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Prognostic Significance of Immune Function Parameters (CD4, CD8 and CD4/CD8 ratio) in Sudanese Patients with Chronic Lymphocytic leukemia

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Abstract

Background: *Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disorder with respect to its clinical course. Accurate identification of prognostic factors is becoming increasingly important in order to determine those patients requiring aggressive treatments. The Rai clinical staging systems is used to define disease extent and predict survival.*
Aim: *to evaluate prognostic significance of CD4, CD8 and CD4/CD8 ratio in Sudanese patients with CLL.*
Method: *We determined immune function parameter in 35 newly diagnosed patients with chronic lymphocytic leukemia (CLL) using flow cytometry and correlated these findings with the clinical data and the subsequent course of the disease.*
Results: *The mean of (CD4/CD8) ratio of 35 patients were (27 males/ 8 females) with the CLL was (0.79±0.08) compared with the mean of 35 normal volunteer (1.96±0.5) (P.value 0.000), they were tested at the same period of time. The decrease of CD4/CD8 ratio observed in stage 0 to IV was statistically significant (P. value < 0.000). In clinical stage III and IV patients presenting with the advanced disease, those who subsequently had more severe course, were found to have at presentation significantly lower CD4/CD8 ratio.*
Conclusion: *CD4/CD8 ratio was decreased in advanced form of chronic lymphocytic leukemia and this simple parameter of immune function seem to be have a huge prognostic value for patients with CLL.*

Keywords: *Chronic lymphocytic leukemia (CLL), T cells, CD4, CD8, CD4/ CD8 ratio.*

Introduction

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western world [1]. Is a lympho-proliferative disease characterized by clonal expansion of B- cells that accumulate in bone marrow and peripheral lymphoid tissues [2].

Patients with CLL suffer from recurrent infections and have an increased incidence of autoimmune diseases, suggesting T-cell dysfunction [1]. The clinical course of early stage CLL is highly variable, with some patients living for decades without requiring treatment whilst others experience a rapidly progressive illness leading to premature death [3].

The expression of CD5, CD19, and CD23 and the absence of FMC7 and CD22 surface markers are routinely used as diagnostic criteria in patients with CLL. However, these markers do not help in determining the aggressiveness of the disease or in predicting the outcome [4].

The staging systems developed by Rai et al. and Binet et al. are the gold standard methods of prognosis assessment in CLL. However, given that these systems cannot identify stable or progressive forms of the disease [5].

Rai stage 0: Lymphocytosis and no enlargement of the lymph nodes, spleen, or liver, and with near normal red blood cell and platelet counts.

Rai stage I: Lymphocytosis plus enlarged lymph nodes. The spleen and liver are not enlarged and the red blood cell and platelet counts are near normal.

Rai stage II: Lymphocytosis plus an enlarged spleen (and possibly an enlarged liver), with or without enlarged lymph nodes. The red blood cell and platelet counts are near normal.

Rai stage III: Lymphocytosis plus anemia (too few red blood cells), with or without enlarged lymph nodes, spleen, or liver. Platelet counts are near normal.

Rai stage IV: Lymphocytosis plus thrombocytopenia (too few blood platelets), with or without anemia, enlarged lymph nodes, spleen, or liver[6].

Doctors separate the Rai stages into low-, intermediate, and high-risk groups when determining treatment options.

Stage 0 is considered low risk. Stages I and II are considered intermediate risk. Stages III and IV are considered high risk [6].

Several biological parameters have been identified as potential prognostic markers of CLL disease, including rapid lymphocyte doubling time, the bone marrow infiltration pattern, cytogenetic data from FISH studies, serum beta-2-microglobulin and thymidine kinase levels, soluble CD23, and IgVh gene mutational status .The immunophenotypic features of the leukemic cells, such as the percentage of cells that express CD38 and ZAP-70s [7].

However, current prognostic factors focus only on the characteristics of malignant B cell clone and do not examine the immune response of the patients [8].

Interactions between the leukemic cells and the native immune system could be a potentially important influence on disease progression. [3]

CLL immune deficiency caused by the both the tumor lesions of lymphoid tissue and the influence of chemotherapy on hematopoiesis, results in the inhibition of the cellular and humoral responses [9].

T cells are key components of the adaptive immune system. Mature T cells are generally considered to express either the CD4 or CD8 co receptor [10]. CD4 and CD8 are a membrane glycoproteins and membrane of immunoglobulin super gene family [11].

T cells from CLL patients respond poorly to mitogens and display defective helper activity, whereas suppressor activities May be either, increased Normal or decreased. Moreover , the total T-cell number is increased, CD4 to CD8 ratio is decreased , and activation markers, such as HLA-DR, CD25, are highly expressed so that this T-cell deregulations plays a role in the hypogammaglobinemia and increased incidence of autoimmunity in CLL[2].

Previous studies have shown that CLL patients have usually increase in CD4 and CD8 cells in early stage of disease and the CD4/CD8 ratio was decrease in advanced form of disease [7][12][13].

In current study, we hypothesized that the presence of incompetent immune system would translate into a poor prognosis and short survival time of patients with CLL and we will show if the numbers of CD4 , CD8 T cells and CD4/CD8 ratio at Diagnosis will predict the clinical course of CLL or not.

Materials and Methods

Patient population

After obtain informed consent, EDTA peripheral blood samples were collected from 35 patients 27 male 8 female(age range 42-90 years) previous diagnosed with CLL and the Rai classification for the estimation of clinical stages was used. 35control samples were collected from apparently healthy individuals at flow cytometer center in Khartoum, Sudan.

Case control study performed in flow cytometer center in Khartoum state in the period from 20 February 2016 to 30 may 2016, and data obtained from patients by using standard questionnaire (with ethical approval from ministry of health) .

The diagnosis of CLL was confirmed in each patient by flow cytometry (EPICS XL Beckman Coulter flow cytometer , Miami ,FL,USA) using fluorescent dye labeled monoclonal antibody for CD5,CD10,CD19,CD20,CD22,CD23 , FMC7 and diagnostic score was determined , ZAP-70 and CD38 used as prognostic markers .

Clinical characteristics of patients including age, sex, and complete hemogram were performed including hemoglobin , red blood cell count, platelet count, total white blood cell count with differential lymphocyte percentage.(As noticed in Table 3)

Methods

Absolute count of CD3 ,CD4 and CD8 were performed by flow cytometry according to the standard protocol from Beckman Coulter using flow cytometer EPICS XL by using monoclonal antibody labeled with fluorescent dyes : anti-CD3- energy coupled dye (ECD) , anti-CD4-fluorescein isothiocyanate (FITC), anti-CD8- phycoerythrin (PE) and using antiCD45-phycoerythrin– tandom dye (PEcy5) .CD45 is used for gating of lymphocyte populations, and with CD3 to exclude monocyte which express CD4 as T helper cells**.**

The number of the CD3, CD4 and CD8 cells was counted and CD4 / CD8 ratio was measured (show figure 4).

Statistical analysis

All data of study population and each parameter were analyzed by using parametric (frequencies, mean±SD, independent t-test, chi square test and one way ANOVA and post hoc analysis were used to assess differences between and within the stages and treatment option risk by using Statistical Package for Social Science SPSS (v.20) statistical computer software program. Comparative analysis of means was performed with significant levels were set at (P. value < 0.05), and the results presented in form of Bars and tables.

Results

All 35CLL patients in the study were classified by Rai stages (Table 1). The largest numbers of patients were in Rai stage IV (n = 10) and Rai stage 0(n = 7).The mean helper to suppressor ratio (CD4/CD8) of 35 patients(27 male/ 8 female) with CLL was 0.79±0.08 which was the mean of 35 normal volunteers tested at the same period of time was 1.96 ± 0.54 (p < 0.00) (Table 2). The absolute

numbers of CD4 + and CD8+ cells, as well as the CD4/CD8 ratio according to the different stages of the disease are shown in (figure 1, 2, and3). Analysis of the data revealed that the decrease of CD4/CD8 ratio observed in stage 0to IV was statistically significant (P .value< 0.00) (Table 2).

But when compare with normal control insignificant, when compared with stage (P. value> 0.9, 0.85 respectively) (Figure1, 2)

The low CD4/CD8 ratio found in advanced stages is due to a decrease of the absolute number of CD4 + cells (Figure 1, 3)

Table 1: Frequency of patients: RaiClassification

Rai Class	Frequency	Percent %
Stage 0	7	20.0
Stage I	6	17.2
Stage II	6	17.2
Stage III	6	17.2
Stage IV	10	28.4
Total	35	100.0

Table 2: CD4 , CD8 and their ratio among study groups

	Sample	Mean	Std. Deviation	P. Value
CD4	Case	29.1486	12.98038	0.001
	Control	21.3543	4.40630	
CD8	Case	52.6114	20.09493	0.001
	Control	40.5571	6.90500	
CD4/CD8 ratio	Case	0.7991	.08395	0.000
	Control	1.9640	.54016	

Table 3: Patient characteristics

Hematological parameter	Mean	Std. Deviation
Age average	64.20	11.704
TWBCs (×109/L)	106.31	100.056
Lymphocyte(×109/L)	81.789	9.1884
RBCs (×106/L)	3.77	1.190
Hemoglobin (g/dL)	11.429	2.7684
Platelet(×103/L)	156.43	88.718

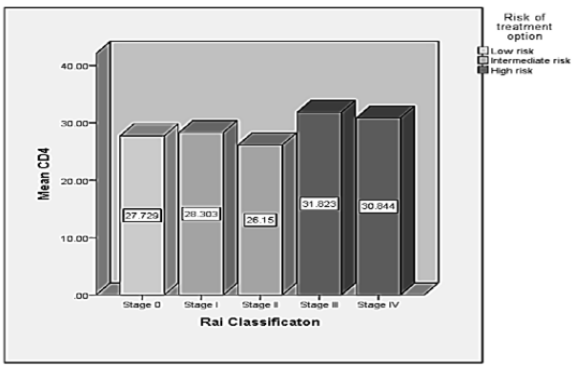


Figure1: The expression of CD4 among patients within different stages of CLL: P.value 0.9 between stages is insignificant

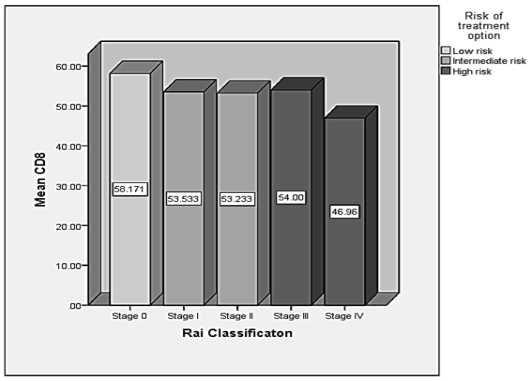


Figure 2: The expression of CD8 among patients within different stages of CLL: P.value 0.8 between stages is insignificant

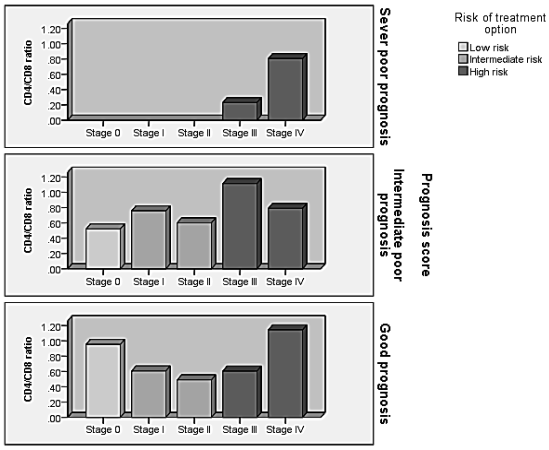
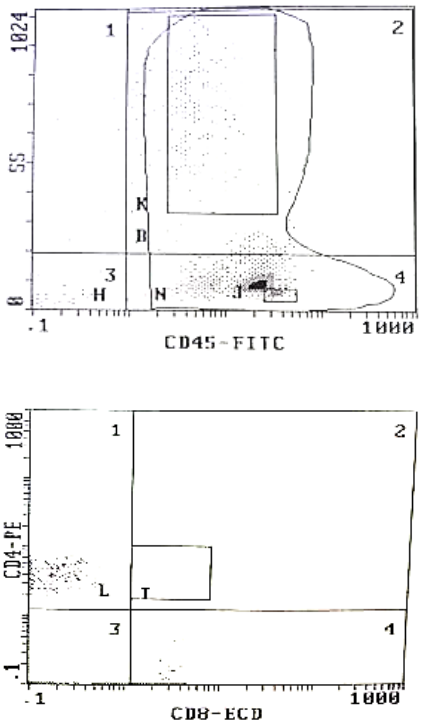


Figure 3: The CD4/CD8 ratio among patients within different stages of Rai staging system of CLL. P.value <0.00 between stages significant



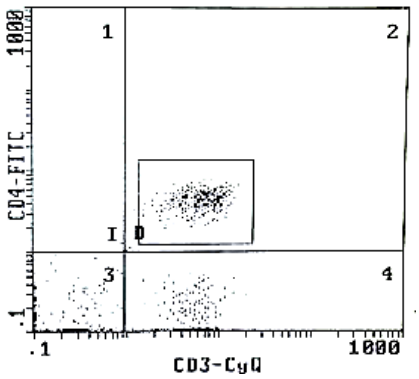


Figure 4: Expression of CD 45 for gating of leukocyte populations, CD3 to exclude monocyte that express CD4, and expression of CD4 and CD8

Discussion

The progression of CLL is associated with quantitative and qualitative changes in the host's immune system. These defects, which include alteration in T-cells, impair the cellular immune response of patients with CLL [14-17]. In this study, we analyzed whether the number of CD4, CD8 T cells and CD4/CD8 ratio at diagnosis correlated with the survival of patients with CLL.

Our study has shown that the absolute number of CD4 correlate with Rai staging show insignificant results between stages in compare with normal controls (figure 1) , but in compare with the absolute number of CD8 cells (figure 2), the absolute number of CD4 cells was decreased.

The correlation of CD4/CD8 ratio with Rai classification in different prognostic groups (good , intermediate poor and sever poor) show CD4/C8 ratio was significantly d0ecreased in patients with CLL in stage III and IV(figure 3) and that indicate the ratio of CD4/CD8 was decreased in advance form of disease and this agree with previous study [13].

The low CD4/CD8 ratio found in advanced stages is due to a decrease of the absolute number of CD4 + cells.

The finding of the present study which is probably of clinical relevance is the fact that immune function parameter at diagnosis seem to have predictive value in patient with CLL in clinical stage III and IV.

Conclusion

CD4/CD8 ratio was decreased in advanced form of chronic lymphocytic leukemia and This simple parameter of immune function seem to have a huge prognostic value for patients with CLL and predict the clinical course of the disease.

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