

Clinico-haematological profile of chronic lymphoproliferative disorders in patients presenting with lymphocytosis

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Abstract

Objective: To assess the spectrum and clinico-haematological profile of chronic lymphoproliferative disorders in patients presenting with lymphocytosis.

Method: The cross-sectional, retrospective study was conducted at the Aga Khan University Hospital, Karachi, and comprised data related to cases of bone marrow aspirate and trephine from January to November 2020. Patients for whom the bone marrow was done for lymphocytosis were studied for the presence of lymphoproliferative disorders, sub-types and patients' characteristics. The diagnosis and classification were based on the World Health Organisation criteria for tumours of haematopoietic and lymphoid tissues. Data was analysed using SPSS 21.

Results: Of the 3,334 bone marrow specimens tested, 103(3%) were related to lymphocytosis. Of these, 84(82%) were diagnosed with lymphoproliferative disorders, while diagnosis remained undetermined in 19(18%) cases. Male:female ratio was 3.6:1 and median age was 60 years (range: 21-85 years). Constitutional symptoms were found in 61(73%) patients. Median absolute lymphocyte count was $45 \times 10^9/L$ (range: 5.3-480). All 84(100%) patients were classified as B-cell lymphoproliferative disorder. Chronic lymphocytic leukaemia was the most common form, 61(73%), and 31(51%) of them presented with advanced stage disease.

Conclusion: A huge majority of patients presenting with lymphocytosis had underlying lymphoproliferative disorders of which B-cell chronic lymphocytic leukaemia was found to be the most common.

Keywords: Lymphoproliferative disorder, Lymphocytosis, Chronic lymphocytic leukaemia, Bone marrow, Pakistan.

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Introduction

Chronic lymphoproliferative disorders (LPDs) are a diverse group of disease, characterised by slow proliferation of lymphocytes in blood-forming tissues, such as the bone marrow, and subsequent release into the blood stream, leading to leucocytosis in adults.¹ Chronic LPDs are derived from the clonal expansion of either B cells, T cells, natural killer (NK) cells or precursors of these cells. It usually presents in the sixth to seventh decade of life, but can be seen in all age groups and has a notable male gender predilection.² The incidence of chronic LPDs varies by race and geographical location, but B-cell LPDs are more common, accounting for 80-85%, than T-cell LPDs that account for <15% worldwide.³ In a population-based study in the United States, chronic lymphocytic leukaemia (CLL) and diffuse large B-cell lymphoma (DLBCL) were reported to be the most common among indolent and aggressive lymphomas, respectively.⁴

In Pakistan, the annual incidence of non-Hodgkins chronic lymphomas is reported to be up to 8.4 per 100,000 and majority are B-cell lymphomas (BCLs).⁵⁻⁷ They manifest clinically with a variety of symptoms depending on their histological subtype, aggressive versus indolent, and the site of involvement, exhibiting significant patient-to-patient variation. Patients may present with localised or generalised lymphadenopathy, hepatosplenomegaly, cytopenias or constitutional symptoms.⁸ However, in some patients, lymphocytosis (absolute lymphocyte count $>5 \times 10^9/L$) with characteristic morphology is the only presenting feature.¹ Although the diagnosis is made on lymph node (LN) or tissue biopsy, in cases presenting with lymphocytosis, blood or bone marrow examination and flow cytometry might be sufficient for making the diagnosis.⁸ Several chronic B-cell and T-cell LPDs present with lymphocytosis, and careful morphological examination of peripheral blood can give the first clue to the type of underlying LPD, followed by bone marrow examination and flow cytometry for definitive diagnosis. Most commonly, CLL presents with lymphocytosis, but up to a third of other chronic LPDs can have high lymphocyte count as well.⁹

There is limited data on the subtypes of chronic LPD with respect to those presenting with lymphocytosis in Pakistan.

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The current study was planned to fill the gap by assessing the spectrum of chronic LPDs in patients presenting with lymphocytosis on bone marrow examination in a tertiary care setting.

Materials and Methods

The cross-sectional, retrospective study was conducted at the Haematology and Transfusion Medicine section of Aga Khan University Hospital (AKUH), Karachi, and comprised data related to cases of bone marrow aspirate and trephine from January to November 2020. After receiving exemption from the institutional ethics review committee, the sample size was calculated with 95% confidence level, taking 7.6% margin of error and estimated population proportion of 19.1%.¹⁰ The sample was raised using non-probability consecutive sampling technique.

Data of bone marrow aspirate and trephine was retrieved from the electronic integrated laboratory management system. Data of patients of all ages and both genders for whom bone marrow was done for the evaluation of persistent lymphocytosis (≥ 3 months) were included and studied for the presence of chronic LPD, sub-types of LPD, presenting symptoms and patient characteristics. Data of patients who were already diagnosed as LPD on LN/tissue biopsy, those for whom the bone marrow was done for staging workup, and those with history of prior chemotherapy was excluded.

Lymphocytosis was defined as a lymphocyte count $\geq 5 \times 10^9/L$.¹ All peripheral blood and bone marrow aspirate smears had been examined after Leishman staining and haematoxylin and eosin (H&E) staining was done on trephine sections. The diagnosis and classification of chronic LPD were based on morphological and immunohistochemical (IHC) criteria proposed by the World Health Organisation (WHO) for tumours of haematopoietic and lymphoid tissues.³ Immunophenotyping by flow cytometry was not performed due to limited resources. The diagnosis had been made by two experienced haematopathologist. IHC panel consisted of lineage-specific markers, including cluster of differentiation

(CD)79a, CD20, CD10, Paired Box 5 (PAX5), CD23 for B-cells; CD3, CD4, CD5, CD8 for T-cell; CD117, CD68, myeloperoxidase (MPO) for myeloid cells. Other markers included were Kiel-67 (Ki67), cyclin D1, B-cell lymphoma 2 (BCL2),

BCL6, CD56, Epstein-Barr virus (EBV), kappa and lambda. A positive control had been included for each IHC for assessing quality staining.

The term "undetermined" was used by the pathologists for cases where in the given bone marrow specimen either the possibility of LPD could not be ruled out or where there was high suspicion of LPD, but was not enough diagnostically.

Direct antiglobulin test (DAT) had been performed in cases with anaemia, which was defined as haemoglobin (Hb) less than the reference range and where peripheral smear findings were suggestive of haemolysis.

Data was analysed using SPSS 21. Data was expressed as mean \pm standard deviation for quantitative variables, while qualitative variables were presented as frequencies and percentages.

Results

Of the 3,334 bone marrow specimens tested, 103(3%) were related to lymphocytosis. Of these, 84(82%) were diagnosed with LPDs. Male:female ratio was 3.6:1 and median age was 60 years (range: 21-85 years). Constitutional symptoms were found in 61(73%) patients. Median absolute lymphocyte count was $45 \times 10^9/L$ (range: 5.3-480). All 84(100%) patients were classified as B-cell LPD. Chronic lymphocytic leukaemia was the most common form 61(73%) (Table 1), and 31(51%) of them presented with advanced stage disease (Table 2). There was no significant association between the stage of the disease and patients' age ($p > 0.05$)

Diagnosis remained undetermined in 19(18%) patients, and in such cases histopathological examination of tissue/LN and immunophenotyping by flow cytometry was advised to determine clonality. The mean age of these patients was 60 ± 11.38 years. Presenting mean lymphocyte count was $35 \pm 32 \times 10^9/L$, Hb 12.9 ± 3.07 gm/dl, platelets $156 \pm 71 \times 10^9/L$, constitutional symptoms were found in 14(87.5%), lymphadenopathy in 5(31.3%) and hepatosplenomegaly in 3(18.8%).

Table-1: Spectrum of chronic lymphoproliferative disorder (LPD) and patient characteristics.

Type of LPD	Median Age (Years)	M/F n	Haemoglobin (gm/dl)	WBC ($\times 10^9$)	Absolute Lymphocyte Count ($\times 10^9$)	Platelet Count ($\times 10^9$)	Constitutional Symptoms n (%)	Lymphadenopathy n (%)	Hepato-splenomegaly n (%)
Chronic Lymphocytic leukaemia	59 (32-85)	48/13	11.2 \pm 2.6	124 \pm 117	109 \pm 109	184 \pm 102	41 (89)	16 (35)	11 (24)
Mantle Cell Lymphoma	60 (40-81)	9/4	8.5 \pm 2.5	53 \pm 58	41 \pm 46	154 \pm 122	11 (85)	5 (45)	6 (54)
Prolymphocytic Leukaemia	67 (60-75)	2/1	9.0 \pm 2.3	193 \pm 204	164 \pm 168	151 \pm 105	2 (67)	2 (67)	1 (33)
Hairy cell leukaemia	56 (52-60)	2/0	6.7 \pm 4.4	13 \pm 0.07	7.4 \pm 2.9	112 \pm 124	2 (100)	2 (100)	1 (50)
Follicular lymphoma	45	1/0	8.1	15	10	70	1 (100)	0	1 (100)
Lymphoplasmacytic Lymphoma	63	1/0	7.6	37	19	104	1 (100)	0	1 (100)
Diffuse Large B-cell Lymphoma	49 (30-68)	2/0	12 \pm 3.8	56 \pm 53	40 \pm 47	243 \pm 4.9	2 (100)	1 (50)	1 (50)
Burkitt's Lymphoma	21	1/0	7.5	20.8	8.6	42	1 (100)	0	0

M: Males, F: Females, WBC: White blood cell count.

Table-2: Distribution of CLL patients according to age and Rai disease stage.

Age (years)	<60	≥60	Total	p-value
Stage 0	7	8	15	0.738
Stage I	6	1	7	0.19
Stage II	5	3	8	0.670
Stage III/IV	14	17	31	0.265
Total	32	29	61	

CLL: Chronic lymphocytic leukaemia.

Of the undetermined cases, strong suspicion of T-cell LPD (T-LPD) was found in 1 (6.25%) case. The patient was a 76-year-old male, with absolute lymphocyte count (ALC) of $98 \times 10^9/L$, had B-symptoms, but no visceromegaly or lymphadenopathy.

Direct antiglobulin test (DAT) was performed on 23 (22.3%) cases. Autoimmune haemolytic anaemia (AIHA) was found in 2 (8.7%) patients; 1 (50%) had CLL and 1 (50%) was among the undetermined cases.

Bone marrow examination was helpful in making the diagnosis in 84 (82%) cases presenting with lymphocytosis.

Discussion

Mature B-cell and T-cell/NK cell neoplasms are quite diverse entities encompassing more than 30 different subtypes of lymphomas alone in the BCL category, according to the 2016 WHO classification.³ These subtypes extremely vary from each other not only in the context of diagnostic parameters, but, most importantly, age group, risk stratification, prognosis and treatment. For instance, some might be indolent where only surveillance is indicated, while others being so aggressive that chemotherapy and haematopoietic stem cell transplant is the only therapeutic option, such as for Burkitt lymphoma. Mature B-cell and T/NK cell lymphoma is conventionally a tissue diagnosis, made by the histopathological evaluation of enlarged LN or of any presumptive neoplastic tissue identified on radiological scans.¹¹ However, a persistently increased lymphocyte count on peripheral blood is a reason of utmost importance to evaluate the bone marrow primarily.

In the present study, bone marrow biopsy was performed for the evaluation of lymphocytosis with the wide range of absolute lymphocyte count, from as low as 5.3 to $480 \times 10^9/L$, whereas the range of patients' age varied from as young as 21 to as high as 85 years. Most chronic and mature LPDs with lymphocytosis present in older age, with few exceptions, like Burkitt lymphoma that usually presents in early childhood or young adults and is one of the most aggressive lymphomas.^{2,12} Its leukaemic variant presents purely as leukaemia with involvement of bone marrow and peripheral blood. In the current study, the diagnosis of Burkitt lymphoma was made in the youngest patient aged

21 years who presented with high lymphocyte count without any palpable lymphadenopathy or visceromegaly.

On presentation, three-fourth of the patients had constitutional symptoms which was higher than earlier reports (5-54.8%).^{1,13} Constitutional symptoms are considered markers of advanced stage disease. It was observed that almost half of the patients of CLL were in advanced stage. Although staging of the other LPDs were not included in the study, it is possible that advance stage contributed to high frequency of constitutional symptoms.

Most chronic LPDs are reported to be predominantly found in men except for follicular lymphoma that has slight preponderance for women. This study also showed male predominance like that reported in local and international studies.^{2,13} This study also showed that male gender was an independent risk factor for lymphoma. This same risk factor is valid for the whites as well, but their male-to-female ratio is comparatively low at 2:1.²

In the present study, CLL was the most common cause of lymphocytosis. The median age, haematological and clinical profiles were found to be comparable with the published data.¹³ The number of patients found to be at advanced stage (stage III/IV) was slightly higher than of those who presented in low-risk and early stage. However, the difference was not significant with respect to age and stage of the disease. This finding is in line with a national study, and stresses that upfront chemotherapeutic options should be offered to these patients.¹³ However, this finding is in disparity with the white racial population who tend to present in early-stage disease on diagnosis.¹⁴ The plausible explanation of more advanced stage disease in the current study could be attributed to delay in seeking medical attention due to financial constraints, lack of diagnostic facilities, genetic variation, and environmental factors. CLL diagnosis does not always require bone marrow biopsy, but flow cytometry is an expensive diagnostic modality and is not readily available in Pakistan. Therefore, physicians prefer to get CLL-specific IHC done on bone marrow biopsy. With easy availability of flow cytometry, decline in bone marrow biopsy for CLL is anticipated.

Mantle cell lymphoma was found to be the second most common cause for lymphocytosis. The updated WHO classification of 2016 recognises classical and leukaemic non-nodal subtypes. The later presents with lymphocytosis and the diagnosis can easily be made by examination of peripheral blood and bone marrow.¹⁵ The frequency of Mantle cell lymphoma was high compared to that reported from India, China and the United States, but the age of presentation was comparable.^{16,17}

Prolymphocytic leukaemia (PLL) is a rare and distinct entity characterised by more than 50% of circulating prolymphocytes exhibiting central prominent nucleolus and the cells are twice the size of lymphocytes. It usually presents with high white cell count and has a relatively aggressive course. The diagnosis is usually made by excluding other lymphomas, as there is no specific marker for PLL. However, combining morphology, immunophenotype and molecular studies reliably confirms the diagnosis. The current study identified three cases of PLL, all presented with hyperleucocytosis, lymphadenopathy and hepatosplenomegaly. All cases were negative for CD5 and CD23. These findings are in concordance with earlier reports.¹⁸

The preponderance of other lymphomas, like follicular lymphoma, DLBCL, hairy cell leukaemia, lymphoplasmacytic lymphoma and Burkitt cell lymphoma, turned out to be quite low in the current cohort. The frequency of these lymphoma might not be truly reflective of their actual incidence, as most of them rarely involve bone marrow, except hairy cell leukaemia and Burkitt cell lymphoma.

Interestingly, two cases of DLBCL were diagnosed which presented with raised lymphocyte count. One of the patients was concomitantly found to have paraspinal mass, the biopsy of which was consistent with DLBCL. Peripheral blood involvement in DLBCL is very rare. This explains low number of DLBCL in the current study. Sovani V. et al also reported a high discordance between bone marrow and peripheral blood involvement.¹⁹

AIHA has a frequent association with non-Hodgkin's as well as Hodgkin's lymphoma. In CLL alone, AIHA has a reported frequency of up to 20% and has a relatively poor prognostic value.²⁰ The current study found a low percentage (2%) of DAT-positive AIHA of which one case was associated with CLL.

The current study has several limitations. Firstly, the diagnosis remained undetermined in 19(18%) cases with lymphocytosis which could represent reactive cause for increased lymphocyte count, such as infections, drugs or autoimmune disorders. Other reasons may include inadequate length of bone trephine section (<1cm) to assess presence of lymphoid infiltration, expression of only pan B-cell (CD20) marker with no expression of other markers (probable marginal zone origin or monoclonal BCL). In these cases, viral serology, autoimmune workup, flow cytometry, cytogenetics and molecular studies could have helped in making a definitive diagnosis.^{21,22} Secondly, suspicion of T-cell LPD was raised in just one case. T-cell LPDs are less common than B-cell LPDs, and show marked

geographical and racial variations. Additionally, neoplastic T-cells show marked morphological variation and are difficult to characterise because of variable or aberrant expression of T-cell antigens and their relationship to the normal sequence of T-cell maturation is far from clear.²³ Most have nodal or extra-nodal involvement and in rare cases involve bone marrow and peripheral blood. Moreover, certain infectious aetiology can simulate lymphoma. Due to these limitations and lack of flow-cytometric evaluation due to cost constraints, the current study applied restrictive diagnostic approach towards these cases and histopathological examination of LN was advised. Lastly, correlation with treatment outcome was not determined.

Despite the limitations, the current study highlighted a few important aspects of chronic LPDs presenting with lymphocytosis. In majority of the cases, bone marrow examination was helpful in making the diagnosis. Since CLL was found to be the commonest entity, referring physicians can alter their diagnostic approach by choosing flow cytometry on peripheral blood rather than opting for invasive procedures. Multidisciplinary approach incorporating all diagnostic modalities should be undertaken to alleviate misdiagnosis and proper characterisation of chronic LPDs and clonality of lymphocytosis.

Conclusion

A huge majority of patients presenting with lymphocytosis had underlying LPDs of which B-cell chronic lymphocytic leukaemia was found to be the most common. Bone marrow examination was found to be helpful in making diagnosis in majority of cases with lymphocytosis.

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Conflict of Interest: None.

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