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TO ASSESS THE CORRELATION OF RAI STAGING WITH EXPRESSION OF CD38 AND ZAP70 IN CLL PATIENTS

Pathology

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ABSTRACT

Background: Chronic lymphocytic leukaemia (CLL) is a set of chronic lymphoproliferative disorder which is characterized by abnormal monoclonal proliferation of mature appearing lymphocytes. CLL/SLL represents approximately 20% of all and is the most common type of leukaemia in the western world in B-cell malignancies.

Aim: To evaluate various haematological parameters in peripheral blood, bone marrow and immunophenotyping.

Method: This study was conducted in the Department of Pathology, Government Medical College and Hospital, Chandigarh. Clinical and laboratory characteristics of fifteen treatment naive CLL cases were studied. Bone marrow examination and flow cytometric immunophenotyping were performed in all patients to confirm the diagnosis. The prognostic factors such as RAI staging, CD38, ZAP70 were correlated.

Results: Nine out of 15 cases (60%) were males and 6 (40%) were females, age ranged between 50-70 years with most common presenting complaint of generalized weakness. Mean Hb was 10.8 ± 2.9 g/dl and ranged from 6.3-14.5g/dl and the median smudge cell percentage was 4% and ranged from (2-43%). The most common pattern in bone marrow was diffuse. Maximum cases were in RAI stage III. No significant correlation found between the clinical stage of CLL and CD38 and ZAP70 markers.

KEYWORDS

Chronic lymphocytic leukaemia (CLL), RAI Staging, ZAP70 marker.

INTRODUCTION:

The neoplasms of mature lymphoid cells include the chronic leukaemia lymphoid neoplasm and non-Hodgkin lymphomas.1 The incidence of CLL in USA is about 3.9 per 100,000 per year. In Indian population median age of diagnosis of CLL is 61 years and males are more affected than females.2 Peripheral blood show increase in small mature looking lymphocytes >5000 µL with typical CLL immunophenotype. Bone marrow aspiration and biopsy are not necessary for the diagnosis although the pattern of infiltration reflects the tumour burden and is of prognostic significance. Morphologically, the lymphocytes are small with clumped nuclear chromatin, inconspicuous nucleolus and scant amount of cytoplasm. Rai et al (1975) and Binet et al (1981) staging systems are the standard clinical staging to estimate prognosis of patients. However, both systems fail to indicate the higher risk of progression among patients in early stages of the disease. [3]

Flow cytometry (FC) is a sophisticated instrument measuring multiple physical characteristics of a single cell such as size and granularity simultaneously. Its working depends on the light scattering features of the cells under investigation, which may be derived from dyes or monoclonal antibodies targeting either extracellular molecules located on the surface or intracellular molecules inside the cell.4 FC analysis is an accepted and essential medical practice in the clinical evaluation of lymphoid neoplasm and in addition to being a fast procedure, it can analyze a broader array of antigens than those detectable by conventional, fixed tissue-based immunohistochemistry. It allows a clear-cut correlation of multiple measurements (antigen expressions, DNA content, light scatter) in individual cells and has ability to quantify both population frequencies and level of antigen expression in individual cells.[5]

CLL has different clinic-hematological features and immunophenotypic expressions that vary from patient to patient therefore studying these groups of the patients can provide clues about the presentations and diagnostic findings that can help in management and prognosis.[6]

MATERIAL AND METHODS:

This prospective study included 15 cases diagnosed as treatment naive CLL. Clinico-haematological work up including complete blood count, bone marrow examination and Rai staging was done and were investigated further with flow cytometry. Zap 70, CD38 were also correlated with the stage of the disease. Patient characteristics were described using qualitative and quantitative measures in the terms of mean±SD and percentages and were compared in different subgroups. Differences between groups were evaluated using Chi-square test for qualitative variables.

Bone marrow aspiration and trephine biopsy were performed under aseptic conditions and touch imprints of the biopsy were made before putting it into 10% buffered formalin fixative. Sections were checked for infiltration and its various patterns were also noted. Bone marrow aspirates 2.0-2.5ml were collected in EDTA/Heparin vaccutainers. The samples were run on the BD FACS Calibur FC. Four colour Calibrite beads bought from BD biosciences were used namely unlabelled, FITC, PE, PERCP and APC and were run one by one in the FC using FACS COMP software. The photomultiplier tube (PMT) adjustments and compensation settings were done automatically by this software and this data was stored in instrument setting files. Lyse – wash-Stain- wash method was used. Along with the test sample unlabelled normal and negative control sample was also run. A customized 4 colour panel was used for all the analysis using FITC (Fluorescein Isothiocyanate), PE (R-Phycoerythrin), PerCP (Perdinin chlorophyll protein complex) and APC (Allophycocyanin) labelled BD Biosciences antibodies including CD10, CD19, CD20, CD25, CD103, FMC7, ZAP70, Cyclin D1, sIg kappa, sIg lambda, CD3, CD4, CD5, CD8, CD45, CD38, CD138, CD11c,CD117, CD34 were used whenever required.

Flow cytometric data were acquired using the Cell Quest Pro software (BD Immunocytometry systems, San Jose, California, USA). Instrument settings were done by opening the instrument setting files prior to acquiring the data. Each tube was run on the instrument one by one and 10,000 or more events were acquired from each tube in the form of dot plot. This data was stored as List mode files and was analysed using Cell Quest Pro software. Gating was done and the cursors were set using the normal cells in the sample as internal control and positive control e.g. B cells for T cell markers and vice versa. A marker was considered positive if >20% of the gated cells were positive. The intensity of the marker was also graded using log scale. If the population of interest fell between 101-102 log, the marker was taken as dimly positive. Accordingly, 102-103 was taken as

intermediate expression and 103-104 log scale as bright expression. IHC was done in some cases of bone marrow infiltration. The final diagnosis was made by careful morphological examination of slides and the pattern of marker expression on FC.

RESULTS:

Out of 15 cases of CLL, 9 out of 15 cases (60%) were males and 6 cases (40%) were females and the average age ranged between 50 to 70 years. A male predominance was also observed with male female ratio of 1.50:1 in them. Five cases (33.33%) out of 15 cases of CLL presented with generalized weakness and 3 cases (20%) presented with loss of appetite. On examination lymphadenopathy were noted in 4 out of 15 cases (26.66%) and cervical lymphadenopathy cases were 3(75%) out of the 4. Four cases (26.66%) out of 15 cases of CLL showed hepatomegaly and 7 cases (46.66%) showed splenomegaly [Table 1]. Hb was less than 8 g/dl in 4 cases (26.66%) and >11 g/dl in 8 cases (53.33%). The mean Hb of 15 cases of CLL was 10.8 ± 2.9 g/dl and ranged from 6.3-14.5g/dl. Absolute lymphocyte count >5x109/L was found in all 15 cases (100%) of CLL. Average TLC of 15 CLL cases were 125.2 x 109/L and ranged from 12.7-1180 x 109/L. Out of 15 cases of CLL, smudge cells >30% was seen in 3 cases (20%). The median smudge cell percentage was 4% and ranged from (2-43%). Prolymphocytes were less than 10% in all 15 cases (100%) of CLL. Out of 15 cases of CLL, platelet count <100 x 109/L was seen in 5 cases (33.33%) and 3 cases (20%) presented with>150 x 109/L. The mean platelet count was 121.8 x109/L and ranged from 3- 196 x 109/L. Two (13.3%) cases out of 15 cases of CLL showed dry tap while 13 (86.66%) cases were hypercellular. None out of 15 cases showed >10% prolymphocytes. Fibroblastic proliferation was noted in 6 out of 15 cases (40%). Nine (60%) out of 15 cases of CLL showed diffuse pattern and 5cases (33.33%) showed nodular- interstitial pattern and 1 case (6.66%) showed interstitial pattern. Grade 0 fibrosis was noted in 12 (80%) cases and grade 1 fibrosis in 2 (13.33%) cases. On flow cytometric analysis on bone marrow all the 15 cases of CLL were subcategorized in CD5+ CD10- group. Matutes scoring was done in all the 15 cases and 4 (26.66%) cases showed 5 score and 11(45.45%) cases out of 15 cases showed score 4. All 15(100%) CLL cases showed moderate positive CD19, moderate positive CD5, and bright positive HLADR. In 8 cases CD23 marker was applied and showed moderate positivity in all the (100%) cases. CD79b was applied in 13 cases and 1 case (7.6%) showed dim positivity. CD10 negativity, Cyclin D1 negativity, FMC7 negativity, CD103 negativity, Tdt negativity, CD4 negativity was seen in all (100%) 15 cases. CD45 was done in 12 cases out of 15 cases and showed moderate positivity in all 12 cases (100%). CLL cases were staged according to Rai staging and were correlated with the prognostic markers CD38 and ZAP70. Six out of 15 cases (40%) were positive for CD38 and 4 out of 15 cases (26.66%) were positive for ZAP70. Out of 15 cases 6 cases (40%) were in stage 3 and 4 cases (26.66%) were in stage 4. [Table 2, Fig 1, 2]. No significant correlation was found between the clinical stage of CLL and prognostic markers.

Table.1. Hepatomegaly and splenomegaly in CLL cases (n=15)

Hepatomegaly		Splenomegaly	
Grades(cm)	No of cases (%)	Grades	No of cases (%)
Not palpable	11(73.33)	Not palpable	8(53.33)
≤ 4 cm	4(26.66)	1-3 cm	6(40)
5-7 cm	0	4-8 cm	1(6.66)
>7cm	0	>8 cm	0

Table.2. Correlation of Clinical stage of CLL with Prognostic marker ZAP70 and Cd38

Markers	Stage					Total	P Value
	0	I	II	III	IV		
ZAP70 Positive	1	0	0	1	2	4	0.617
ZAP70 Negative	3	0	1	5	2	11	
CD38 Positive	1	0	0	4	1	6	0.363
Total	4	0	1	6	4	15	
CD38Negative	3	0	1	2	3	9	

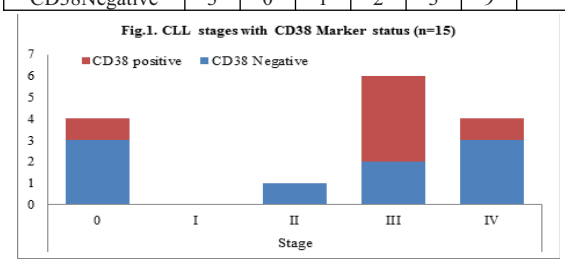


Fig.1. CLL stages with CD38 Marker status (n=15)

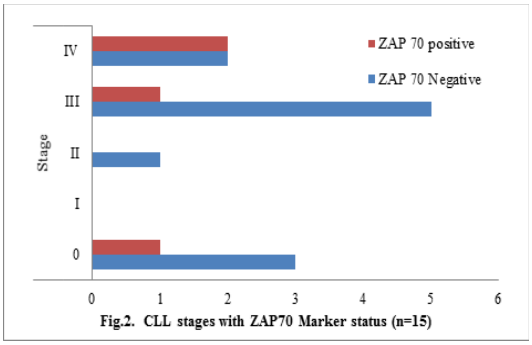
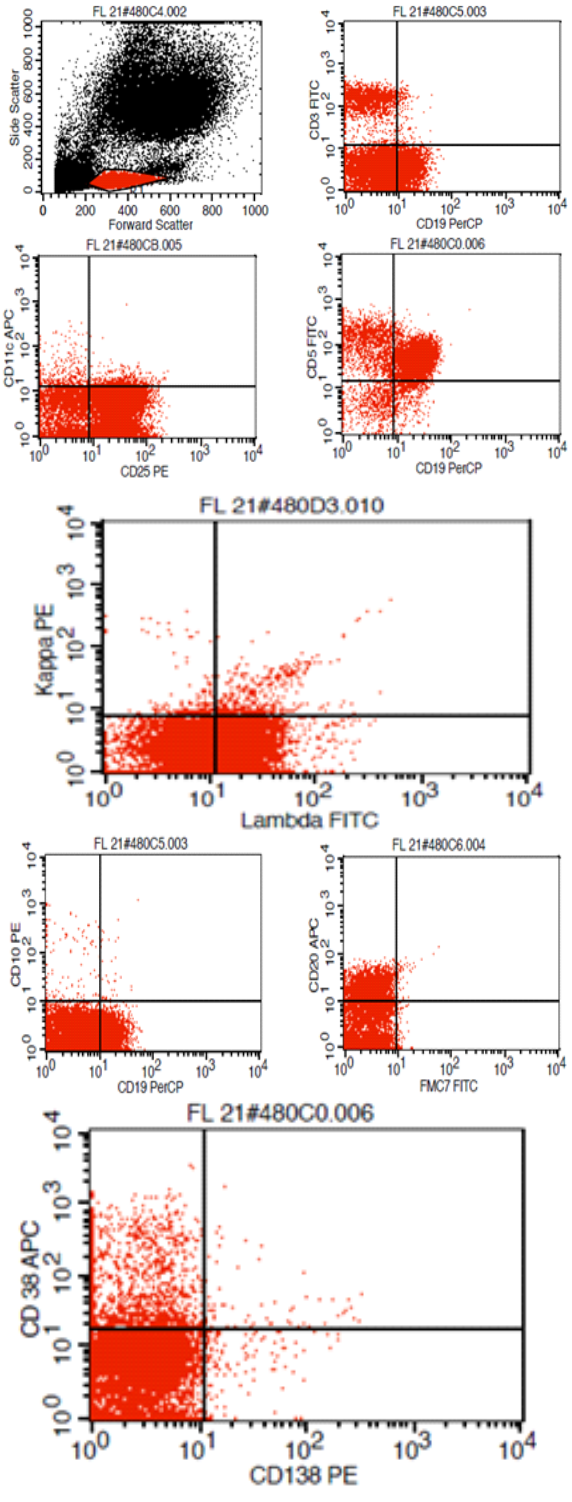


Fig.2. CLL stages with ZAP70 Marker status (n=15)



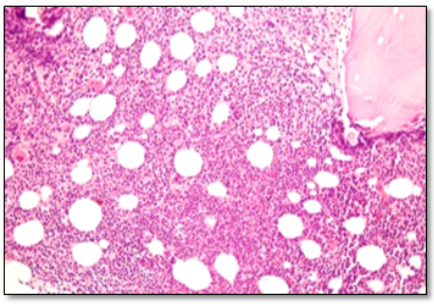


Fig. 3

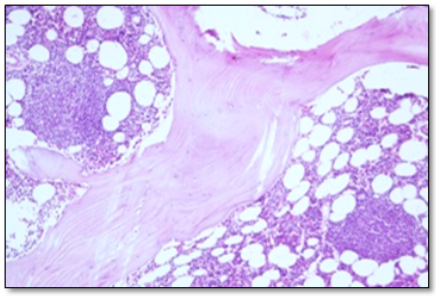


Fig. 4

Fig.3 Diffuse infiltratation pattern in CLL (MGG 200x) and nodulo interstitial pattern in Fig4.(MGG 100x)

DISCUSSION:

In the Indian subcontinent incidence of CLL is 1.7-8.8%. CLL in India is different from the west as it occurs at younger age, have larger spleen and present in high risk Rai stages. It is possible that this more aggressive disease in India may be due to differences in the biology of CLL in India.6 Male predominance was seen, with male female ratio of 1.5:1 which is similar to studies done by Agarwal et al in 2007 where median age of presentation was 61 years with a range from 34-83 years.2 Another study by Sall et al and Jaksic et al showed concordance with our study with median age of 61 and 62 years respectively.[7,8]

Out of 15 cases of CLL in our study 5 cases (33.33%) presented with weakness, 4 cases (26.66%) with lymphadenopathy, 4 cases (26.66%) showed hepatomegaly and 7 cases (46.66%) showed splenomegaly. Study by Agarwal et al showed most common presenting complain as weakness in 57 cases (60%) out of 95 cases which was in concordance with our study.2 The mean Hb of 15 cases of CLL was 10.8 ± 2.9 g/dl and ranged from 6.3-14.5 g/dl. Sall et al studied 40 CLL cases and the mean haemoglobin level was 9.5 g/dl and ranged from 3.9 to 15.2 g/dl which was similar to our study7. Absolute lymphocyte count >5x109/L was found in all 15 cases (100%) of CLL with average TLC of 125.2x109/L and ranged from 12.7-1180x109/L. Sall et al also showed high average lymphocytosis of 186.68x109/L which ranged from 5.03 to 869 x109 /L. Prolymphocytes count in all their cases were below 15% which was similar to our study.7 Smudge cells >30% was seen in 2 cases (20%) with median smudge cell percentage of 4% that ranged from 2-43% in our study which is less than the smudge cell percentages studied by Nowakowski et al, Gogia A et al which was 28% and 29.6% respectively. This was probably due to low sample size of our study.[9,10]

Out of 15 cases of CLL, the mean platelet count was 121.8 x109/L and ranged from 3- 196 x 109/L which was less than that of study by Saxena et al where mean platelets count were 197.8±103.2 x109 /l and ranged from 71-560 x 109/L. Another study by Faria JRD et al showed platelet count <100 x 109/L was seen in 15(20.5%) out of 73 CLL cases which was similar to our study where platelet count <100 x 109/L was seen in 5 cases (33.33%).6,11 Nine (60%) out of 15 cases of CLL showed diffuse pattern and 5 cases (33.33%) showed nodular-interstitial pattern and 1 case (6.66%) showed interstitial pattern which was in concordance with the study by Gafar et al which also showed diffuse pattern as the most common pattern of infiltration.[12]

All 15 cases of CLL were subcategorized in CD5+ CD10- group. All 15(100%) cases showed CD19 moderate positivity, CD5 moderate positivity, HLA DR bright positivity and TDT negativity. In 8 cases CD23 marker was applied and showed moderate positivity in all the 8 (100%) cases. CD79b was applied in 13 cases and 1 case (7.6%) showed dim positivity. Dim Kappa restriction was seen in 10 (66.66%) cases out of 15 cases. 2 (13.33%) cases out of 15 cases showed Dim Lambda restriction. Matutes scoring was done in all the 15 cases and 4 (26.66%) cases showed 5 score and 11 (73.33%) cases out of 15 cases showed score 4 which is similar to study by Dewan K et al, in which CD5 positivity was seen in 90.5% (19 of 21 cases), CD23 positivity was seen in 95.2% (20 of 21 cases). Weak Sm Ig positivity was seen in 100% cases and FMC7 positivity was seen in 33.3% (7 of 21 cases), however FMC 7 was negative in all my cases, and 20% (3 out of 15cases) were negative for both kappa lambda this may be due to paucity of sample.13 No aberrant expression was noted in our study.

Fifteen cases of CLL were staged according to Rai staging and were correlated with the prognostic markers CD38 and ZAP70. Six out of 15 cases (40%) were positive for CD38 and 4 out of 15 cases (26.66%) were positive for ZAP70. Out of 15 cases of CLL in our study, 6 cases (40%) were in stage III and 4 cases (26.66 %) were in stage IV which is similar to a study by Kinawy et al who studied 40 cases of CLL and maximum no of cases were in stage III (20%) and 15 (37.5%) were stage IV. Fourteen Out of 80 cases studied by Gogia et al maximum ZAP70 positivity was seen in 9(11.2%) cases in Rai 0 and I (p=0.24). This is different from our study which showed Zap 70 positivity maximum in Rai stage IV(p=0.61). Also maximum no of our cases, CD38 positivity i.e. 4 cases (26.66%) each was seen in stage 0 and IV (p=0.36) in our study which also was different to study by Gogia et al who had maximum no of cases in Stage II (p=0.13).10 However ZAP70, CD38 failed to be statistically significant with the stage of CLL in our case.

CONCLUSION:

Nine out of 15 cases (60%) were males and 6 cases (40%) were females and the average age ranged between 50 to 70 years with most common presenting complaint of generalized weakness. The mean Hb of 15 cases of CLL was 10.8 ± 2.9 g/dl and ranged from 6.3-14.5g/dl and the median smudge cell percentage was 4% and ranged from (2-43%). The most common pattern of infiltration in the bone marrow was diffuse pattern. Most of our cases were in advanced clinical stage. No significant correlation was found between the clinical stage of CLL and CD38 and ZAP70 markers.

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