GUIDELINES FOR DIAGNOSIS AND TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA

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1 Introduction and definition

The traditional definition of chronic lymphocytic leukemia (CLL) requires for diagnosis only elevated number of morphologically defined lymphocytes in the peripheral blood (> 15 x 10^9 / L) and bone marrow (> 30%), regardless of the possible infiltration of lymphoid and other organs.[1] Other characteristics of neoplastic lymphocyte populations (such as immunophenotype, cytogenetic and molecular features), were not fully known, and attempts to morphologically distinguish between typical CLL and "lymphosarcoma cell leukemia" were not precise enough so that morphological classification and/or subclassification has not received general support.

It should be emphasized that the morphological definition of disease as a requirement for the diagnosis of the disease is still present. Thus, chronic lymphocytic leukemia includes all variants of typical and atypical small and medium-sized lymphocytes, and excludes prolymphocytic and lymphoblastic leukemias.[2]

In parallel with the development of immunology and the rapid growth in diagnostic technology at the cellular and subcellular level, there was a modification of the traditional approach, so that today the predominant and widely accepted definition is based on a typical immune phenotype of neoplastic lymphocyte populations (WHO definitions and iwCLL).[3-6] This enabled nosological entity so defined to include with typical B-CLL also the monoclonal B-lymphocytosis and small lymphocytic lymphoma. Thus, it expanded the scope of nosological entity defined by identical phenotype to conditionally non-leukemic forms. At the same time the remaining, far less common forms of lymphocytic leukemias in clinical practice had to be classified outside the typical B-CLL.

This approach has now gained widespread support because it allows a simple and unbiased classification. In a typical CLL phenotype, three variants are present ordered by prevalence (1) **MBL**, (2) **B-CLL**, (3) **SLL** (Figure 1), and in addition (4) outside of the typical CLL phenotype, which includes variants of clonal B-lymphocyte leukemia and rare T-lymphocyte variant of chronic lymphocytic leukemia. All entities that are characterized by absolute lymphocytosis regardless of typicality morphology and immunophenotype are together Chronic lymphocytic leukemia syndrome (see Table 1);

To classify the patients in whom the typical immunophenotype verified clonal B lymphocytes in the peripheral blood, bone marrow or lymph node (Figure 1) a key role has (a) quantification (absolute numbers of clonal B lymphocytes in the peripheral blood) and (b) the distribution of tumor mass, which allows consistent unbiased classification into three basic categories. In recent years, there has been some changes in the definition of quantitative criteria, so it is now generally accepted that the patient is diagnosed (1) monoclonal B-lymphocytosis (MBL) if the number of clonal B-lymphocytes in the peripheral blood of less than 5 x 10^9 / L, and no lymphadenopathy greater than 1.5 cm, nor splenomegaly, anemia or thrombocytopenia. If the number of clonal B-cells is greater than 5 x 10^9 / L, then the patient is diagnosed as (2) B-CLL, regardless of the presence or absence of infiltration of organs. If indeed lymph node greater than 1.5 cm with a typical phenotype is present, and the number of clonal cells in the peripheral blood does not exceed 5 x 10^9 / L, the disease is classified as (3) SLL (small lymphocytic lymphoma). [3, 5-14]

Since the diagnosis does not automatically mean the indication for treatment (in principle, it is necessary to meet additional criteria), the monitoring of patients without therapy may document changes and the transition from one of the above categories to other categories. Therefore, the disease is definitely classified at a point just before the start of therapy (base line), based on the above criteria, given the distribution of the tumor mass, on B-CLL or SLL. This is justified on the ground that under the current knowledge it is not necessary to treat MBL. Classifying patients after treatment for obvious reasons is not acceptable.

Epidemiology. Chronic lymphocytic leukemia (B-CLL) is the most common type of leukemia in Western countries, the incidence is estimated to more than 5 per 100,000 people annually. The median age at diagnosis is growing globally, so that now exceeds 70 years. The disease is nearly twice as common in men. [15-20] It is further estimated that up to 1/3 of patients at diagnosis there is an indication to start therapy, while others should be observed (more than 2/3 patients). Of this number at least half (1/3 of all patients) never require treatment. These estimates should be validated by our country, and hopes are placed in the Register organized by Krohem.

2 Diagnostic procedure

The diagnostic process can be conditionally divided into several sections (steps, phases) with respect to the different objectives to be achieved. In Table 2., the basis for the decision is specified, the main criteria for classification and possible categories to which the classification in this section should lead.

2.1 Setting the suspicion and the referral of patients to hematologist.

Today, the most frequent finding leading to suspicion to CLL is absolute lymphocytosis during routine blood examination auto-increment (70-80%), and less frequently (20-30%) is due to the finding of organomegaly (swollen lymph nodes and / or spleen) or symptoms associated with CLL. As noted above, the input to the system is a lymphocytic morphology (lymphoblasts were excluded, as well as the predominant finding, over 55%, of prolymphocytes).

2.2 The diagnosis and differential diagnosis

The activities that follow are directed toward defining the cell type, enabling the diagnosis (type of disease) and differential diagnosis. It is possible (and necessary) to make the diagnosis of a typical B-CLL on the basis of morphology and flow cytometry in the peripheral blood sample and to distinguish it from other entities in the CLL syndrome. For the diagnosis of B-CLL phenotype (typical phenotype) the following is required: The restriction of sIg light-chain expression of low intensity, CD5 +, CD19 +, CD20 low, CD23 +, CD10 - (see later). Basic hematological clinical findings & blood count allow quantification of the tumor mass in the peripheral blood and lymphoid organs, which allows the classification of entities that meet the diagnostic criteria for (1) B-CLL (presence in the blood of more than 5 x 10^9 / L clonal cells) or (2) SLL (less then 5 x 10^9 / L clonal cells in blood and present lymphadenopathy greater than 1.5 cm), and (3) MBL (less then 5 x 10^9 / L clonal cells in blood and no lymphadenopathy or symptoms). MBL (monoclonal B-lymphocytosis), is the counterpart of MGUS (monoclonal gammopathy of unknown significance) in the diagnosis of myeloma. So, a different size of the tumor mass between B-CLL and MBL and between MBL and SLL is critical. Today it is considered that these entities are different manifestations of the same disease, and, if they meet the criteria of quantitative disease called B-CLL/SLL, approximately 90% of patients meet the criteria for B-CLL, and about 10% for SLL. MBL prevalence increases with age and is estimated to be around 6% in population aged over 60 years. As these people do not have any lymphocytosis nor characteristic symptoms, only a very small number is processed by flow cytometry, so they remain in the vast majority undetected.[12, 21, 22] This is the reason why in the diagnostic procedure for B-CLL the expected percentage is not listed. Typical panel of investigated epitopes that allows classification of patients who meet the criteria for the syndrome CLL (B-CLL and other lymphoproliferative disease with lymphocytosis) is presented in Table 3.

It is evident that for the diagnosis of type of disease, a very small number of tests is enough, because if the result is positive in the peripheral blood it is not necessary analyze the bone marrow or lymph nodes, although these tests have their place in further diagnostic procedures. However, the development of medical technology and the increasing availability of ultrasound machines sets a new standard, and lymphadenopathy are increasingly assessed by ultrasound examination of lymph nodes in the peripheral regions, and certainly in organomegalies in the abdomen. For the diagnosis of SLL it is recommended to do node biopsy to establish the diagnosis and to distinguish between MBL and SLL the CT of thorax, abdomen and pelvis can be useful. In the case that according to the above criteria disorder is classified as MBL, and there are peripheral cytopenia, bone marrow analysis can point to strong infiltration of B-CLL cell clone, so in this case it is possible to diagnose B-CLL even without quantitative criteria in the peripheral blood.

It should be remembered that this type of disorder classification based only on the immune phenotype is not using morphological, cytogenetic, nor molecular or other characteristics of the disease that today show significant association with prognosis. Therefore, it is not excluded that in the near future a new paradigm will emerge, in which the classification of nosological entities is based on the new features (like today for example, in the case of acute leukemia, where an increasingly important role in the classification have the cytogenetics and molecular investigations).

2.3 Evaluation of the stage / extent of the disease

After the diagnosis of the type of disease, the evaluation of disease stage or extent follows. This is in principle made on the basis of clinical and hematological evaluation, under the criteria set out in Tables 4., 5. and 6. Clinical stages represent a simple tool for the clinical assessment of the disease extent, but also allow assessment of other important features such as prognosis prediction, indications for therapy, and can be used for stratification in clinical trials (see later). The basis of these systems is the assumption that the disease is gradually progressing and expanding. Therefore, the patients who have advanced disease have a higher tumor load and more extensive disease. [1, 23, 24]

The clinical stages according to Rai and Binet are shown on tables 4 and 5. Those staging systems assess tumor size by simple parameter estimates of the size of the tumor mass (without precise quantification of the affected compartments) along with the parameters for the assessment of bone marrow failure. In doing so, the greatest contribution to prognostic power has the failure of the bone marrow. Note that it is not a direct sign of tumor size but an indirect one. The prognostic power is relatively weak if the failure of the bone marrow is excluded.

Estimate of the size of the tumor mass (TTM) is different in that it quantitatively evaluates the tumor mass in 3 major cell compartments, regardless of bone marrow failure (Table 6). Quantitative character enables unbiased monitoring of disease progression and is a very convenient tool for the assessment of therapeutic response (see later). In addition, in most patients who have affected both peripheral blood and lymphoid organs it is possible to determine the type of distribution of the tumor mass by comparing with TM_1 with TM_2 +TM_3. [25] This feature of TTM system enables us to estimate the dynamics of disease, progression and response to therapy (see later).

2.4 Prognostic factors at diagnosis

The next step is the classification of patients into prognostic groups a priori. The basis for the decision with respect to classification into given prognostic category are primarily prognostic factors that can be determined immediately at diagnosis, and refer to the additional characterization of neoplastic cells. For some of them it is characteristic that in principle do not change during the evolution of the disease as they relate to the mutational status of IgVH genes, but this is, probably due to the complexity, unsuitable for routine clinical practice. Therefore, tests that have a high correlation with mutational status are used, but beyond that they may have an independent prognostic power. The most widely used tests are expressions CD38 and ZAP-70 by flow cytometry and immunohistochemistry, with the higher value was found to correlate with poorer prognosis.[26-41] Cytogenetics and molecular tests (FISH) to determine: del (11q22-23), +12; del (13q14), del (17p13) showed a strong independent prognostic effect. These tests have proven to be useful to stratify patients into groups that react differently to certain drugs, so they are obligatory performed just before therapy to assess the percentage of cells affected by the change. If the percentage of cells with del (17p) is more than 20%, the patients are classified into group with a deletion of p53 genes.[42]

On the other hand, a number of additional factors more related to patient's state also have a strong predictive power, so a number of different parameters are often used, and multivariate analyzes study their impact on prognosis. Because of the above, we distinguish three types of factors, given the causal connection or disconnection with B-CLL. First, those who are associated with B-CLL clonal neoplastic diseases (size distribution, the growth rate of tumors, mutational status, CD38, ZAP-70, FISH, response to therapy, etc.) [1, 23, 24, 35-38, 42-47] ; second , those who are associated with organ failure (mixed groups, they may be due to both the very undelying neoplastic diseases and consequences of associated diseases, such as anemia, thrombocytopenia, whose ethiology should be carefully evaluated), and third, those factors that are associated with the patient and not directly with neoplasms (age, gender, comorbidity).[31, 48-51]

On the basis of strong independent predictors composite prognostic indices are calculated. They show very good prognostic power with respect to the length of survival. However, the stratification of patients for treatment that is adapted to risk one should be careful, because the composite index usually consist of the factors that belong to each of the above mentioned three groups. In fact, if the poor prognosis is associated with the first group of factors, then it can justify the use of more aggressive therapy. If, on the other hand, the poor prognosis is associated with a third group of factors, then this points to a very poor ability to receive aggressive therapy, and in fact suggests

the less aggressive treatment. Similarly, the prognostic nomogram (Wierda, 2007), which demonstrated strong overall predictive power, but as a guideline for therapy is not suitable because of its strong dependence on factors from a third group. [43, 52]

2.5 Indications for treatment / the criteria for initiation of therapy

The basis for the decision is based solely on an assessment of the parameters that are associated with neoplastic B-CLL clone.[3] Therefore, all the parameters that can be caused independently of the neoplasm, it is necessary to carefully evaluate the extent to which parameters are associated with neoplastic clone (eg fever, anemia, etc.). Criterion parameters can be classified into three distinct types: 1. Quantitative parameters for which is defined threshold consensus that is considered to justify the initiation of treatment, such as anemia, defined by a certain level of hemoglobin, thrombocytopenia, defined by platelet count, organomegaly defined by size of the spleen or lymph nodes. 2. Monitoring data to enable assessment of trends, eg progressive cytopenia, progressive lymphadenopathy and / or splenomegaly, increased leukocyte count or TTM values (see the previous section). It should be emphasized that only the measurement of dynamic parameters can directly evaluate progression of disease, as opposed to the a priori estimate of a possible evolution described in the previous section (see fourth Prognostic features at diagnosis). Here, however, we should point out certain difficulties and ambiguities in quantitative measurement of the dynamic parameters, especially in the early stages of the disease. This imprecision in a priori definition of the criteria for progressiveness, despite a very attractive concept, often leads to delay a decision until the moment when it reaches the absolute value of the threshold that is set up as described under number one. 3. Qualitative criteria of the occurrence of symptoms, threatening organ damage and the like, and which is considered to be the result of neoplastic disease activity.

Definition of the indication for treatment is of practical use, as direct the treatment to the causes of the problems. If, for example, as an indication for therapy a strong weight loss (more than 10% in 6 months) is accepted, then the success of therapy criterion should be an appropriate recovery of body weight, rather than the reduction in the number of lymphocytes. Likewise, the presence of unfavorable prognostic factors alone may not be sufficient justification for the start of therapy, although it is an open and important research issue. Therefore, on the basis of conducted clinical research, it is generally accepted today that asymptomatic patients do not require treatment until becoming symptomatic (with active / progressive disease). [3]

It should be noted that the mere indication simultaneously indicate immediate treatment goals (!). Today, we seek to combine threshold criteria with dynamic criteria, as shown in Table 7. Observe that it uses a number of criteria, the criteria of threshold, dynamics and qualitative changes. Although it is sufficient to indicate treatment the presence of only one of the criteria, it is important that this criterion is compelling, and the presence of multiple criteria makes decision certainly easier. For dynamic criteria it is especially important, so it is good to compare the growth trend of the tumor mass with trends of deepening anemia and / or thrombocytopenia. The indication for treatment (according to KROHEM guidance) should be documented in patient records.

2.6 The schedule of activities and assessments

Table 8 shows the general scheme of tests that are used in pretreatment work-up, in therapy monitoring and after therapy. For about third of patients indications for therapy are present, so the pre-treatment work-up is done immediately at diagnosis. Other patients are observed after diagnosis until meeting the criteria for initiation of treatment (Figure 2). At that point the pre-tretment complete work-up is performed.

Half of the patients being followed (1/3 of the total) never reaches the criteria for initiation of treatment and the other half reaches the criteria, but in a different period of time, from several months to more than 10 years. Therefore pretherapeutical monitoring takes a different time. It is possible to distinguish several specific clinical questions that need to be answered (see Table 9). These are following detection of the disease (1), differential diagnosis (2), evaluation of the extent of disease (3), assessment of prognostic groups and (4), and the monitoring of the clinical parameters that serve as criteria for initiation of treatment (5). Immediate pre-therapeutical work-up (6) provide a definite classification of the disease, a definitive assessment of prognostic parameters as well as the general state of the patient, and any associated illnesses including infection status. After that, monitoring of the course of therapy follows (7) and at the end of therapy (EOT) the evaluation of achieved responses (8). After that as

a rule the steps are repeated, again monitoring without therapy (5 ') and then, in case of the need for a new line of therapy repete pre-therapeutical work-up (6') followed by the monitoring of treatment (7') and evaluation of therapy (8').

Table 9 shows more elaborate evaluation of patients by the above sections and goals. In Croatia, it is possible to do all these tests, but everything can not be done in all centers. For investigations that can not be done at the local center, it is possible to send patients for work-up in one of the centers where it is possible to do the necessary tests. This will offer to all patients in Croatia equal agreed standard for diagnosis and treatment.

The first three **groups (1-3)** are related to the clinical examination and routine hematological tests, to be used to determine the clinical stage and TTM, ie, **groups (4-5)**. An electronic calculator is available online to estimate the size of the tumor mass, the distribution of the tumor mass and growth rate of the tumor mass (see later).

Groups (6-9) include cytological examination, flow cytometry, cytogenetics, molecular testing and histology. Although, according to the new definitions for the diagnosis of B-CLL peripheral blood finding is sufficient, in certain cases it is necessary to perform at diagnosis examination of bone marrow and lymph nodes for the cytological, histological and immunophenotypic and molecular work-up, as shown in Table 9.

Cytological examination of peripheral blood is readily available, and is routinely used in all stages of processing, including monitoring before and during therapy. Cytological bone marrow aspiration is mandatory at diagnosis, immediately before institution of therapy and after the therapy. Lymph node cytology is performed in case of clinical indications.

Flow cytometry and immunohistochemistry. For the *diagnosis* of B-CLL it is necessary to confirm the typical immunophenotype (restriction of light-chain with sIg expression of low intensity, CD5 +, CD19 +, CD20 low, CD23 +) in peripheral blood lymphocytes by flow cytometry. For the diagnosis and assessment of the extent of involvement and the individual compartment, the lymph node(s) and the bone marrow can be sampled. Recommended basic diagnostic panel for flow cytometry is: kappa / lambda, CD19, CD20, CD5, CD23, CD10, and for the immunohistochemistry method is: CD3, CD5, CD10, CD20, CD23, CyclinD1. After diagnosis confirmation it is necessary to determine the main *prognostic markers* CD38 and / or ZAP-70 on peripheral blood lymphocytes, since the results are not yet validated in other lymphoid compartments. Immunophenotyping must be repeated before the start of treatment on the peripheral blood sample, and the samples from other compartments if there has been a significant increase in tumor mass in given compartment (i.e. possible clonal evolution or transformation).

Upon completion of treatment, immunophenotyping of peripheral blood and bone marrow should estimate the *minimal residual disease* (MRD). Recommended basic panel CD19/CD5/κ/λ which is used at diagnosis, for MRD shows a relatively low sensitivity, so it is advisable to use one of the more sensitive fourfold combinations, such as CD19/CD5/CD20/CD79b. Given the many new insights into the biology of B-CLL, the above basic panels can be expanded with additional markers.

Cytogenetics or molecular tests (FISH) to determine: del (11q22-23), +12, del (13q14), del (17p13). These basic panel should be performed at diagnosis, just before the start of therapy (and other lines of treatment), due to the possibility of selection and evolution of the malignant clone, acquisition of new changes during the progression of the disease and treatment, and impact on treatment choice (eg del17p). The main panel can be expanded with other probes.

Bone marrow biopsy is necessary to make the diagnosis, and in case of clinical indication a biopsy of the *lymph node* may be needed, mandatory in the case of suspicion of transformation (Richter's syndrome).(53)

Groups (10-11) are routine laboratory tests. As a rule, serum biochemical tests include CMP ("Comprehensive metabolic panel"), which includes 14 basic parameters (glucose, Ca, albumin, total protein, Na, K, Cl, bicarbonate, urea, creatinine, AST, ALT, ALP, bilirubin), and along with it and LDH, serum proteins electrophoresis, IgG, IgA, IgM, and beta-2-microglobulin.

Group 12 includes imaging methods. Similar information can be obtained by different imaging methods. Today, ultrasound is used to evaluate abdominal lymphadenopathy and splenomegaly, in addition to the more commonly used ultrasound to verify the peripheral lymph nodes, (which is in principle possible to evaluate by the clinical

examination) and also estimate the size of the spleen. It is believed, however, that MSCT is still the gold standard for assessment of thoracic lymphadenopathy and to evaluate abdominal and pelvic lymph nodes. We recommend the use of MSCT in pre-treatment work-up, and the according to clinical indication.

Groups (13-14) are routine cardiological and infectology investigations. Anticipated aggressive therapy requires determination of HIV, hepatitis B and C and possibly other viral status.

Group 15 includes all tests that can occur clinically indicated.

Group 16 represents the scale for the assessment of the cumulative impact of comorbities, and is used for therapeutic stratification. Based on data collected in groups 1-15 estimated involvement of certain organ systems is made, and given the strength of disorder a score is calculated. The most widely used is modified CIRS (Cumulative Illness Rating Scale). [(48)] Electronic versions and simulations are prepared (Figure 2 and Figure 3). Online calculators are present on the web, for now either at http://www.ttm-cll.org or KROHEM web site http://www.krohem.hr .

Group 17 is reserved for research and investigations can be increased at will. Depending on the planned research protocol, it is possible to predict the research taking samples at any time point.

3 Therapy individualization - defining goals and strategies, and stratifying patients

At the end of diagnostic procedure described in sections 1-6, there is a need to make a decision about therapy.

Decision is based on the integration of the factors related to the neoplasm on the one side, with the factors related to the patient on the other side. In practical terms, on the basis of a diagnostic procedure which determines the type and subtype, and after stratifying patients according to age and comorbidity, the general principles are applied to a specific patient and realistic therapeutic goals are set. In doing this wishes should be balanced with possibilities. Specifically, there is no doubt that the highest goal of any treatment should be cure. Although it is reasonable to set a highest possible goal (i.e. hierarchically: cure; or molecular response; or complete hematologic response; or complete clinical remission; or partial remission; or stable disease and like), the essential questions for each goal are: (1) is it within reach? and (2) is the risk acceptable? This risk depends on the ability of patients to receive treatment, i.e. on the assessment of general health status. The overall approach is essentially based on clinical judgment and the expertise of hematologist with special interest in CLL seems to be warranted, as shown in a retrospective survey of the outcome of patients with CLL managed by either a haemato-oncologist specializing in CLL or in another haematological malignancy showed an improved overall survival for patients managed by CLL-experts after adjusting for age, gender, stage and lymphocyte count at diagnosis.[(54)]

Selection of the type of treatment, as well as strategies of therapy must be consistent with the realistic goals set.[(55)] If the answer is yes to both above questions (i.e. within reach & acceptable risk) then we are opting for a strategy that should lead to long-term remission and cure. Proven effective therapy is applied, now mostly chemo-immunotherapy, and if that is not enough, the therapy is increased in intensity (step-up), including allo stem cell transplantation. Efforts are made to eradicate the disease, often to the limits of tolerance, and the effect is measured by negativisation of minimal residual disease (MRD). However, the therapy must be carefully monitored for the occurrence of toxicity and care should be taken to avoid forcing the same treatment if it does not achieve the intended results and is too toxic. If necessary, one should adjust the objectives to match the possibilities and to choose a less ambitious goal. Special attention should be put on the immunosuppression that occurs with untreated disease, but which can significantly deepen by forcing the strong antilymphocytic drug therapy.

If however, from the beginning the risk of a currently available “curative” treatment is estimated unacceptable, a less aggressive therapeutic strategy should be choosed to control the disease. Thus, the feasible therapy goal becomes to achieve remission, or stabilization of disease or maintenance of quality of life, but not MRD negativity. In

this case the less "aggressive" therapeutic strategies are applied in order to achieve the desired (or possible) level of response. Therefore, in this setting the least therapy to achieve the desired goal applies.

It is therefore on the one hand the clinical assessment of the nature of the disease which can be transformed into an assessment of the risk of the disease. On the other hand it is possible to estimate the tolerance of each form of therapy, which primarily depends on the general state of the organism. In general, we are comparing the two risks: (1) the risk of disease and (2) the risk of treatment. It is clear that the risk of treatment should reasonable lower than the risk of the disease.

- The risk of disease. For patients in early, stable and asymptomatic disease clinical trials carried out so far showed that the standard chemotherapy (regardless of the general condition of the patient) is greater than the risk of the disease. In patients who fulfill criteria to start therapy the greater is the risk of the disease and the therapy is warranted. Moreover, when the forecast is worse according to CLL prognostic factors, it is reasonable to apply the "aggressive" therapy (although this therapy generally causes more toxicity), because the risk of the disease in this case is greater than the risk of therapy.

  In addition, some variants of disease are less responsive to therapy in general or to a specific individual treatment, or vice versa some variants may respond better. For example, it is now generally accepted that patients that have a p53 gene deletion /del (17p)/,(up to 10% of all patients) have a very poor prognosis, associated with a very poor therapeutic response to otherwise effective anti lymphocyte therapy. [31] (42) In recent years, emphasizes that patients with del (11q), (up to 20% of all patients) have a high relapse rate, and therefore poorer prognosis, although unlike del (17p) respond very well to FCR treatment.[(56)]

- The risk of the therapy, i.e. tolerance is highly dependent on the general condition of the patient and the presence of associated diseases, which is often associated (though not exclusively) with the patient's age.

  A patient is classified as capable (fit, Go Go), when there is a low comorbidity score (eg CIRS-G <6). Although age does not enter into the calculation for CIRS-G, it is known that age is a very important factor, and it should be taken into account. It is common to impose an age limit for stratification in therapeutic groups. Today the limit is set at 70 years, and in accordance with expected prolongation of life has a tendency to increase. Patient aged over 70 years can be considered capable for receiving aggressive therapy, if is in excellent health, without substantial comorbidity. However, at that age only small number of patients qualifies as fit.

  Patients older than 70 years (as a rule), and younger if they have significant comorbidities are classified as unfit for aggressive therapy (unfit, Slow Go, frail), but it is still the goal of therapy to achieve remission. Some authors recommend inclusion of third groups (No Go) in addition to Go Go and Slow Go, for those patients for whom is only symptomatic therapy possible, although such stratification is not generally accepted.

  Although this concept is very good and important, problems can occur if the criteria are applied in different ways, which is why some clinical studies have different patients in each stratum, significantly reducing the credibility of the comparison. We are witnessing the current dispute between the American and European authors, which is based exactly on this aspect of selection of patients. Therefore, we believe that this aspect should be based on clear unbiased criteria.

On the basis of the two above-mentioned principles patients are today usually stratified into four strata with respect to therapy: (1) without the deletion of the p53 gene with a good general condition (capable for aggressive therapy), (2) the deletion of the p53 gene with a good general condition (capable for aggressive therapy), (3) without the p53 gene deletions with poor general condition (incapable for aggressive therapy), (4) the deletion of the p53 gene with a poor general condition (incapable for aggressive therapy). [(13, 14, 55, 57)] In other words, these strata may in principle represent the combination of age (high correlation with fitness) and risk (high correlation with TP53 abnormality). However, for the reasons stipulated above the chronological age cut-off should not be rigid, to allow elderly patients in good health to enjoy the benefit of more aggressive treatment and vice versa spare younger patients with co-morbidity of unwarranted therapy associated risks.

4 Therapeutic procedure

4.1.1 Therapeutic recommendations based on evidence / clinical trials

The treatment for B-CLL consist of antineoplastic therapy and supportive measures. Antineoplastic measures consist of chemotherapy, therapeutic antibodies, radiotherapy, stem cell transplantation methods and recently a fast development of newer agents that include proteasom inhibitors, novel class of immunomodulators (ImiDs), and particularly interesting BCR signal transduction inhibitors that are very promising. This recommendations are based on Phase III clinical trials, in some cases on evidence from earlier phase trials, and on the approved agents and therapies in Croatia. Combination therapy is as a rule more efficient then monotherapy. Newer agents are already in clinical trials, and first results of Phase III are expected by 2015. If new treatments that are now recruiting patients in various phases of clinical trials will meet the expectations we may see a major break-through and new therapeutic paradigm in the next few years.

The role of chemotherapy. The effectiveness of therapy is systematically investigated, and the choice of treatment is based on the conducted randomized clinical trials that compare the individual therapeutic strategies. Although this procedure is unavoidable, in practice it is still a bottleneck in the overall clinical trials, covering less than 15% of all clinical trials. Reasons for this are manifold - from administrative, logistics, high costs, which requires generous sponsoring (so today academic trials are exception), to methodological reasons that are partly due to a long period of time required to perform the trial. Chemotherapy is an oldest example. It often happens that the standard therapy that has been used as the comparator treatment in the meantime change, so it is questionable whether the overall benefits of such an experiment, because it leaves open the question of what would be the comparison of the investigated treatment with new comparator. Paradoxically, regulators accept drugs for registration on the basis of the Phase III trials, which are made with the comparators in very low doses [58-60], while claiming at the same time that the therapeutic success of such therapies is extremely modest. The best example is that chlorambucil as a comparator in these trials was used in very low doses, while the published randomized trials of more cooperative groups (IGCI, EORTC, UK) showed that middle and especially high doses have significantly greater effectiveness.[61-64] Also beyond randomized trials, a good Croatian experience from the KBC Zagreb author's group was reported at ASH and aroused notable interest.[65]

Because of the above one should take into account the fact that not infrequently, new treatments based on the results of early phase clinical research are promoted without clear scientific evidence, sometimes (unjustifiably) bypassing published results in well-known international journals.[66] Thus, it is less about the evidence based on facts and more on the opinion of some authorities, leading to variety of recommendations, with different therapeutic approaches.

The role of therapeutic antibodies. The results of clinical trials published in recent years have undoubtedly shown than addition of rituximab to chemotherapy in all combinations in which tested leads to significantly higher response compared with the chemotherapy alone. Therefore, it is generally accepted that the addition of rituximab is a new gold standard in treating CLL.[56, 67-70]

The role of newer agents. Studies of the pathogenesis of the disease have revealed the importance of signal transduction via the B-cell receptor (BCR), but also an important role of microenvironment. This helped to define new therapeutic targets, which are very intensively studied in clinical trials.[71-77] Results so far are encouraging, and we can expect that this treatment modality will change the current standard treatment concepts. Research therapies are not subject to these guidelines, but we want to emphasize that we believe that it is justified to include our patients in clinical research.[78, 79]

The role of allogeneic stem cell transplantation. Allogeneic transplantation proved to be an opportunity for long-term control of the disease, so it should be considered and incorporated as potential part of therapeutic strategies (for now it is recommended after the second-line therapy, while exploring strategies by moving allotransplantation in the early phases of treatment). This is especially true of younger patients and in patients with a mutation of the p53 gene and the gene ATM, where it seems justified as elective non-myeloablative allogeneic transplant. [57, 80]

Maintenance therapy may have an important role to play[81], but to date there is no convincing evidence based on randomized clinical trials.

The therapeutic effect is a consequence of total therapeutic interventions, including antineoplastic and supportive measures that are particularly important.

4.2 Criteria for evaluation of response to therapy

Table 10 shows the criteria for assessment of therapeutic effect. The criteria generally used the same grounds, based on the estimation of parameters of the tumor mass in different compatrments on the one hand, and the parameters for the assessment of myelopoiesis on the other hand. The criteria are somewhat different in the NCI / iwCLL criteria [3, 6] and the criteria described below (IGCI, EORTC) [61-63, 82, 83] Due to the continuous quantitative character assessment of responses based on the determination of TTM score, it is possible modify criteria for each specific KROHEM research. It should be noted that none of the usual recommendation does not require immunopoietic recovery assessment, although it is known that drug-induced immunodeficiency component can last for years after the treatment was applied. Should we keep general oncologic definition of complete remission as the absence of all signs of disease along with the recovery of failure(s), the number of published complete remission would be drastically smaller. Protracted therapy induced immunodeficiency is very important from a clinical point of view as a target for prophylaxis and / or treatment of possible frequent infections. Also, new agents cause marked increase in blood lymphocytosis, while in the same time a very marked shrinkage of lymph nodes occur. NCI criteria are suboptimal for monitoring of the disease response[19, 84], while TTM scoring system is much better measuring redistribution of clonal cells among compartments.

4.3 Antineoplastic therapy - treatment options

There are several treatment options. Therapeutic recommendations summary for **first- and second-line treatment** in major therapeutic stratification groups are shown on Table 11.

4.3.1 Management of patients with no accepted criteria for therapy

If the patient is not showing any signs of active / progressive / symptomatic disease, the therapy is not recommended, but the patient is monitored and reviewed without therapy. This view is based on evidence collected in randomized trials during the 80-ies of the last century, when it was shown that chlorambucil based treatment does not contribute to longer survival, moreover, despite the relative ease of controlling symptoms and achieving clinical remission, overall survival was marginally worse.[61, 82, 85] This is the paradox compared to the general oncologic doctrine that the treatment should be applied as soon as possible and is a very important research issue in order to determine ways and treatments that would prolong the survival of these patients. This unfortunately requires a very long period of monitoring and trials that have been started, or are planned, will not be able to give an answer to these important questions for a long time. Often advocated concept implies that for the success of the therapy the absence of minimal residual disease should be achieved. A deeper remission, and consequent cure of disease, of course, would have a better chance if the treatment is applied earlier in the course of the disease. This is opposite from current practice, that is waiting till full blown symptomatic disease and / or development failure symptoms. Paradoxically, we wait while disease progresses and then when the disease is advanced we do not apply stabilization strategies, but instead we choose the therapies with the aim of achieving molecular remission.[43, 86]

In asymptomatic early stage patients with poor risk prognostic factors benefits of early versus delayed treatment are currently being evaluated in randomized trials using FCR (GCLLSG-CLL7), or lenalidomide (NCI: OSU-2011C0005).

4.3.2 Initial treatment (first-line treatment)

The **first-line treatment** relates to previously untreated patients. All patients in standard care must have clinical indication for treatment initiation (i.e must fulfil criteria stipulated in section 2.5). Recommendation depends on the risk associated with B-CLL (High or Low) and patient general condition (Fit or Unfit). Each stratum will be discussed separately.

4.3.2.1 Initial treatment for fit patients with no TP53 abnormality. (Fit+Low risk)

As a rule the patients are younger than 70 years, the therapeutic goal is to be set high, to full remission and prolonging survival and even a possible cure.

FCR (fludarabine, cyclophosphamide, rituximab) is recommended as a standard initial therapy for previously untreated fit patients outside clinical trials.[56] According to the DCLLSG CLL8 protocol, 6 cycles at intervals of 28 days if the patients tolerate the treatment well, and after EOT, no further treatment is anticipated, only follow-up visits. However, if the patient's tumor is responding well, but a prolonged myelotoxicity occur, the intervals between the cycles are prolonged and / or reducing the total dose per treatment cycle. At the end of treatment (after 6 cycles) treatment is stopped and the calculation of the total cumulative dose applied and is expressed as the intensity of doses per month during the period of the therapy administration. Patients who progress after 1 cycle of FCR or who have stable disease after 2 cycles have high risk disease and should be managed accordingly.

In patients who are unsuitable for fludarabine therapy we suggest chlorambucil ± rituximab. It is important to apply chlorambucil in high enough doses (HD-CLB: 8-10 mg/m2 per day, an average of 15 mg per day continuously until complete response or occurrence of toxicity), according to the protocol IGCI CLL02 or EORTC-CLL-3.[62, 83] This dose of chlorambucil per month is 6-7 times higher than the dose (40 mg/m2 every 4 weeks), which was used as a comparator in the registration trials for fludarabine, alemtuzumab and bendamustin. As a rule, chlorambucil monotherapy at high doses is given at least 4 weeks. In combination with rituximab the application can be modified to fit each month chlorambucil therapy breaks. In combination with rituximab dose of chlorambucil may be reduced in number of days (for example, 10 days), or the like, so that treatment is cyclical, not continuous. Also the daily dose can be modified.

Other alternatives are shown in the table, it should be noted that some of the drugs are not registered in Croatia.

Phase III studies comparing FCR with FR [87], FCMR[88] and BR[89], and are in progress based on Phase II studies that have demonstrated the efficacy these combinations. At present B (Bendamustine) is not (yet) approved in Croatia, although is approved by FDA end EMA.

4.3.2.2 Initial treatment for fit patients with a TP53 abnormality. (Fit+High risk)

We are talking about patients who have a poor prognosis and are characterized by poor response to therapy based on fludarabine and probably alkylating drugs. There have been no randomized studies specifically for patients with high risk CLL. We recommend the induction with FCR[56] or A (alemtuzumab)[59, 90] or combination therapy with alemtuzumab and pulsed high-dose glucocorticoids (A+HDMP) that achieves response rates and PFS superior to those achieved with FCR or alemtuzumab alone.[91] Consequently, A+HDMP should be regarded as the induction regimen of choice. This regimen is associated with a significant risk of infection requiring that meticulous attention should be paid to antimicrobial prophylaxis and supportive care. Routine antimicrobial prophylaxis with oral co-trimoxazole, aciclovir and itraconazole and monitoring for CMV reactivation is recommended. Induction therapy should be followed by elective allogeneic stem cells transplantation in suitable patients, since the duration of remission following alemtuzumab containing regimens is relatively short. Alternatives are shown in the table.

4.3.2.3 Initial treatment for unfit patients with no TP53 abnormality. (Unfit+Low risk)

Majority of patients belong to that group. As a rule, the patients are older than 70 years, with co morbidity therefore not capable tolerate aggressive therapeutic approach, and therefore it's necessary to modify the therapeutic goal and choose remission or stabilization of disease with a well-preserved quality of life.

We as a standard recommend chlorambucil in medium or high doses, nowadays often with rituximab.[62, 92] Chlorambucil remains widely used in the UK for patients considered unfit for intensive therapy. A meta-analysis of trials comparing chlorambucil to fludarabine did not show advantage of fludarabine.[93] The problem of dose is discussed in section 4.1.1.. Also in UK LRF CLL4 trial in which chlorambucil was administered at a dose of 10 mg/m^2/day for 7 days every 4 weeks initially for 6 months extending to 12 months, in patients still responding after 6 months treatment.[64]

As alternative we suggest CVP + R or lower doses of F or FA or FCR, while part of alternative drugs is not registered in Croatia.

A recent phase III study randomized patients to chlorambucil or bendamustine[60] showed a higher response rate and longer PFS for the bendamustine arm. The ORR and PFS in the chlorambucil arm was lower than in the IGCI CLL-02 and the EORTC CLL01 and UK LRF CLL4 trial but comparisons between these studies are hampered by the use of different chlorambucil dose regimens and differing inclusion criteria. Bendamustin is not at present approved in Croatia.

A higher overall response rate (80% v 66%) was achieved with the combination of chlorambucil and rituximab compared to a historical control arm derived from patients receiving single agent chlorambucil in the UK CLL4 trial [94]. A similarly high overall response rate of 91% was obtained in 117 patients, of whom 26% were over the age of 70 years, treated with bendamustine and rituximab.[89]

Phase III trials of chlorambucil or bendamustine in combination with an anti-CD20 antibody are in progress.

In view of the efficacy of FC and FCR in CLL, based on small non randomized phase II studies have evaluated dose-reduced regimens in patients considered unfit for full dose treatment, larger randomized studies with prolonged follow up are in progress to determine the efficacy of dose-reduced FC or FCR.[95]

4.3.2.4 Initial treatment for unfit patients with with the TP53 abnormality.(Unfit+High risk)

These patients are not suitable for aggressive therapy nor for allogeneic transplantation and generally have a very poor prognosis.

We recommend A (alemtuzumab)[59] ; or HDMP (High Dose Methyl Prednisolon)[91, 96] + R ; or R HD (High Dose Rituximab)[97] ; and as alternative CLB + R, HDDex[98], CFAR[99], OFAR[100]. Part of alternative drugs is not registered in Croatia.

4.3.3 Treatment for relapsed CLL

This relates to previously treated patients. Again, they should fulfill criteria for re-treatment, essentially the same as described in the section 2.5. The situation here is much more complex, since in addition to four major therapeutic strata special attention should be paid to previous treatment(s) (type of treatment, number of treatment lines and the period that had lapsed from previous treatment etc.). In principle, with the exception of very late relapses, the patients require more therapy to achieve less response.

Since the vast majority (>90%) of all CLL first-line treated patients have no TP53 abnormality, their treatment allocation was essentially dependent on their general condition (Fit or Unfit). Thus, the fit patients received more aggressive treatment aiming MRD negativity (hopefully the eradication of the disease) while the unfit patients receive less aggressive treatment that is less likely to achieve MDR negativity and consequently the therapeutic aim is less ambitious.

The relapse is, therefore, primarily linked to the therapeutic stratum. It is an indicator of respective therapy failure. In principle, the longer the period to relapse the more effective first line treatment was.

General principles of therapeutic strategies in relapsing / refractory patients is shown on table 11. The relapsing patient in early relapse (less than one year for chlorambucil, and, less than 2 or according to some authors 3 years for FCR) is considered refractory, and if is fit, a step up with or more aggressive treatment may be warranted. That includes new drugs or combinations followed if feasible to an allo stem cell transplantation. If the patient is unfit, the therapy should be changed to alternative in the same range of agressivity and / or included in clinical trial(s). If the treatment result in remission of long duration, it is reasonable to try in relapse again the same treatment that has proved effective. Therefore, such a therapy may be repeated in function of achieved duration until the duration of remission is not shortened to one year, after which it is justified to go to the second line therapy.

For this reason relapses will be described as a function of first-line stratification therapeutic failures. However, in all patients in relapse TP53 status should be checked to assess whether the risk grade has changed in comparison to front-line stratification. For patients that change the risk status alemtuzumab based treatment should be considered.

4.3.3.1 Patients relapsing from the Fit+Low risk stratum

In this patient group majority of patients are supposed to be treated with FCR like therapy. However, since FCR therapy have been accepted as gold standard for this group of patients recently, a number of patients have been treated with alkylators, others with fludarabine monotherapy. The number of these patients will be drastically lower from now on. If the relapse appears after a long period of time (more than one year for chlorambucil, and more than 2 or according to some authors 3 years for FCR) and the evaluation before new line of therapy shows that the patient have not acquired a *TP53* abnormality can be expected to respond to a further course of their initial therapy. Although the PFS is usually shorter than after initial therapy and repeated courses often lead to drug resistance. However, re-treatment with the previous therapy is not recommended in patients whose initial treatment was according to today standards sub-optimal or if a new treatment becomes available, showing to be superior to the initial therapy.

Patients relapsing late (at least 2 years after FC, FCR or similar regimens) who have not acquired a *TP53* abnormality, remain fit enough for fludarabine-based treatment and in whom there is a clinical indication for treatment, should receive FCR. Since there are no Phase III studies of patients relapsing after FC or FCR, this recommendation is based on studies that analyzed FCR in comparison to historical control treated with FC. [101]

A non randomised phase II study of bendamustine and rituximab[89] has shown response rates that are inferior, so further studies are required to evaluate the role of bendamustine in combination with an anti-CD20 antibody in fit patients with relapsed disease

Patients relapsing early are considered refractory, and different strategy applies. Step up of therapy may be warranted as described above. As a standard we can recommend combination of alemtuzumab and fludarabine reinduction and and elective allogeneic stem cells transplants. The outcome of fludarabine-refractory patients treated with chemotherapy is generally poor. Regimens that include fludarabine and alemtuzumab have activity in patients refractory to either agent alone but responses are not durable and the risk of infectious complications is high.[101-103]

For those patients belonging to this first-line therapeutic stratum but who were for various reasons treated with chlormbucil or fludarabine monotherapy, a step up seems apropriate to FCR or like, regardless if they relapse late or early. If the patient at the time of relapse changes to unfit stratum, the relapse treatment described in respective section applies.

4.3.3.2 Patients relapsing from the Fit+High risk stratum

Those patients are at particularly high risk. The management of high-risk CLL (both in fit & unfit patients i.e. in patients dealed this and in 4.3.3.4 section) is controversial and poses considerable therapeutic challenges. No Phase III clinical trials were performed in this group of patients. Accordingly, early input from a centre with a specialist interest in CLL or inclusion in a clinical trial is strongly recommended.

If the fit patients are not allo transplanted, a reinduction should be considered with different combination (including ofatumumab) and if successful proceed to transplantation. For patients for whom allogeneic transplantation is not an option, re-treatment with alemtuzumab should be considered in those patients who relapse more than 12 months after initial treatment.[104]

4.3.3.3 Patients relapsing from the Unfit+Low risk stratum

Over 50% of all treated patients belong to this group. In this group less aggressive treatment was applied in front-line due to the fact that those patients are not likely to tolerate FCR. Obtained response is less likely MRD negative and relapses are expected in the wide range from early (less than 1 year) or late relapse.

In case of early relapse patient is considered refractory to given treatment and a change of therapy is suggested. Since the patient is most likely again unfit to tolerate FCR the treatment with a change to treatment of acceptable toxicity is indicated. Based on the Phase two trials B+R is a good candidate for patients treated font-line with CLB+R. In Croatia bendamustin is not at present approved in Croatia in spite of FDA and EMA approval, but this will hopefully be changed soon. Entry into trials which include bendamustine an anti-CD20 antibody is strongly recommended. For patients previously treated with chlorambucil without rituximab, a CLB+R is recommended. For rare patients in this group who improved their general condition FCR should be given, on the basis of good results in REACH study [(105)] or FA.[(103)]

In case of a late relapse the patients relapsing after chlorambucil can be retreated with chlorambucil. The therapy may be repeated several times (in function of achieved duration) until the duration of remission is not shortened to one year, after which it is justified to go to the second line therapy. Other options for patients who are refractory to chlorambucil and unable to tolerate myelosuppressive therapy include high dose steroids, alone or in combination with rituximab, and alemtuzumab.

4.3.3.4 Patients relapsing from the Unfit+High risk stratum

Those patients are generally of very poor risk. The management of high-risk CLL is controversial and poses considerable therapeutic challenges. Accordingly, early input from a centre with a specialist interest in CLL and / or inclusion in clinical trials is strongly recommended.

Also in this group of patients there are no phase III clinical trials performed. We should consider to control the disease with alemtuzumab in combination with pulsed high dose glucocorticoid or alternatively with a therapy that has not been used in the first line.

Treatment options for both fit & unfit patients who fail or relapse early after alemtuzumab-based therapy are limited. Ofatumumab[(106)] is the treatment of choice, other options include lenalidomide[(107)], high-dose steroids with or without rituximab[(108)] or radiotherapy. Steroids given at conventional dose can provide useful short-term disease control and improve CLL-related symptoms. The choice of therapy depends on patient fitness, previous treatment and drug availability.

4.3.4 Consolidation / Maintenance therapy

The observation that an MRD negative remission is associated with prolonged progression free survival both in previously untreated[(109)] and relapsed[(110)] patients. This has lead to studies of additional treatment in patients with residual disease post therapy.

The use of alemtuzumab following initial therapy with fludarabine-based regimens has led to an improved CR rate, MRD eradication and prolonged PFS, but the potential for infective complications necessitates careful attention to the timing of consolidation therapy and to antimicrobial prophylaxis and treatment [(111-113)] For this reason the consolidation and maintenance antibody therapy therapy should only be offered in clinical trials as the clinical benefit versus the risk of morbidity is still uncertain.

Autologous stem cell transplantation has recently being evaluated in Phase III studies that showed that gains of a similar magnitude might have been achieved with chemo-immunotherapy. [(114)] Therefore, autologous stem cell transplantation is not recommended as part of standard care in CLL.

4.3.5 The role of allogeneic transplantation

Allogeneic stem-cell transplantation provides the best opportunity of achieving long term disease-free survival for patients with high-risk CLL, including those with TP53 abnormalities, indicating that 17p deletion loses its adverse prognostic significance in this therapeutic context. [(80)]

A comparison of registry data suggests that reduced intensity conditioning (RIC) transplants may be superior to myeloablative transplants – the reduction in disease control using a reduced intensity approach is more than compensated for by the reduction in TRM. Recent data suggest that the outcomes following transplants from fully matched unrelated donors are identical to those following transplants from sibling donors and will increase the donor pool.[114] Analysis of prospective trials of allografting in CLL suggests that not being in remission has greater adverse prognostic significance than the number of lines of prior therapy. The patients with co-morbidities have significantly inferior outlook for OS, EFS and NRM.[115]

Allogeneic stem-cell transplantation should be considered as consolidation therapy for all fit patients with high-risk CLL and should ideally be performed in the setting of a secure remission. Suitable patients should be discussed with a transplant centre at the earliest opportunity.

Current indications for allogeneic stem cell transplantation inculde: (1) relapse within 6 months of purine analogue therapy; (2) relapse within 24 months of intensive therapy including purine analogue /alkylator combinations, chemoimmunotherapy or autologous transplantation; (3) patients with *TP53* loss / mutation ideally after maximal response to *TP53* independent therapy; (4) Patients not fulfilling the above criteria who are in second or subsequent relapse with at least one other commonly recognized adverse feature as follows: (a) bone marrow failure according to Binet criteria; (b) unmutated *IGHV* genes, high expression of ZAP70 or CD38; (c) deletion of 11q.

4.3.6 The role of radiotherapy

Radiotherapy can provide effective palliation in cases with symptomatic bulky lymphadenopathy and should be offered to patients for whom chemo-immunotherapy has been ineffective or is contra-indicated. Low doses of external beam radiotherapy (2 x 2Gy) can be highly effective in this situation and a higher dose (30 Gy in 2-3 Gy fractions) may be required in patients with transformed aggressive disease or those known to have a *TP53* abnormality. [116]

4.3.7 Management of lymphomatous transformation

The diagnosis of lymphomatous transformation requires histological confirmation. Depending on the histological sub type of lymphomatous transformation, patients who are suitable for intensive therapy should receive regimens currently employed for either primary diffuse large B cell lymphoma (Richter syndrome) or Hodgkin's lymphoma. Younger patients who achieve a good response are candidates for allogeneic stem cell transplantation.

There have been no randomized trials on the treatment of aggressive lymphomas developing in CLL. The outcome of CLL patients with lymphomatous transformation is significantly poorer than that of patients presenting with de-novo lymphomas with a similar histology.[117] The overall response rate is even for those receiving chemotherapy and rituximab is less than 50% with the median survival of 8 months. Of the patients who achieved a remission, those who underwent allogeneic stem cell transplantation had a longer survival than those receiving no additional therapy or those who underwent allogeneic or autologous transplantation for relapsed or refractory disease.[118] In a separate analysis of 18 patients who developed Hodgkin's lymphoma the overall response rate to "Hodgkin like"chemotherapy was 44**%** with median overall survival of 9 months.[119] More recently an overall response rate of 50% was achieved in 20 patients with lymphomatous transformation treated with a combination of Oxaliplatin, fludarabine, cytarabine and rituximab. The median response duration was 10 months.[100] Options are limited for patients unable to tolerate intensive therapy but palliation might be achieved using a high dose steroid regimen.

4.3.8 Treatment of SLL

The biological similarities between SLL and CLL are so close that a similar response to treatment could be expected. This is supported by MDACC single centre retrospective study.[120] Indications for, and choices of treatment are the same as for CLL. Rare patients in whom SLL is diagnosed following biopsy of an enlarged lymph node in the absence of detectable disease at any other site, may be offered local radiotherapy with curative intent.

4.4 Autoimmune complications in CLL

Autoimmune complications are common in CLL occurring in 10-20% of patients.(121) These almost exclusively target blood cells, most commonly red blood cells. The haemolytic anaemia (AIHA) is predominant and immune thrombocytopenia (ITP) is 4-5 times less common. A bone marrow aspirate is usually required to confirm the diagnosis of autoimmune cytopenia.

AIHA or ITP should be treated before deciding whether therapy for CLL is needed. First line therapy is prednisolone. Second line therapies for patients intolerant of or refractory to steroids, include cyclosporine, intravenous immunoglobulin (ITP), thrombopoietin mimetic agents (ITP), low-dose cyclophosphamide, rituximab, alemtuzumab and splenectomy. CLL treatment may be initiated to control recurrent or refractory AIHA/ITP. Rituximab –containing regimens are recommended in patients who do not have a *TP53* abnormality. If AIHA/ITP develops during CLL treatment the same regimen should only be used again in that patient with extreme caution and if no effective alternative is available. Autoimmune neutropenia usually responds to GCSF.

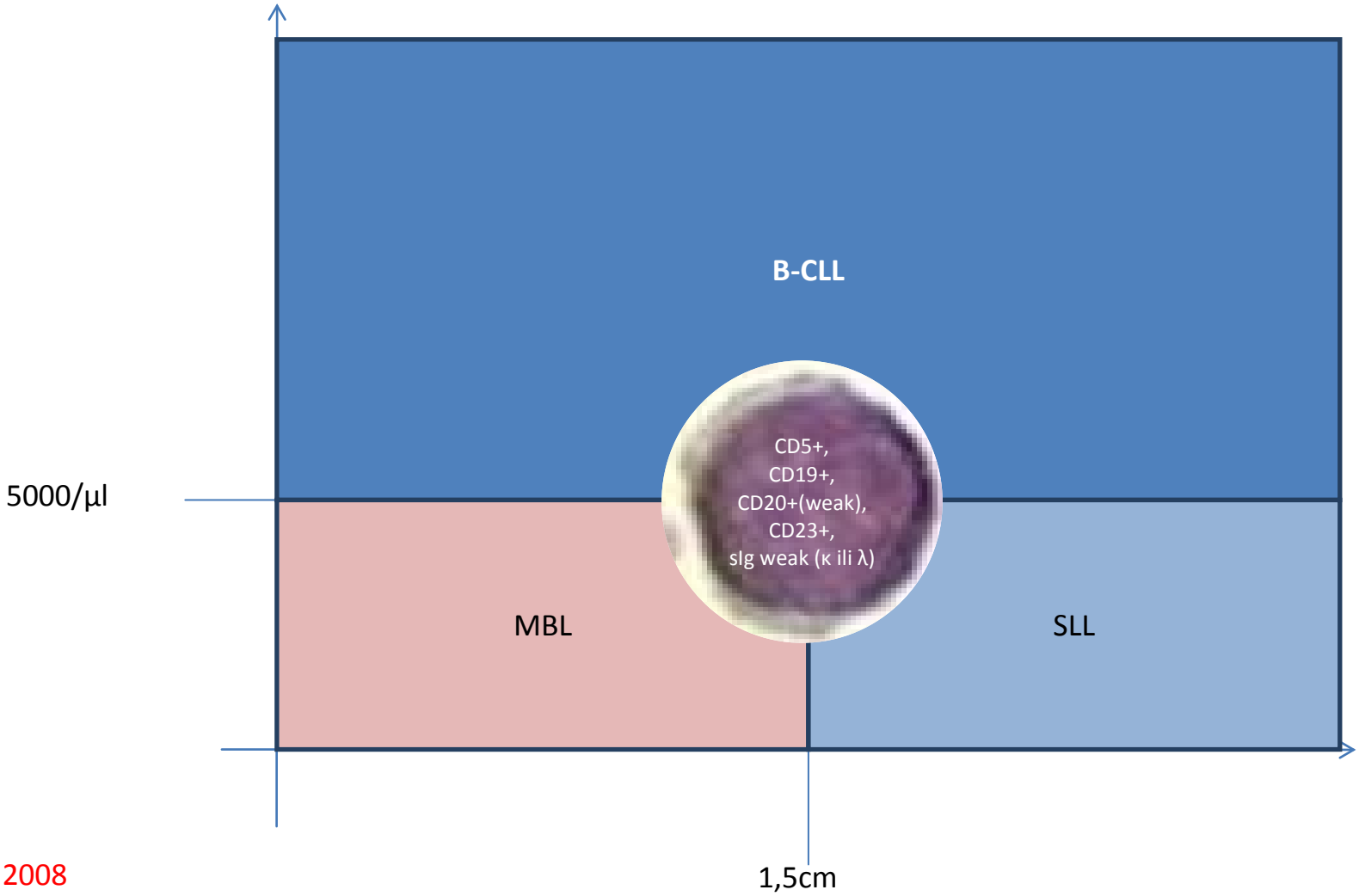
4.5 Supportive therapy

This part of the guidelines will be taken from the protocols and guidelines developed by the KROHEM Working Group's for supportive therapy in hematology.

Table 14 shows the basic characteristics of supportive therapy in B-CLL. This covers the area of vaccination, anti-infective prophylaxis, respiratory recurrent infections requiring IV antibiotics and hospitalization, immunoglobulin replacement therapy and blood transfusions.

5 Figures and tables

5.1 Figure 1 Classification of B-CLL typical phenotype entities: B chronic lymphocytic leukemia; MBL: monoclonal B lymphocytosis; SLL: small lymphocytic lymphoma, the ordinate shows the absolute number of clonal cells typical phenotype



5.2 Table 1 Differential diagnosis of leukemic chronic lymphoproliferative disease entities

WHO Classification	Entity	% incidence of total leukemic number (>5x 10^9/L)	% leukemic (>5 x 10^9/L) of total entity number	% lymphoma of total entity number	Median survival (months)	Main clinical features of each entity
B-neoplasms (about 94%)	CLL / SLL	80	90	10	>72	Progressive infiltration of small lymphocytes in BM, PB, LN, spleen and other organs
	FL	5	5	95	96-144	Leukemic indolent folicullar lymphoma
	MCL	<2	20	80	36-60	Leukemic mantle cell lymphoma
	SLMZ / SLV	<2	50	50	120	Leukemic splenic B-cell marginal zone lymphoma; leukemic lymphocytes may have vilous shoots
	MALT	<1	10	90	120	Leukemic lymphoma associated with mucose lymphatic tissue (gastrointestinal or respiratory system)
	PLL	<2	100	0	36	Hyperleukocytosis, marked splenomegaly, an aggressive course
	HCL	<2*	100*	0	>80	Pancytopenia, splenomegaly, reticulin marrow fibrosis, a favorable response to splenectomy
T-neoplasms (about 6%)	PLL	<4	95	5	6-12	Frequent skin infiltration, hepatospleno-megaly, CNS infiltration, an aggressive course
	TLLA(HTLV-1+)	<1	30	70	5-13	Skin Infiltration, organomegaly, hypercalcemia, presence of HTLV-1 virus, an aggressive course
	Sezary / Mycosis fungoides	<1	5	95	>120	Leukemic form characterized by the finding of typical Sezary cells; cutaneous form Mycosis fungoides is much more common
	VGL	<1	100	0	60	BM & PB infiltration with large granular lymphocytes (LGL); granulocytopenia
	TOTAL	100				

Legenda: CLL – chronic lymphocytic leukema; SLL – small lymphocytic lymphoma; FL – follicular lymphoma; MCL – mante cell lymphoma: SLMZ / SLV – splenic marginal zone lymphoma / splenic vilous cells lymphoma; PLL – prolymphocytic leukemia; HCL – hairy cell lekemia; ATLL – Adult T-cell leukemia/lymphoma; LGL – leukemia of granular lymphocytes; BM – bone marrow; PB – peripheral blood; LN – lymph node; LGL – large granular lymphocytes; * quantitative exception for leukemia diagnosis.

5.3 Table 2 Steps and aims in diagnostic process that lead to definition of therapeutic goal and strategy

	Steps and aims	Basis for decision	Criteria	Targeted categories of classification
1	Disease detection; Patient – hematologist contact	• Lymphocytosis (70-80%) and/or • Lymph nodes/spleen enlargement (20-30%)	• PE and hematology lab	
2	Diagnosis and differential	• B-cell clone of typical B-CLL phenotype (in PB and/or BM and/or LN) • Other lymphoproliferative disease with lymphocytosis	• Morphology + characteristic immuno-phenoptype with quantifications • Difference in phenotype and/or morphology	• CLL (72%) • SLL (8%) • MBL • B (14%): FL, SLMZ/SLML, MCL, PLL, MALT, TL • T (6%): PLL, TLLO, Sezary, LGL
3	Disease extent assessment	• Clinical and lab (hematology) assessment	• Rai, • Binet, • TTM	• 0, I, II, III, IV • A, B, C • Continuous parameter of the size and distribution of tumor mass
4	Classification in prognostic groups apriori	• PFs (apriori) @ dg	• Prognostic markers (CD38 and ZAP), FISH(4), Prognostic indices, mutation status of IgVH genes	• Good prognosis / • Bad prognosis
5	Indications for therapy	• Quantitative treshold criteria • Dynamic criteria • Qualitative criteria	• Anemia, thrombocytopeina, organomegaly, lymphocytosis • Trend of tumor load increase (DT), progressive cytopenias, • Presence of symptoms	• Advanced stsge vs early disease • Rapidly progressive vs stable, slowly progressive disease • Symptomatic vs asymtomatic *Early, stable, asymptomatic disease = observation only* *Any other = indication for therapy*
6	Immediate pre-treatment evaluation and comorbidity & general condition assessment	• Clinical and laboratory assessment	• Updating and completing work-up • CIRS	• Fit / • unfit
7	Individualiztion of therapy Therapy aim and strategy definition	• B-CLL related factors • Patient related factors	• Difference in advancement and progressiveness, p53 • General condition and co-morbidity	*Stratification (2x2):* • Without Del (17p) vs Del(17p) • Fit / unfit (frail) *Aim and strategy (in interactioiwiths 4 strata):* • Eradication or • Disease control

Comment: table shows diagnostic steps. Steps 1-4 are made in single visit. Each step is different with respect to aim, decision criteria as well as the extent of work-up. Last column describes proposed classification categories. Only about 1/3 of paients have indication for therapy at diagnosis. Others are followed-up repeatedly until criteria for therapy are reached (step 5). This period may vary in another third of all patients (a half of those followed) while in remaining third of all patients the criteria are never reached. The scope of work-up is different in each step (see Table 8), pre-treatment being the most complete aiming to provide all of the necessary elements for patients stratification and definition of therapeutic goal. The overall goal of diagnostic process is to enable individualization of therapy, definition of therapeutic aim and strategy, by implementing general principles on individual case.

5.4 Table 3 Differential diagnosis of clonal B lymphoproliferative diseases based on immunophenotype

Markers	Application	B-CLL (80-90%)	Other lymphoproliferative diseases with lymphocytosis (10-20%)				
Disease:		CLL/SLL	PLL	HCL	MZL	FL	MCL
Clonality (κ/λ)	D,M	+	+	+	+	+	+
CD19	D,M	++	++	+++	++	++	+++
CD20	D,M	weak	+++	+++	++	++	++
CD5	D,M	++	-/+	-	-/+	-	++
CD23	D	++	-/+	-	-/+	-	-
CD10	D	-	-/+	-	-/+	++	-
sIg	D	weak	+++	+++	++	++	++
CD79b	M	-	++	++	++	++	++
Dodatni	D		CD11c,CD25,				
	D,M		CD103				Cyclin D1
	P	CD38, ZAP-70					

Legend: CLL – chronic lymnphocytic leukemia; SLL – small lymphocyte lymphoma; PLL – prolymphocytic leukema; HLC - hairy cell leukemia; MZL – marginal zone lymphoma; MZL – limfom marginalne zone; FL – follicular lymphoma; MCL – mantle cell lymphoma.
Application: D – diagnostic; M – MRD (minimal residual disease); P - prognosis

5.5 Table 4 Clinical stages according to Rai (Rai KR et al. Blood 1975,46(2):219-234.)

STAGE	DESCRIPTION	RISK
0	Lymphocytosis, limfociti u perifernoj krvi > 15 x 10^9/L and > 40% lymphocytes in bone marrow	Low
I	Stage 0 with enlarged lymph node (nodes)	Intermediate
II	Stage 0-I with splenomegaly, hepatomegaly or both	Intermediate
III*	Stadij 0-II with hemoglobin < 110 g/L ili hematocrit < 0.33	High
IV*	Stadij 0-III with platelets < 100 x 10^9/L	High

*immune cytopenias do not fit in this stage definition

5.6 Table 5 Clinical stages according to Binet (Binet JL et al. Cancer 1981;48(1):198-206.)

STGE	DESCRIPTION	RISK
A	Hemoglobin ≥ 100 g/L and platelets ≥ 100 x 10^9/L and < 3 involved regions**	Low
B	Hemoglobin ≥ 100g/L and platelets ≥ 100 x 10^9/L and ≥ 3 involved regions	Intermediate
C*	Hemoglobin < 100g/L and/or platelets < 100 x 10^9/L and any number of involoved regions	High

*immune cytopenias do not fit in this stage definition

**The five lymphoid areas comprise: uni or bilateral cervical, axillary and inguinal lymphoid, hepatomegaly and splenomegaly

5.7 Table 6 Assesment of the tumor mass size: TTM-score (Jakšić B, Vitale B. Br J Haematol. 1981 Nov;49(3):405-13.)

COMPARTMENT	REPRESENTATIVE	SIZE
TM_1 – bone marrow and peripheral blood	Lymphocyte count (peripheral blood)	$\sqrt{\lvert ly \rvert}\ x\ 10^9/\mathrm{L}$
TM_2 - lymphn nodes	Diameter of largest palpable node	cm
TM_3 - spleen and liver	palpable spleen(below left costal margin)	cm
TTM :		$TM_1+TM_2+TM_3$

Legend: |ly| - absolute number of lymphocytes; TTM – Total Tumor Mass score

Tumor Mass Distribution (TD) is calculated as quantitative parameter according to formula: $TD = \frac{TM_1}{TTM}$.

Doubling Time of TTM (DT) is calculated as quantitative parameter according to formula: $DT = \frac{M\ x\ TTM_{beg}}{TTM_{end} - TTM_{beg}}$

where TTM_{beg} is size of TTM at the beginig of period M, TTM_{end} is TTM size at the edne of period M M, M is interval between TTM_{beg} and TTM_{end} in months.
M should be at lease 3 months.

Electronic calculator i available online at http://www.krohem.hr

5.8 Table 7 Criteria for active (progressive / symptomatic) disease

	Criterion	Treshold	Dynamics	Qualitative	Comment
1	Hemoglobin	< 100 g/L	Trend (worsening)		The level of anemia that is used in determining clinical stages according to Rai and Binet.
2	Patelets	< 100 x 10^9/L	Trend (worsening)		The level of thrombocytopenia that is used in determining clinical stages according to Rai and Binet.
3	High tumor mass	TTM > 15	TTM DT < 12 mj		Below 9 is not a sufficient criterion, between 9 and 15 is a "gray zone", above 15 is an indication present. Lymphocyte count in itself is not a sufficient criterion, except in extreme cases (TTM > 15 = Lymphocytes > 225x10^9 / L)
4	Massive splenomegaly	> 6 cm below LCM or US > 20 cm	(progressive)	Pain	Usually they are combined, but not necessarily. Dynamic parameters involved in TTM
5	Massive lymph nodes	> 10 cm	(progressive)	Pain	Usually they are combined, but not necessarily. Dynamic parameters involved in TTM
6	The threat of organ function			Clinical judgement	For example, compressive symptoms
7	B simptoms defined as any one or more of the disease-related symptoms or signs:	• Unintentional weight loss >10% /6 months; or • Sifnificant fatigue (ECOG PS 2 or worse); or • Fever >38°C for 2or more weeks without evidence of infection; or • night sweats for more than 1 month without evidence of infection			Usually they are combined, but not necessarily. The proposed system has long been used, particularly in lymphomas, and is well validated. The presence of B-symptoms is important and indisputable element of therapeutic indications. It suffices that one is present, but there may be several present simultaneously.
8	Autoimmune anemia or thrombocytopena			Poorly respon-sive to standard therapy	Standard therapy does not imply anticancer drugs, but includes corticoids

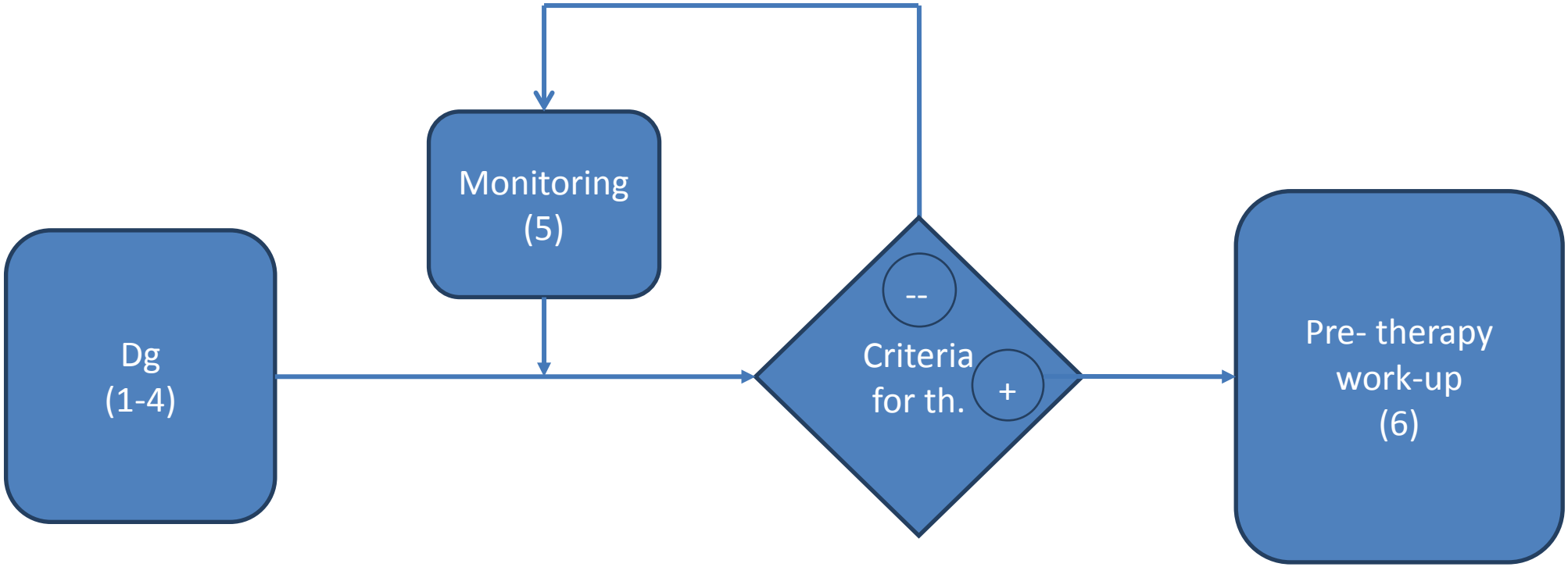
Hypogammaglobulinemia, monoclonal or oligoclonal paraproteinemia, or absolute lymphocyte count, do not in themselves constitute an indication for therapy. It is out of 8 groups of criteria theoretically possible to identify 11 individual indications based on exceeding a threshold, 3 dynamic evaluation of continuous quantitative parameters, where individual trends can be compared and thereby gain additional derived criteria, and 4 qualitative assessments. Although in principle sufficient presence of at least one indication, we should avoid making decisions on an isolated indication. It is clear that a larger number of indications further reinforces the decision to begin treatment. It is possible to decide that the patient needs to document the presence of at least two or more of the above indications for active (progressive / symptomatic) disease. The indication for treatment (according to KROHEM guidance) should be documented in patient records!

5.9 Table 8 General schedule of investigations before, during and after therapy

		1-4	5*	6	7	8	(5'→6') (7',8')
		@ diagnosis	Monitoring visits to meet criteria for treatment	Pre-treatment work-up	First line of treatment follow-up	EOT evaluation	Before new line of treatment; then repeat 7' and 8'
1	History	+	+	+	+	+	+
2	PE	+	+	+		+	+
3	Hematology	+	+	+	+	+	+
4	Stage	+	+	+			
5	TTM	+	+	+	+	+	+
6	Cytology	+	+	+	+	+	+
7	Flow Cytometry	+		+		+	+
8	FISH CLL(4)PB/BM	+		+			
9	Histology	+[b]		+[b]			+[b]
10	Biochemistry	+	+	+	+	+	+
11	Urine	+		+		+	+
12	Imaging	+	+	+		+	+
13	Cardiology	+		+			+
14	Infectology			+			+
15	Other clin ind	+	+	+	+	+	+
16	CIRS	+		+			+
17	Research	+	+	+	+	+	+

* It is estimated that in 1/3 of patients indications for treatment is present immediately upon diagnosis, so in one episode stages 1-6 are completed. In two thirds of patients after diagnosis no indication for therapy is present, so they are just monitored without therapy. Half of them will never require specific therapy, whereas in the other half in due time (from a few months to several years, and even decades) the indications for therapy appears. Monitoring protocol frequency varies depending on the clinical condition from several weeks to several months, or even one year if the situation is stable, without change. However, in the emergence of new circumstances, it is necessary to check- up early. In any case, the phases 1-4 are done in the same episode, and the transition to the next phase depends on the time required for the appearance of indications for therapy; [b]preferred, but not required tests;

1.1 Figure 2 Different volume of work-up, depending on the stage of the diagnostic procedure. At the begining, after suspicion (1); it is mandatory to make tests to confirm the diagnosis and differential dg. (2); to evaluate the extent of the disease (3); and assess the prognostic group (4). If no criteria for therapy are met, patients are monitored by simple indicators (5) to meet the criteria for therapy, when it comes to comprehensive pretherapeutical processing (6) which form the basis for the decision on therapeutic strategy. (See Table 8 and Table 9)



5.10 Table 9 Detailed schedule of investigations before, during and after therapy finition of response to treatment

No		1	2	3	4	5*	6	7	8	(5'→6') (7',8')
		Detection *(%) = expected incidence*	Diagn. - Diff.dg: • *within CLL phenotype* • *other entitetes*	Extent / stage	Prognostic groups	Indication for therapy	Comprehensive pre-treatment (base line) evaluation	First line therapy	EOT evaluation	Before new line of th (6'); then repeate 7' & 8'
	Points of reference:	• Lymphocytosis (70-80%) • Enlarged node/spleen (20-30%)	• Morph. & • Immunophenotyp; Quantif. Clon. Ly & organomegalies: • >5= CLL (>90%) • <5+LN>1.5) = SLL (<10%) • <5=MBL	Rai, Binet TTM	• Mutat. Status Surogats (CD38, ZAP); • FISH (4); • Progn index;	(a) Quantitative, „treshold"; (b) Dynamic, monitoring (c) Qualitative;	Clin & Lab investigation; CIRS, CCI; GoGo vs SlowGo	• Standard, • Alternative, • Experimental	Response: • Clinical, • Hematol, • Immunol, • MRD	• Standard, • Alternative, • Experimental
	Note:			*Data derived from (2)*	*Supplement the basic routine tests*	*If there is not enaugh (a) or (c), systematic moni-toring is needed*	*When the indication is present (5)*	*Follow-up :short panel, evaluate tolerance*	*Precisely document response*	*Repeate (6') then follow-up and re-evaluation (7' & 8')*
1	Anamnesis									
1.1.	History	+				+	+	+	+	+
1.2.	Symptoms	+				+	+	+	+	+
1.3.	PS (ECOG)	+				+	+	+	+	+
2	Status (PE)									
2.1.	General		+			+	+		+	+
2.2.	organomegaly	+	+			+	+	+	+	+
2.3.	Other		+			+	+	+	+	+
3	Hematol									
3.1.	WBC	+	+			+	+	+	+	+
3.2.	Differential	+	+			+	+	+	+	+
3.3.	RBC		+			+	+	+	+	+
3.4.	Platelets		+			+	+	+	+	+
3.5.	Rtc		+			+	+	+	+	+
3.6.	ESR		+			+	+		+	+
3.7.	Coombs				+		+			+
4	Stage									
4.1.	Rai			+						
4.2.	Binet			+			+			
5	TTM			+		+	+	+	+	+
6	Cytology									
6.1.	PB		+			+	+	+	+	+
6.2.	BM		+[a]		+		+		+	+[a]
6.3.	LN		+[a]				+[a]			+[a]
7	Flow Cytom									
7.1.	PB-CLL[basic]		+							
7.2.	PB/BM[CD38,ZAP70]				+		+			
7.3.	CLL[ext(LN/KS)]				+[b]		+[b]		+[b]	
7.4.	PB/BM[MRD]								+	

No		1	2	3	4	5*	6	7	8	(5'→6') (7',8')
8	FISH C$^{LL(4)PB/BM}$				+		+			+
9	Histology									
9.1.	BM				+		+		+	+
9.2.	LN		+[a]				+^{Richter}			+[a]
10	Biochemistry									
10.1.	CMP				+	+	+	+	+	+
10.2.	Ser Elforeza				+	+	+		+	+
10.3.	immunoglobuni				+	+	+		+	+
10.4.	B-2-MG				+	+	+		+	+
10.5.	CRP				+	+	+		+	+
10.6.	LDH				+	+	+		+	+
11	Urine				+		+		+	+
12	Imaging									
12.1.	Chest X-ray				+					+[a]
12.2.	US LN regions		+			+[e]	+		+	+[a]
12.3.	US Abdomen		+			+	+		+	+[a]
12.3.	CT/MRI $^{abd+tor}$		+[d]				+[b]		+[b]	
12.4.	PET-CT						+^{Richter}			+^{Richter}
13	Cardiology									
13.1.	EKG				+		+			+
13.2.	Echo card						+			+[a]
14	Infectology									
14.1.	HBSAg						+			
14.2.	HBCAg						+			
14.3.	HBV-PCR						+[c]			
14.4.	HCV						+			
14.5.	CMV						+			
14.6.	EBV						+			
14.7.	HIV						+			
15	Other$^{clin\ ind}$									
15.1.	Clin ind[x]				+	+	+	+	+	+
16	CIRS				+		+			+
17	Research									
17.1.	New 1,2..				+	+	+	+	+	+

[a]if the other steps do not lead to the desired result, [b]preferred but not mandatory investigations [c]if proven HBAg, it is necessary to quantify the number of copies, [d]to distinguish MBL from LML, [e]according to clinical judgment.

5.11 Table 10 Definition of response to treatment (NCI updated guidelines, Blood 2008)

	Parameter	CR[1]	PR[1]	PD[1]
NCI / IWCLL	Group A			
	Lymphadenopaty [2]	There is not one > 1.5 cm	Decrease ≥ 50%	Increase ≥ 50%
	Hepatomegaly	No	Decrease ≥ 50%	Increase ≥ 50%
	Splenomegaly	No	Decrease ≥ 50%	Increase ≥ 50%
	Lymphocytosis in blood	< 4 x 10^9/L	Decrease ≥ 50% from baseline	Increase ≥ 50% from baseline
	Bone marrow[3]	Normocellular, < 30% lymphocytes, No B-lymphoid nodules. Hypocellular marrow defines CRi.	50% reduction of marrow infiltration or B lymfoid nodules.	
	Group B			
	Platelets	>100 x 10^9/L	>100 x 10^9/L or increase ≥ 50% from baseline	Reduction of ≥ 50% from baseline as a result of CLL
	Hemoglobin	>110 g/L	>110 g/L or increase ≥ 50% from baseline	Reduction of > 2 g/dL from baseline as a result of CLL
	Neutrophils[3]	>1.5 x 10^9/L	>1.5 x10^9/L or > 50% improvement from baseline	
TTM* (EORTC / IGCI / KROHEM)	TTM	TTM <2 (lymphocitosis <4x10^9/l, no lympdadenopaty, no organomegaly)	TTM decrease ≥ 50% and TTM<9	TTM increase ≥25%
	BM function	Platelets > 100 x 10^9/L Hemoglobin >110g/l, Neutrophils >1,5x10^9/l	– no BM failure – BM failure	

Group A criteria define tumor mass, group B criteria define hematopoetic system (or bone marrow) function.

[1] CR (complete remission): all criteria must be present, and patients must be without general symptoms associated with CLL; PR (partial remission): at least two criteria in group A plus one in group B must be present; SD (stable disease) is the absence of progressive disease (PD) if at least PR is not reached; PD (progressive disease): at least one criterion from gropus A or B must be present.

[2] The sum of the products of multiple lymph nodes (as evaluated by means of CT in clinical trials or physical examination in general practice).

[3] These parameters are irrelevant for certain types of responses.

* To monitor the dynamics of the disease (both progression and responsel to therapy) **TTM score** (described in the clinical stage) **is very convenient**, because is the only clinical system that is based on continuous, quantitative parameter easy to apply, validated in thousands of patients in various international clinical trials. To estimate the doubling time (DT) is more reliable than just the number of lymphocytes, because it can compensate for changes in the distribution of the occurrence of the tumor mass as after administration of corticosteroids or TKIs, when it may be an increase in the number of leukocytes while reducing nodes or spleen. This point is recently brought up and discussed in the literature poiniting out that NCI based criteria are inadequate for monitoring TKI therapy, stressing the need for different approach. (lit…) When assessing the response to therapy the complete remission (CR) is assessed equally in TTM and NCI based criteria, but TTM shows the advantage in assessing partial remission (PR) by comparing the total tumor mass before and after treatment, so it is possible to set a minimum threshold for minimal remission (MR), eg reduction of > 25%, partial remission (PR) > 50%, very good PR > 75% and more. Likewise, TTM based criteria are more accurate and without bias estimate the stable disease (SD) and progressive disease (PD). Continuous quantitative character of TTM size allows comparison of trends between the group criterion A (TTM) and group B (function residual normal hematopoiesis). It is possible to evaluate the beneficial antineoplatic effect of the therapy independently of toxic effect on hematopoiesis.

5.12 Table 11 Proposed algorithm summary for first-and second-line CLL therapy (modified from Hallek, ASH 2009; iwCLL 2011, NCCN 2012; BCSH 2012; Croatian experience)

Stage	General condition	Molecular cytogenetics	First line of treatment		
			Standard	Alternative*	Not registred in Croatia
Asymptomatic ; Binet:A-B ; Rai 0-II; TTM<9 (15)	Irrelevant	Irrelevant	Nothing	Only in clinical trial: to treat high-risk patients	
Binet C, Rai III-IV; TTM>15; Or symptomatic disease (regardless of stage)	Fit	No del(17p)	FCR, CLB±R	FR, FA, FCA, CLB±Pred	BR, PCR, B, combinations with new experimental drugs **
		Del(17p)	FCR, A or FA or HDMP+R →alloSCT (elective)	CHOP+R, HyperCVAD, HDDex or CFAR	B, combinations with new experimental drugs
	Unfit	No del(17p)	CLB±R	Lower dose F or FA or FCR, CVP+R,	CLB+GA101, B, combinations with new experimental drugs
		Del(17p)	A or HDMP+R or HD R	CLB+R, HDDex, CFAR, OFAR,	B, combinations with new experimental drugs

Relapse	General condition	Molecular cytogenetics	Second line of treatment liječenja		
			Standard	Alternative	Not registred in Croatia
Early (< 1 year) = refractory disease	Fit	Irrelevant	A or FA or FCR→alloSCT	CHOP+R, HypeCVAD, EPOCH+R, OFAR, Ofa	BR, Fla, Len, combinations with new experimental drugs
	Unfit	Irrelevant	Change therapy, if possible include in clinical trial	CLB+R, lower dose F or FA or FCR, CVP+R, Ofa, CFAR, OFAR, CLB+R;for del 17p: A, HDDex, HDMP+R	BR, B, Len, combinations with new experimental drugs
Late (> 1 year)	Fit & Unfit		Repeat first line		

* Partially tested in Phase III trials;

CLB = chlorambucil; F = fludarabine; C = cyclophosphamide; A = alemtuzumab; R = rituximab; B = bendamustin; Allo SCT = allogeneic stem cell transplantation, P = pentostatin, GA101 = new anti CD20 antibody, Fla. = flavopiridol, Len = lenalidomid ; HyperCVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone); HDMP (high dose methylprednisolone); HDDex, (high-dose dexamethasone); EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin); CFAR (cyclophosphamide, fludarabine, alemtuzumab, rituximab); OFAR (oxaliplatin, fludarabine, cytarabine, rituximab); Ofa = ofatumumab; CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone); CVP (cyclophosphamide, vincristine, prednisone); ** Newer experimental drugs include thyrosin kinase inhibitors, new monoclonal antibodies, Bcl-2 inhibitors and the like.

5.13 Table 12 Proposed algorithm for first-line CLL therapy in 4 therapeutic strata, with estimate proportion of patients in each stratum (modified from Hallek, ASH 2009; iwCLL 2011, NCCN 2012; BCSH 2012; Croatian experience).

Stage %	General condition %	Molecular cytogenetics %	First line of treatment	
			Standard %	Alternative **
Asymptomatic ; Binet:A-B ; Rai 0-II; TTM<9 (15) 33	Irrelevant	Irrelevant	Nothing	
Binet C, Rai III-IV; TTM>15; or symptomatic disease (regardless of stage) 66	Fit 40	No del(17p) 36	FCR 33 CLB±R 3	FR, FA, FCA, CLB±Pred
		Del(17p) 4	FCR 1 ↘ A 1 → alloSCT FA 1 ↗ (elective) HDMP+R 1 ↗	CHOP+R, HyperCVAD, HDDex or CFAR
	Unfit 60	No del(17p) 54	CLB±R 50 B*±R 4	Lower dose F or FA or FCR, CVP+R,
		Del(17p) 6	A 2 HDMP+R 2 HD R 2	CLB+R, HDDex, CFAR, OFAR,

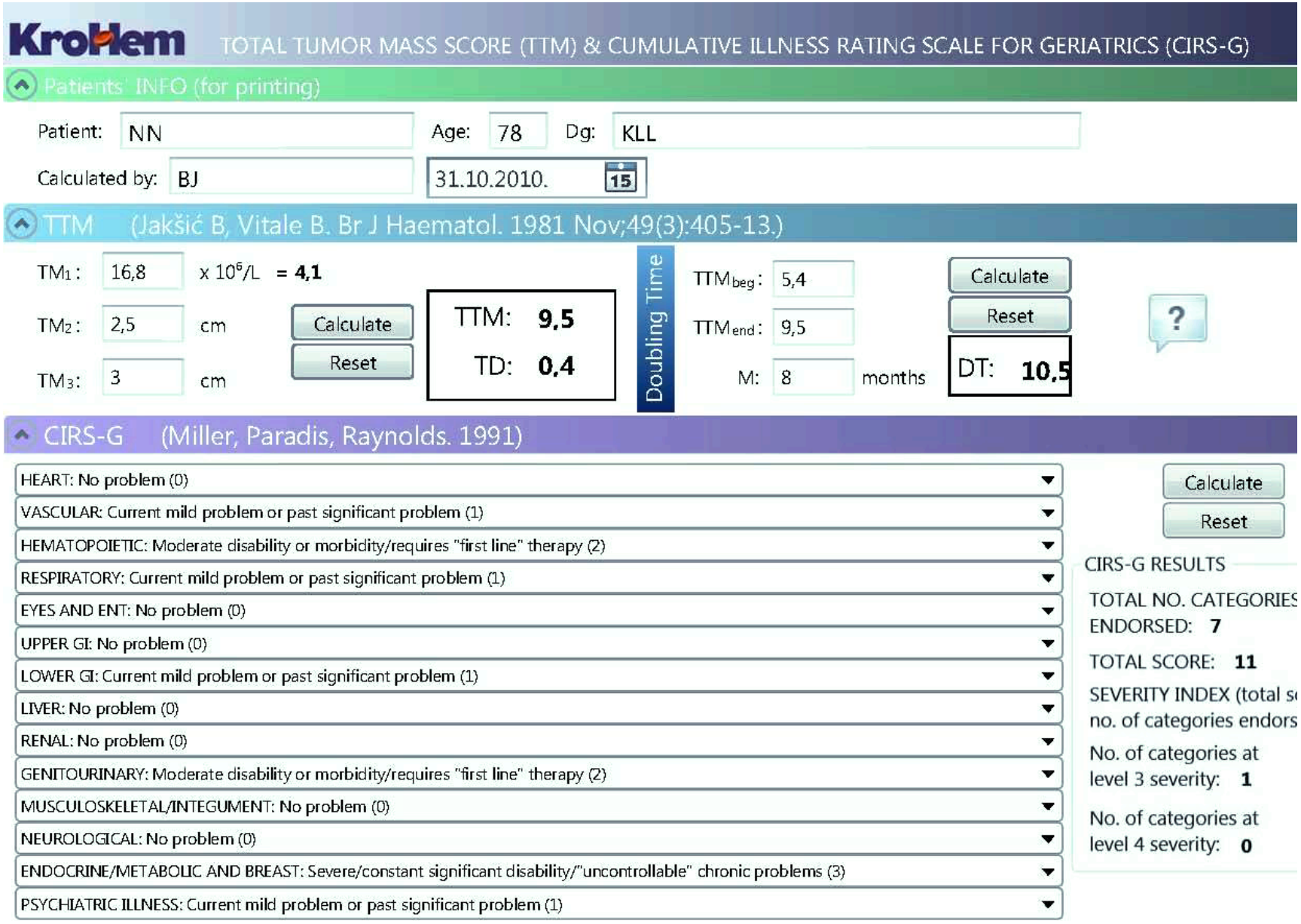
** Partially tested in Phase III trials;

CLB = chlorambucil; F = fludarabine; C = cyclophosphamide; A = alemtuzumab; R = rituximab; B = bendamustin (*at present not approved in Croatia); Allo SCT = allogeneic stem cell transplantation; HyperCVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone); HDMP (high dose methylprednisolone); HDDex, (high-dose dexamethasone); EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin); CFAR (cyclophosphamide, fludarabine, alemtuzumab, rituximab); OFAR (oxaliplatin, fludarabine, cytarabine, rituximab); Ofa = ofatumumab; CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone); CVP (cyclophosphamide, vincristine, prednisone);

5.14 Table 14 Supportive therapy in patients with B-CLL

	Problem	Recommendations
1	Vaccination	• Annual vaccination against influenza. Care must be taken of the fact that the recovery of B-cell system after anti CD20 antibody therapy lasts about 9 months so that the response to vaccination in this period is inadequate. • Pneumococcal vaccine every 5 years • Avoid all live vaccines, including Zoster
2	Antiinfective prophylaxis	• For patients receiving purine analogues and / or alemtuzumab, and in period after that following prophylaxis is recommended: ✓ Herpes viruses (acyclovir) ✓ PCP (sulfamethoxzole/trimethoprim) • Special attention in patients receiving alemtuzumab should be paid to the problem of CMV reactivation. Athough there is no common position in the literature, majority recommend that prophylactic ganciclovir is prescribed if viremia present. The viral load levels must be monitored every few weeks.
3	Respiratory infections requiring IV antibiotics and hospitalization	• Apply appropriate antibiotic therapy. Apply appropriate antibiotic therapy. • Determine serum IgG, and if the value is less than 5 g / L: ✓ Apply monthly IVIG 0.3-0.5 g / kg ✓ Adjust the dose so that the value is maintained above 5 g / L
4	Immunoglobulin replacement therapy	• should be considered as a means of reducing the incidence of bacterial infections in patients with a low serum IgG level who have experienced a previous major or recurrent minor bacterial infections despite optimal anti bacterial prophylaxis. • The goal should be to reduce the incidence of infection and the immunoglobulin dose should be adjusted accordingly. • Patients should be reviewed regularly to evaluate the effectiveness of immunoglobulin replacement therapy and whether there is a continuing need for treatment. • Patients who develop serious and/or recurrent infections despite antimicrobial prophylaxis and immunoglobulin replacement should be managed in conjunction with a microbiologist, infectious diseases specialist and/or immunologist.
5	Blood transfusion	• The use of irradiated blood products should be considered in the following situations: indefinitely in patients treated with a purine analogue, following bendamustine until more evidence emerges about the risk of transfusion-associated graft - versus host disease, following alemtuzumab and for 3 months post conditioning with chemotherapy or immunotherapy (6 months afer total body irradiation) for patients undergoing autologous transplantation.

5.15 Figure 3 Examples of electronic calculator web interface to determine the size of the tumor mass, distribution and growth dynamics with the electronic calculator for CIRS-G.



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