Clinico-Hematological Evaluation of 130 Chronic Lymphocytic Leukemia Patients in the Central Anatolia Region in Turkey

Türkiye Orta Anadolu Bölgesinde Kronik Lenfositik Lösemili 130 Olgunun Klinik ve Hematolojik Yönden Değerlendirilmesi

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ABSTRACT Objective: In this study, the clinico-hematological features, the treatment modalities, and the survivals of 130 patients with chronic lymphocytic leukemia (CLL) were evaluated. **Material and Methods:** The medical records of 130 patients diagnosed with CLL in Eskişehir Osmangazi University Medical Faculty, Department of Internal Medicine, Division of Hematology between 1987 to 2006 were retrospectively evaluated. **Results:** Eighty five of 130 cases (65%) were men and 45 (35%) were women (male/female ratio, 1.9). The mean age at the time of diagnosis was 64 ± 0.8 years (range 43-84). Cervical lymph node enlargement was the most common finding. Forty one percent of patients had interstitial bone marrow involvement. Flow cytometric analysis revealed kappa positive CLL in 85/130. During the follow-up period, Richter's syndrome developed in 2 (2%) cases. Sixteen patients were treatment naïve for CLL. A response to treatment (complete or partial) was obtained in 58% of patients after initial therapy. Overall survival was 89.7 months in Binet stage A, 77.9 months in stage B and 63.7 months in stage C ($p > 0.05$). Forty-one (53%) patients died during the follow up. Most of the CLL-related deaths were due to infections (38%) and most of the CLL-unrelated deaths were secondary to cardiovascular causes (30%). **Conclusion:** The demographic features of our CLL patients were not different from those in other countries. Anemia, thrombocytopenia, elevated white blood cell count, elevated lymphocyte count and elevated bone marrow lymphocyte infiltration were negative prognostic factors since these parameters were significantly common in our late-stage patients.

Key Words: Leukemia, lymphocytic, chronic, B-Cell

ÖZET Amaç: Bu çalışmada, 130 kronik lenfositik lösemi (KLL)'li olgunun klinik ve hematolojik özellikleri, tedavi şekilleri ve yaşam süreleri değerlendirildi. **Gereç ve Yöntemler:** Eskişehir Osmangazi Üniversitesi Tıp Fakültesi İç Hastalıkları Anabilim Dalı Hematoloji Bölümünde 1987 ve 2006 yılları arasında KLL teşhisi konulan 130 olgunun tıbbi kayıtları retrospektif olarak incelendi. **Bulgular:** Hastaların 85 (%65)'i erkek ve 45 (%35)'i kadın (erkek/kadın oranı, 1.9) idi. Tanı sırasında yaş ortalaması 64 ± 0.8 (43-84) yıl idi. Servikal lenfadenopati fizik muayenede en sık saptanan bulguydu. Hastaların %41'inde interstisyel tip kemik iliği tutulum mevcuttu. Akış sitometrik analizle 85/130 olguda kappa tip KLL saptandı. Takip sırasında 2 (%2) olguda Richter sendromu gelişti. 16 olguda KLL'ye yönelik herhangi bir tedavi uygulanmadı. Başlangıç tedavisine hastaların %58'inde yanıt (tam veya kısmi) elde edildi. Toplam survi Binet evre A'da 89.7 ay, evre B'de 77.9 ay ve evre C'de 63.7 ay idi ($p > 0.05$). Olguların 41 (%53)'i takip sırasında yaşamını yitirdi. KLL-ilişkili ölümlerin çoğu enfeksiyonlara (%38) ve KLL-ilişkisiz ölümlerin çoğu kardiyovasküler nedenlere (%30) bağlı idi. **Sonuç:** KLL'li olgularımızın demografik özellikleri diğer ülkelerden farklı değildi. Anemi trombositopeni, beyaz küre yüksekliği, lenfosit yüksekliği ve artmış kemik iliği lenfosit infiltrasyonu ileri evre olgularımızda daha sık olup negatif prognostik faktörler olarak gözlenmiştir.

Anahtar Kelimeler: B-hücreli kronik lenfatik lösemi

Chronic lymphocytic leukemia (CLL) is a clonal lymphoproliferative disorder characterized by proliferation of morphologically and immunophenotypically mature lymphocytes.[1] CLL is primarily a disease of older adults, with more than 90% occurring in persons older than 50 years; however, CLL has also been described in young adults.[2,3] Asymptomatic disease is seen in about 25% of patients.[1] CLL diagnosis requires high peripheral blood absolute lymphocyte count, CD20, CD19/CD5, CD23 positivity and FMC-7 negativity by flow cytometry, as well as bone marrow (BM) lymphocyte infiltration higher than 30%.[4] The clinical staging systems were proposed by Rai et al and Binet et al and the current diagnosis of CLL is based on the revised guidelines of the criteria originally proposed by the National Cancer Institute--Sponsored Working Group.[4-6] Clinical stage, lymphocyte doubling time, bone marrow biopsy pattern, β 2 microglobulin levels, new prognostic markers such as ZAP-70, CD38 or mutational status of immunoglobulins have impact on prognosis.[1,7-9] In this study, we evaluated the clinico-hematological features, the treatment modalities, and the survivals of patients who were diagnosed with CLL in our centre. We aimed to determine the survival and factors affecting prognosis in our study cohort.

MATERIAL AND METHODS

130 patients diagnosed with CLL in Eskişehir Osmangazi University Medical Faculty, Department of Internal Medicine, Division of Hematology between 1987 to 2006 were retrospectively evaluated. The diagnosis of CLL was based on the history, physical examination, peripheral blood smears, immunophenotyping, and BM examination. The clinical and hematological data analysed at initial diagnosis included age, sex, physical examination findings (lymphadenopathy, hepatomegaly, splenomegaly), laboratory findings (hemoglobin, leucocyte count, lymphocyte count, platelet count, erythrocyte sedimentation rate, albumin, C-reactive protein, fibrinogen, serum immunoglobulin levels, β 2 microglobulin levels, direct Coombs test, hepatitis B and hepatitis C serology), clinical staging (Rai and Binet staging), bone marrow lymphocyte infiltration and pattern of bone marrow biopsy. IgG levels <600 mg/dL were considered hypogammaglobulinemia. Flow cytometric immunophenotypic analysis (CD5, CD19, CD20, CD23, CD38, kappa or lambda light chain expression) of bone marrow was performed in all patients. Additionally, survival time was recorded.

Statistical Analysis

Statistical analysis was performed using SPSS 16.0 software for Windows and differences with a value of $p< 0.05$ were considered significant. The Shapiro-Wilk test was performed for testing normality. Parametric tests were used for variables normally distributed, and non-parametric tests were used for variables not normally distributed. Spearman's rank correlation coefficient was calculated to determine the correlation between Rai and Binet classification stages and hemoglobin, platelet count, absolute lymphocyte count, white blood count, hypogamaglobulinemia, bone marrow lymphocyte infiltration, and erythrocyte sedimentation rate. Pearson correlation coefficient was calculated to determine the correlation between age, hemoglobin, serum albumin level, β 2 microglobulin and CD 38 expression. ANOVA was used to test differences of means of hemoglobin and platelet count between Binet stages and Tukey HSD post hoc test was used to determine which particular groups differ. Overall survival of CLL patients according to Binet stages was estimated using the Kaplan-Meier method, and survival distributions were compared using the log-rank test. Follow-up periods of patients <65 and ≥65 years were compared using the Kaplan-Meier method and log-rank test. The chi-square test was used to evaluate the difference between the distribution of number of patients <65 and ≥65 years according to Rai and Binet classification stages. Results were expressed as mean (SD).

RESULTS

85 of 130 cases (65%) were men and 45 (35%) were women (male/female ratio, 1.9). The mean age at the time of diagnosis was 64 ± 0.8 (range 43-84).

Six cases (4%) were younger than 50 years of age, 58 cases (45%) were between 50 and 65 years of age, 66 cases (51%) were older than 65 years of age.

Anemia was seen in 36% and thrombocytopenia was detected in 18% of cases at initial diagnosis. The mean white blood cell count was 70.9 x $10^3/\mu L$ (range: 3.4-480 x $10^3/\mu L$) and the mean absolute lymphocyte count was 48.7 x $10^3/\mu L$ (range: 1.4-430 x $10^3/\mu L$). The demographic and laboratory data are shown in Table 1. Cervical lymph node enlargement was the most common finding (Table 2).

The distribution of patients according to Rai classification system were 20% stage 0, 13% stage I, 35% stage II, 21% stage III, and 11% stage IV. The corresponding figures for the Binet stages were 34% stage A, 37% stage B, and 29% stage C. The distribution of patients within age groups is shown in Figures 1 and 2. Of 70 patients whose bone marrow involvement patterns were available, 29 (41%) had interstitial involvement. Bone marrow lymphocyte infiltration was between 30-50% in 13%, 50-75% in 34%, 75-100% in 53% of patients. Flow cytometric analysis in 85 out of 130 patients revealed kappa positive CLL in 67% and lambda positive CLL in 34% of cases.

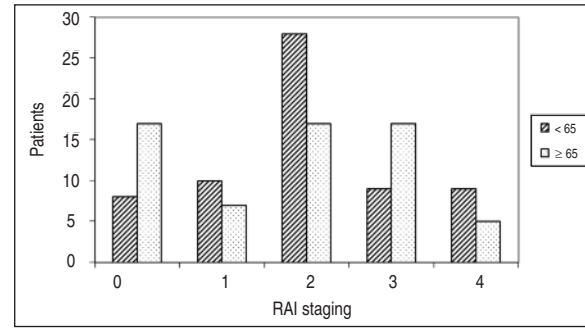

FIGURE 1: Rai staging within age groups.

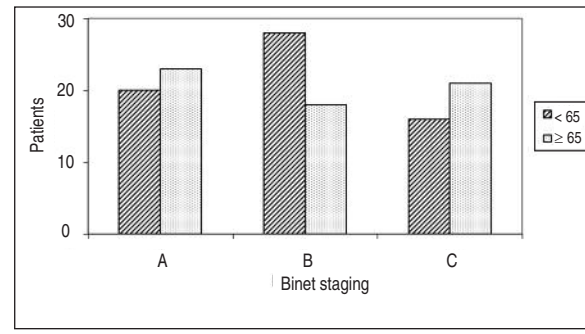

FIGURE 2: Binet staging within age groups.

TABLE 1: Demographic and laboratory data.

Male	85 (65%)
Female	45 (35%)
Median (range) age	64 (43-84)
Anemia (g/dL, mean ± SD)	11.7 ± 0.23
Thrombocytopenia (x109/L, mean±SD)	196.8 ± 9.1
Bone marrow involvement	
Interstitial	29 (41%)
Diffuse	25 (36%)
Noduler	9 (13%)
Mixed	7 (10%)
Kappa CLL	56 (67%)
Lambda CLL	29 (34%)
HBsAg positivity	6 (8%)
HCV positivity	1 (1%)
Direct Coombs test positivity	2 (2%)

TABLE 2: Physical examination findings in two groups.

	<65 years n= 64	≥65 years n= 66
Cervical lymph nodes	49 (77%)	38 (56%)
Axillary lymph nodes	36 (56%)	27 (41%)
Inguinal lymph nodes	24 (38%)	16 (24%)
Splenomegaly	44 (69%)	28 (42%)
Hepatomegaly	39 (61%)	32 (49%)

Six out of 75 cases were positive for HBsAg, while HCV was positive in only one case. Direct Coombs test was positive in 2% of 86 cases. 85% (100 of 130 cases) of the patients had hypogammaglobulinemia. During the follow-up period, 8 cases (6%) had CLL-prolymphocytic leukemia, 2 (2%) cases had squamous cell carcinoma, 1 (1%) case had breast cancer, 1 (1%) case had gastric cancer, while Richter's syndrome developed in 2 (2%) cases (Table 3).

16 patients have not received any treatment for CLL. First-line therapies included chlorambusil plus prednisolone in 71%, cyclophosphamide plus vincristine plus prednisolone in 5%, cyclophosphamide plus adriamycine plus vincristine plus prednisolone in 5% and fludarabine based protocols in 3% of pati-

TABLE 3: Clinical findings accompanying CLL.

	N	%
Autoimmune hemolytic anemia	2	3
Hypogammaglobulinemia	64	65
CLL/PLL	8	6
Secondary malignancies	4	3
Richter syndrome	2	2

ents. A response to treatment (complete or partial) was obtained in 58% of patients after initial therapies.

The median follow-up period for 76 patients followed up regularly was 91 months (range, 62-115 months) in patients <65 years of age and 59 months (range, 43-74 months) in patients ≥65 years of age (Figure 3). Forty-one (53%) of these died during the follow up. Overall survival was 89.7 months in Binet stage A, 77.9 months in stage B and 63.7 months in stage C (p> 0.05). Most of the CLL-related deaths were due to infections (38%) and most of the CLL-unrelated deaths were secondary to cardiovascular causes (30%).

Comparison of laboratory parameters according to Rai classification stages revealed that patients with anemia (p< 0.001), thrombocytopenia (p< 0.001), hypogamaglobulinemia (p< 0.05), high white blood count (p< 0.001), high absolute lymphocyte count (p< 0.05) and bone marrow lymphocyte infiltration (p< 0.01) were in late stages (III + IV).

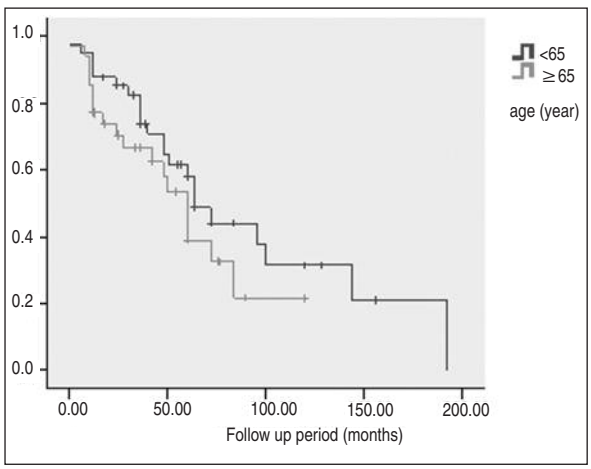

FIGURE 3: The survival curves of CLL patients according to age (<65 and ≥65).

When the same analyses were carried out according to Binet classification, patients with anemia (p< 0.001), trhombocytopenia (p< 0.001), high white blood cell count (p< 0.01), high absolute lymphocyte count (p< 0.001), elevated erythrocyte sedimentation rate (p< 0.05), high bone marrow lymphocyte infiltration (p< 0.05) had late stages. Serum albumin level was lower in older age and anemic patients (p< 0.05), and β 2 microglobuline was higher in patients with older age (p< 0.05) and higher CD38 expression (p< 0.05).

DISCUSSION

In this retrospective study, there were more male CLL patients than were female patients (male/female ratio 1.9). Similarly, male patients were predominant in other CLL series also.[10,11] CLL primarily affects older people and the mean age at diagnosis is approximately 70.[10,12] The disease is very rare in patients aged less than 50 years as in our study.[10]

Most of our cases had cervical lymphadenopathy at initial diagnosis, which was followed by splenomegaly, hepatomegaly, axillary lymphadenopathy and inguinal lymphadenopathy. The earliest signs of CLL were unexplained absolute and persistent lymphocytosis, mild enlargement of the cervical, supraclavicular, and/or axillary lymph nodes and splenomegaly in most patients.[1] In a study by Agrawal et al splenomegaly, hepatomegaly and lymphadenopathy were the first findings in order of frequency.[13] The frequency of anemia and thrombocytopenia in our study was 35% and 19%, respectively. The high frequency of anemia may be explained by the late admission of our CLL patients to hematology centers. Therefore, our CLL patients were diagnosed at later stages although studies published in recent years report that CLL has been being diagnosed mostly at the early stages of the disease.[10-12]

Diffuse bone marrow involvement, which is considered a poor prognostic marker in some CLL series was observed more frequently in our late stage patients.[1,8,14] Mixed pattern of BM involvement was reported to be more common in the series of Agrawal et al while diffuse pattern was more com-

mon in the series of Zengin et al.[13,15] However, interstitial pattern of BM involvement was the most frequent type in our study cohort.

Flow cytometric analysis revealed more kappa positive CLL patients (kappa/lambda ratio 1.9/1) in our study in accordance with the previous literature.[10] CD38 expression more than 30% by flow cytometry is considered a negative prognostic parameter in CLL patients.[8,16] However, we observed that 59% of patients with CD38 expressing cells were in early stages. This finding may be explained by the lack of difference between survival periods of patients in early and late stages. Immunologic activity was reported to be deteriorated in CLL; likewise, approximately half of our patients had hypogammaglobulinemia, which was more frequent than previously reported in literature.[17] Autoimmunity is frequent in CLL; Coombs positive autoimmune hemolytic anemia was reported in 1-10% of CLL patients at initial diagnosis and up to 25-35% as the disease progresses.[1,11,18,19] The frequency of autoimmune hemolytic anemia at the time of diagnosis in our CLL patients was lower compared to previous reports in the literature.

PLL, secondary malignancies, and transformation to lymphoma (Richter syndrome) were reported in patients with CLL.[11,20,21] The frequency of CLL/PLL, secondary malignancies including squamous cell carcinoma, breast cancer, gastric cancer and transformation to lymphoma in our study was similar to that reported in the literature.

Many clinical, morphological and biochemical parameters have predictive value in determining prognosis in CLL.[12] Thrombocytopenia and anemia were the most important prognostic markers in the study of Binet et al.[5] In the study by Orfao et al, anemia, thrombocytopenia, hypogammaglobulinemia, absolute peripheral lymphocyte count, serum uric acid level, the histopathologic patterns of bone marrow biopsy and the percent of lymphocytes in bone marrow were significant prognostic factors.[14] Serum albumin level was determined to be an independent prognostic factor by Levis et al.[22] β 2 microglobulin, soluble CD23 and lymphocyte doubling time were evaluated as independent prognostic factors by Zwiebel et al.[20] In published studies by Eichhorst et al, Rai et al and Durig et al, new prognostic markers CD38 and ZAP-70 were evaluated.[8,16,23] In our study; patients with anemia, thrombocytopenia, hypogammaglobulinemia, elevated white blood cell count, lymphocyte count and bone marrow lymphocyte infiltration were at later stages according to the Rai staging system. On the other side, patients with anemia, thrombocytopenia, high white blood cell count and absolute lymphocyte count, elevated erythrocyte sedimentation rate, high bone marrow lymphocyte infiltration were at later stages according to the Binet system. Serum albumin level was lower in older and anemic patients. β 2 microglobulin levels were higher in elderly patients and in patients with high percentage of CD38 expression. Age, LDH, β 2 microglobulin, CD 23 and CD 38 did not have any prognostic significance.

Presence of symptoms, disease activity, stage of disease, anemia, thrombocytopenia, massive and/or progressive splenomegaly, massive and/or progressive lymphadenopathy, progressive lymphocytosis and autoimmune anemia and/or thrombocytopenia are the criteria for treatment in CLL.[8] In the study of Agrawal et al 56% of 95 patients were treated with protocols containing chlorambucil.[13] In another Turkish study by Pamuk et al 48% of 200 patients were treated at the time of diagnosis.[10] In our study, there were more patients requiring therapy at initial diagnosis than previously reported in literature, which may also be explained by the late admission of CLL patients to hematology centers in our region.

The clinical course of CLL is extremely variable due to the heterogenity of the disease, with survival ranging from months to several years and is largely dependent on the clinical stage.[1] The median survival of our patients was 60 months. The median survival was longer in early stages than in late stages but the difference was not statistically significant. The median survival was 47 months in another study from our country, by Pamuk et al.[11]

In conclusion, the demographic features of our CLL patients were not different from those in other countries. Anemia, thrombocytopenia, elevated white blood cell count, elevated lymphocyte count and elevated bone marrow lymphocyte infiltration were negative prognostic factors since these parameters were significantly common in our late-stage patients.

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