal when the patient was diagnosed. The means of the other biochemical parameters studied (TGO, TGO, urea, creatinine, uric acid, LDH, GGT, alkaline phosphatasis) were also normal.

- It was noticed that the mean onset age of CLL is slightly decreasing compared to the data from the specialized literature and the mean age in the more advanced stages of the disease is lower for women compared to men.
- In our geographical area, we have a smaller percentage of patients diagnosed in an early stage of the disease.
- The stage of the disease when diagnosed is correlated with the level of blood cholesterol and the infiltration of bone marrow with lymphocytes.
- Most of the patients show various degrees of obesity, arterial hypertension, comorbidities, which hinder the therapeutic decision and influence the survival rate.
- Due to the fact that many patients are asymptomatic, they do not show palpable peripheral adenopathies or hepato – splenomegaly, we have to notice the increasing role of the association of normal hemoleucogram with the immunophenotyping or immunohistochemical examination in order to diagnose the early stages of the disease.

Study 2- Evaluation of clinical – biological patients in dynamics and identification of certain predictive factors

Material and method

The identification data, gender, age, stage of the disease, data related to the biochemical and hematological status before and after the application of the chemotherapy treatment were taken from the monitoring charts of the patients from the batch of 74 CLL patients.

The batch was divided in two groups: group I – patients who did no receive chemotherapy treatment and group II – patients who received chemotherapy. The stages of the disease were encoded with 0 for 0, I and A, with 1 for II and B, with 2 for III, IV and C.

The results were statistically evaluated through the mean, median value, standard deviation, minimum and maximum. The univariable comparisons were performed through the log-rank test. The categorical and continuous variables were evaluated using the $\chi 2$ test. All the *p* were considered statistically significant at a value lower than or equal to 0.05.

Results and conclusions

Group I

Demographic data

Group I includes a number of 30 patients who did not benefit from chemotherapy treatment. In this group, the mean diagnosis age was 66.70±11.67 years and the distribution according to genders is the following: 22 women (73.3%) and 8 men (26.7%). The patients of this batch had a mean stage of the disease of 0.066±0.253.

Biochemical and hematological profile

In this batch, the cholesterol is directly correlated with the level of triglycerides (*r=0.257*) and reversely correlated with the LDH (*r = -0.303*) and with the stage of the disease (*r = -0.253*), but I did not identify correlations infiltration degree of the bone marrow with lymphocytes, hepato – splenomegaly, number and dimensions of adenopathies, hemoglobin and number of platelets.

Group II

Demographic data

Group II includes a number of 44 patients who benefited from chemotherapy treatment. In this group, the mean diagnosis age was 67.45±9.51 years and the distribution according to genders is the following: 18 women (40.9%) and 26 men (59.1%). The mean stage of the disease in the batch of treated patients is 0.860±0.639.

Biochemical and hematological profile

Following the chemotherapy treatment applied, in our batch of patients the hematological and biochemical parameters suffered statistically significant variations of the cholesterol (*p=0.028*), urea (*p= 0.019*), uric acid (*p <0.001*) and obviously white cells (*p<0.001)* and lymphocytes (*p <0.001*). No statistically significant variations were registered for any of the other parameters studied.

The level of the serum cholesterol increased (*p=0.028*) from a mean value of 180.16±42.2 mg/dl before applying the chemotherapy treatment, to the mean value of 196.53±51.93 mg/dl, registering a mean increase of 27.17±45.72 mg/dl. The triglycerides also registered an increase from a mean value of 157.16±82.85 mg/dl to the mean value of 167.44 ± 105.76 mg/dl, however, the increase is statistically insignificant. (*p=0.204*).

The serum cholesterol value before the chemotherapy treatment is reversely correlated with the stage of the disease (*p=0.040*), for the early stages of the disease the mean value is 204.95±56.53 mg/dl, and for the advanced stages of the disease it decreases to a mean value of 180.53±37.48 mg/dl.

The blood cholesterol before treatment is directly correlated with the level of triglycerides (*r=0.436*), stronger than in the batch without treatment and reversely correlated with the stage of the disease (*r=-0.265*) and with the medullar infiltration percentage with lymphocytes (*r=-0.467*), but it is not correlated with the level of the LDH. The cholesterol before treatment is also directly correlated with the hemoglobin (*r=0.290*) and with the number of platelets (*r=0.307*) before the treatment. The more advance the stage of the disease is and implicitly the higher the tumor mass, the more reduced the blood cholesterol level is. This finding complies with the data from the literature and with the hypothesis regarding the consumption of the cholesterol by the other tumor cells for their own proliferation.

The cholesterol measured after the treatment is reversely correlated with the stage of the disease (*r=-0.245*) and with the number of adenopathies (*r=-0.242*) and directly correlated with the level of triglycerides (*r=0.280*), hemoglobin (*r=0.245*) and number of platelets (*r=0.272*).

The entire batch

For the entire batch of patients, with the mean age of 67.15±10.3 years, the cholesterol is reversely correlated with the stage of the disease (*r=-0.291*) and with the hepatomegaly (*r=-0.234*) and directly correlated with the triglycerides (*r=0.318*). The correlation of the cholesterol with the hepatomegaly is statistically significant (*p=0,048*).

In the entire batch of patients, we notice statistically significant differences between the cholesterol before applying the treatment for the patients considered in the active stage of the

disease and who have a higher tumor mass (*p=0.008*). In the sub-batch of the untreated patients, the cholesterol has higher values (mean value 211.93±56.11) compared to the sub-batch of patients who will undergo chemotherapy treatments (mean value 180.16±42.2).

I did not find correlations of the cholesterol with the splenomegaly, number and dimensions of adenopathies.

- In conclusion, the blood cholesterol decreases with the increase of the tumor mass and the activity of the disease. The triglycerides are correlated with the cholesterol in all the stages of the disease ad they follow its variations. The cholesterol could be a parameter for monitoring the response to therapy and an evolutionary marker of the disease.

Study 3. Current treatment in chronic lymphocytic leukemia

Material and method

I worked with the monitoring charts of the 47 CLL patients, out of which I extracted the data related to age, gender, stage of the disease when diagnosed, metabolic status (obesity, diabetes mellitus), arterial hypertension, existence of associated comorbidities, timeframe without treatment, type of treatment previously administered, type of treatment administered, time to obtain the response to treatment, type of response obtained, relapses, treatment of relapses, number of relapses, complications, second neoplasia, death and death causes, biochemical status (cholesterol, triglycerides, transaminases, GGT, uric acid, creatinine, alkaline phosphatasis, LDH) and hematological status (absolute number of white cells, absolute number of lymphocytes, hemoglobin, number of platelets, medullar infiltration with lymphocytes) when the patient was diagnosed/administered the treatment and upon 2-3 months after the administration of the treatment.

The patients in stages 0, I and A of disease were classified in category 1, the patients from stages B and II in category 2 and the stages III, IV and C in category 3.

The survival without disease progression and the global survival (OS) were calculated from the administration date of the first treatment until the moment of progression or death. The survival curves were obtained using the Kaplan-Meier method. The univariable comparisons were performed through the log-rank test. I used the Cox regression test for survival in the multivariable analysis. The categorical and continuous variables were evaluated using the $\chi 2$ test. All the *p* were considered statistically significant at a value lower than or equal to 0.05. Only the factors which were statistically significant ($p \leq 0.05$) in the

univariable analyses were used in order to develop multivariable models. In addition, the results were statistically evaluated through the mean value and standard deviation.

Results and conclusions

Evaluation of the response to treatment

The evaluation of the response to treatment is performed after 6 therapy cycles, based on the criteria defined by the CLL study group of the National Cancer Institute, NCI, through clinical examination, periodical laboratory examinations (hemoleucogram and medullar biopsy), thorax x-ray and abdominal ultrasound or computer tomography examination, only for the patients in full remission.

Layering of the batch according to the necessity of the therapy

In the batch of 74 patients studied, a number of 30 patients were in incipient stages of the disease who did not require therapy, but only surveillance ("*watch and wait*") and a number of 44 patients had stages of disease which imposed therapy with chemotherapy medication. The necessity to apply the therapy is statistically significant directly correlated with the stage of the disease ($p<0.0001$), but it is not correlated with the gender ($p=0.291$) or age of the patients ($p=0,954$).

For the patients who needed treatment, there is a male predominance: 26 men (59%) compared to 18 women (18%).

The timeframe without treatment, defined as the timeframe from the diagnosis up to the application of a treatment, had a mean value of 16.3 ± 28.3 months. This parameter is reversely correlated with the stage of the disease ($p=0.005$). The average timeframe without treatment is longer for women (33.87 months) compared to men (12.9 months) in the overall batch of patients ($p=0.06$).

From the batch of chemotreated patients, the mean timeframe without treatment is 26.36±38.66 months for women and 9.38±17.23 months for men ($p=0.272$).

The timeframe without treatment decreases from the incipient stages of the disease (44.83±41.25 months) to the intermediary (11.5±20.56 months) and advanced stages (0.16±0.31 months), where the therapy is practically imposed when the patient is diagnosed ($p=0.010, r=-0.512$).

The therapeutic options in the batch studied were the following:

- Treatment with monoclonal antibodies + chemotherapy (alkylating agents, purinic analogues) ± corticosteroids: FCR, FCD-R
- Treatment with anthracyclines and purines: FC, FCD, Litak, FC-Mabcamph

- Polychemotherapy treatment with alkylating agents ± corticosteroids: COP, CVP, CHOP, Leukeran- corticosteroids
- Polychemotherapy with monoclonal antibodies: COP-R, CHOP-R

Only the chemotherapy treatments and not the surgical treatments were considered.

The monoclonal antibodies + chemotherapy treatment was mainly applied for the patients from stages II and B of disease (11 patients), the anthracyclines + purines treatment was mainly applied for the patients in stages 0, I and A (6 patients). The patients in stages II and B and almost equally the patients from 0, I and A (4 and respectively 4 patients) benefited from polychemotherapy with alkylating agents ± corticosteroids, and the type of polychemotherapy with monoclonal antibodies was applied in stages II, B and III, IV, C (6, respectively 4 patients).

The patients in the early stage of the disease mainly benefited from anthracyclines and purines treatments (50%) and more reduced treatments with monoclonal antibodies and chemotherapy (25%) or polychemotherapy and alkylating agents ± corticosteroids (25%).

In the stages with intermediary risks, the monoclonal antibodies and chemotherapy treatments (44%) and polychemotherapy with monoclonal antibodies (24%) predominate, compared to the treatment with anthracyclines and purines (16%) or alkylating agents ± corticosteroids (16%).

The stages of disease with increased risk benefited from polychemotherapy and monoclonal antibodies in 57%, anthracyclines and purines treatment 29%, polychemotherapy with alkylating agents ± corticosteroids treatments 14% and no patient who benefited from the association of anthracyclines and purines.

Most of the patients benefited from a single line of treatment (29 patients, 65.9%), a smaller number benefited from 2 lines (12 patients, 27.3%) and 1 patient (2.3%) benefited from 3, 4 and respectively 5 lines. The number of the chemotherapy lines is reversely correlated with the duration of the response to therapy (*r=-0.253)* and statistically significantly directly correlated with the survival rate (*r=0.299*, *p=0.048*) and relapses of the disease (*r=0.469, p=0.002*), but it is not correlated with the response time to therapy (*p=0.882*).

From the batch of 44 patients who received treatment, a number of 40 patients could be evaluated in terms of the response to therapy. The initial overall response rate to therapy was 97% (full and partial remission), out of which 25% partial response and 72% full response. The mean survival of the patients with response to therapy was 51.88±25.74 months. The

mean duration of the response to treatment is 21.13±15.19 months, with extreme values between 0 months and 50 months.

Correlations between the type of chemotherapy treatment and the response to treatment

The patients treated with monoclonal antibodies in association with chemotherapy (alkylating agents, purinic analogues) ± corticosteroids (treatment type 1) compared to the patients treated with anthracyclines and purines (treatment type 2) show statistically significant differences in terms of the timeframe without treatment (*p=0.040*), duration of survival (*p=0.042*) and stage of the disease (*p=0.022).* Among the other parameters studied, namely: the number of treatment lines, the response time to treatment, the duration of the response, the necessity to administer erythropoietin, the occurrence of relapses, the positive Coombs test, the risk of death or the diagnosis age, there are no statistically significant differences between the 2 types of treatments.

It is noticed that the patients who received the treatment type 1 have a medium response time (6.8) which is 2.3 months longer than the patients with treatment type 2 (4.5 months). The patients with type 1 rank were in the stages 0, I, II, A, B of the disease, while the patients with type 2 of treatment rank in more incipient stages, 0, I, A. After type 1 therapy, a percentage of 12.5% of the patients required the stimulation of erythropoietin, compared to 20% after type 2 therapy.

In the batch of patients who received polychemotherapy associated with alkylating agents ± corticosteroids (treatment type 3) and the patients with polychemotherapy associated with monoclonal antibodies (treatment type 4), we notice statistically significant correlations for the response time to treatment (*p=0.010*), necessity to administer erythropoietin (*p=0.013*) and stage of the disease when diagnosed (*p=0.038*).

The response time to treatment is more reduced after the type 3 of treatment (2.75 months) compared to type 4 (7.20 months), the stage of the disease when diagnosed is more advanced for the patients with type 4 of treatment (mean and advanced stages) compared to type 3 where we have patients in all the stages (from incipient to advanced). A percentage of 70% of the patients treated with type 4 treatment required the administration of erythropoietin, compared to only 12.5% after the type 3 treatments.

Correlations between the chemotherapy treatment and the variations of the biochemical and hematological parameters

Following the chemotherapy treatment applied, in our batch of patients certain hematological and biochemical parameters suffered statistically significant variations: cholesterol (*p=0.028*), urea (*p= 0.019*), uric acid (*p <0.001*) and obviously the white cells

(*p<0.001*) and lymphocytes (*p<0.001*). The variations are not statistically signification for all the other parameters studied.

Type 1 of chemotherapy treatment was applied for 16 patients, mainly in stages II and B of the disease (11 patients, 69%) (*p= 0,010*). The biological resonance of this type of treatment manifests through statistically significant variations of the values of LDH (*p=0.035*), creatinine (*p<0.001*), uric acid (*p<0.001*), white cells (*p<0,001*), platelets (*p<0,001*), before and after the treatment. The variations of cholesterol, triglycerides, TGO, TGP, urea, GGT, hemoglobin, platelets are statistically insignificant.

The analysis of the batch of patients (10 patients) who received chemotherapy with type 2 treatment reveals the fact that they were administered in particular to the patients with incipient and intermediary stages of the disease, without statistically significant differences between the 2 stages of the disease (*p= 0.754*). In stages 0, I and A, a number of 6 patients (60%) received this type of treatment and in stages II, B other 4 patients (40%) received this treatment. After the type 2 treatment, we notice that the uric acid (*p<0.001*), GGT (*p=0.044*), white cells (*p=0.026*) and lymphocytes (*p=0.028*) presented statistically significant differences before and after the treatment. The other parameters: cholesterol, triglycerides, TGO, TGP, creatitine, urea, hemoglobin, platelets had no significant variations.

The chemotherapy treatment with type 3 treatments was administered for a number of 8 patients, in all the stages of the disease (*p= 0.417*). Only the uric acid showed statistically significant differences between the start and the end of the treatment (*p<0.001*).

The 10 patients who benefited from type 4 chemotherapy ranked in the medium and advanced stages of the disease (*p= 0.754*). In this batch, statistically significant biological variations were registered for the uric acid (*p< 0.001*), white cells (*p< 0.001*) and lymphocytes (*p< 0.001*).

Relapses

From the batch of 40 patients treated who were monitored post chemotherapy, a number of 7 patients (17%) showed relapses of the disease. The survival without the progression of the disease was 20.8±16.43 months.

Most of the patients had a single relapse (5 patients, 71%), only 2 patients (29%) had 2 relapses. The mean age of the patients who relapsed is 61.88 ±7.52 years.

No statistically significant differences were registered between the patients with various stages of the disease in terms of the relapse frequency. There are no statistically significant differences in terms of the relapse frequency between the chemotherapy treatment of type 1 and 2 (p=0.200), or between types 3 and 4 (p=0.333).

The treatment of relapse was performed with type 1 treatment for a number of 4 patients (57%), type 2 of treatment for 1 patient (19%) and type 3 of treatment for 2 patients (27%). Following the treatment of relapses, we obtained 43% full remissions (3 patients), 29% partial remissions (2 patients), 14% progressive disease (1 patient) and 1 patient (14%) is still under treatment.

No correlations were registered between the incidence of relapses and the free treatment timeframe (*p=0.421*), response time (*p=0.317*), duration of response (*p=0.165*) and overall survival (*p=0.052*). There is no correlation between relapses and deaths (*p=0.176*).

Complications

Hemolytic anemia

From the total patients studied, a number of 4 cases, representing 5.40%, showed at least a flare of hemolytic anemia, with positive Coombs test. One of the patients had 2 flares of hemolytic anemia in the evolution of the disease.

Cytopenias

The presence of cytopenias (anemia or thrombocytopenia) is associated with the administration of the chemotherapy treatment (*p=0.001*). If in the batch of 30 untreated patients most of them do not show cytopenia (25 patients, 83%), in the batch of 41 treated patients predominate the patients with peripheral cytopenia (32 patients, 78%).

In the batch of treated patients, 30% patients associate the anemia and thrombocytopenia, 35% show only thrombocytopenia and 12.5% show only anemia.

There is no statistically significant correlation (*p=0.870*) between the various types of chemotherapy treatment and the presence of cytopenias (anemia, thrombocytopenia). The incidence of the anemia is correlated with the stage of the disease (p=0.012), but the thrombocytopenia is not correlated (p=0.665).

Infections

In our global batch (74 patients), 28% of the patients had at least one infectious episode throughout the evolution of the disease.

In the sub-batch of chemotreated patients (44 patients), 38.6% (17 patients) showed infections throughout the disease. Most of them (8 patients, 47%) had a single type infectious complications (microbial, viral, fungal), 3 patients (18%) showed all 3 types, equally 3 patients (17%) had microbial and fungal infections, 2 patients (12%) associated microbial and viral infections and a single patient (6%) associated viral and fungus infections.

The most frequent infectious complications are the microbial infections, representing almost half (48%) of the total infectious complications, then the fungal infections (28%) and the viral infections (24%).

In the batch of untreated patients (30 patients), the frequency of the infections is much more reduced (4 patients, 13.3%) and no multiple infectious episodes are registered. According to the types of infections, the classification is the following: we have 3 patients (10%) with 1 episode of microbial infection, 1 patient (3.3%) with 1 episode of viral infection, and 1 patient (3.3%) with fungal infection. One of the patients had two successive different types of infection (microbial and fungal).

Although the infectious complications are rare among the untreated patients, we can notice that in this batch also predominate the microbial complications (60%), compared to the viral (20%) or fungal complications (20%).

The second neoplasia

It was noticed that a number of 9 patients (12%) of the total 74 CLL patients studied developed a second neuplasia throughout the evolution. The mean age of the patients who developed a second neoplasia is 65.22 ±5.38 years, and the distribution according to genders of these patients is equal. The mean timeframe from the administration of the treatment up to the occurrence of the second neoplasia is 49.33 ± 20.5 months.

From the batch of treated patients, 9 patients evolved towards the second neoplasia (20%).

As type of cancer, skin cancers are registered at the second neoplasia (67%), with location on the face, thorax or in the auricular area, bronchopulmonary cancer (22%) and ovarian cancer (11%).

One of the patients also developed a third neoplasia, which, in the general batch, represents a percentage of 2% and 1% in the batch of treated patients. The third neoplasia also proved to be, similar to the second neoplasia, a skin cancer. The third cancer occurred 82 months after the administration of the first chemotherapy treatment.

From the 9 patients diagnosed with the second cancer, 6 patients (67%) survive and 3 patients died (33%). All the three deceased patients showed skin cancer (2 patients) and respectively bronchopulmonary neoplasm (1 patient).

The second neoplasia is more frequent for the patients who received the type 2 treatment (40%) and type 4 treatment (20%).

The presence of the second malignity is not correlated with the survival ($p=0.883$) or with the stage of the disease ($p=0.088$).

Deaths

From the overall batch studied, a number of 16 patients (22%) died throughout the study, the highest number of deaths being in 2014 (4 deaths, 25%) and 2012 (4 deaths, 25%).

From the sub-batch of treated patients (44 patients), 12 deaths were registered (27%), and in the sub-batch of untreated patients (30 patients), only 4 deaths were registered (13%).

Analyzing the batch of untreated patients, we notice that all the deceased patients are women, with the mean age of 73.25±7.97 years, they are in the 0, I or A stage of the disease, and the causes of death are the following: in 2 case the acute pulmonary edema, for the third patient the cerebrovascular accident and finally, the sepsis of unmentioned etiology.

For the patients who received chemotherapy, the deaths occurred for a number of 7 men (58%) and 5 women (42%), the mean age of the group was 68.58±8.71 years and the stage of the disease was I, A, B, C or IV. The causes of death in this group were the following: 5 patients died of sepsis of various etiologies, 5 patients due to cardiac causes (acute myocardial infarction, arrhythmias, cardiac failure), 1 patient due to a pulmonary malign tumor on the left upper lobe, with multiple secondary disseminations and another patient due to the blastic transformation of a chronic myeloproliferative syndrome. The mean age of the patients deceased due to infectious causes was 66.8±5.38 years.

Most of the deceased patients from the chemotreated batch were in the B stage of the disease (42%) and the most frequent cause of death (42%) was the sepsis, with a microbial infectious complication as starting point.

Survival

In our overall batch of CLL patients, the median survival is 43 months. In the sub-batch of untreated patients, the survival is 28 months, and in the treated batch, the survival is 52.5 months. Taking into consideration the fact that in the sub-batch of untreated patients there is a large number of patients diagnosed in the last year and who benefited from a shorter monitoring period, it is inadequate to interpret the data.

The survival rate at 5 years is 35.2% in the overall batch and 47.6% in the batch of patients who received chemotherapy.

Analyzing the median survival according to the stages of the disease, we notice that in the incipient and intermediary stages of the disease, the median survival is equally good, regardless of the type of treatment administered. In the stages of aggressive disease, the best median survival is insured by the regimes which associate the Rituximab.

The survival charts and death hazard charts at 5 years for the CLL patients reveal a higher death risk in the first 10 months from the diagnosis, then this risk decreases. The

statistical survival and death hazard at 5 years analysis does not highlight statistically significant differences between the two genders (*p=0.305).*

In the early stage of the disease, the statistical analysis and the aspect of the survival curve indicate that there are no statistically significant differences between the types of administered treatment (*p=0.656*), but in the intermediary and advanced stage of the disease there are statistically significant differences between the types of treatments administered (*p=0.050* and respectively *p=0.047*).

- It is noticed that women have a more benign and longer evolution of the disease compared to men, and the relapses are more frequent for men.
- Two thirds of the patients show cytopenia in the evolution of the disease, probably autoimmune, which does not influence the prognosis and it is not correlated with the administration of chemotherapy medication.
- The infections have a high rate of incidence and they influence the morbidity and mortality.
- The occurrence frequency of the second malignity is increased after 4 years of evolution of the disease. The second neoplasia had an increased frequency for the patients treated with purinic analogues and anthracyclines. The most frequent second neoplasia was the skin cancer.
- Any newly occurred symptom, apparently unrelated to the basic disease, has to be carefully investigated, because it raises the suspicion of a second primary neoplasia. The risk of death is higher in the first 10 months from the diagnosis of the disease. There are no significant differences between genders in terms of the survival and the death hazard at 5 years. The most frequent causes of death are the infection and the second neoplasia.
- The association of Rituximab in the cytostatic treatments of the patients with advanced stage of the disease improves the median survival, the mean time without the progression of the disease, the mean survival duration and the response rate to treatment.

General conclusions

1. In our geographical area, the median onset age of CLL is slightly decreasing compared to the data from the literature and the number of patients diagnosed in earlier stages of the disease is smaller.
2. Women have a lower and gentler evolution of the disease, with fewer relapses. However, the mean age in the more advanced stages of the disease is lower for women compared to men.
3. Due to the fact that a large part of patients are asymptomatic, they do not show peripheral adenopathies or palpable hepato – splenomegaly, we have to underline the increasing role of the hemoleucogram correlated with the immunophenotyping examination or immunohistochemistry for the early diagnosis of the disease.
4. The stage of the disease when diagnosed is correlated with the level of the blood cholesterol and the infiltration of the bone marrow with lymphocytes. The blood cholesterol decreases when the tumor mass and the activity of the disease increase.
5. The triglycerides are correlated with the cholesterol in all the stages of the disease and they follow its variations.
6. The cholesterol can be an evolutionary marker of the disease and a monitoring parameter of the response to therapy, rapidly and easy to evaluate, with minimum costs.
7. The cytopenias are a frequent event in the evolution of the disease, but they are not correlated with the type of treatment administrated and they do not influence the disease prognosis.
8. The infections are a frequent complication and they represent a main cause of death.
9. The second main cause of death is the occurrence of the second cancer, more frequent upon 4 years from the administration of the first chemotherapy treatment and for the patients who benefited from purinic analogues and anthracyclines.
10. Any newly occurred symptom, apparently unrelated to the basic disease, has to be carefully investigated, because it raises the suspicion of a second primary neoplasia.
11. The association of Rituximab in the chemotherapy treatments improves both the mean time without the progression of the disease, the mean survival duration and the response rate to treatment and the median survival of the patients with advanced disease.

Innovations of this study

- It is the first study from Romania and Eastern Europe which evaluates the relation between cholesterol, triglycerides and the volume of the tumor mass when the patient is diagnosed, as well as the relation with the response to treatment for the patients with chronic lymphocytic leukemia.
- We propose the cholesterol as an evolutionary factor of the disease and a monitoring parameter of the response to treatment due to the rapid, convenient evaluations and with minimum costs in any laboratory from the country.
- We obtained statistically significant data regarding the correlations between various demographic, clinical – biological factors, survival rates and the response to therapy.
- We proved the efficiency of associating the Rituximab with the chemotherapy treatment in certain disease conditions.