Chronic Lymphocytic Leukemia. 
From Diagnosis to Treatment Decision

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REZUMAT

Leucemia limfaticã cronicã. De la diagnostic la decizia terapeutãcă

Leucemia limfaticã cronicã reprezintã o limfoproliferare cronicã indolenãa cu o evoluþie extrem de heterogenã adesea grevatã de recãderi greu de gestionat. Investigaþiile clinice æi paraclinice complete încã de la debut contribuie semnificativ la alegerea opþiunii terapeutice cele mai potrivite æi a momentului iniþierii tratamentului specific. Schemele chimioterapice clasice æi-au dovedit în timp eficienþã. Completarea sau schimbarea acestora cu agenþi terapeutici noi, în paralel cu îmbunãtãåirea tehnicii de investigare a dus la creæterea semnificativã a procentului de remisiuni complete obåinute precum æi a supravieåuirii fãrã progresie de boalã.

O monitorizare riguroasã a pacienþilor atât pe parcursul tratamentului cât æi postchimioterapie, prin depistarea bolii minime reziduale, creæte æansa depistãrii precoce a cazurilor neresponsive sau a recãderilor. Terapia de linia a doua corect æi la timp instituitã putând oferi o prelungire a supravieåuirii prin obåinerea unei noi remisiuni complete.

Cuvinte cheie: leucemia limfaticã cronicã, factori de prognostic, chimioterapie

ABSTRACT

Chronic lymphocytic leukemia is an indolent chronic lymphoproliferation with a highly heterogeneous evolution often burdened by difficult unmanageable relapses. Complete clinical and laboratory investigations since the debut contribute significantly to choosing the most appropriate therapeutic option and the time of initiation of specific treatment. Classical chemotherapeutic schemes have proven their effectiveness in time. Completing or changing them with new therapeutic agents, while improving investigative techniques, led to a significant increase in the percentage of achieved complete remission and survival without disease progression.

A rigorous monitoring of patients both during treatment and post-chemotherapy, the detection of minimal residual disease, early detection increases the chance of relapse or unresponsive cases. Second-line therapy instituted properly and on time can provide an extension of survival by getting a new complete remission.

Key words: chronic lymphocytic leukemia, prognostic factors, chemotherapy

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Chronic lymphocytic leukemia (CLL), haematological described since the early nineteenth century, is considered a haematological indolence, but of-a-time there was found that its evolution can be extremely varied.

Most of the patients were over 60 years at the time the diagnosis was established, and this may be due to decreased immune competence with age. Males are affected 2 times more frequently than the female, the male percentage: female being 2:1. Fewer than 10% of cases occur in adults and in children below 40 years old have been few reported cases of CLL. (1,2)

Environmental factors do not seem to play a major role in pathogenesis, so it is the only one of leukemia that is not associated with the exposure to ionizing radiation. (3,4). Nor the chemical or alkylating agents have etiologic role in CLL. The involvement of viruses in LLC etiology has not yet been proven, but the infection with a lymphotropic retrovirus RNA such as Human T-cell Leukemia / Lymphoma Virus (HTLV-I), is involved in the onset of rare forms of leukemia - T-cell leukemia / lymphoma of the adult. (1)

Genetic factor remains the most important in the etiopathogenesis of LLC and it is proven by the increased family incidence (8.1% of the cases), first degree relatives of patients with CLL having a health risk 3 times higher for CLL and other malignant lymphoproliferation. For the families of patients with CLL it was found an increased incidence of autoimmune diseases and solid cancers. (2,5,6)

Symptoms of the disease are due to both bone marrow and other tissue infiltration of malignant cells, as well as alterations in the humoral and cellular immunity. CLL is characterized by a state of immune deficiency, the patients presenting autoimmune manifestations, particularly autoimmune hemolytic anemia and thrombocytopenia.

Most of the times (40% of cases), patients are asymptomatic, the disease being diagnosed by accident at a routine examination, which reveals lymphocytosis in peripheral blood and they may have slightly lymphadenopathy and splenomegaly.

In advanced stages of the disease, early symptoms include: asthenia, fatigue, fever, sweating, especially at night, and weight loss, which occur in a very short period of time, clinical manifested anemia, and bleeding/hemorrhagic syndrome. (7)

The complains, frequently met in the onset, are related to the occurrence of lymph nodes/adenopathies or splenomegaly and viral infections (eg, Herpes zoster), bacterial (eg Pneumococcus), fungal (eg, Candida, Cryptococcus), which are installed under immunosuppression related to the disease evolution. It may be noted a susceptibility to the recurrent infections and having an evolving train, complicated and resistant to the conventional therapies. (8,9,10)

The adenopathies are presented in 80% of the cases, typically generalized, having elastic consistency, softly, being movable, painless, symmetrically, non-adherent to the in depth plans and without skin changes and no tendency to the fistulization.

Splenomegaly may occur in 50-70% of the cases, often dominating the clinical picture. It is generally elastic and painless. Due to the elastic character, as in the adenopathies, it does not determine the compression disturbances to the adjacent organs.

Hepatomegaly occurs often after the splenomegaly, being found in 50% of the cases, the character of the liver being also elastic, the lower edge is rounded off, and this is globally increased, homogenous and diffuse, interested by the process.

In the advanced stages of the disease appear the paleness, fatigue, palpitations, dyspnea, angina, due to anemia and mucocutaneous hemorrhagic manifestations as an expression of thrombocytopenia.

Neurological manifestations in the patients with CLL are very rare and often correlated with elevated leukocytosis over 800.000/μl when there may occur the phenomena of hyperviscosity. (8,9,10,11,12)

Laboratory data

The hemogram/blood count is suggestively altered for the diagnosis of chronic lymphatic leukemia. Characteristically there is the lymphocytosis, over 5.000/μl, often than 100.000/μl, and the lymphocytes are apparently morphologically normal. There is possible the presence of an anemia with the hyporegenerative look, if its mechanism is the infiltration of bone marrow by the lymphocyte cells, or microcytic hypochrome if the mechanism is feriprivia by repeated bleeding, but often looks hemolytic by the auto-hemolytic component in approximately 10% of the cases. Platelets can be normal or low by splenectomy in the context of splenomegaly, or also autoimmune one by the anti-thrombocyary auto-antibodies. Usually these pathogenic factors are intricate.

In the peripheral blood smear may be noticed some morphological characteristics of the malignant lymphocytes: are small lymphocytes, mature, with
little cytoplasm, basophilic one, having the characteristic presence of nuclear Gumprecht shadows that are remnants of lymphocyte nuclei. Sometimes there is a small percentage of <10% for the young elements (prolymphocytes and lymphoblasts). (fig. 1)

Examination of the bone marrow is essential for the diagnosis, to determine the degree of bone marrow infiltration by the malignant lymphocytes. In the osteomarrow biopsy examination, lymphocytic infiltrate character is an indicator of clinical status and prognosis. You can see four types of lymphocyte infiltration: nodular, interstitial, mixed and diffuse. Nodular and interstitial infiltrates are found in the early stages of the disease and have a better prognostic significance, whereas diffuse infiltration is in advanced stage of the disease. If the lymphoid tissue takes up more than 50% of the bone, patients tend to have cytopenia. Erythroblastic insularly or diffuse hyperplasia is suggestively for the autoimmune hemolytic anemia. (8,9,10,11,12,13) (fig. 2).

Immunophenotyping

The immunophenotypic studies are essentially in the diagnosis of chronic lymphatic leukemia being a useful and quick method to recognize both phenotypes characteristic of different neoplasms types of lymphoid B-cell and identifying some target elements for the immuno-modulatory therapy and in the evaluation of residual disease. Following to the flowcytometrical exam, phenotypic patterns are seen helping in B lymphocyte cell biology understanding, and bringing the predictions on disease evolution. (14, 15)

The immunophenotyping with monoclonal antibodies reveals the presence of CD5 antigen. In 95% of the cases, lymphocytes appear as belonging to the line B, being found the specific markers of B line: surface Ig (SIg), CD19, CD20, CD21, the rest of 5% being T line having the specific markers: CD2, CD3, CD4, CD7, CD8. (8,9,10,11,12) (fig. 3).

By the cytogenetic tests can detect chromosomal abnormalities with total apart importance in terms of the evolution of a case of chronic lymphocytic leukemia. Genetic mutations (13q-, 11q-, trisomy 12, 17p-, 14q +) can be identified at the diagnosis, but also for the refractory or relapsed disease, the case which in can be changed the cytogenetic picture and may appear new mutations with unfavorable connotations, often influencing and changing the therapeutical scheme. (2,8,9,10)

Lymph node biopsy is not a routine examination. The appearance is small cell lymphoproliferation. Lymph node biopsy making is mandatory at that time when is suspected lymphomatous transformation of CLL within Richter syndrome.

A series of laboratory tests are useful and necessary for evaluation of initial hematologic status in the patients newly diagnosed with CLL. Values may be elevated for the lactate dehydrogenase, aβ2-microglobulin, values changes of the bilirubins and serum protein electrophoresis, investigation of the hemolytic processes by the Coombs tests, all of these factors in turn relate to prognosis and subsequent development of the disease.

Radiological examination, abdominal ultrasound and computed tomography are useful imaging investigations performed in order to determine supra- and sub-diaphragmatic lymph nodes/adenopathies, localization and volume of these important elements.
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**Figure 3.** Peripheral blood sample flow cytometry; (BD FACSCanto II Software v 6.1.3, using standardized and compatible reagents); WBC = 62,580/μl with 82% mature lymphocytes: (a) lymphocyte subpopulation: CD19 + B lymphocytes (90%); (b) and (c) monoclonal lymphocytes CD19 + (sIgk + dim) and coexpression of CD5 + CD23 +. (Coltea Clinic Hospital - Hematology Laboratory)

**Figure 4.** CLL staging. Rai and Binet staging.

*Related to the staging are used two classifications Rai and Binet, widely well-known, easy to be applied for, based on clinical and laboratory factors. (16,17) (fig. 4).

Once the diagnosis of CLL was set, the physician will have to decide how to treat the patient and what
the optimal time to initiate the therapy is. Standard indications for initiation of specific treatment are related to the presence of anemia, thrombocytopenia, major or painful splenomegaly, lymphadenopathy, symptomatology related to the disease (weight loss, fatigue, fever, night sweats), lymphocytes doubling time <6 months, transformation in more aggressive forms - pro-lymphocytic or Richterian one. (18, 19)

Criteria to treatment start may differ between clinical experience and conduct of clinical trials, so active disease should be clearly documented for choosing a treatment protocol. (table 1)

The obtained results of CLL therapy are much improved, especially in advanced cases of the disease or relapses with the advent of new chemotherapeutic agents, biological therapy representing a wide range of interesting and encouraging results. (table 2)

The choice of treatment is closely related to the development of modern investigative techniques that detect and comprise the patients in certain risk groups depending upon the unfavorable prognostic factors. (table 3)

**Therapeutical decision**

The most majority of the patients with CLL does not require specific treatment, those with asymptomatic lymphocytosis in early stages may remain in hematologic clinical observation - "watch and wait". At the onset, number of leukocytes in itself is an indication of the treatment beginning of the treatment, other than at very high values (WBC> 50,000/mmc) or when there is the danger of hyperviscosity syndrome appearance and in most of the cases this situation concurs, most of the times, with advanced disease stage (Rai III - IV). (20). Patients with recurrences can also be monitored without therapy until symptoms associated with the disease will occur with damage to blood counts, discomfort caused by lymphadenopathy or hepatosplenomegaly, recurrent infections or associated autoimmune diseases. (20)

If the initiation of chemotherapy is necessary, were created different strategies of the risk treatment tailored according to patient characteristics (age, comorbidities, performance status), as well as to the therapeutic goal. (21) (table 4)

Response assessment should include a thorough physical exam and evaluation of peripheral blood and bone marrow. Response criteria set out by the International Workshop and NCI-WG guidelines

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**Table 1. Criteria for active disease**

1. At least one of the following symptoms must be present:
   - Weight loss ≥ 10% in the last six months.
   - Extreme fatigue (performance index ≥ 2; unable to perform daily activities).
   - Fever or temperature greater than 38°C within ≥ 2 weeks without evidence of infection.
   - Night sweats without evidence of infection

2. Demonstration of bone marrow failure manifested by the development or worsening of anemia and/or thrombocytopenia

3. Hemolytic anemia and/or thrombocytopenia with inefficient response to corticosteroids.

4. Severe splenomegaly (> 6 cm below the costal edge) or progressive splenomegaly.

5. Large nodes masses (> 10 cm) or progressive lymphadenopathies.

6. Progressive lymphocytosis with an increase of > 50% in a period of two months, or a lymphocyte cells doubling time of <6 months.

7. Marked hypogammaglobulinemia or the emergence of a monoclonal protein. In the absence of any of the above criteria for demonstrating active disease will not be sufficient to produce a therapeutic protocol.

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**Table 2. Therapeutical schemes**

**Previous therapeutic methods**
- Monotherapy glucocorticoids, alkylating agents
- Polychemotherapy chlorambucil + prednisone, COP, CHOP, fludarabine-containing chemotherapy combinations: FC, FCM

**Recent therapeutic methods**
- Immunetherapy + Anti CD 20 + FC
- Polychemotherapy Anti CD 20 + FC + anti CD23
- Immunetherapy Anti CD 52 monoclonal antibodies
- Radio-immunetherapy
- Transplant of the stem cells (auto and allo-transplant)

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**Table 3. Prognostic factors in chronic lymphocytic leukemia**

**Traditional negative prognostic factors**
- Patients with advanced stages
- Lymphocytes doubling time <12 months
- Initial absolute number of lymphocytes > 50,000/mmc

**Recent adverse prognostic factors**
- CD38 positive
- ZAP 70 positive
- IgVH mutational status
- Positive thymidine kinase
- Cyto genetic abnormalities (mutations in p53, 12 trisomy or 17p, 13q, 11q deletions)
- Abnormal karyotype
- Beta2-microglobulin
and are used even today. (12, 22,23)

Complete remission implies fulfilling of all criteria following a period of at least 2 months: absence of lymph nodes/adenopathies on physical examination and radiological techniques, absence of splenomegaly and hepatomegaly on physical examination and radiological techniques, ≥ 1.500/μL leukocyte, platelets count > 100,000/μL, hemoglobin > 11.0 g/dL, or focal nodular lymphoid infiltrates compatible with complete remission, bone marrow lymphocytosis < 30%.

Partial remission is considered that could allow the detection of agents with biological effect. To consider partial remission must be met first three criteria and one or more of the following criteria left for at least 2 months: decrease of ≥ 50% decrease in peripheral blood lymphocytes from pretreatment value, reduction of ≥ 50% in lymphadenopathy, reducing the size of the liver and / or spleen ≥ 50 %, leukocytes number ≥ 1.500/μL or improvement with 50% from baseline, platelets count > 100,000/μL or 50% improvement from baseline, hemoglobin > 11.0 g/dL or 50% improvement from baseline without transfusions. NCI -WG guidelines suggest that patients with complete remission with persistent nodules (complete nodal remission) should be carefully investigated, they having a shorter time to disease progression compared with those in complete remission without nodular infiltrate. The recommendation is that these patients to be included in the category of those with the partial remission.

The progressive disease is characterized by at least one of the following: the increase ≥ 50 % of the products sum of at least 2 lymph nodes at two consecutive measurements in a period of two weeks (at least one lymph node ≥ 2 cm), the emergence of a new palpable lymph, increase ≥ 50% in the absolute number of circulating lymphocytes (≥ 5,000/μL) aggressive histologic transformation (eg Richter ‘s syndrome or prolymphocytary leukemia with pro lymphocytes > 55 %). In the absence of these criteria, a decrease in Hb concentration ≥ 2 g / dL or decrease ≥ 50% in platelets count and / or the number of granulocytes shall not take apart the patient from continued monitoring. In this case it is recommended to perform bone marrow aspiration and bone marrow biopsy to determine the cause of blood cells suppression. Patients who can not be categorized as being in the complete or partial remission and who have not progressive disease, are considered to have stable disease. Treatment results are considered benefically from the clinical point of view comprise complete remission, nodal complete remission and partial remission; the rest of the results, for example the stable disease without response, progressive disease and death from any cause, shall be deemed as the failure of therapy. Duration of response should be measured from the time the patient presented a maximum response until the disease progression. (12, 23)

**CONCLUSIONS**

Chronic lymphocytic leukemia is considered an indolent hemopathy, often with an insidious onset and diagnosis in routine medical examinations. Although it offers many alternatives, the path in treating a case of CLL is sometimes difficult, the moment of treatment begining in accordance with the identification of prognostic factors at diagnosis and also various treatment regimens toxicity alters the LLC evolution. But the final goal is to achieve a lasting complete remission. Increasing importance is the determination of a minimal residual disease. Flow cytometry and quantitative real-time PCR represent techniques and high accuracy in the detection of minimal residual disease (24). The tests can be performed both in peripheral blood and bone marrow aspirate, the latest version being preferred in patients whose regimens were used monoclonal
antibodies. It is still questionable usefulness of further specific therapies in patients with presented minimal residual disease although it has been demonstrated that their prognosis is more reserved and greater risk of relapse.

REFERENCES