Management of chronic lymphocytic leukemia: practice guidelines from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation

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Objectives. The Italian Society of Hematology (SIE) and two affiliate societies (SIES and GITMO) commissioned a project to develop clinical practice guidelines for the treatment of chronic lymphocytic leukemia (CLL).

Methods. Key questions in the management of patients with CLL were formulated by an Advisory Committee and approved by an Expert Panel of eight senior hematologists. After a systematic review of the literature, recommendations for disease-specific and supportive therapies were formulated and graded according to the supporting evidence. Explicit consensus methods were used for providing recommendations for questions with incomplete or potentially biased evidence.

Results. It is recommended that therapy is commenced in patients with CLL when at least one of the following are present: B-symptoms, progressive/obstructive lymphadenopathy or organomegaly, rapid lymphocyte doubling time, anemia or thrombocytopenia (of new onset, worsening or steroid-resistant). It is recommended that patients without co-morbidity should receive fludarabine plus cyclophosphamide, whereas elderly patients with co-morbidity should receive oral chlorambucil. Younger patients with unfavorable biological risk factors should be considered for high-dose chemotherapy and autologous or allogeneic stem cell transplantation within approved clinical trials. Patients either relapsing rapidly after, or non-responsive to, first-line chlorambucil should receive fludarabine-containing regimens. Patients either relapsing soon after or not responding to fludarabine-based chemotherapy should be considered for schedules including non-cross-reactive agents, such as alemtuzumab, possibly followed by high-dose chemotherapy and autologous transplantation in the context of a clinical trial or by allogeneic stem cell transplantation.

Conclusions. We describe the results of a systematic literature review and an explicit approach to consensus techniques which resulted in recommendations for the key therapeutic decisions in patients with CLL.

Key words: chronic lymphocytic leukemia, clinical practice guidelines, systematic review, chemotherapy.

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Chronic lymphocytic leukemia (CLL) is the most common adult leukemia, accounting for 24% of all leukemias. The median survival of patients with CLL ranges from 5 to 10 years, but a trend to a longer survival has become evident in the last years, partially due to earlier diagnosis. Infections and secondary cancers continue to be the principal causes of death in these patients.

New therapeutic strategies and prognostic markers have recently been developed, which may lead to uncertainty and variability in the clinical management of CLL. Therefore, the Italian Society of Hematology (SIE) and two affiliates societies, the Italian Society of Experimental Hematology (SIES) and the Italian Group for Bone Marrow Transplantation (GITMO), commissioned a project to develop clinical practice guidelines for the therapy of CLL. The recommendations were developed through a systematic search of evidence and formulated according to explicit methods for consensus development, after balancing the health benefits and risks. The project was intended to support the clinical practice of hematologists, oncologists and internists who care for patients with CLL.

Design and Methods

Methods

Organization and Design

The organization and design of this project have been reported in a previous paper on guidelines from SIE, SIES and GITMO.[1] The first search of evidence bases was performed in July 2004, but updated literature searches were continued during the project duration. The grading system chosen is the one designed by the Scottish Intercollegiate Guideline Network (SIGN). The draft rec-

ommendations were reviewed by an external panel of two internationally recognized experts in the field and by the presidents of the SIE, SIES and GITMO scientific societies. The present guidelines were developed according to the best quality criteria, and adhere to the AGREE items with a few exceptions. These guidelines are reported according to the Conference on Guideline Standardization checklist. Updating of the present guideline is expected in 2008.

Definitions

The expert panel agreed on the disease defintions to be used in the present guidelines (Table 1).[2,3] CLL stage was defined according to Binet and Rai (Table 2).[4,5] Clinical response was defined according to the revised NCI-WG revised criteria (Table 3).[6]

Results

Indications to start therapy and pre-treatment evaluations

Patients with early stage CLL (Rai 0-I, Binet A) do not experience a survival advantage if they receive chemotherapy with chlorambucil at diagnosis prior to disease progression when compared to deferring treatment until progression. This conclusion is based on a meta-analysis of randomized studies (level 1+),[7] and it is, therefore, recommended that chemotherapy is only initiated for patients with advanced and/or active disease. Ongoing studies are testing the clinical benefit of early treatment with fludarabine-based chemotherapy in a specific population of patients with stage A CLL and poor risk biological features. As well as the traditional clinical parameters, several novel markers have been demonstrated to have prognostic importance, independently of clinical stage.[8] Such markers include the presence of unmutated immunoglobulin genes (IgV_H), the expression of zeta-associated protein of 70 kD (ZAP-70), and the expression of CD38 (level 2+ to 3).[9,10] The identification of recurrent chromosomal abnormalities, as demonstrated by fluorescence *in situ* hybridization (FISH), has been shown to be an important marker providing relevant information on the progression of the disease and response to therapy (level 2+ to 3).[11] The determination of the aforementioned prognostic markers is desirable in all patients but cannot be a mandatory requirement of clinical pratice for a variety of reasons, including the lack of harmonization and standardization of the tests; furthermore, as yet, there is no evidence that altering therapy on the basis of these factors confers an advantage to the patient. The decision to treat a patient should not be taken on the basis of these biological and molecular markers. However these factors are useful in predicting the outcomes for individual patients and may be useful in selected patients prior to commencement of therapy. Therefore, it is recommended

Table 1. Definitions.

- Chronic lymphocytic leukemia: a chronic lymphoproliferative disorder in which a diagnosis should be proven by documenting an absolute lymphocytosis of greater than 5,000 lymphocytes/μL in the peripheral blood. The lymphocytes should appear mature, with less than 55% of atypical cells (eg. prolymphocytes), should express CD5, CD19, CD20, CD23, surface immunoglobulin with light chain restriction, with coexpression of CD5/CD19 or CD20, and with a low density of surface immunoglobulins (sIg).[2]
- Prolymphocytic transformation: CLL with more than 55% prolymphocytes in the peripheral blood or bone marrow.[3]

Table 2. Staging systems for CLL.

System	*Risk level*	*Stage*	*Clinical features at diagnosis*
Rai[4]	Low	0	Blood and marrow lymphocytosis
	Intermediate	I	Lymphocytosis and lymphadenopathy
		II	Lymphocytosis and splenomegaly or hepatomegaly
	High	III	Lymphocytosis and anemia (hemoglobin < 11 g/dL)
		IV	Lymphocytosis and thromboctopenia (platelets < 100,000/μL)
Binet[5]		A	Blood and marrow lymphocytosis and less than three areas of palpable lymphoid involvement
		B	Same with three or more areas of palpable lymphoid involvement
		C	Same plus anemia or thrombocytopenia

Table 3. Categorization of clinical responses of CLL.[6]

	Definition
Complete Response (CR)	All the following criteria need to be verified for at least 2 months: normal physical examination and absence of symptoms AND no infiltrates or nodules at bone marrow biopsy AND less than 30% bone marrow lymphocytes AND lymphocyte count in peripheral blood lower than 4×10^3/μL AND neutrophil count higher than 1.54×10^3/μL AND platelet count higher than 100×10^3/μL AND hemoglobin value higher than 11 g/dL.
Partial Response (PR)	All the following criteria need to be verified for at least 2 months: over 50% decrease in size of nodes, liver and spleen AND neutrophil count higher than 1.5×10^3/μL AND platelet count higher than 100×10^3/μL or improved by at least 50% AND hemoglobin value higher than 11 g/dL
Nodular Partial Response (nPR)	All CR criteria are fullfilled, except for persisting lymphoid nodules at bone marrow biopsy.

that the most informative prognostic markers (FISH and V_H mutational analysis or equivalent) are studied for all patients in clinical trials and in selected patients who are being considered for intensive or targeted therapy.

Bone marrow biopsy and lymph node biopsies are not required for either the diagnosis of typical B-CLL or for prognostic assessment. However, both procedures are recommended in the presence of atypical clinical, morphologic and cytometric features, i.e. suggesting a differential diagnosis. Bone marrow and lymph node biopsies are also recommended during the follow-up to exclude histological transformation into aggressive non-Hodgkin's lymphoma (see the section "*Therapy for transformed disease*").

Recommendations

The indications for initiation of disease-specific therapy in CLL include the presence of at least one of these features: B symptoms (i.e. fever, sweats, extreme fatigue, or weight loss), progressive enlargement of lymph nodes or hepatosplenomegaly, obstructive adenopathy, development or worsening of thrombocytopenia or anemia, immune hemolysis or thrombocytopenia not responsive to steroids, rapid lymphocyte doubling time (grade B).

Before starting first-line treatment, the physician needs to obtain the following information: peripheral blood count, morphological examination of blood smear (typical or atypical CLL), immunophenotypic analysis (CD5/CD19, CD20, CD5/CD23, sIg κ/λ and CD19/CD38), serum biochemistry (serum lactate dehydrogenase, β_2-microglobulin), direct Coombs' test, and imaging of adenomegalies, assessed either by total body computed tomography or by the combination of chest X-ray and abdomen ultrasound (grade D).

In patients without co-morbidity further biological parameters associated with aggressive disease and independent of the disease stage should be assessed (Table 4). In patients who do not need to start therapy, the monitoring strategy should include physical examination (every 3-6 months), hematologic evaluation including lymphocyte doubling time and biochemistry including serum immunoglobulin levels (every 3-6 months), abdominal ultrasound (every 6-12 months), chest X-ray (when informative at diagnosis). Monitoring of basic immunophenotypic analysis is not strictly necessary (grade D).

First-line therapy

Chlorambucil

Chlorambucil was the standard first-line therapy for CLL until the appearance of purine analogs. It has been suggested that long-term toxicity, such as secondary cancers, might be slightly increased in patients treated with chlorambucil, as compared to patients who do not receive therapy (level 1-). In spite of its use for many decades, there is not a commonly accepted standard dosing schedule for chlorambucil: possible chlorambucil schedules are indicated in Table 5. Comparative, randomized and non-randomized studies showed that higher chlorambucil doses induce a higher response rate and a longer overall

Table 4. Biological parameters associated with aggressive disease.

- Aberrations in chromosomes 11 (11q-) or 17 (17p-)
- Lack of somatic mutations in the expressed immunoglobulin V_H-genes (IgVH unmutated profile)
- Expression of cytoplasmic ZAP-70

Table 5. Currently used therapy schedules.

Fludarabine-based regimens	*References*
F	
fludarabine 25 mg/m²/day iv (or 40 mg/sqm/day orally) for 3 days	12,16,48
fludarabine 25 mg/m²/day iv for 5 days every 4 weeks for 6 cycles	
FND	49
fludarabine 30 mg/m²/day iv for 3 days	
mitoxantrone 10 mg/m²/day iv day 1	
dexamethasone 20 mg orally daily for 5 days	
to be repeated every 28 days for a maximum of 6 cycles	
FN	62
fludarabine 30 mg/m²/day iv for 3 days	
mitoxantrone 10 mg/m²/day iv day 1	
to be repeated every 28 days for a maximum of 6 cycles	
FC	50
fludarabine 25 mg/m²/day iv for 3 days	
cyclophosphamide 250-300 mg/m²/day for 3 days	
to be repeated every 28 days for 5-6	
FAND	51
fludarabine 25 mg/m²/day iv for 3 days	
Ara-C 700 mg/m² for 3 days	
novantrone 10 mg/m² the first day	
dexamethasone 10 mg iv twice daily for 3 days	
to be repeated every 28 days	
FCM	52
fludarabine 25 mg/m²/day iv for 3 days	
cyclophosphamide 200 mg/m²/day for 3 days	
mitoxantrone 6 mg/m²/day iv day 1	
to be repeated every 28 days for 5-6 for a maximum of 6 cycles	
Chlorambucil therapy	
Low-dose chlorambucil	
40 mg/m² on day 1 every 28 days	12
0.4 mg/Kg on day 1 every 2 weeks	53
0.4-0-8 mg/Kg every month	54
to be continued for at least 6 months	
Intermediate/high dose chlorambucil	
30 mg/m² on day 1 and 15 each month	55
12 mg/m²/day on day 1 to 7 each month	56
10 mg/m²/day on day 1 to 6 each month	57
to be continued for at least 6 months	
Chemoimmunotherapy	
FR	19
Fludarabine 25 mg/m² d 1-5 for 6 cycles	
Rituximab in 1st cycle: 50 mg day 1, 325/m² day 3 , 375/m² day 5	
then Rituximab 375 mg/m² day 1 of cycles 2-6. 12 mg/m²/day	
on days 1 to 7 each month	
FCR	19
Fludarabine 25 mg/m²	
Cyclophosphamide 250 mg/m² days 1-3 every 5 weeks for 6 cycles	
Rituximab 375 mg/m² on day 0 of the 1st cycle,	
then 500 mg/m² on day 1 of cycles 2-6	
Alemtuzumab	58,59,60,61
30 mg iv or sc three times a week, starting with two	
administration of 3 and 10 mg, respectively, for 12-18 weeks	

survival, but also, as expected, higher hematologic toxicity than standard doses (level 1+). There is no evidence that the association of chlorambucil with steroid therapy has a greater efficacy or equal safety.

Purine analogs

Two large randomized studies (level 1+)[12,13] and a meta-analysis of randomized studies,[14] proved that fludarabine, used as single agent and compared with chlorambucil at standard doses, induced higher complete response rates and improved patients' quality of life, irrespectively of age. Response rates were further improved by fludarabine-containing combination regimens, especially by the fludarabine plus cyclophosphamide combination (Table 5), which was shown to have a higher efficacy also in subgroups at higher risk, such as patients with del(17) or p53 mutation.[15] Thus far, no difference in median overall survival has been observed.[15]

Retrospective cohort studies showed that fludarabine did not increase the rate of secondary cancers, as compared to the rate in untreated patients. However, fludarabine has some drawbacks, such as the increased risk of opportunistic infections, the occurrence of fludarabine-associated autoimmune hemolytic anemia (AIHA), and decreased stem cell mobilization, although data supporting this remain controversial. It is recommended that fludarabine should not be combined with steroids as there is no evidence for increased clinical efficacy whereas there is the potential for increased risk of infections. The available evidence reported a 2-8% rate of AIHA in patients receiving fludarabine chemotherapy, the AIHA frequency being much lower in patients receiving the fludarabine-cyclophosphamide combination. Patients who develop Coombs' positive autoimmune hemolysis during fludarabine therapy should not be re-exposed to fludarabine.

It is considered that complete response rates and quality of life are relevant outcomes upon which to base therapeutic decisions and therefore first-line therapy with combined fludarabine and cyclophosphamide is recommended, with dose adjustment according to renal function. However, it is considered that the toxicity and side effects of such therapy and the effects on quality of life might counterbalance the potential efficacy in elderly unfit patients or in patients with comorbidities. Therefore, the Panel recommended first-line chlorambucil in such patients.

Cladribine as single agent produced high complete response rates, with event-free survival and overall survival rates similar to those obtained with chlorambucil; the association of cladribine with cyclophosphamide did not improve overall response rates or the median duration of response.

Combination chemotherapy without purine analogs

A large meta-analysis of ten randomized studies proved that first-line treatment with COP, CHOP, CMP or CAP resulted in more rapid and/or frequent responses, but did not prolong progression-free or overall survival, as compared to treatment with chlorambucil, with or without prednisolone.[7] Furthermore, first-line CAP and CHOP therapy were inferior to fludarabine, since they induced lower overall and complete responses (level 1++).[16]

Immunotherapy and chemo-immunotherapy

Only one study has reported on the use of rituximab monotherapy as first-line treatment for CLL: it reported a low complete response rate (9%) and an average response duration of 18 months (level 2+).[17] A few studies published in abstract form reported the application of first-line rituximab plus cyclophosphamide, while the concurrent administration of fludarabine and rituximab has been more thoroughly investigated. The latter strategy induced complete responses in up to half of the patients and, in a retrospective analysis compared to historical controls, it increased the 2-year overall survival rate from 81% to 93% and the 2-year progression-free survival rate from 45% to 67%, as compared with fludarabine alone (level 2+, 2++, 1+).[18-20] Rituximab combined with both fludarabine and cyclophosphamide (FCR) achieved even higher complete response rates, i.e. 68% with a number of molecular responses also reported (Table 4).[21] The addition of rituximab to fludarabine and cyclophosphamide was not apparently associated with increased hematologic and infectious complications in indolent lymphoproliferative diseases. In the absence of any randomized controlled trials it is considered that the addition of rituximab to chemotherapy cannot yet be recommended as first-line treatment of patients with CLL.

A few studies investigated alemtuzumab (Campath) as first-line single agent therapy (Table 5). A randomized trial is currently comparing frontline alemtuzumab with chlorambucil in Rai stage I-IV patients with evidence of progressive disease. Interferon-α (IFN-α) showed limited activity as a single-agent both in patients with advanced disease, and in untreated patients with early disease. However, the studies applying this strategy were mostly non-comparative and low-quality. A randomized phase II study found no difference in response rate from the addition of interferon to fludarabine (level 2++).

Stem cell transplantation

Young patients with high-risk advanced disease have a low probability of remaining in remission for a prolonged period after first-line chemotherapy or chemo-immunotherapy. Therefore it is reasonable to consider therapy aimed at obtaining the highest rate of molecular remissions and the longest response duration. Autologous stem cell transplantation (SCT) is a feasible frontline approach in 50-70% of patients, and is associated with 1-5% transplant-related mortality (TRM). This procedure can achieve a high rate of long-lasting complete responses and also a relevant proportion of molecular responses.

However, no plateau has been shown in survival curves, and secondary myelodysplastic syndromes/acute myeloid leukemias were reported in 6-8% of the patients.

As far as allogeneic transplant is concerned, no cohort studies have specifically addressed its use as first-line therapy for young CLL patients: only 12 cases were reported in the EBMT database from 1992 to 1999. It is considered that the current evidence does not support front-line autologous or allogeneic SCT in clinical practice.

Recommendations

Low-risk, i.e. without unfavorable biological risk factors, younger patients and selected elderly patients, i.e. with a very good performance status and free of co-morbidities, are recommended to receive first-line therapy with fludarabine plus cyclophosphamide (Table 3) in order to achieve the best possible response (grade A).

Adjustment of fludarabine dose according to renal function is suggested (grade C). Corticosteroid use alongside fludarabine should be limited to selected cases, i.e. patients with autoimmune cytopenia (grade D). Younger patients with unfavorable biological prognostic factors should be considered for high-dose chemotherapy and autologous or allogeneic stem cell transplantation, which might achieve a durable good quality complete remission. However, it is recommended that first-line autologous or allogeneic stem cell transplantation is performed only within approved clinical trials (grade C).

Patients who are not candidates for or who have contra-rindications to fludarabine-based therapy should receive chlorambucil in order to pursue the control of symptoms and a good quality of life, while preserving overall survival (grade B). The association of steroids with chlorambucil may be recommended only in the case of autoimmune complications or the presence of systemic symptoms (grade C).

Combination chemotherapy not containing purine analogs, either with or without anthracyclines, such as COP, CHOP or CAP, cannot be recommended as first-line therapy (grade A).

In the absence of clear advantages in efficacy and toxicity, single agent alemtuzumab as first-line treatment cannot be recommended, so far, outside of approved clinical trials.

Monitoring the response to first-line therapy

The techniques for assessing minimal residual disease (MRD) are currently undergoing critical evaluation and standardization. New technologies such as four-color flow cytometry and real time quantitative polymerase chain reaction (PCR) analysis can determine whether patients in complete remission by NCI-WG criteria have detectable MRD. Eradication of MRD with alemtuzumab therapy in previously treated patients is associated with prolonged survival.[22] However, the Panel agreed that the current evidence is not sufficient to define the value of MRD assessment as an end-point for routine clinical practice.

Recommendations

Patients in clinical complete remission after fludarabine-based therapy should have the following parameters assessed at the end of the therapy and during follow-up (grade D): physical examination (every 3-6 months), and peripheral blood count (every 3-6 months).

Minimal residual disease assessment, performed by direct quadruple staining with CD5, CD19, CD20 and CD79b expression on bone marrow lymphocytes, is not recommended in clinical practice since the eradication of minimal residual disease cannot be recommended as a therapeutic end-point outside of clinical trials (Grade D).

Maintenance therapy

Maintenance therapy with alemtuzumab proved to increase time to progression and survival.[23,24] Discordant results were reported for infectious complications.[23,24] Maintenance treatment with rituximab also produced a small improvement in progression-free survival. A single study demonstrated a beneficial effect of interferon-α given as maintenance therapy in CLL patients who had achieved a response to chemotherapy. However, in a retrospective study and two randomized trials (level 2+, 1+), including patients responsive to fludarabine, no significant benefits were observed in terms of response duration.

Recommendations

Maintenance interferon-α is not recommended in patients achieving a response to chemotherapy (grade B). On the basis of current evidence, the use of alemtuzumab or rituximab as maintenance therapy cannot be recommended.

Second-line therapy

Purine analogs (fludarabine-naive patients)

Patients with late relapse have a high response rate to a repetition of their first-line treatment, such as chlorambucil. In contrast, patients who do not respond or relapse early after first-line chlorambucil usually do not get any benefit from retreatment: an overall response to fludarabine-based regimens can be obtained in nearly half of the patients and a complete response in up to one fourth of them. Higher response rates (up to 86%) can be achieved with fludarabine plus cyclophosphamide. In chlorambucil-refractory patients, cladrabine induced a 64% response rate; however, the lack of evidence prevents the formulation of specific recommendations on the use of purine analogs other than fludarabine.

Anthracycline-containing combination chemotherapy did not prove to be superior to fludarabine as second-line treatment for chlorambucil-refractory patients.

Purine analogs (fludarabine-pretreated patients)

Around 40-67% of patients who relapse after first-line single agent fludarabine may respond to retreatment with

fludarabine or fludarabine plus cyclophosphamide. However, fludarabine-refractory patients, i.e. those who did not respond or had early (within 6 months) progression after the end of treatment, have a poor prognosis, achieve limited response rates with fludarabine plus cyclophosphamide, and have a short survival.

Higher response rates can be achieved with fludarabine, cyclophosphamide and rituximab with response rates up to 72% (complete response >13%). The overall survival is also improved with the above regimen, as compared with historical control patients treated with fludarabine plus cyclophosphamide or single agent fludarabine.[25] In a phase II study reported only in abstract form, the combination of fludarabine, cyclophosphamide, rituximab and alemtuzumab produced clinical responses in over half of patients with a median survival of 21 months. Good clinical outcomes were also reported following combination therapy with fludarabine and alemtuzumab,[26] or filgastrim and alemtuzumab.[27] Pentostatin-based chemotherapy and combination with rituximab have also been tested in fludarabine-refractory patients, but only a few studies have been conducted. Inconsistent data were reported on the efficacy of cladrabine in this clinical setting. The Panel agreed that the balance between risk and benefits of second-line combination regimens in fludarabine-pretreated patients has been insufficiently explored in clinical trials. The Panel therefore recommends enrolling patients in clinical trials or, for older patients, maintaining a conservative attitude using chlorambucil.

Immunotherapy

Immunotherapy with rituximab as a single agent did not result in clinical efficacy in patients with previously treated CLL, even at high doses: the overall response rate was lower than 46% and the complete response rate below 4%. Alemtuzumab as a single agent was effective in inducing clinical remissions in about one third of previously treated CLL patients, and also showed clinical efficacy in the subset of fludarabine-refractory patients and in non-responsive patients with p53 mutations.[28]

Stem cell transplantation

High-dose chemotherapy and autologous SCT has been reported as second-line therapy in large retrospective studies which appeared to show an incremental 5-year overall survival (77% vs 44%) as compared with that in historical patients treated with conventional chemotherapy (level 2++). However, no plateau in survival curves is reported after autologous SCT, and molecular remissions after autologous SCT are short-lasting.[29] An improvement of 5-year overall survival (45% vs 27%) was observed after allogeneic SCT, as compared with standard chemotherapy, and the advantage was maintained also in fludarabine-refractory patients. Moreover, patients after allogeneic transplant have a realistic chance of cure, or at least prolonged survival, at 10 years (level 2++).[30,31] Due to the high TRM, allogeneic SCT had a worse 3-year overall survival than autologous SCT (78% vs 54%). However, transplant modalities (e.g. stem cell donor, conditioning intensity, *in vivo* purging with monoclonal antibodies, prophylaxis of graft-versus-host disease) may greatly influence TRM and post-transplant relapse rates. Therefore, the clear survival advantage conferred by autologous SCT might not be applicable to specific patients.

Recommendations

Patients relapsing within 6 months from first-line therapy should be treated as recommended for refractory patients (grade D).

Patients with late relapses (beyond 6 months from the end of first-line treatment) should be considered for further treatment according to the recommendations for first-line therapy (see above) (grade D).

Younger patients refractory to first-line fludarabine plus cyclophosphamide should be considered for stem cell transplant procedures, after disease debulking with schedules including non-cross-reactive agents and monoclonal antibodies, within the frame of controlled clinical studies (grade B). Those patients with no donor and/or not suitable for autologous or allogeneic stem cell transplantation should be considered for experimental drugs within clinical trials.

Patients in whom first-line fails should receive fludarabine or fludarabine-containing regimens (grade A).

Therapy for transformed disease

Richter transformation

Richter transformation occurs when CLL evolves into an aggressive diffuse large B-cell lymphoma: this has been reported to occur in about 3–10% of CLL patients and is associated with rapid lymph node enlargement, fever, and weight loss. Different combination chemotherapy regimens have achieved short-lasting responses and a survival of less than 12 months. Immunotherapy, radioimmunotherapy and allogeneic SCT have been reported in only a few patients.

Prolymphocytic transformation

Prolymphocytic transformation, which has been reported to occur in 10% of CLL patients, is characterized by a poor prognosis and massive splenomegaly. Fludarabine, alemtuzumab and rituximab have been successfully used in this clinical setting. Only a few cases have been reported to have received successful autologous or allogeneic SCT.

Recommendations

The treatment of Richter transformation should be the same as that for aggressive non-Hodgkin's lymphoma (grade D). Allogeneic stem cell transplantation is recommended in younger cases (grade D).

Patients with prolymphocytic transformation may receive fludarabine-based regimens and monoclonal antibodies (grade D).

Supportive anti-infectious therapy

Infections are the most frequent complications of CLL, accounting for up to 50% of all CLL-related deaths. Higher infection rates are reported to be associated with advanced stage, higher patients' age, number of previous treatments, type of treatment, use of steroids, use of alemtuzumab in refractory CLL, poor response to therapy, hypogammaglobulinemia with low IgG levels, persistent and severe neutropenia, low CD4 lymphocyte counts and renal dysfunction. The spectrum of infections in patients treated with standard-dose chemotherapy is mainly bacterial; Gram-negative bacteria are a major cause of pneumonia. Opportunistic infections typically associated with T-cell dysfunction, including listeriosis and *P. carinii* pneumonia (PCP), have been described in patients receiving alemtuzumab or fludarabine. Opportunistic infections are more frequently described in refractory patients and in patients receiving steroids, either concurrently or subsequently to purine analogs. Herpesvirus infections, especially varicella-zoster virus (VZV), are frequent in patients treated with fludarabine or alemtuzumab. Most VZV infections are dermatomal, however, morbidity may be significant. Finally, cytomegalovirus (CMV) reactivation has been reported in 10-25% of patients treated with alemtuzumab.

Vaccinations

No controlled studies have evaluated the clinical efficacy of anti-infectious vaccinations in patients with CLL. Modest and short-lasting serological responses to a variety of vaccines (influenza vaccine, pneumococcal polysaccharide vaccine, *Haemophilus influenzae b* conjugate vaccine and tetanus toxoid antigen) have been reported. Booster vaccine doses were also of little value. A more effective antibody response has been recorded in younger patients, with less advanced disease and normal, or almost normal, immunoglobulin levels (i.e. IgG level >700 mg/ dL). Moreover, in CLL patients, vaccines containing live attenuated viruses could be unsafe because of the disease- and therapy-related immunosuppression.

It is deemed that the available evidence is insufficient to establish a clinical benefit of anti-infective immunization in CLL patients. Therefore, the Panel did not formulate any specific recommendation.

Intravenous immunoglobulins

One randomized trial (level 1+) and further phase II studies showed that intravenous immunoglobulins are associated with a reduced incidence of bacterial infections in patients with CLL with hypogammaglobulinemia and/or a history of recurrent infections;[32] therefore, it is considered appropriate to recommend intravenous immunoglobulins in this clinical subgroup.

Anti-infectious prophylaxis

No clinical trial has been reported that specifically addresses anti-bacterial prophylaxis in CLL patients. In cancer patients with granulocytopenia after cytotoxic therapy, anti-bacterial prophylaxis reduces mortality: in particular, fluoroquinolones reduced infection-related mortality and the rate of clinically documented infections, as reported by two meta-analyses of randomized controlled trials enrolling patients with lymphoma or any cancer (level 1++).[33,34] In a cohort of neutropenic patients with hematologic malignancies, a GIMEMA randomized trial showed a superior efficacy of prophylactic ciprofloxacin, as compared with norfloxacin.[35] Despite the above evidence derived from studies enrolling patients with several different malignancies, prophylaxis is not generally recommended for neutropenic CLL patients. However, the Expert Panel judged that both antibacterial and anti-protozoal prophylaxis were appropriate in patients who had a very high infectious risk due to disease-related or therapy-related factors. In patients treated with fludarabine, anti-protozoal prophylaxis is reported to be the only significant predictor of infections. Trimethoprim-sulfamethoxazole was chosen as the recommended agent, since it is also effective against *Listeria*, *Legionella*, *Nocardia spp.* and other bacterial infections known to occur in patients with CLL. Since this drug is relatively well tolerated, it is suitable for long-term use, for instance in patients who have long-lasting lymphocytopenia after therapy with fludarabine or alemtuzumab. Other protozoal diseases may be more easily prevented by informing patients treated with fludarabine to avoid food stuffs that can contain *Listeria monocytogenes* (unpasteurized milk, some cheeses, raw vegetables, and undercooked poultry or meat).

Therapies for CLL may also increase the risk of viral infections, therefore, the Expert Panel recommended anti-herpetic prophylaxis in those subgroups of patients known to have depressed T-cell immunity. A high rate of CMV reactivation is specifically reported during alemtuzumab therapy, and antiviral prophylaxis is widely used; however, controlled studies are still onging and the clinical benefit of such a strategy is still unknown. Therefore, it is recommended that close monitoring of clinical and laboratory signs of CMV reactivation (antigenemia, PCR-based CMV DNA detection assays) should be performed in all CLL patients treated with alemtuzumab. The Expert Panel also deemed that patients with clinical and laboratory evidence of CMV infection should be promptly removed from therapy and treated with gancyclovir or foscarnet.

Granulocyte colony-stimulating factor

Some trials indicate that therapy with granulocyte growth factors (G-CSF, GM-CSF) is effective in reducing the duration and severity of therapy-related neutropenia in CLL patients; however, the efficacy of these growth factors in reducing infection rate is uncertain.

Due to the scarcity of evidence specifically concerning

CLL patients, it was considered appropriate to apply high-quality evidence from other cancer populations. In particular, there are two meta-analyses of randomized controlled trials: the first (level 1++) considered an overall cancer population with chemotherapy-induced febrile neutropenia and reported that colony-stimulating factors hastened neutrophil recovery and shortened hospitalization, marginally reducing infection-related mortality (level 1++);[36] the second (level 1+) considered patients with non-Hodgkin's lymphoma undergoing chemotherapy and reported that colony-stimulating factors prevented febrile neutropenia and infections.[37] Indeed, current international guidelines recommend primary prophylaxis in patients with a high risk of febrile neutropenia and/or specific risk factors, including decreased immune function, pre-existing neutropenia due to disease, extensive prior chemotherapy, more advanced cancer, and poor performance status.[39,39]

Recommendations

Patients treated with purine analogs should receive anti-infection prophylaxis in the presence of additional risk factors for infection, such as: age older than 65 years, previous cytotoxic therapy, poor response to therapy, corticosteroid therapy (either previously, concomitant or subsequent), persistent and severe neutropenia, low CD4 lymphocyte count, low IgG levels, renal dysfunction. Pneumocystis carinii prophylaxis should be recommended for previously treated and refractory patients and to patients exposed to corticosteroids concomitantly with fludarabine or before and after fludarabine. Steroids should be avoided unless otherwise indicated (grade D).

Prophylaxis should be continued for at least 12 months and, thereafter, while T-cell lymphocytopenia persists (CD4 count lower than 400 cell/μL) (grade D).

Acyclovir prophylaxis should be considered for CLL patients at higher risk for herpetic infections, who are those with a marked T-cell reduction (CD4 count lower than 400 cell/μL) and/or some additional risk factors such as: older age, recurrent herpetic infection, and low serum IgG levels (grade D). Although the duration of prophylaxis remains uncertain, it should be continued as long as these risk factors persist (grade D).

Patients treated with alemtuzumab should receive anti-protozoal prophylaxis with trimethoprim-sulfamethaxazole (or nebulized pentamidine) and anti herpes simplex virus prophylaxis. Prophylaxis should continue while T-cell lymphocytopenia persists (grade D).

Patients treated with alemtuzumab should undergo close monitoring of clinical and laboratory signs of CMV reactivation (grade D). Patients with laboratory evidence of CMV infection should be promptly removed from therapy and treated with appropriate anti-CMV therapy (grade D). In the presence of severe therapy-related neutropenia (neutrophil count below 500 cells/μL) quinolone antibiotics and granulocyte growth factors should be considered in order to prevent iatrogenic infections (grade A).

Intravenous immunoglobulins should be considered for patients with low serum IgG levels and sino/pulmonary infections (grade B).

Therapy for cytopenias

Antibody-mediated cytopenias

The incidence of autoimmune cytopenias is significantly higher in CLL patients than in the general population, with a prevalence in CLL patients ranging from 10% to 20%. Autoimmune hemolytic anemia (AIHA) is not always associated with a poor outcome, except for some sporadic fatal cases. Prednisone (1-1.5 mg/Kg) is effective in controlling hemolysis, especially when warm autoantibodies are present. Similarly, chlorambucil combinations with steroids can induce a remission of hemolysis. *High-dose intravenous immunoglobulins may be an effective rescue therapy when an immediate, although transient, increase of hemoglobin level may be lifesaving.* Monoclonal antibodies, mainly rituximab, have been successfully used in over 92 reported cases of AIHA, 20 of which were CLL-related: a complete response in anemia was achieved in 21% of the patients receiving rituximab monotherapy (overall response rate 69%) and in 40% of the patients receiving combination therapy, especially with steroids. However, the use of monoclonal antibodies in this clinical setting is still investigational, being mainly supported by anecdotal evidence. *In unresponsive patients, cyclosporine-A and splenectomy can be considered: infectious and thrombotic risks of the above therapeutic choices should be carefully considered.*

CLL-related autoimmune thrombocytopenia occurs in 1-2% of patients: prednisone is effective in most cases. High-dose intravenous immunoglobulins, cyclosporine-A, or other cytotoxic therapy may be suggested is steroids fail. Splenectomy was reported to produce a durable improvement in platelet count in CLL patients; however, its specific efficacy for CLL-related autoimmune thrombocythopenia has never been investigated.

CLL-related pure red-cell aplasia is a rare complication which possibly responds to prednisolone therapy, alemtuzumab, cyclosporine, and rituximab.

Non-autoimmune anemia

From 9% to 29% of CLL patients have a moderate-to-severe non-autoimmune anemia, which has a negative prognostic impact since it correlates with advanced disease and is associated with patients having a poor response to chemotherapy. Non-autoimmune anemia, however, might be also associated with chemotherapy. Erythropoietin can be effective in improving hemoglobin and preventing transfusions in CLL patients undergoing chemotherapy, as supported by several randomized trials.[40] The largest randomized trial was reported only in abstract form and did not select patients based on erythropoietin deficiency: 150 IU/kg of erythropoietin-α three time a week resulted in a response rate of 47% in

the treated group, with a corresponding increase in quality of life.[41] Two trials selected patients with relative erythropoietin deficiency. Only one of the two trials, however, compared erythropoietin-β to transfusions alone: in the 125 enrolled CLL patients, the risk of requiring transfusion and severe anemia were significantly reduced by 43% and 51%, respectively. Quality of life also improved, paralleling the hemoglobin increase. In this clinical setting, an erythropoietin-β dose of 30,000 IU given subcutaneously once weekly was at least as effective as 10,000 IU twice weekly.

Splenectomy

Splenectomy has been employed for several years in CLL patients with advanced disease in order to improve cytopenias: a systematic review of published case series allowed the selection of ten studies enrolling 292 patients and reporting the hematologic outcome after splenectomy. Anemia and thrombocytopenia improved in most patients after splenectomy, although, no survival benefit was reported in a case-controlled study of 55 splenectomized patients compared with 55 matched patients receiving fludarabine.

Recommendations

Patients with advanced stage disease (Rai III-IV) undergoing chemotherapy should receive epoetin (erythropoietin α or β at least 30,000 IU per week or darbopoietin at least 150 μg per week), if a relative erythropoietin deficiency has been proven (Grade A).

Autoimmune hemolytic anemia or autoimmune thrombocytopenia should be treated with prednisone before starting disease-specific chemotherapy (grade C). In patients with autoimmune hemolytic anemia or autoimmune thrombocytopenia not responding to corticosteroid therapy and to disease-specific chemotherapy, a second-choice treatment can be used (i.e. cyclophosphamide-based regimen, rituximab, high-dose intravenous immunoglobulins, with or without splenectomy) (grade D).

Splenectomy is also recommended for patients without transformed disease, but with a severe cytopenia (platelet count below 50×10^9/L, neutrophil count below 1.0×10^9/L, transfusion-dependent anemia unresponsive to erythropoietin) and/or symptomatic splenomegaly refractory to chemotherapy (grade D).

Prevention and treatment of tumor lysis syndrome

Following initiation of cytotoxic therapy, some metabolic derangements may occur in a portion of patients with CLL due to the rapid destruction of malignant cells and the abrupt release of intracellular ions, nucleic acids, proteins and their metabolites into the extracellular space, possibly causing hyperuricemia and hyperkalemia: this set of derangements is defined *tumor lysis syndrome*. *Clinical tumor lysis syndrome* occurs when laboratory evidence of tumor lysis syndrome is accompanied by renal failure, arrythmia, or seizures. Clinical tumor lysis syndrome is associated with a 15-20% mortality, which is directly related to renal failure.

Overall 43 cases of tumor lysis syndrome occurring in CLL patients being treated with fludarabine or rituximab have been reported in medical literature since 1990. Indeed, tumor lysis syndrome is reported in less than 1% of CLL patients treated with fludarabine and also receiving hydration and allopurinol. Evidence (level 2++) from studies enrolling patients with non-Hodgkin's lymphomas supports the use of hydratation plus an hypouricemic agent, either allopurinol or rasburicase: the latter agent prevents uric acid production and thus has a much higher efficacy than allopurinol.

Once clinical tumor lysis syndrome has developed, rasburicase is very effective and cost-effective in controlling hyperuricemia and uric-acid-related nephropathy.

Recommendations

CLL patients receiving fludarabine-containing regimens and/or monoclonal antibodies (i.e. rituximab, alemtuzumab) should have their renal function and uric acid monitored (grade D). Patients in whom a rapid lysis of a large number of lymphocytes is anticipated should receive prophylaxis with hydration plus allopurinol (grade D). Patients with asymptomatic hyperuricemia preceding or developing during cytolytic therapy should receive rasburicase (grade D).

Rasburicase is recommended for the treatment of hyperuricemia in clinical tumor lysis syndrome (grade C).

Discussion

The recent discovery of novel prognostic markers and the proposal of new biological and transplant therapies for CLL has led to a general discussion on the optimal management for this disease. Clinical practice guidelines should support clinicians, especially regarding novel costly therapies and/or treatments associated with a high risk, by providing evidence-based patient management pathways and recommendations for specific relevant clinical questions.

In this work, all the pieces of evidence on the key question on therapy of CLL were collected and evaluated for both their quality and overall consistency. Experts in the field judged whether the body of evidence was sufficient to provide any recommendation for clinical use of a specific therapy. The amount, type (full paper versus abstract form), quality (study design, type of outcomes assessed, duration of follow-up, directness) and consistency of the different pieces of evidence were the issues ascertained in this phase. The conceptual ground of this process is that the relative benefit-to-risk balance of valuable therapies results from a partially subjective process that involves interpretation of and consensus on the evidence by the members of an expert panel. The theoretical value of the experts' consensus approach to influencing practice is the

assumption that such acknowledged experts have an implicit and comprehensive command of the scientific and practical information that would yield the most appropriate recommendations. The present guidelines suggest the use of NCI criteria to identify patients needing treatment at diagnosis. The same recommendation was provided by the British Committee for Standards in Haematology,[42] and by the result of an International Workshop on CLL (IWCLL).[43] It should be noted that NCI criteria were published nearly 10 years ago and that they are currently undergoing an international revision procedure whose results are expected to be reported shortly. Our recommendation to spare patients in initial and stable phase of the disease from therapy is based on strong evidence although the randomized trials leading to this recommendation included only chlorambucil therapy. So far, there are no reported experiences with purine analog therapy in this setting of patients and only a small study with rituximab treatment, which did not result in a clinical advantage. In the near future, the conclusion of large randomized trials from French and German cooperative groups exploring the possible advantage of early therapy in patients in the initial phase of the disease but with biological adverse prognostic factors will give new indications for this subset of CLL cases.

The panel recommended a preferential use of fludarabine plus cyclophosphamide combination as first-line therapy for the majority of patients, although the use of chlorambucil therapy was considered appropriate in cases with significant co-morbidity. The present guidelines agree with the National Cancer Institute Working Group guidelines and other ones, reported before year 2003, in recommending the use of purine analogs in first-line treatment of younger CLL patients.[42,44-47] However, no other guidelines strongly recommended the association chemotherapy of fludarabine with cyclophosphamide as do these guidelines which took into account the most recent evidence.

The use of transplant procedures in CLL is a rapidly developing issue. High dose chemotherapy with autologous stem cell transplantation now has a low risk of short term mortality; unfortunately, a prolonged follow-up of autotransplanted cases has clearly demonstrated a constant trend to relapse. Thus, at the moment, the panel's recommendations were to consider these therapeutic approaches for young cases at high risk, and within the frame of controlled studies.

In conclusion, the main issues of CLL therapy have been reviewed and relevant recommendations have been proposed; these may be helpful in guiding physicians through the huge amount of information that has been produced in recent years. However, continuously developing knowledge on the biology and treatment of CLL suggests that patients with this disease should be increasingly treated in specialized institutions. In addition, it is reasonable to foresee early updating of the present guidelines as new evidence-based information appears.

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