RESEARCH ARTICLE

Chronic Lymphocytic Leukemia and Prognostic Factors

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Abstract

Background: The clinical course of individual chronic lymphocytic leukemia (CLL) is highly variable and clinical staging systems do not help us to predict if and at what rate there will be disease progression in an individual patient diagnosed with early stage disease. Recently, several important observations related to other prognostic factors including lymphocyte doubling time (LDT), β_2-microglobulin (β_2-MG), and percent of smudge cell in peripheral blood smears, cytogenetic and molecular analysis have been made. The aim of this study was to evaluate a range of prognostic factors in our CLL patients. Design and methods: Seventy patients with CLL were enrolled. Prognostic factors of disease including Binet staging, LDT, β_2-MG, ESR, LDH, percent of smudge cell in peripheral blood smear, absolute lymphocyte count, and conventional cytogenetic (CC) analysis were evaluated at diagnosis, and the patients were followed up to determine their outcome. We compared factors with each other and with Binet staging and prognosis. Results: Enrolled patients aged 37–85 years at diagnosis or during follow up. There was no relationship between serum LDH level (P=0.3), ESR (P=0.11), percent of smudge cells in peripheral blood smear (P=0.94), and absolute lymphocyte count (P=0.18) with the stage of disease and prognosis, but the β_2 macroglobulin level (p<0.0001), LDT (p<0.001) had direct and significant relation with staging and outcome. In 19% of patients cytogenetic alteration were seen. Conclusion: The detection of cytogenetic alteration only using the CC method is not sufficient and we need to use FISH, but because FISH study is an expensive method not available in all areas, instead we believe that β_2 MG can be applied in its place as a good prognostic factor for CLL at diagnosis and during follow up. We suggest to add it to Binet staging for prognostic subgrouping of CLL.

Keywords: Chronic lymphocytic leukemia - prognostic factor - β_2 MG - cytogenetic

Introduction

Chronic lymphocytic leukemia (CLL) is the frequent type of leukemia in the elderly individuals, but about a third of patients are below 60 age at diagnosis, and accounting for 30% of adult leukemia (Doneda et al., 2003). Rai et al and Binet et al staging systems are the standard clinical staging to estimate prognosis of patients (Rai et al., 1975; Binet et al., 1981). But, there is heterogeneity in the course of the disease among individual patients within a single stage group. The clinical staging systems (Rai and Binet) do not help us to predict who in the good prognosis group and early stage of disease at diagnosis will develop progressive disease (Kröber et al., 2002). Therefore, there is a need to identify markers that may help to refine outcome prediction for CLL patients. In addition, a risk versus benefit evaluation based on individual disease characteristics would be desirable. Clinical patient characteristics such as age, gender and performance status; laboratory parameters reflecting the tumor burden or disease activity such as lymphocyte count, lactate dehydrogenase (LDH) elevation, bone marrow infiltration pattern or lymphocyte doubling time (LDT); serum markers such as soluble CD23, β_2-microglobulin (β_2-MG) or thymidine kinase (TK) (Hallek et al., 1999), and genetic markers of tumor cells are important in the outcome of disease. Recently, several important observations related to the biologic significance of ZAP-70 overexpression, V_H mutational status, disrupted p53 function, and chromosomal aberrations have led to the ability to identify patients at high risk for early disease progression and inferior survival (Byrd et al., 2004; Glassman and Hayes, 2005).

The aim of this study is to identify the correlation between clinical characteristic, laboratory parameters and chromosomal aberrations using CC, with the prognosis of disease in our patients.

Materials and Methods

In a prospective study 70 patients with chronic lymphocytic leukemia between 2008-2011 were enrolled. The diagnosis of CLL was based on mature lymphocytosis more than 5×10^6/mL in peripheral blood and more

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Zahra Mozaheb et al

than 30% in bone marrow cytology and in some cases flowcytometry was done and confirmed the disease by existing CD5 and CD23. Patient clinical characteristic including: anemia, splenomegaly, lymphadenopathy and fever were recorded; and laboratory parameter including: absolute lymphocyte count, smudge cell percent, serum lactate dehydrogenase (LDH) level, lymphocyte doubling time serum marker such as ß$_2$-microglobulin (ß$_2$-MG), and caryotype analysis were performed and analyzed alone or in combination.

About forty five percent of patients (32 cases) had received no treatment; the rest were treated with alkylating agents or purine analogs as first-line therapy. Thirty-seven patients were studied at diagnosis and 33 during follow-up. The mean follow-up period was 37 (8-130) months.

Serum ß$_2$-MG was measured by 2-step ELISA Reader Stat Fax 2010. The reference range for ß$_2$-MG was <3.5 mg/l. For cytogenetic analysis peripheral blood culture was performed using (RPMI 1640) harvested after 72 hours at 37 degree of centigrade and processed by standard cytogenetic methods. Caryotype carried out on slides and stained by G-banding methods, and then whenever possible, 20-25 metaphases were analyzed and described. Symptomatic patients (stage C or B) were treated at the time of diagnosis. In the remaining cases indications for treatment were clinical stage progression from stage A to B or C, a rapid LDT shorter than 6 months. The front-line treatment was fludarabin or an alkylating agent, usually chlorambucil with or without low doses of corticosteroids. A chi-squared test and exact test were applied to compare categorical variables between subsets of patients. If the P-value was ≤0.05 an effect was considered statistically significant.

Results

Among 70 patients 66% (46) were men and 34% (24) were women, the male/female ratio was 1.9; their ages at the time of diagnosis ranged from 37 to 85 years (median=67 years), about 65% of patients were between 60 to 80 years old and 20.5% of patients are diagnosed before the age of 60. At the time of enrollment, 26 (37%) patients were at Binet stage A, 18 (26%) at stage B, 26 (37%) at stage C. The most common job in our patients was farming. Some major prognostic factor which evaluate in this study is shown in the Table 1.

ß$_2$-microglobulin was measured in 65 patients (93%), which the serum level of ß$_2$-MG were less than 3.5 mg/l in 23% (n=16), between 3.5-7 mg/l in 27% (n=19) and more than 7 mg/l in 43% (n=30) of them. In Binet stage A, 11 patients had ß$_2$-MG more than 3.5mg/l, while in Binet stage C in 26 patients it was more than 3.5 mg/l (P<0.0001). LDH was measured in all patients, it was normal in 80% (n=56) and in 20% (n=14) was above the normal. ESR was more than 40 only in 6% (n=3) of patients. There is no relationship between the serum LDH level (P=0.3) and ESR (P=0.11), the percent of smudge cell (P=0.94), and the absolute lymphocyte count more than 50000/μl (P=0.18) with the stage of disease and its prognosis. But there is significant relationship between the serum level of ß$_2$-MG with the stage of disease, their prognosis and treatment (p<0.0001) (Table1). In addition the lymphocyte doubling time were measured in 57 patients, as we can see in Table 1, 31.5% (18) of patients with LTD>12 month were in stage A (p<0.0001), and 30% (17) of patients with LTD<12 month were in stage C (p<0.001). There was a significant relationship with serum ß$_2$-MG and LTD (p<0.0001) (Table 3). The percent of smudge cell in peripheral smear were accounted in 47 patients, and we cannot find any relation between the percent of smudge cell and the level of ß$_2$-MG and stage of disease (P=0.38).

Table 2 shows the different level of ß$_2$-MG in various Binet stage, as we can about 54% (13) of patients who had ß$_2$-MG less than 3.5 mg/l were in Binet stage A, and 73% (19) who had ß$_2$-MG more than 7 mg/l were in Binet stage C. Based on the level of ß$_2$-MG, only about 6% (2) of patients with ß$_2$-MG less than 3.5 mg/l needed to treat,

Table 1. Correlation of Binet Staging and Prognostic Factor

Binet stage	M/F	LDT< 12m	LDT> 12m	ESR> 40	Lymphocyte >50,000	Smudge cell>10%	β$_2$mic> 3.5	LDH >400	abnormal karyotyping
A	17/ 9	4	18	0	7	8	11	4	4
B	7/ 9	10	7	0	5	6	12	6	0
C	18/ 8	17	1	3	13	7	26	4	4
Total	46/24	31 (57)	26 (57)	3 (70)	25 (70)	22 (47)	49 (65)	14 (70)	8 (42)
p value	0.8	0.001	0.0001	0.11	0.13	0.94	0.0001	0.3	0.2

*LDT: Lymphocyte Doubling Time

Table 2. Correlation between ß$_2$-MG and Binet Stage and Treatment

| | | | Binet Stage | | | | Treatment plan | |
			A	B	C	Total	Need to treat	Observe
ß$_2$-MG	0-3.5	Count	13	3	0	16	2	14
		% within Stage	54.20%	20%	0.00%	24.60%	5.70%	54%
	3.6-7	Count	8	4	7	19	11	7
		% within Stage	33.30%	26.60%	27%	29.20%	31.50%	27%
	>7	Count	3	8	19	30	23	5
		% within Stage	12.50%	53.30%	73%	46.20%	65.70%	19%
Total		Count	24	15	26	65	35	26
		% within Stage	100.00%	100.00%	100.00%	100.00%	100%	100%

Table 3. The Correlation between ß₂-MG and Lymphocyte Doubling Time

ß₂-MG	Lymphocyte Doubling Time(month)				Total
	<6	6-12	12-24	>24	
0-3.5	0	1	6	4	11 (24%)
3.6-7	3	3	5	3	14 (30.5%)
>7	16	2	2	1	21 (45.5%)
Total	19 (41.3%)	6 (13%)	13 (28%)	8 (17%)	46 (100%)

p value=0.0001

Table 4. Patients Characteristics and Karyotypingy

Case	Age /sex	Binet stage	WBC 10⁹/L	LYM-PHOCYT%	LDH	ESR	ß₂-MG	Karyo-type	Time point	OS mo
1	M/74.0	C	100	90	304	60	11.4	NL	Dx	48
2	M/70.0	C	12.6	73	376	25	12.8	NL	Dx	45
3	M/83.0	C	109	70	690	35	7.8	NL	F/U	50
4	M/60.0	C	67	79	419	28	10	NL	F/U	48
5	F/66.0	C	40	74	705	39	12.4	NL	F/U	20
6	M/71.0	A	26	87	493	5	2.9	NL	Dx	12
7	F/60.0	C	120	80	721	21	6	NL	n/a	12
8	M/68.0	C	14.9	81	328	30	7.5	NL	Dx	24
9	M/59.0	C	21.6	71	362	9	4.7	NL	F/U	27
10	M/63.0	C	21.6	81	308	5	3.4	NL	Dx	26
11	F/75.0	C	91.9	74	360	18	11.3	NL	F/U	12
12	M/n/a	C	55.1	76	278	7	12	NL	Dx	34
13	M/64.0	C	55.1	76	278	7	12	Addᵃ	Dx	15
14	F/80.0	C	21.7	64	305	61	5.9	NL	Dx	84
15	F/84.0	A	23.7	80	329	4	3.9	NL	Dx	30
16	M/57.0	B	33.6	80	427	8	2.1	NL	Dx	12
17	M/60.0	A	25	60	289	2	1.4	Delᵇ	Dx	30
18	F/78.0	C	31.3	82	n/a	9.5	9.2	NL	Dx	n/a
19	M/56.0	A	63	70	344	10	5.6	Delᶜ	F/U	30
20	M/50.0	A	18.6	70	330	7	6.3	NL	F/u	45
21	F/80.0	A	34.5	87	295	20	8	NL	F/U	60
22	M/83.0	C	15.1	65	243	6	11.7	NL	Dx	72
23	F/80.0	B	140	67	414	20	9.8	NL	Dx	34
24	M/66.0	B	185	98	485	22	2.6	NL	F/U	42
25	F/85.0	C	77.4	88	405	17	5.8	Delᵈ	Dx	38
26	F/65.0	A	16.6	63	245	7	11.2	NL	Dx	38
27	M/71.0	A	57.5	75	225	26	3.8	NL	Dx	48
28	M/68.0	A	15.5	61	247	5	2.8	NL	Dx	40
29	M/63.0	A	24.2	80	289	7	9.8	NL	Dx	45
30	M/65.0	B	78	90	395	n/a	10	NL	Dx	24
31	M/55.0	C	65	77	341	32	10	Delᵉ	Dx	25
32	F/80.0	A	89	87	258	7	3	NL	F/u	38
33	M/58.0	A	39.4	93	176	38	5.6	NL	Dx	72
34	F/58.0	A	20	85	267	8	4.4	NL	Dx	33
35	F/53.0	A	44	60	376	1	2.5	NL	F/u	40
36	M/62.0	A	35	60	244	22	7.6	Addᶠ	F/u	64
37	F/37.0	A	48	85	320	5	1.9	NL	F/u	130
38	M/70.0	C	5	64	173	25	8.5	NL	F/u	100
39	F/65.0	A	86	70	173	25	8.5	NL	F/u	105
40	M/53.0	A	91.6	80	105	11	5.6	NL	Dx	48
41	M/70.0	C	85	70	250	23	7	7q+	Dx	n/a
42	M/80	A	64.4	70	450	20	7.4	Delᵍ		12

ᵃAdd 10p15.3, ᵇDel 17p(13.3), ᶜDel 7q21.11, ᵈDel 17q25.3, ᵉDel 9q3.3.3, ᶠAdd 8q24.3, ᵍDel 13q14.3

but about 66% (23) of those who had ß₂-MG more than 7 mg/l needed to treat.

The correlation between ß₂-MG and lymphocyte doubling time is illustrated in Table 3. In 84 % (16) of patients who had LDT less than 6 month, the ß₂-MG level was more than 7 mg/l (P=0.0001).

The Patient characteristics and karyotyping of 42Cll patient is shown in Table 4. Genetic aberrations were found in 19% of them by using CC. The abnormalities were as follows: deletion (del) 17p, addition 8q, trisomy 7q, double del 7q & 14q, del 9q, del 17q, addition 10p and del 13q. patients who had cytogenetic alteration, were in stage A or C based on Binet staging at diagnosis and their ß₂-microglobulin were more than 5 (5.6-12), all of them were treated with chlorambucil or fludarabin, even those patients who were in stage A, except two patients with del (17p and 13q).

Discussion

The standard clinical staging to estimate CLL prognosis are the clinical staging systems developed by Rai et al. (1975) and Binet et al. (1981). However, there is heterogeneity in the course of the disease among individual patients within a same stage (Kröber et al. 2002). Recent advances in risk stratification for patients with CLL have made it clear that one approach does not fit for all patients with CLL. For some patients CLL is an indolent disease that never progresses to require treatment. However, when CLL patients become symptomatic, the Overall Survival with older therapy is quite short, and less than 6 years (Dighiero et al., 1998). There has been several studies on clinical and biological factors of potential prognostic relevance that may add to the classic assessment. Among these are (1) clinical patient characteristics such as gender, age and performance status; (2) laboratory parameters reflecting the tumor burden and disease activity such as lymphocyte count, bone marrow infiltration pattern, LDH elevation, or lymphocyte doubling time (LDT) (Rozman and Montserrat, 1995); (3) serum markers such as ß₂-microglobulin (ß₂-MG), soluble CD23 or thymidine kinase,(Hallek et al., 1999) and (4) genetic markers of tumor cells such as genomic aberrations, gene abnormalities (p53 and ATM) (Montserrat, 2002), the mutation status of the variable segments of immunoglobulin heavy chain genes (V$_H$), or surrogate markers for these factors (CD38, ZAP-70, LPL, etc.) (Oscier et al., 2002; Crespo et al., 2003). In this study we evaluate the correlation between some prognostic factor in CLL patients with their Binet staging and prognosis of patients.

In this study the median age at diagnosis is 65 years for men and 72 years for women, and the median age for intire group were 67. About 6% of patients was younger than 50 years (4.5% in men). According to Surveillance, Epidemiology, and End Results (SEER) database in the US, the median age at diagnosis is 70 years for men and 74 years for women (Dighiero and Hamblin, 2008)), and the median age of all patients at diagnosis of CLL is around 70 years old in western countries (Moreno and Montserrat, 2010). Therefore, the median age difference between men in western countries studies was more than the difference in women. These data may support the idea that environmental factors which are more common in men and in our area like chemical exposure have an important

role in the pathogenesis of disease (Mozaheb et al., 2011), and gene also is important. In the study in nijer in sixty patients there was female preponderance (M:F ratio, 1:3), and the median age was 56 years, with 15% were below 40 years (Omoti et al., 2007), it is completely different from our study and Western studies, the reason was not fined .

Based Binet staging men were poor prognosis than women. In Rai study they believe that both sex and age were shown to be poor predictors of survival after adjustment for stage (Rai et al., 1975). But as we can see in Table 1, 39% of men and 33% of women were in stage C at diagnosis, and there is no significant difference between male and female for Binet stage (P=0.8). Also there was no correlation between the ß_2-MG level in men (P=0.19) and women (P=0.23). 45% of all patients at diagnosis based on Binet staging did not need to treat, but during follow up 20% again needed treatment, which 61.5% had ß_2-MG more than 3.5 mg/l.

The other evaluated prognostic factor was ß_2-MG. ß_2-MG is a serum marker and correlates with disease stage and tumor burden in patients with CLL. In 65 patients ß_2-MG was measured, in 49 (75%) of them it was more than 3.5mg/l which the Binet stage in 53% was C, in 25% B, and 22% in A (P=0.0001) (Table 1). In 16 patients ß_2-MG was less than 3.5 mg/l, which 81% of them was in stage A. 2(6%) patients with ß_2-MG less than 3.5 mg/l and 35 (94%) patients with ß_2-MG more than 3.5 mg/l had needed to treat. Nobody in stage C have ß_2-MG less than 3.5 mg/l (Table 2). Based on these data patients with the ß_2-MG less than 3.5 mg/l were low risk group and more than3.5 mg/l were high risk. In the CLL1 trial of the German CLL Study Group CLL patients with Binet A disease are stratified into a high risk if they have a LDT <12 months and/or a diffuse bone marrow infiltration pattern and a TK level >7 U/L and/or ß_2-MG level >3.5 mg/L(Furman 2010). Zwiebel study showed that, several factors, including ß_2-microglobulin, soluble CD23, and lymphocyte doubling time were particular promise as independent prognostic factors (Montserrat, 2002). The other study by measuring serum ß_2-microglobulin in 22 patients with B chronic lymphocytic leukemia and 15 healthy age matched control subjects, showed that the ß_2-MG mean value in the CLL group was significantly higher than the control group, and there was a positive correlation between ß_2-MG and the clinical stage in both CLL staging system(Di Giovanni, Valentini et al. 1989). A retrospective study of 302 untreated patients from the MD Anderson Cancer Center found ß_2-MG was the strongest predictor of 5-year survival on multivariate analysis which controlled for performance status, age, and stage(Shanafelt et al.,2004)). Authors who analyzed the serum level of ß_2-MG and sCD23 showed that the presence of increased serum levels of ß_2-MG /sCD23 and diffuse BM histology signified high-risk disease, whereas the absence of any adverse variable was associated with prolonged survival (Molica et al., 1999). In the study by using a multivariate model incorporating β-2M and IL-6, only ß_2-MG was identified as a significant predictor of survival (Lai et al., 2002). In a MD Anderson cancer center study revealed that in previously treated CLL patients, ß_2-MG was a significant prognostic indicator for response to

therapy, time to treatment failure and overall survival (Kay et al., 2007). Although, ß_2-MG determination could exhibit a lower predictive power particularly at the early disease stages compared to the newer biological markers, such as IgVH gene status, ZAP-70 and CD38 (Mainou-Fowler et al., 2004), but based on these studies it seems that ß_2-MG is the best prognostic factor for CLL staging subgroup.

In Table 3 we can see in 55% of patients who have LTD<12 month, the Binet stage was C, on the other hand 69% of patients who have LTD>12 month, were in Binet stage A (P=0.001), the overall survival of patients who have LTD<12 was less than patients with LTD>12, even patients that was in stage A. Also in a study Molica S. et al. in 99 previouly untreated CLL patients showed clear differences n the life expectancy of patients with LDT of less than or equal to 12 months compared with those with LDT of more than 12 month, and concluded that it is useful in the clicical management of CLL (Molica and Alberti 1987). The other studies in young adults with CLL revealed that LDT were prognostically useful (Montserrat et al., 1991; Dhodapkar et al., 1993).

The absolute lymphocyte count more than $50000/\mu$l were seen in 25 (19%) patient, which 13 (52%) patients were in stage C, 5 (20%) in stage B and 7 (28%) in stage A (Table 1). Therefore the difference was not significant (P=0.13). But in univariate analysis the following prognostic factors were significant for a shorter PFS: TK (P<0.001), LDT (P=0.001), lymphadenopathy (P=0.002), ß_2-MG (P=0.006), absolute lymphocytes (P=0.004), unfavorable genomic aberrations (11q⁻, 17p⁻, +12q) (P<0.001) (Byrd et al., 2004).

No correlation was demonstrated between ESR and serum level of LDH with stage of disease (P=0.11, P=0.3), and ß_2-MG (P=0.87), and LTD (P=0.48, P=0.69). Although, the level of LDH is an important prognostic factor that is predictive of survival in Non Hodgkin's Lymphoma(Gordon et al., 1995), but it seems it don't have any correlation with prognosis in CLL patients. Smudge cell in peripheral smear of 47 patients was evaluated, in 22 (47%) of patients was more than 10% of total lymphocyte, but there is no difference in stage A, B, and C with each other (P=0.94) (Table 1).

Karyotyping chronic lymphocytic leukaemia cells in our patients identified alteration only in 19% (8) of patients, 50% was in stage C and 50% in stage A, but most of them (75%) after a short period of time need to treat. Because of this low incidence of alteration we cannot find any relation between special cytogenetic alteration and patient's prognosis. In the Armand B et al study the result of conventional cytogenetic analysis showed that 28% of patients had abnormal karyotype by CC, which 6 patients had complex caryotype and two had abnormalities in 6q (Glassman and Hayes, 2005). The other study showed that about 50% of CLL cases had clonal aberration by CC (Ripollés et al., 2006). Our result from conventional cytogenetic analysis were very lower than other studies, the reason may be related to this fact which most laboratories are unable to satisfactorily bring chronic lymphocytic leukaemia cells into mitosis by CC.

In conclusion we believe that addition of the ß_2-MG level, which can be a reliable predictor for survival used

at diagnosis or during the course of the disease, to the Binet or Rai staging allows segregation of patients into prognostic subgroups. Undoubtedly incorporation of other factors specially cytogenetic abnormalities will give greater refinement, but we think in our area ß$_2$-MG can be a better marker, because conventional caryotyping was positive in rare patients and FISH techniques are very expensive and not available in all regions for routine usage.

Acknowledgements

References

Binet J, A Auquier, ONE AUTHOR, et al (1981). A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer*, **48**, 198-206.

Byrd J C, S Stilgenbauer, ONE AUTHOR, et al (2004). Chronic lymphocytic leukemia. *ASH Education Program Book*, **?**, 163-83.

Crespo M, F Bosch, ONE AUTHOR, et al (2003). ZAP-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *New England J Med*, **3488**, 1764-75.

Dhodapkar M, A Tefferi, ONE AUTHOR, et al (1993). Prognostic features and survival in young adults with early/intermediate chronic lymphocytic leukemia (B-CLL): a single institution study. Leukemia: official journal of the Leukemia Society of America. *Leukemia Res Fund*, **7**, 1232.

Di Giovanni S, G Valentini, ONE AUTHOR, et al (1989). Beta-2-microglobulin is a reliable tumor marker in chronic lymphocytic leukemia. *Acta haematologica*, **81**, 181-5.

Dighiero G, T Hamblin (2008). Chronic lymphocytic leukaemia. *The Lancet*, **371**, 1017-29.

Dighiero G, K Maloum, ONE AUTHOR, et al (1998). Chlorambucil in indolent chronic lymphocytic leukemia. *New England J Med*, **338**, 1506-14.

Doneda L, M Montillo, ONE AUTHOR, et al (2003). Interphase fluorescence in situ hybridization analysis of del (11)(q23) and del (17)(p13) in chronic lymphocytic leukemia: a study of 40 early-onset patients. *Cancer genetics and cytogenetics*, **140**, 31-6.

Furman R R (2010). Prognostic markers and stratification of chronic lymphocytic leukemia. *ASH Education Program Book*, **?**, 77-81.

Glassman A B, K J Hayes (2005). The value of fluorescence in situ hybridization in the diagnosis and prognosis of chronic lymphocytic leukemia. *Cancer genetics and cytogenetics*, **158**, 88-91.

Gordon L I, J Andersen, ONE AUTHOR, et al (1995). Advanced diffuse non-Hodgkin's lymphoma. Analysis of prognostic factors by the international index and by lactic dehydrogenase in an intergroup study. *Cancer*, **75**, 865-3.

Hallek M, I Langenmayer, ONE AUTHOR, et al (1999). Elevated serum thymidine kinase levels identify a subgroup at high risk of disease progression in early, nonsmoldering chronic lymphocytic leukemia. *Blood*, **93**, 1732-7.

Kay N, S O'brien, ONE AUTHOR, et al (2007). The role of prognostic factors in assessing 'high-risk' subgroups of patients with chronic lymphocytic leukemia. *Leukemia*, **21**, 1885-91.

Kröber A, T Seiler, ONE AUTHOR, et al (2002). V$_H$ mutation status, CD38 expression level, genomic aberrations, and survival in chronic lymphocytic leukemia. *Blood*, **100**, 1410-16.

Lai R, S O'Brien, ONE AUTHOR, et al (2002). Prognostic value of plasma interleukin-6 levels in patients with chronic lymphocytic leukemia. *Cancer*, **95**, 1071-5.

Mainou-Fowler T, H M Dignum, ONE AUTHOR, et al (2004). The prognostic value of CD38 expression and its quantification in B cell chronic lymphocytic leukemia (B-CLL). *Leukemia & lymphoma*, **45**, 455-2.

Molica S, A Alberti (1987). Prognostic value of the lymphocyte doubling time in chronic lymphocytic leukemia. *Cancer*, **60**, 2712-6.

Molica S, D Levato, ONE AUTHOR, et al (1999). Clinico-prognostic implications of simultaneous increased serum levels of soluble CD23 and β$_2$-microglobulin in B-cell chronic lymphocytic leukemia. *Eur J Haematol*, **62**, 117-22.

Montserrat E (2002). Classical and new prognostic factors in chronic lymphocytic leukemia: where to now? *The Hematology J*, **3**, 7-9.

Montserrat E, F Gomis, ONE AUTHOR, et al (1991). Presenting features and prognosis of chronic lymphocytic leukemia in younger adults [see comments]. *Blood*, **78**, 1545-51.

Moreno C, E Montserrat (2010). Genetic lesions in chronic lymphocytic leukemia: what's ready for prime time use? *Haematologica*, **95**, 12-5.

Mozaheb Z, A Aledavood, ONE AUTHOR, et al (2011). Distributions of major sub-types of lymphoid malignancies among adults in Mashhad, Iran. *Cancer epidemiol*, **35**, 26-9.

Omoti C, O Awodu, ONE AUTHOR, et al (2007). Chronic lymphoid leukaemia: clinico-haematological correlation and outcome in a single institution in Niger Delta region of Nigeria. *Int J Laboratory Hematol*, **29**, 426-2.

Oscier D G, A C Gardiner, ONE AUTHOR, et al (2002). Multivariate analysis of prognostic factors in CLL: clinical stage, IGVH gene mutational status, and loss or mutation of the p53 gene are independent prognostic factors. *Blood*, **100**, 1177-4.

Rai K R, A Sawitsky, ONE AUTHOR, et al (1975). Clinical staging of chronic lymphocytic leukemia. *Blood*, **46**, 219-34.

Ripollés L, M Ortega, ONE AUTHOR, et al (2006). Genetic abnormalities and clinical outcome in chronic lymphocytic leukemia. *Cancer Genetics and Cytogenetics*, **171**, 57-4.

Rozman C, E Montserrat (1995). Chronic lymphocytic leukemia. *New England J Med*, **333**, 1052-7.

Shanafelt T D, S M Geyer, ONE AUTHOR, et al (2004). Prognosis at diagnosis: integrating molecular biologic insights into clinical practice for patients with CLL. *Blood*, **103**, 1202-10.