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Prognostic significance of serum immunoglobulins in B- chronic lymphocytic leukemia

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BACKGROUND: *Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder of mature appearing B lymphocytes which are at the intermediate stage of differentiation. The disease is frequently accompanied by low levels of gamma globulins and different classes of immunoglobulins. In this study we analyzed the levels of immunoglobulins in different stages of B-CLL and its prognostic implication on survival.*

METHODS: *Between November 1999 and December 2000, 66 patients with CLL were treated at the Institute of Hematology. In this group of patients, 36 were males and 30 females, with median age of 61.2 year (range 37-75 years).Serum gammaglobulin level was quantitated by electrophoresis on cellulose acetate and photodensitometry. Serum immunoglobulins were quantitated by radial immunodiffusion using Immunoplates (Behring Institute, FRG).*

RESULTS: *According to Binet staging system 26(39.2%) were in stage A, 15 (22.6%) stage B and 25 (37.8%) patients in stage C. Low levels of immunoglobulins were found: IgG in 14 patients (21.2%), IgA in 21 (31%) and IgM in 16 patients (24.2%). At least one immunoglobulin was decreased in 77.2% patients.*

CONCLUSION: *The prolonged clinical course of B-CLL is usually complicated by hypogammaglobulinemia. Reduced survival was significantly influenced by low level of IgA (p=0.07). The intravenous administration of immunoglobulins could signifantly contribute to the reduction of infection frequency and improvement in the quality of life.*

KEY WORDS: *Leukemia, B-cell, Chronic; Immunoglobulins; Tumor Markers, Biological*

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INTRODUCTION

B-CLL is frequently accompanied by decreased levels of serum immunoglobulins which increases patients susceptibility to infections (1,2). Low levels of IgA, IgM and IgG occur in 30%, 30% and 10% of B-CLL patients respectively at the time of diagnosis. The appearance of decreased levels of immunoglobulins is a continuos process developing spontaneously during the untreated course of the disease. As the clinical stage of the disease advances there is a trend for a more frequently decreased levels of immunoglobulins. In other low grade B-cell malignancies such as small lymphocytic lymphoma and hairy cell leukemia hypergammaglobulinemia is, on the contrary, often present (1-5). Patients with advanced stage of the disease, heavier bone marrow infiltration or high absolute lymphocyte count tend to be hypogammaglobulinemic even at diagnosis (1,6). The prognostic significance of immunoglobulin levels was analyzed by studying their influence on survival.

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PATIENTS AND METHODS

Investigation was done in the group of 66 patients with B-CLL who were treated at the Institute of Hematology, Clinical Center of Serbia from January 1990 to December 2000. There were 36 males (54.0%) and 30 females (46.0%). The diagnosis of B-CLL according to NCI and IWCLL was established (6,7). Immunophenotyping of peripheral blood lymphocytes was done by flow cytometry with a panel of monoclonal antibodies : CD45, CD38, CD3, CD2, CD5, CD19, CD20, CD22, CD23, CD10, CD25, SmIg, kappa and lambda. Clinical and laboratory characteristics of the patients are shown in Table 1. The distribution of the

patients according to Binet staging system revealed 26 patients (39.3%) in stage A, 15 patients (22.7%) in stage B, and 25 patients (38.0%) in stage C.

Serum gamma globulin and immunoglobulin measurement

Serum gamma globulin was quantitated by electrophoresis on cellulose acetate and photodensitometry. The gamma globulin level was considered to be decreased when it fell below 8.0 g/l. Serum immunoglobulins were quantitated by radial immunodiffusion using Immunoplates (Behring Institute, FRG). Normal ranges in our laboratory were defined as IgG 6.0 to 15.0 g/l, IgA 0.80 to 4.5 g/l and IgM 0.50 to 2.8 g/l. Immunoglobulin levels were considered to be decreased when their values were below the normal range.

Statistical methods

Actual survival curves were plotted according to the Kaplan-Meier method (8). Different curves were statistically compared using the log-rank test.

Table 1. Clinical findings in B-CLL

N^0 (male/female)	77(43/34)
Age (median)	61,2 years
Age range	38-75 years
Febrility	19(24,97%)
Haemorrhagic syndrome	5(6,9%)
Splenomegaly	39(50,64%)
Hepatomegaly	23 (29,87%)
Stage (Binet)	
A	26 (39,2%)
B	15 (22,6%)
C	25(37,8%)

Table 2. Laboratory findings in B-CLL

	median	range	SD
Hemoglobin (g/l)	124	63-171	24.23
WBC $x10^9$/l	33.0	11-370	65.15
Platelets $x10^9$/l	195	3-420	107.60
ALC $x10^9$/l	24.5	6.4-340.4	61.55
Percent of lymphocyte bone marrow infiltration(%)	61.2	38-100	38.50
IgG (g/l)	10.19	5.00-18.20	2.92
IgA (g/l)	1.40	0.30-5.80	1.32
IgM (g/l)	0.82	0.10-2.05	0.43

WBC-white blood cells
ALC-absolute lymphocyte count

RESULTS

We analyzed a group of 66 patients, 36 males and 30 females, median age of 61.2 years. The clinical and morphological diagnoses were confirmed by immunophenotyping. Immunophenotypic analysis showed the following results:CD 45 +(97%), CD5+ (82%), CD19+(80%), CD20+(73%), CD23+(695), CD38 + (25%) and CD25-, CD3-, CD2-, SmIg (38%), kappa (27%), lambda (10%).

Decreased levels of gammaglobulin and different immunoglobulins were observed with the following frequency: gamma globulins in 34 patients (51.5%); IgG in 14 (21.2%); IgA in 21 (31 %); and IgM in 16 patients (24.2%). At least one immunoglobulin was decreased in 77.2 % patients. The relationship between levels of immunoglobulins and clinical stages is shown in Table 3. As the clinical stage advanced, there was a trend for a more frequent decreased levels of gammaglobulins.

Table 3. Frequency of decreased levels of immunoglobulin classes in B-CLL and its relationship with clinical stages

	percent	N^0 of pts.	Stage (percent)		
			A	B	C
IgG (<6,0 g/l)	21.2	14/66	21.4	32.6	46.0
IgA (<0,80 g/l)	31.2	21/66	19.6	21.9	57.6
IgM (<0,50 g/l)	24.2	16/66	21.9	29.2	49.1

Pathohistological examination of bone marrow showed interstitial infiltration in 10 patients(15.1%), nodular in15 (22.7%), combination nodular-interstitial in 19 (28.9%) and diffuse infiltration in 22 patients (33.3%). Statistically significant correlation between type of bone marrow infiltration and stage of the disease was found. The heaviest diffuse lymphocytic infiltration of bone marrow was in stage C of the disease according to Binet ($p<0.05$)

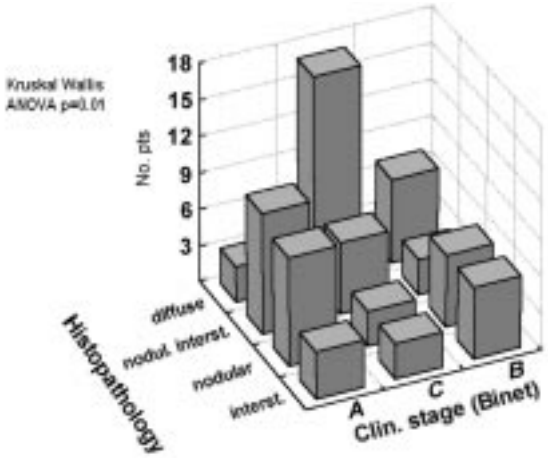

Figure 1. Correlation between clinical Stage of the disease and type of lymphocytic bone marrow infiltration (more advanced stages of the disease have a diffuse bone marrow infiltration)

(Figure 1).

Survival studies

Correlation between serum level of IgG and clinical stage of the disease showed that the lowest levels of IgG were in patients with stage C. The level of IgG significantly correlated with the stage of the disease ($p<0.016$) (Figure 2). A significant correlation between level of IgA and clinical stage of the disease was also found ($p<0.0106$). As the stage of the disease advanced there was a trend for more decreased level of IgA. The same finding was observed for IgM.

Survival was not statistically different between the patients with normal and decreased level of IgG ($p=0.07$) (Figure 3). Decreased level of IgA was associated with reduced survival

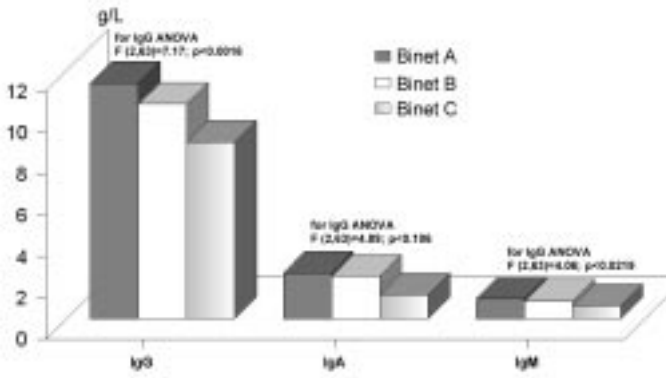

Figure 2. Correlation between serum levels of immunoglobulins and clinical stage of the disease according to Binet. There were correlation between clinical stage of the disease and level of immunoglobulins

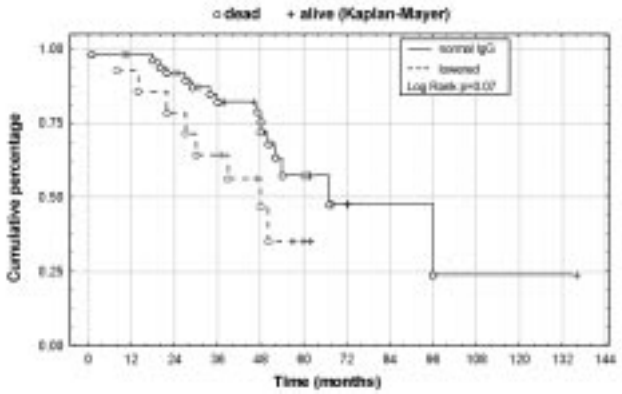

Figure 3. Survival probability according to the serum concentration of IgG (p=0.07)

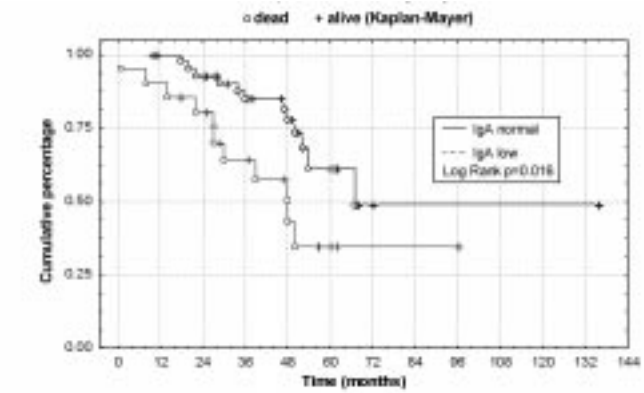

Figure 4. Survival probability according to the serum concentration of IgA (p=0.016)

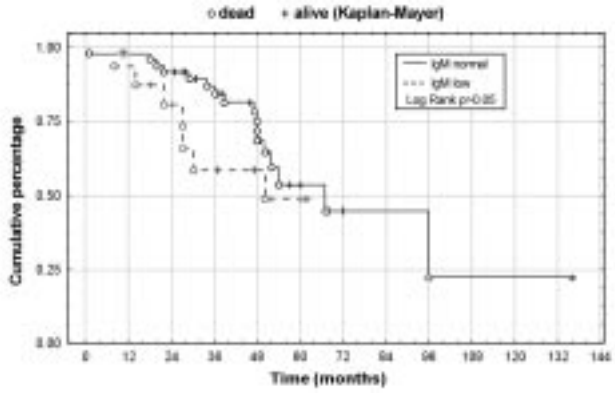

Figure 5. Survival probability according to serum concentration of IgM (p>0.05)

(p=0.016), (Figure 4).

There was no difference in survival between patients with normal and low level of IgM (p>0.05) (Figure 5).

DISCUSSION

The prevalence of hypogammaglobulinemia in patients with B-CLL depends on time and stage of the disease (1,9). Almost all patients with this disease eventually develop hypogammaglobulinemia. Consistent with hypogammaglobulinemia and T-cell abnormalities, patients with CLL have impaired antibody and cell-mediated immunity to recall antigens.

Despite its low frequency at the time of diagnosis, the appearance of hypogammaglobulinemia seems to be a continuous process during the clinical course of B-CLL, reaching 50% and 75% at 5 and 9 years of follow up, respectively. IgA and IgM levels are more frequently reduced as it was in our series of patients.

The appearance of hypogammaglobulinemia was more common in cases with diffuse infiltration of bone marrow and advanced stage of the disease (B and C).

Low levels of gamma globulin predispose to development of infections, especially bacterial (9-12). Furthermore, infections have been shown to occur more frequently in B-CLL patients with hypogammaglobulinemia than those with normal gammaglobulins. Infections are a frequent cause of complications and mortality. But hypogammaglobulinemia and hypoimmunglobulinemia is not the only factor which accounts for increased tendency to infections in this disease. T cell dysfunction, granulocytiopenia, monocytopenia, impaired complement activity, cytotoxic therapy contribute to infections (9-16).

Decreased levels of immunoglobulins has been suggested as prognostic factor for survival from indirect observations as it is more frequently found in advanced stage of the disease and in heavier lymphocytic bone marrow infiltration. Study by Rozman demonstrated a significant association between initially decreased levels of gammaglobulins, IgG and IgA and shortened survival. In this study the most significant is the level of IgA, whereas IgM levels lack prognostic significance (1,16-23).

According to Rozman et al. predominant IgA deficiency leads to an increased frequency of respiratory infections. This hypothesis would fit with the fact that non-CLL patients with selective IgA-deficiency suffer frequent respiratory infections, and respiratory tract infections are particularly frequent in CLL patients (1).

Although patients with CLL have a relative granulocytopenia and monocytopenia, the absolute numbers of circulating neutrophils seems to be normal during the most of disease course. During the course of the disease and after chemotherapy severe neutropenia can be acquired and can contribute to deaths from infection. If patients have hypogammaglobulinemia initially they often

have aggressive or advanced disease.

The pathogenic mechanism of hypogammaglobulinemia in B-CLL still remains controversial. Decreased immunoglobulin levels probably result from impaired B-cell function. Decreased in vitro immunoglobulin synthesis to polyclonal mitogens or antigens has been reported (1). According to Sampalo A. et al. B-CLL cells play a direct role in Ig production. They have found that B-CLL cells inhibit the spontaneous IgG secretion by bone marrow plasma cells in coculture of bone marrow immunoglobulin secreting - cells with autologous B-CLL cells. This inhibitory effect is proportional to the number of B-cells. This effect is operative through CD95-CD95L (24).

Hypergammaglobulinemia, usually polyclonal, is present in 15% of patients, especially in females and elderly persons over 60 years of age. A monoclonal immunoglobulin peak, usually of IgM isotype, is found in 5% of B-CLL patients. This paraprotein is usually the same type as that on the surface of the leukemic cells. This percentage is even higher when high resolution technique are used as agarose gel electrophoresis and immunofixation.

CONCLUSION

Hypogammaglobulinemia in B-CLL is also related to autoimmune complications. Prolonged clinical course of B-cell is often complicated by hypogammaglobulinemia and infections as well as with autoimmune destruction of blood cells. B-CLL patients have elevated levels of autoantibodies such as rheumatoid factor, antibodies against DNA. It seems that CD5+ cells are associated with these antibodies(25). Because of these findings intravenous administration of immunoglobulins significantly contributes to the reduction of infection and impairment of quality of life (2).

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