ASSOCIATION OF 13Q14 DELETION WITH CLINICO-LABORATORY PARAMETERS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

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Summary. A deletion of chromosome band 13q14 (13q-) detected by fluorescence in situ hybridization (FISH) analysis is the single most common cytogenetic abnormality in patients with chronic lymphocytic leukemia (CLL). In our study we disccused the incidence of molecular-cytogenetic deletion of 13q14 marker in B-CLL in order to determine the association of this aberration with clinical manifestation, laboratory parameters and poor disease prognosis. Patients with 13q14 aberrations were significantly older. They had ZAP (+) status, rapid disease progression and therapy dependency. A determination of 13q14 genetic abnormality should, therefore, be included in the investigations of the prognostic factors of B-cell chronic lymphocytic leukemia.

Key words: *13q14 deletion, chronic lymphocytic leukemia, Fluorescence in situ hybridization, ZAP70.*

INTRODUCTION

B-cell chronic lymphocytic leukemia (B-CLL) is a chronic lymphoproliferative disorder with an indolent clinical course, occasionally aggressive and, in some instances, ending with lethal outcome in a few months. Determination of prognostic factors should be done in the early stages of the disease, as this is essential for predicting the disease evolution and therapeutic decision making [2, 4]. Two staging systems are usually being applied in determining the prognosis of the disease – Rai's (1975) and Binet's (1981). Although they are easily applicable in

clinical practice, they can not explain the different prognosis of patients being in the same stage of the disease, genesis of the pancytopenia, biological heterogeneity, the degree of malignancy, as well as potential evolution of the disease.

Introducing of cytogenetic analysis in routine diagnostics led to the establishment of new prognostic indicators of oncological diseases. Genetic pathways defined at the chromosomal level are mirrored by genetic changes at the level of the gene. Genomic deletions are found frequently in cancers and are believed to contribute to their development and progression through inactivation of tumor suppressor genes [1, 3, 7, 11].

One of the most frequent cytogenetic anomalies in B-CLL is a deletion of the long arm of chromosome 13 (13q-), in particular deletion/translocation of chromosome band 13q14. Monoallelic 13q14 deletions are present in nearly 70% of patients with CLL, while biallelic and mosaic monoallelic-biallelic deletions affect almost 19% of the patients [6, 13, 17]. According to some investigators there is a correlation between 13q deletion and a better prognosis concerning survival of the patients – 134 months for patients with the aberration versus 111 months for patients with a normal karyotype [4, 5, 16, 18].

The region contains two essential tumor-suppressor genes – RB1 and BRCA2. Both are involved in the regulation of cell cycle and DNA-repair after damages [17]. Taking into consideration that normal regulation of cell cycle is of great importance for response to many chemotherapeutics, the disturbance of any of these genes could be associated with therapy resistance and poor prognosis. Hemizygous deletion of RB1 was detected in 21-30% of the cases with B-CLL; no deletion of another tumor-suppressor gene BRCA2 was established. There is no data about reduced survival in patients with RB1 deletion [13]. However, another marker telomeric to the RB1 locus, was discovered to be deleted in 45% of the cases with B-CLL, suggesting that a new candidate tumor-suppressor gene, involved in B-CLL genesis may be present [1, 11]. Further investigations are needed in order to elucidate its role in leukemogenesis.

The objective of our study was to determine the incidence of molecular-cytogenetic deletion of 13q14 marker in B-CLL in order to determine the association of this aberration with clinical manifestation and laboratory parameters, and to define the contribution of the aberration to the poor prognosis.

MATERIALS AND METHODS

Patients. A cohort of 18 patients, diagnosed over a 3-year period with B-CLL according to the revised criteria of the National Cancer Institute-sponsored Working Group (NCI-WG) on Chronic Lymphocytic Leukemia (CLL), was included in the study. All patients were classified according to the Rai staging system. The poor prognosis was determined if stage progression or dependence of chemotherapy occurred. The progression-free period ranged between 0 and 52 months, progres-

sion/chemotherapy dependence occurred in 0-12 months. The laboratory parameters – white blood cells (WBC), lymphocytes, CD38 and ZAP70 status – were examined and are shown in Table 1 together with personal and stage data.

Lymphocyte separation and preparation. Peripheral blood was taken in a vacuumtainer, containing heparin, the blood sample was mixed 1:1 with NaCl 0.9%, then slowly dropped to Lymphoprep for separation of the lymphocytes. Lymphoprep contains different high polymeric compounds, which support the agglutination of the erythrocytes. After sedimentation of the upper layer of the lymphocytes, they were treated with hypotonic solution of KCl, then fixed three times in fresh cold fixative (25ml cold acetic acid + 75ml methanol) and finally white sediment was resuspended and applied on the slide.

Table 1. Clinical and laboratory characteristics of B-CLL patients, included in the study

N	Age (yr)	Rai Stage	WBC(x109/l)	CD38	ZAP70	Progression
1	52	I	20	(-)	(-)	No
2	56	II	50	(-)	(-)	No
3	56	0	55	(-)	(-)	No
4	62	0	25	(-)	(-)	No
5	62	I	69	(-)	(-)	No
6	65	0	18	(-)	(-)	No
7	65	II	30	(-)	(-)	No
8	65	I	45	(-)	(-)	No
9	66	IV	20	(+)	(-)	No
10	67	IV	40	(+)	(+)	No
11	68	0	34	(-)	(-)	After 12 m., chemotherapy initiated after 16 m.
12	68	II	70	(-)	(-)	No
13	72	I	70	(-)	(-)	No
14	72	III	74	(+)	(+)	Chemotherapy initiated in the beginning
15	72	III	150	(-)	(-)	After 3 m., chemotherapy initiated after 4 m.
16	74	II	20	(+)	(+)	After 12 m., chemotherapy initiated after 14 m.
17	74	IV	40	(-)	(+)	Chemotherapy initiated in the beginning
18	74	I	100	(-)	(-)	After 12 m., chemotherapy initiated after 24 m.

Fluorescence in situ hybridization (FISH). Fluorescence in situ hybridization is a rapid method, highly specific and sensitive for evaluation of particular genetic aberrations. It represents direct visualization of fluorescence labeled DNA sequence on interphase or metaphase nuclei. Here we have used FISH for detection of copy number of locus-specific identifier D13S25 at 13q14. Prior to hybridization the slides

were treated with protease at 37oC for 3-30 min, then fixed in 1% paraformaldehyde and dehydrated in ethanol with increased concentrations. FISH was performed using a locus-specific probe for D13S25 (13q14) labeled with SpectrumOrange (Vysis, USA). Denaturation of the DNA was carried out at 75°C for 5 minutes (probe) or 5 minutes (slides). The probe was applied to the slides and hybridized overnight in a moist chamber at 37°C. The post-hybridization washes were performed as described in "LSI procedure" (Vysis). Slides were counterstained with DAPI in antifade. The presence of 1 copy per cell in more than 10% of the lymphocytes was considered indicative of a deletion. The presence of 2 copies per cell in more than 10% of the lymphocytes was considered a normal state. Presence of 1 big and 2 small signals per cells were considered to be rearrangement of the locus.

Statistical analysis. Comparisons of quantitative variables among patient groups were made by one-way analysis of variance. A comparison of qualitative data was performed by means of the Chi-Square and T-test. All statistical tests were two-sided. Only probability values less than 0.05 were considered statistically significant.

RESULTS

13q14 copy number in B-CLL. Lymphocyte preparations of 18 patients with B-CLL from different stages were analyzed for deletion/rearrangement of 13q14 marker D13S25 by FISH. The frequency of 13q14 deletion was 55.6%, the rearrangement of 13q14 was observed in 1 out of 18 cases (5.6%). The number of cases with rearrangement of 13q14 was not enough for statistical analysis.

13q14 copy number and stage of B-CLL. The relationship between 13q14 copy number and stage of B-CLL is summarized in table 2. There was no difference between frequencies of 13q14 deletion in early compared to advanced stages (Stage 0-1 – 55.6%, Stage 2-4 – 55.6%) (Table 2).

Table 2. The frequency of 13q14 copy number in different stages of B-CLL

	Normal **n, %**	**Del 13q14** **n, %**	**Rearrangement 13q14** **n, %**	**Total**
Stage 0-I	4 44.4	5 55.6	0	9
Stage II-IV	3 33.3	5 55.6	1 11.1	9
Total	7 38.8	10 55.6	1 5.6	18

13q14 copy number and age of the patients with B-CLL. There was statistically significant difference between the frequencies of 13q14 deletion in the patients under 70 years of age (33.3%) compared to the patients over the age of 70 years (100%) ($p < 0.01$) (Table 3).

Table 3. The frequency of 13q14 copy number in different age groups of patients

	Normal n, %	**Del 13q14 n, %**	**Rearrangement 13q14 n, %**	**Total**
Age <70	7 58.3	4 33.3	1 8.3	12
Age >70	0	6 100.0 (p < 0.01)	0	6

13q14 copy number and laboratory parameters. We investigated the possible correlation between the copy number of 13q14 and some laboratory parameters, which have an important value in B-CLL – WBC count, CD38 and ZAP70 expression. The results are represented in Table 4.

There was statistically significant association between 13q14 deletion and high WBC count (p < 0.05) (Table 4).

There was no association of 13q14 deletion with CD38 status, as well as with ZAP70 status (Table 5 and 6).

Table 4. Copy number of 13q14 and WBC count

WBC (x109/l)	**Normal n, %**	**Del 13q14 n, %**	**Rearrangement 13q14 n, %**	**Total**
WBC < 40	3 42.9	3* 42.9	1 14.2	7
WBC = 40-70	4 66.7	2* 33.3	0	6
WBC > 70	0 0.0	5* 100.0	0	5

*p < 0.05

Table 5. Copy number of 13q14 and CD38 status.

	Normal n, %	**Del 13q14 n, %**	**Rearrangement 13q14 n, %**	**Total**
CD38 (+) cells	1 20.0	3 60.0	1 20.0	5
CD38 (-) cells	6 46.2	7 53.8	0	13

Table 6. Copy number of 13q14 and ZAP70 status

	Normal n, %	**Del 13q14 n, %**	**Rearrangement 13q14 n, %**	**Total**
ZAP70 (+)	1 25.0	3 75.0	0 0.0	4
ZAP70 (-)	6 42.9	7 50.0	1 7.1	14

Table 7. 13q14 deletion and poor prognosis in relation to ZAP70 status

		Normal %		Del 13q14 %	
Progression/chemotherapy	ZAP70 (+)	1 16.7	0	5* 83.3	3** (60.0)
	ZAP70 (-)		1 (100.0)		2 (40.0)
Non progression/chemotherapy	ZAP70 (+)	6 54.5	1 (16.7)	5* 45.5	0**
	ZAP70 (-)		5 (83.3)		5 (100.0)

*p < 0.12; **p < 0.03

13q14 copy number and poor prognosis (stage progression or dependence of chemotherapy). There was a trend for a higher frequency of 13q14 deletion in the cases with poor prognosis (p < 0.12) (Table 7). It is important to note that among the patients with 13q14 deletion and poor prognosis, there was statistically significant higher frequency of positive ZAP70 (p < 0.03) (Table 7).

Combined analysis of parameters with potential prognostic significance. We analyzed the prognostic value of parameters with a potential prognostic significance (known as “risk”-factors) – 13q14 deletion, high WBC count, positive ZAP70 status and age > 70 years (Table 8). The results showed that poor prognosis is strongly associated with the combination of 3 of the considered parameters (p < 0.019) (Table 8), whereas most of the non-progressed cases had only one of these factors.

Table 8. Prognosis in relation to the presence of one or more “risk”-factors – 13q14 deletion, high WBC count, positive ZAP70 status and age > 70 years

	Progression/chemotherapy n, %	Non progression/chemotherapy n, %
Without “risk”-factors	1* 25.0	4 75.0
With 1 of “risk”-factors	0* 0.0	5 100.0
With 2 of “risk”-factors	0* 0.0	1 100.0
With 3 of “risk”-factors	5* 83.3	1 16.7

*p < 0.019

The distribution of the patients with 3 “risk”-factors according the stage system of Rai was as follow: Stage I – 1 patient, Stage II – 1 patient, Stage III – 2 patients, Stage IV – 1 patient.

DISCUSSION

B-CLL has the most favorable outcome compared to other hematological cancers. Patients with smoldering CLL usually do not receive any therapy. Factors influencing the prognosis are still unclear. Chemotherapy is indicated if a progressive clinical course is observed, including anemia, thrombocytopenia, progressive lymphadenopathy or splenomegaly.

FISH is one of the most powerful and widely used prognostic tools in patients with CLL even though it evaluates a small number of genetic defects. Deletion of 13q14 is reported to be the most common chromosomal abnormality in B-CLL patients. A gene with putative tumor suppressor function has been intensively searched at this region; however, recent studies suggest the existence of cluster of genes as being more likely [1, 11].

Here we have determined the prognostic significance of some molecular factors in B-CLL, using FISH technique for 13q14 deletion and combined analysis of this aberration with WBC, CD38 and ZAP70 status.

We have established high frequency of 13q14 deletion in B-CLL patients – 55.6%. Our result is similar to the published data indicating a 45-56% incidence of 13q14 deletion in B-CLL [6, 12, 18]. According to Hernández et al, the high numbers of 13q14 losses are associated with a worse outcome and biological differences in patients with B-cell chronic lymphoid leukemia [10].

Prognostic value of 13q14 deletion or rearrangement has been analyzed for their ability to predict disease progression. The results suggest significant differences in 13q14 copy number between patients in two age groups, with prevalence of high copy numbers in advanced age (over 70 years) ($p < 0.01$). Deletion and rearrangement of 13q14 (60% and 20 %, respectively) occurred principally in CD38 (+) leukemic cells. Clinical significance of this relation is controversial and unclear. According to some authors, CD38 expression is transient and variable during the clinical course of the disease in 30% of B-CLL [9, 14, 15].

In our study, in most cases del(13)(q14) was accompanied by ZAP70 (+) cell expression. The association between del 13q14 and ZAP70 has had tendency for progression of disease and needs for applying chemotherapy (Tabl.7). Similar to our data is the published by Wiestner A. et al [19]. Prognosis of CLL patients having a combination of 13q14 deletion, ZAP70 (+) status, high WBC and age over 70 years (3 or more “risk” factors) was poor and 83% of them developed progression, compared to those without risk factors – 25%, $p < 0.019$.

CONCLUSION

Patients with 13q14 aberrations were significantly older. They had ZAP (+) status and developed rapid disease progression and therapy dependence.

A determination of 13q14 genetic abnormality should, therefore, be included in the investigations of the prognostic factors of B-cell chronic lymphocytic leukemia.

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