ASSOCIATION OF DELETION 13Q14 WITH CLINICOPATHOLOGIC FEATURES IN CHRONIC LYMPHOCYTIC LEUKEMIA

Ali Ahmed, Ch Altaf Hussain, Mohammad Abdul Naeem*, Hamid Saeed Malik, Maria Khan*, Saqib Hussain Korejo

Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Combined Military Hospital Lahore/National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To determine the frequency of Del 13q14 in Chronic lymphocytic leukaemia, to compare its association with clinic-pathologic features and to define the contribution of this abnormality to the prognosis.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology, Armed Forces Bone Marrow Transplant Centre and Oncology department, Combined Military Hospital Rawalpindi, from Apr 2017 to Jul 2018.

Methodology: A total of 56 newly diagnosed cases of CLL were included in the study. Patients were diagnosed on the basis of National Cancer Institute Working Group guidelines for diagnosis of CLL. After detailed history and thorough clinical examination; complete blood counts, biochemical profile, bone marrow examination, immunophenotyping on bone marrow or peripheral blood samples were done and Interphase FISH studies on blood or bone marrow specimens for detection of Del 13q14 were performed. Clinico-pathological features of CLL patients with Del 13q14 were compared with other cytogenetic abnormalities.

Results: The frequency of Trisomy 12 was found to be 37.5%. Most of CLL patients with Del 13q14 were aymptomatic and were diagnosed on routine workup. The WBC count and Absolute lymphocyte count was slightly lower in patients with Del13 q14 lower when compared with the CLL patients without 13q14 Deletion. Most of the patients with this aberration presented in early stage (Binet stage A) and this association of Del 13q14 with Binet stage was statistically significant (p<0.05). However, no association was found between 13q14 deletion and ZAP70 as all of our patients were negative for this marker. Many patients with Del 13q14 did not require chemotherapy at diagnosis and during follow up as compared to patients without del13q14, and this association was statistically significant (p<0.05). The study also showed that disease progression in patients with deletion 13q14 become significantly less as compared to patient without Del 13q14.

Conclusions: The deletion of 13q14 influence clinical outcome of patients with CLL and was found to be associated with favourable prognosis. A determination of Del 13q14 should therefore be included in the investigations of the prognostic factors of B-cell chronic lymphocytic leukemia. This can prevent unwanted chemotherapy in these patients.

Keywords: Chronic lymphocytic leukaemia, Fluorescence in situ hybridization, Del 13q14, Trisomy 12.

INTRODUCTION

Chronic lymphocytic leukaemia is a chronic lymphoproliferative disorder characterized by a clonal proliferation of mature lymphocytes[1] with distinct immunophenotype (CD5+, CD19+, CD20+, CD23+ and weak sIg). Although, not very common in our region, it is most common form of leukaemia in the Western world[2,3]. CLL is an adult leukemia with incidence increasing with advancing age, and median age at diagnosis of around 70 years. Men are more commonly affected than women, it has male to female of 2:1[4,5].

Clinical course of the disease is highly variable, Most of the patients are asymptomatic while others may have painless generalized lymphadenopathy or less frequently patients may presents with bacterial and viral infections. In some patients CLL may develop into more aggressive Richter's syndrome or Hodgkin's lymphoma[6].

The Rai and Binet staging systems were developed 3 decades back, but still are the backbone for estimating prognosis and indicating therapy. These are based on presence or absence of absolute lymphocytosis, lymphadenopathy, organomegaly (splenomegaly and hepatomegaly) and/or anemia and/or thrombocytopenia[7].

Beside clinical stages (Rai and Binet), Lymphocytes doubling time, IGHV gene mutational status, immunophenotypic prognostic markers (ZAP70, CD49d and CD38) and cytogenetic chromosomal abnormalities are also used to predict the prognosis of the disease. Determination of prognostic factors should be done at the time of diagnosis to predict disease evolution and therapeutic decision making[8].

Chromosomal abnormalities have been observed in many patients at diagnosis[9]. Conventional cytogene-

Correspondence: Dr Ali Ahmed, Haematology Department, Combined Military Hospital, Lahore Pakistan

Received: 15 Jan 2019; revised received: 21 Jan 2020; accepted: 24 Jan 2020

tic analysis reveal Chromosomal Aberrations in only 40-50% of patients. However, Fluorescence in situ hybridization (FISH) analysis on interphase cells can detect chromosomal changes. In approximately 80% of patients with CLL, with the most well-known being Del 13q14.3, Del 11q22.3, trisomy 12, Del 17p13.1 and Del 6q23.

Deletion 13q14 means deletion of a region present on the q (long arm) of 13th chromosome and 14 refers to "region 1, band 4". So, the entire locus is read as "thirteen q one four".miR-15a and miR16-1 are located in this region, these micro RNAs have tumora suppressor function and their expression is frequently decreased in CLL patients. Besides these micro RNAs, other genes located in 13q, such as DLEU7, also cooperate in the tumoral suppressor activity. In addition, 13q lossesmay also involve RB1 tumour suppresor gene. Overall, Del 13q14 in CLL results in loss of tumour suppressor function.

Patients having Del 13q as the sole deformity shows better prognosis, while patients with Del 11q22 or Del 17 phave a poor expectancy and those with trisomy 12 are associated within termediate survival time[10].

The study was conducted to determine the frequency of Del 13q14 in chronic lymphocytic leukemia, its association with clinic-pathologic features and to define the contribution of this abnormality to the prognosis.

METHODOLOGY

It was a cross-sectional study conducted in Haematology department AFIP, AFBMTC and Oncology department CMH Rawalpindi from April 2017 to June, 2018. About 56 newly diagnosed cases of B-CLL of either gender and all ages were included who were presented during the study period. Patient were diagnosed as CLL as per National Cancer Institute Working Guidelines for CLL (Lymphocytosis >5 x 10^9/L, CD19+, CD5+, CD23+, CD20 weak positive and expression of either kappa or lambda), were included using non-probability consecutive sampling technique. Patients already taking treatment and patients who lost to follow up were excluded from the study. Detailed history and examination were done, and investigations were performed. All the patients were classified according to BINET staging system. Fish analysis was performed to detect specific cytogenetic abnormalities. The Laboratory parameters, WBC count, Hb levels, Platelet count, CD38 and ZAP70 status were examined alongwith personal and BINET stage data. Patients were followed up (mean follow up 12 months) fordisease progression or dependence of chemotherapy. Progression of disease here is defined as Stage progression, Increase in B2 microglobulin, LDT<12 months.

Flourescent In Situ Hybridization analysis: Interphase FISH studies were performed on blood or bone marrow specimens processed by standard methods for cultured samples. 3ml sample was collected in sodium heparin tube. It was cultured at 37°C. PHA for 24 hours and then after adding 200ul colchicine for 1 hr. It was then centrifuged at 1500 rpm for 8 min. Supernant was discarded and KCL was added followed by centrifugation. 3 washes were given with glacial acetic acid and methanol (3:1). The clear pellet was placed on the slide. Slide was prepared for FISH by treatment with ascending concentration of alcohol followed by saline sodium citrate (SSC solution). The slide was air dried in a dark place. 10ul of Meta system XL DLEU/TP53 locus-specific probe (13q14) was applied on the target slide. Cover slip was applied and sealed with rubber cement. Denaturation at 74°C for 5 min, hybridization at 37°C for 18 hrs and co-denaturation at 74°C for 5

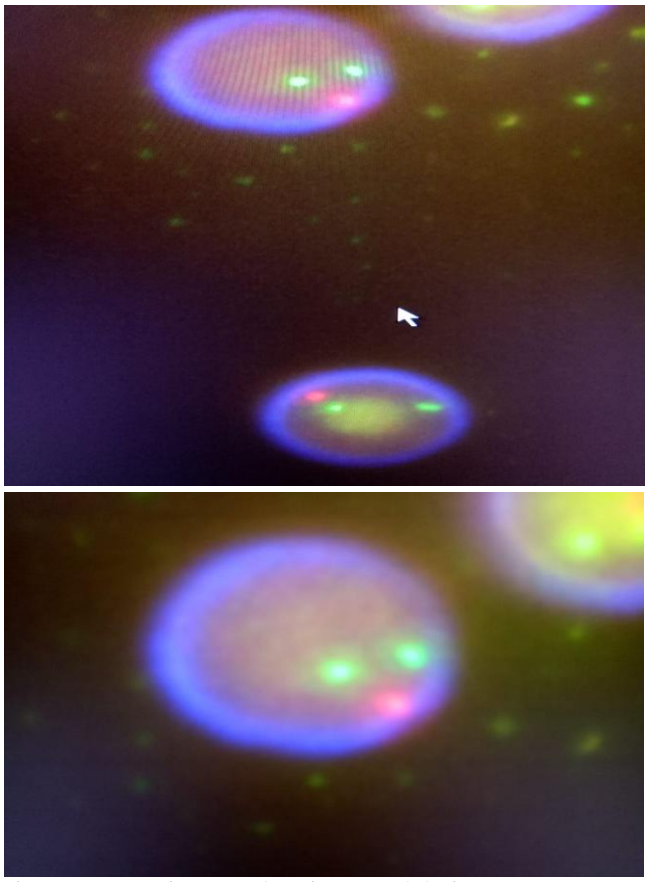

Figure-1: FISH images showing 13q deletion.

were done. The slide was washed and air dried. 10 to 20 ul of DAPI-2 counter stain was added and it was frozen at -20°C for 1 day. A total of 500 nuclei were analyzed per probe set using a fluorescent microscope with an orange green spectrum filter. Positive Del 11q22 was denoted by one green signal and two orange signals. FISH analysis is shown in fig-1.

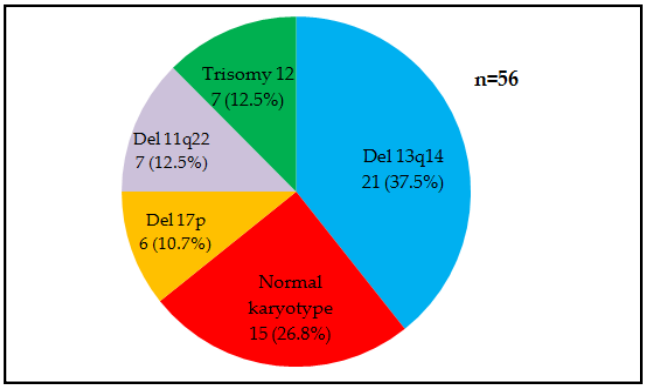

Figure-2: Frequency of Cytogenetic abnormalities (n=56).

Interpretation: The probe (Metasystem XL CLL probe kit) contains two vials of locus specific probes for most common cytogenetic abnormalities for CLL. One of the vial, after hybridization, in normal cell showed 2 orange specific for 13q14 region, 2 green for chromosome 12, and 2 blue signals for 13q34, white cells having 13q14 deletion showed two green (2G), two blue (2B) signals and only 1 orange (1O) suggesting deletion 13q14.

Collected data was entered and analyzed by SPSS-20. Test of normality (kolmogrove and shipro test) were applied. Descriptive statistics (mean IQR) was computed for quantitative non-parametric variables. Frequency and percentages were computed for categorical variable. Test of significance (Pearson chi-square test for qualitative variables and Mann Whitney U test for quantitative variables) was applied to find out the *p*-value ($p<0.05$ was taken as significant).

RESULTS

A total of 56 patients were included in the study. Age of patients ranged between 38-87 years. Out of these, 45 (80.4%) were males and 11 (19.6%) were females. Deletion 13q14 was detected in 21 (37.5%) CLL patients. Most of patients with Del 13q14 were asymptomatic and presented without B-symtpoms, lymphadenopathy and organomegaly. Most of them were diagnosed incidentally on routine blood counts. Clinical characteristics, blood counts, staging and prognostic markers in patients with Del 13q14 are summarized in table-I. CD38 expression was positive in 10 (47.6%) cases. ZAP 70 was negative in all cases.

Laboratory parameters between patients of CLL with and without Del 13q14 were compared. There

Table-I: Clinico-pathologic features of CLL patients with Del 13q14 (n=21).

Symptoms	n(%)
Asymptomatic	14 (67%)
Fever	7 (33%)
Weight loss	-
Physical Findings	
Lymphadenopathy	11 (52.4%)
Splenomegaly	7 (33.3%)
Hepatomegaly	3 (14.3%)
Mean Blood Counts on Presentation	
WBCs count (x 10^9/L)	106 (179-9.3)
ALC (x 10^9/L)	92.86 (234-8.4)
Haemoglobin conc (g/dl)	11.03(15.1-4.9)
Platelets (x 10^9/L)	164 (352-54)
Binet Stage	
A	15 (71.4%)
B	01 (4.8%)
C	05 (23.8%)
CD38 expression	10 (47.6%)
ZAP70	-
Disease progression	08 (38%)
Chemotherapy dependence	08 (38%)

was significant difference among median values of hemoglobin concentration, WBC, Absolute lymphocyte Count (ALC) and Platelet count. Clinico-pathological features with and without Del 13q14 in CLL are summarized in table-II. Patients with Del 13q14 were slightly older as compared to other cytogenetic abnormalities. Median age of diagnosis in patients with Del 13q14 was around 60 years and with other cytogenetic abnormalities were 59 years. However, no significant associationwas found between Del 13q14 and age of the patients.Patients with Del 13q14 presented with low WBC counts as compared to other cytogenetic abnormalities, but this association was not statistically significant.

While assessing Binet stage system, it was found that total 28 patients were staged in Binet stage-A, 5 in Binet stage-B and 23 in Binet stage C. There was a statistically significant association ($p<0.05$) of del 13q14.

Binet staging, ALC (×10^9/L), hemoglobin (g/dl) and B-symptoms showed statistically significant difference with frequency of deletion of 13q14 ($p<0.05$) (table-II).

Table-II: Clinico-pathological features with 13q14 Deletion and without 13q14 Deletion and comparison of median values of lab parameters.

		Deletion with 13q14 Deletion (n=21) (Mean ± SD)	Without 13q14Deletion (n=35) (Mean ± SD)	*p*-value
Age (Range)		58.0 ± 0.56 (48-74 years)	57.0 ± 0.31 (38-87)	
BINET staging	Stage A	15 (71.4%)	13 (37%)	0.046
	Stage B	1 (4.7%)	4 (11%)	
	Stage C	5 (23.8%)	18 (51%)	
ALC (x 10^9/L), Median (IQR)		43.1 (8.4-190)	87.17 (8.4-234)	0.011
WBCs count x10^9/L, Median (IQR)		68.48 (9-204)	99.45 (9.3-234)	0.221
Hemoglobin (g/dl), Median(IQR)		9.92 (7.7-15.10)	111.5 (4.9-15.2)	0.015
Platelets x 10^9/L, Median(IQR)		126.95 (54-352)	84.69 (50-383)	0.106
B-Symptoms (Fever, weight loss)				
Yes		7 (33%)	22 (62.9%)	0.032
No		14 (67%)	13 (37.1%)	
Physical Examination				
Splenomegaly		7 (33%)	8 (22.9%)	0.867
hepatomegaly		3 (14.2%)	4 (11.4%)	
CD38 (Positivity)		10 (45.5%)	24 (68.6%)	0.102

As discussed earlier, all the patients were followed up for a period of 1 year for progression of disease (Stage progression, increase in B2 microglobulin, LDT<12 months) and chemotherapy dependence. Results obtained in patients with deletion of 13q14 were compared with other cytogenetic abnormalities and it was seen that most of the patients with 13q14 deletion did not require chemotherapy at diagnosis and during follow up as compared to other patients in which others abnormalities, and this association was statistically significant (p<0.05). The present study also showed that disease progression in patients with deletion 13q14 became significantly less as compared to patient without Del 13q14 and this association was also statistically not significant (table-III)

Table-III: Relationship of Deletion 13q14 and Disease Progression and chemotherapy dependence and comparison with other cytogenetic abnormalities.

Del 13q14 and Disease Progression				**R.R (95% CI)**
	Disease Progression			
	Yes, n (%)	**No, n (%)**	***p*-value**	
Del 13q14	05 (23.8)	16 (76.2)	0.001	0.34 (0.15-0.77)
Without Del 13q14	24 (68.7)	11 (31.3)		
Total	29 (51.8)	27 (48.2)		
Del 13q14 and Chemotherapy Dependence				**R.R (95% CI)**
	Chemotherapy Dependence			
	Yes, n (%)	**No, n (%)**	***p*-value**	
Del 13q14	05 (23.8)	16 (76.2)	0.001	0.34 (0.15-0.77)
Without Del 13q14	24 (68.7)	11 (31.3)		
Total	29 (51.8)	27 (48.2)		

DISCUSSION

Chronic lymphocytic leukemia has the most favorable outcomes compared to other malignant hematological disorders. Chemotherapy is indicated if a progressive clinical course is observed, including anemia, thrombocytopenia, progressive lymphadenopathy or splenomegaly[11-16]. Many factors discussed earlier influence the prognosis of patients with CLL including chromosomal abnormalities. FISH is one of the most powerful and widely used prognostic tools in patients with CLL and deletion of 13q14 is reported to be the most common chromosomal abnormality in B-CLL patients.

In the present study, deletion of 13q14 was present in 37.5% of the CLL patients. In another study by Rowntree *et al*, deletion of 13q14 was demonstrated in 51.5% of the 64 CLL patients[16]. Some studies have reported that 13q14 deletion is mostly associated with advanced age[5,11]. The present study revealed a patient age range of 38-87 years, with mean age of 60 years whereas Mahmood *et al* have reported a mean age of 64.18 years with age range of 45–75 years[17]. In another study by Teimori *et al* reported mean age as 61.73 years which is almost same as our findings[7]. The present study revealed male to female ratio of 45/11. Similarly the study by Mahmood and Teimori *et al* showed simi-lar findings, males were predominantly affected by CLL[7,17].

Ouillette *et al* studied prognostic significance in chronic lymphocytic leukemia. They concluded that hat 13q14 deletions have varying effects on CLL clini-

cal behavior, with 13q14 deletions associated with aprognostic change at small level. Mehes *et al*[18] suggested that patients with del 13q14 usually presents with moderately high WBC count and normal Hb levels and platelet count, however in our study patients with Del 13q14 presented with Lower WBC count as compared to patients without Del13q14.

Prognostic significance of 13q14 deletion has been documented by many researchers, which suggest that Del 13q14 is associated with good prognosis, so the results of the present study have been supported by many studies. Similar studies were found by Shuhua YiHeng LiZengjun *et al*[19] and other authors[11,20-24]. Orlandi *et al* conducted studies by taking 13q14 deletion as sole diagnostic factor with good prognosis in chronic lymphocytic leukemia[22]. Progression of disease was relatively slow in patients with 13q deletion and this has been reported by many researchers like Gunnarsson *et al* reported, that patients with biallelic deletion (13q) had better survival as compared with patients with monoallelic deletion 13q[23].

CONCLUSIONS

The deletion of 13q14 influence prognosis of patients with CLL and is usually associated with favourable outcome. A determination of 13q14 genetic abnormality should, therefore, be included in the investigations of the prognostic factors of B-cell chronic lymphocytic leukemia. This can lead towards patient improvement and prevent chemotherapy.

CONFLICT OF INTEREST

The study has no conflict of interest to declare by any author.

REFERENCES

1. Byrd JC, Flynn JM. Chronic lymphocytic leukemia. Abeloff's Clinical Oncology: Elsevier; 2014: 1958-78.
2. Frenzel LP, Reinhardt HC, Pallasch CP. Concepts of chronic lymphocytic leukemia pathogenesis: DNA damage response and tumor microenvironment. Oncol Res Treat 2016; 39 (1-2): 9-16.
3. Rodrigues CA, Goncalves MV, Ikoma MR, Lorand-Metze I, Pereira AD, Farias DL, et al. Diagnosis and treatment of chronic lymphocytic leukemia: recommendations from the Brazilian Group of Chronic Lymphocytic Leukemia. Revista brasileira de hematologia e hemoterapia 2016; 38(4): 346-57.
4. Burger JA, Tedeschi A, Barr PM, Robak T, Owen C, Ghia P, et al. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. N Engl J Med 2015; 373 (25): 2425-37.
5. Hayakawa K, Formica AM, Colombo MJ, Shinton SA, Brill-Dashoff J. Loss of a chromosomal region with synteny to human 13q14 occurs in mouse chronic lymphocytic leukemia that originates from early-generated B-1 B cells. Leukemia 2016; 30(7): 1510-19.
6. Tsimberidou AM, Keating MJ. Richter syndrome: biology, inci-dence, and therapeutic strategies. Cancer 2005; 103(2): 216-28.
7. Teimori H, Ashoori S, Akbari MT, Naeini M, Hashemzade Chaleshtori M. FISH Analysis for del6q21 and del17p13 in B-cell Chronic Lymphocytic Leukemia in Iranians. Iranian Red Cres-cent Med J 2013; 15(2): 107-12.
8. Wu SJ, Lin CT, Agathangelidis A, Lin LI, Kuo YY, Tien HF, et al. Distinct molecular genetics of chronic lymphocytic leukemia in Taiwan: clinical and pathogenetic implications. Haematologica 2017; 102(6): 1085-90.
9. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullin-ger L, et al. Genomic aberrations and survival in chronic lympho-cytic leukemia. N Engl J Med 2000; 343(26): 1910-16.
10. Glassman AB, Hayes KJ. The value of fluorescence in situ hybridization in the diagnosis and prognosis of chronic lymphocytic leukemia. Cancer Genet Cytogenet 2005; 158(1): 88-91.
11. Alexandrova K, Velizarova M, Dimova I. Association of 13q14 deletion with clinico-laboratory parameters in B-cell chronic lymphocytic leukemia (B-CLL). Acta Medica Bulgarica 2012; 39: 73-81.
12. Aoun P, Blair HE, Smith LM, Dave BJ, Lynch J, Weisenburger DD, et al. Fluorescence in situ hybridization detection of cyto-genetic abnormalities in B-cell chronic lymphocytic leukemia/ small lymphocytic lymphoma. Leuk Lymphoma 2004; 45(8): 1595-603.
13. Goorha S, Glenn MJ, Drozd-Borysiuk E, Chen Z. A set of commercially available fluorescent in-situ hybridization probes effi-ciently detects cytogenetic abnormalities in patients with chronic lymphocytic leukemia. Genet Med 2004; 6(1): 48-53.
14. Flanagan MB, Sathanoori M, Surti U, Soma L, Swerdlow SH. Cytogenetic abnormalities detected by fluorescence in situ hybridization on paraffin-embedded chronic lymphocytic leukemia/ small lymphocytic lymphoma lymphoid tissue biopsy speci-mens. Am J Clin Pathol 2008; 130(4): 620-27.
15. Bagir EK, Acikalin A, Alsancak P, Paydas S, Gurkan E, Ergin M. Prevalence of cytogenetic abnormalities in chronic lymphocytic leukemia in the southern part of Turkey. Indian J Cancer 2017; 54(3): 572-75.
16. Rowntree C, Duke V, Panayiotidis P, Kotsi P, Palmisano G, Hoffbrand A, et al. Deletion analysis of chromosome 13q14. 3 and characterisation of an alternative splice form of LEU1 in B cell chronic lymphocytic leukemia. Leukemia 2002; 16(7): 1267.
17. Mahmood R, Khan SA, Altaf C, Malik HS, Khadim MT. Clinico-hematological parameters and outcomes in a cohort of chronic lymphocytic leukemia patients with Deletion 17p from Pakistan. Blood Res 2018; 53(4): 276-80.
18. Mehes G. Chromosome abnormalities with prognostic impact in B-cell chronic lymphocytic leukemia. Pathol Oncol Res 2005; 11(4): 205-10.
19. Yi S, Li H, Li Z, Xiong W, Liu H, Liu W, et al. The prognostic significance of 13q deletions of different sizes in patients with B-cell chronic lymphoproliferative disorders: a retrospective study. Int J Hematol 2017; 106(3): 418-25.
20. Ouillette P, Erba H, Kujawski L, Kaminski M, Shedden K, Malek SN. Integrated genomic profiling of chronic lymphocytic leuke-mia identifies subtypes of deletion 13q14. Cancer Res 2008; 68(4): 1012-21.
21. Parker H, Rose-Zerilli MJ, Parker A, Chaplin T, Wade R, Gar-diner A, et al. 13q deletion anatomy and disease progression in patients with chronic lymphocytic leukemia. Leukemia 2011; 25(3): 489-97.
22. Orlandi EM, Bernasconi P, Pascutto C, Giardini I, Cavigliano PM, Boni M, et al. Chronic lymphocytic leukemia with del13q14 as the sole abnormality: dynamic prognostic estimate by interphase -FISH. Hematol Oncol 2013; 31(3): 136-42.
23. Gunnarsson R, Isaksson A, Mansouri M, Goransson H, Jansson M, Cahill N, et al. Large but not small copy-number alterations correlate to high-risk genomic aberrations and survival in chronic lymphocytic leukemia: a high-resolution genomic screening of newly diagnosed patients. Leukemia 2010; 24(1): 211-15.
24. Davids MS, Vartanov A, Werner L, Neuberg D, Dal Cin P, Brown JR. Controversial fluorescence in situ hybridization cytogenetic abnormalities in chronic lymphocytic leukaemia: new insights from a large cohort. Br J Haematol 2015; 170(5): 694-703.