VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 63, No. 4, pp. 485–489

ISSN 0015–5659
www.fm.viamedica.pl

# The influence of chemotherapy on bone marrow in patients with chronic lymphocytic leukaemia

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[Received 6 August 2004; Revised 14 October 2004; Accepted 14 October 2004]

*In chronic lymphocytic leukaemia (CLL) bone marrow trephine biopsy (BMT) is not required for diagnosis but can have a significant prognostic value and can be used for the detection of the minimal residual disease (MRD) and for assessment of the effectiveness of the treatment applied. The aim of the study was to evaluate the morphological changes in bone marrow after treatment with purine nucleoside analogues cladribine and fludarabine. Bone marrow trephine biopsy was taken routinely from 15 patients with CLL. Bone marrow trephine biopsy was performed on every patient before as well as after chemotherapy. The number of cell elements of the marrow (the degree of atrophy), the patterns of bone marrow infiltration, the presence of reticulin and collagen fibres and the disturbances in bone marrow stroma were assessed. The infiltration of bone marrow by neoplastic cells was observed in all the patients before administration of chemotherapy. The infiltration was followed by an increase in the number of reticulin fibres. After the treatment a regression of the reticulin fibres was observed with the lessening of the infiltration. After the treatment the levels of marrow infiltrate were decreased. Increased hypoplasia of the bone marrow was observed after the chemotherapy.*

**Key words: bone marrow trephine biopsy, lymphoid infiltration, reticulin fibrosis**

## INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common form of leukaemia in adults to occur in Western societies [10]. For its diagnosis bone marrow trephine biopsy (BMT) is not required. However, histopathological assessment of bone marrow can be of significant prognostic value and is strongly recommended by the National Cancer Institute-Sponsored Working Group on CLL guidelines [3]. In clinical studies BMT is performed before the start of treatment as well as after the treatment. It is a useful diagnostic device for the assessment of the minimal residual disease (MRD) and the effectiveness of the applied treatment [1, 6, 14]. Histopathological examination allows for assessment of the influence of chemotherapy on haematopoietic cell lineages and helps to assess the haematopoietic inductive microenvironment (HIM), for example the degree of reticulin fibrosis (increase in the number of argentophylic fibres) and collagen fibrosis (increase in the number of collagen fibres) of the bone marrow [5]. Bone marrow trephine biopsy is used for the assessment of bone marrow involvement. In CLL there are 3 types of different infiltration patterns of leukaemic cells that can be recognised in BMT specimens: nodular, diffuse, and interstitial [9]. Nodular or interstitial patterns may be grouped together and described as non-diffuse.

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In the last 20 years new therapeutic methods in CLL have emerged due to the introduction of the new generation of drugs which can be used in single or combination chemotherapy. New purine nucleoside analogues (PNA) are such agents and this group consists of: 2'-deoxycoformycin (pentostatin), 2-chlorodeoxyadenosine (2-CdA, cladribine) and fludarabine (FAMP). Many studies have proved their effectiveness in terms of the overall response (OR) to the treatment and even the achievement of complete remission (CR) [7, 12]. Assessment of nodular partial remission (nPR) in the bone marrow may be performed with the use of BMT simultaneously with CR assessment after the treatment [1, 14].

The aim of the study was to evaluate the morphological changes in bone marrow after PNA treatment (2-CdA or FAMP).

## MATERIAL AND METHODS

Using the Jamshidi type needle, bone marrow trephine biopsy was taken routinely from the postero-superior iliac spine from 15 previously untreated patients with CLL in the 2nd, 3rd or 4th clinical stage of the disease according to the Rai staging system. Bone marrow trephine biopsy was performed in every patient before as well as after chemotherapy. The group studied included 6 females and 9 males aged 35 to 72 years. FAMP was administered to 3 patients and 2-CdA to the remaining 12. Bone marrow tissue material was fixed in so-called "Oxford solution". After fixing (48–72 hours), the oval-shaped fragments of bone marrow from 1.5 to 2.5 cm in length and 1 mm in diameter were put into paraffin bars. Thin sections at 5 $\mu$m were cut by the use of microtom. Haematoxylin and eosin were applied in routine staining techniques [15]. The number of cell elements of the marrow (the degree of atrophy), the presence of lymphoid infiltration and the presence of argentophilic and collagen fibres was assessed. For the assessment of reticulin and collagen fibrosis Gomori's silver impregnation and the Azan method were applied. The degree of reticulin fibrosis was measured according to Kundel's criteria [8] in the Baumeister modification [2] and expressed in grades, where N was defined as single argentophylic reticulin fibres: Grade 1 — insignificant, delicate, diffused foci of argentophylic fibres with single reticular foci; Grade 2 — visible reticular foci of argentophylic fibres in few microscopic fields; Grade 3 — diffuse reticulin, visible wide argentophylic fibres without collagen fibres; Grade 4 — collagen fibrosis. The morphometric examination was carried out using the image analysis set DP 12. Statistical analysis of the results was assessed by the Wilcoxon matched pairs test and the Mann-Whitney U test using the computer program Statistica Pl. The study was approved by the Bioethical Committee of the Medical University of Bialystok N°R-I-003/200/2003.

## RESULTS

Before the administration of chemotherapy the infiltration of bone marrow by neoplastic cells was observed in all the patients. In 13 (87%) patients the marrow was infiltrated in a diffuse pattern (Fig. 1) and in 2 patients in a nodular pattern (13%). After the treatment diffuse infiltration persisted in 4 patients, and only in 2 patients was the presence of single neoplastic cells observed. The nodular infiltration persisted in 9 patients (Fig. 2). In 9 cases statistically significant differences ($p < 0.05$) were

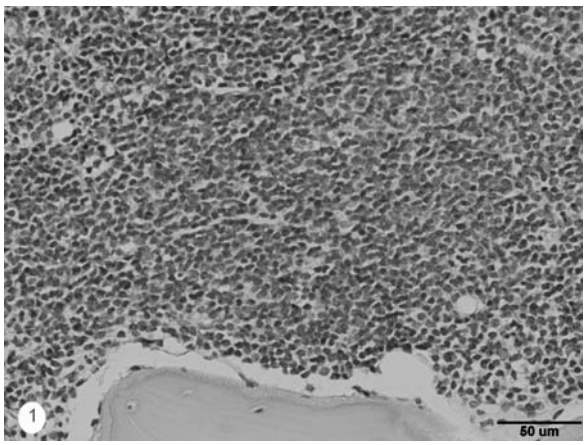


**Figure 1.** BM trephine section, CLL, diffuse infiltration. H-E. Paraffin-embedded.

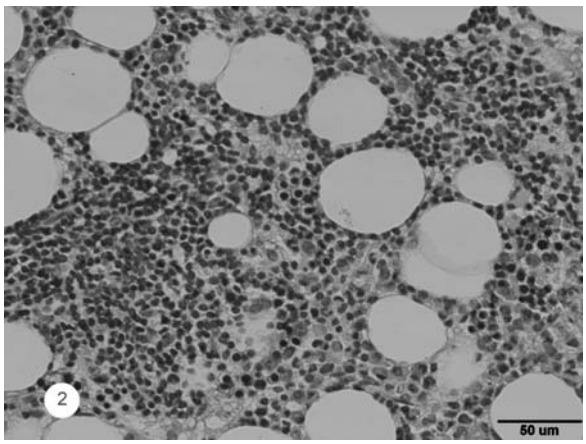


**Figure 2.** BM trephine section, CLL, nodular partial remission. H-E. Paraffin-embedded.

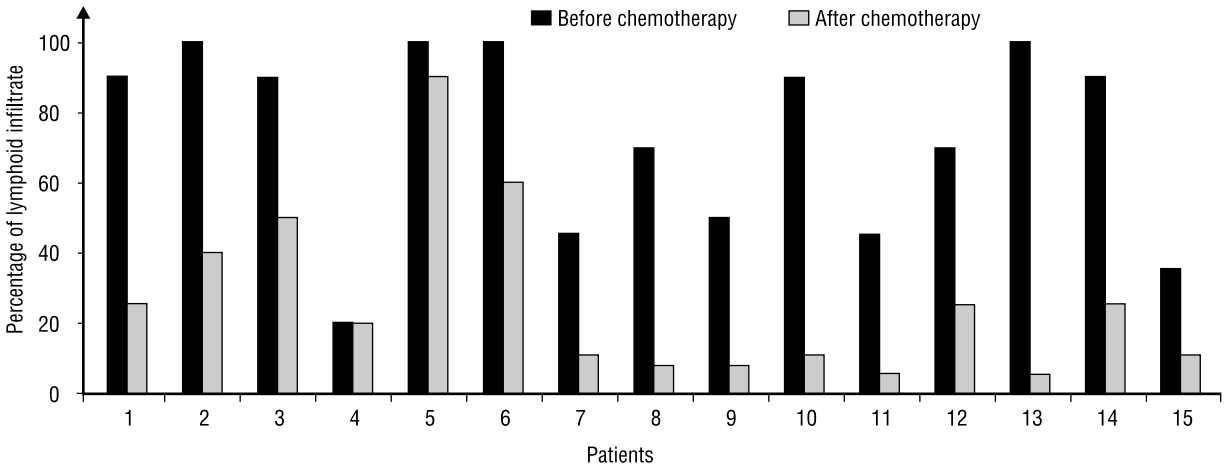


**Figure 3.** The level of lymphoid infiltration of bone marrow in patients with CLL before and after chemotherapy.

observed between the extent of the infiltration before and after the treatment (Fig. 3). Before the treatment the infiltration was accompanied by an increase in the number of reticulin fibres. In 6 patients (40%) Grade 1 fibrosis was diagnosed, while Grade 2 (Fig. 4) was observed in 3 patients (20%). After the treatment the regression of argentophylic fibres was noticed in 8 patients and in the remaining persons features of Grade 1 were observed. Only in 1 patient (7%), diagnosed Grade 3 of reticulin fibrosis, was the presence of collagen fibrosis noticed after the treatment (Fig. 5). After the treatment Grades 2 and 3 were not observed (Fig. 6). In 8 patients features of haematopoietic cell deficiency was noticed before the chemotherapy and it either persisted or appeared after the treatment in 11 patients. Inhibition of haematopoietic cell lineage maturation was assessed in 6 patients.

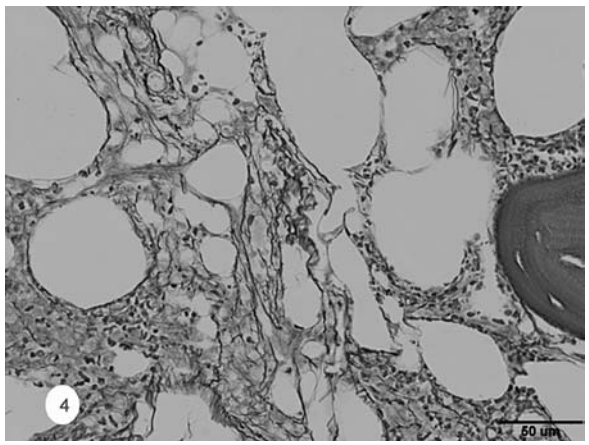


**Figure 4.** BM trephine section, CLL, showing Grade 2 reticulin deposition. Gomori. Paraffin-embedded.

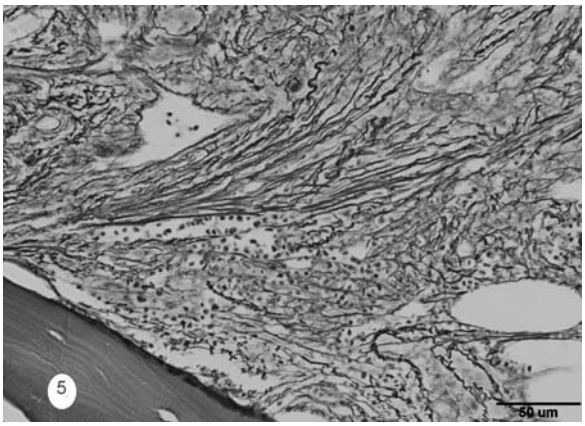


**Figure 5.** BM trephine section, CLL, showing Grade 4 collagen deposition. Azan. Paraffin-embedded.

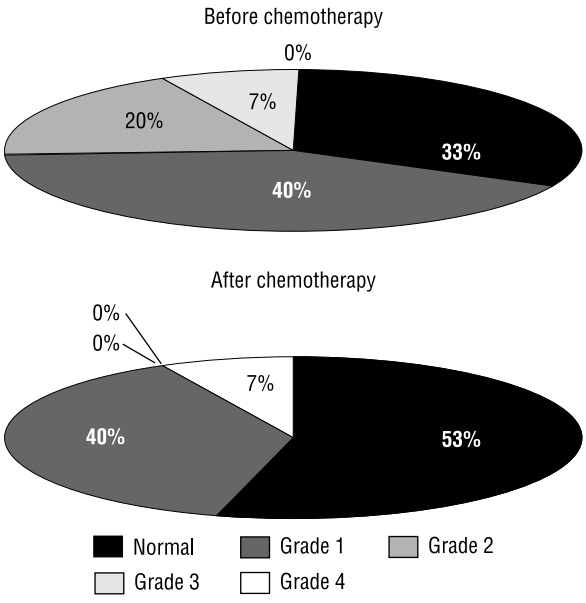


**Figure 6.** The frequency of occurrence of reticulin and collagen fibrosis in patients with CLL before and after chemotherapy.

## DISCUSSION

The new generation of anti-neoplastic drugs such as PNA is highly active in CLL [10]. Patients with CLL are considered in nPR when they are in remission after chemotherapy. In studying the significance of the levels of bone marrow lymphoid infiltration in CLL with nPR after treatment with nucleoside analogues Oudat et al. [11] observed nodular infiltration of bone marrow in 82% of patients and mixed infiltration in 18% (nodular and interstitial). Before treatment diffuse infiltration he had only observed in 36% of the patients and non-diffuse infiltration in 64%. In our study 73% of the patients responded to the treatment and, although no infiltration with leukaemic cells was observed in 13% of the patients, nodular infiltration was seen in 60%. These values were of statistical significance ($p < 0.05$). This finding would seem to be of particular importance because the diffuse infiltration was observed in 87% of the patients before treatment. The patients with diffuse infiltration tend to develop a more advanced form of the disease and have a relatively poor prognosis in comparison with those patients with non-diffuse infiltrative patterns [1, 13]. Thus it seems especially important that the treatment applied has caused nPR in most cases. Nodular partial remission is frequently seen in CLL patients. The significance of the level of bone marrow infiltration with lymphoid cells in the form of nPR is not fully understood [11].

Reticulin fibrosis after the treatment is reversible and rebuilding of the proper bone marrow structure occurs. However, in some cases in which collagen fibres are present only partial regression is possible. Reticulin fibrosis frequently occurs in areas of infiltration of the lymphatic system and collagen fibrosis is often present [1, 14]. In our study the infiltration was followed by an increase in the number of reticulin fibres in most of the patients. After the treatment a regression of the reticulin fibres with lessening of the infiltration was observed. Only in 1 patient with Grade 3 reticulin fibrosis diagnosed prior to the treatment was a sudden progression after treatment observed along with the appearance of collagen fibrosis. However, this patient had diffuse infiltration of the bone marrow before the treatment and did not respond to the treatment while the infiltration persisted.

Purine nucleoside analogues are not free of side-effects. Myelosuppression and infections are among most common [4, 12]. In our study an increased hypoplasia of bone marrow was observed after the treatment. Moreover, in a few patients inhibition of haematopoietic cell lineage maturation was observed. These changes may lead to persisting thrombocytopoenia, neutropoenia and anaemia in patients administered purine analogues. Apart from quantitative changes in the haematopoietic system, qualitative changes appear through the disturbance of the platelet function, especially through impairment of their antibacterial activity. These effects of PNA may be responsible for frequent infections, apart from the immunosuppressive qualities of those drugs.

Bone marrow trephine biopsy is a more sensitive method for the detection of infiltration in B cell disorders than bone marrow aspirates but flow cytometry may, in turn, be slightly more sensitive than BMT in detecting MRD in CLL [14]. However, BMT is especially useful for the initial analysis of the effects of administered chemotherapy on bone marrow and helps in assessing the inhibition of haematopoietic cell lineage maturation, the degree of atrophy and the presence of reticulin and collagen fibrosis after chemotherapy.

## ACKNOWLEDGEMENTS

The research was supported by the Grant N° 3-13580 from the Medical University of Bialystok.

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