

Association of serum protein electrophoresis with clinicopathological characteristics and its prognostic relevance in chronic lymphocytic leukaemia patients

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Abstract

Objective: To assess the association of serum protein electrophoresis abnormalities with clinicopathological characteristics, and its impact on overall survival in chronic lymphocytic leukaemia patients.

Methods: The prospective study was conducted at Haematology and Immunology departments of the University of Health Sciences, Lahore, Pakistan, from 2019 to 2022, and comprised newly diagnosed chronic lymphocytic leukaemia patients. Lactate dehydrogenase and beta-2 microglobulin levels were measured by spectrophotometric principle, whereas serum protein electrophoresis was determined through commercially available capillary electrophoresis systems. Patients were followed up for 2 years post-diagnosis. Data was analysed using SPSS 21.

Results: Of the 50 patients, 40(80%) were males and 10(20%) were females. The overall mean age was 60±11 years. Serum protein electrophoresis was available for 40(80%) patients, and, among them, 12(30%) patients had abnormal levels, while 29(72.5%) required treatment. Overall response rate was 25(86.2%), and median two-year overall survival was 16.5 months (95% confidence interval: 10-20 months).

Abnormal serum protein electrophoresis was significantly associated with Binet stage C, lower mean haemoglobin levels and higher median levels of lactate dehydrogenase and beta-2 microglobulin ($p<0.05$). Regarding overall survival, the survival curves of chronic lymphocytic leukaemia patients with normal and abnormal serum protein electrophoresis status differed significantly ($p=0.04$).

Conclusion: Abnormal serum protein electrophoresis could be considered a surrogate marker for advanced chronic lymphocytic leukaemia disease.

Key Words: CLL, Serum protein electrophoresis, Overall survival, Pakistan.

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Introduction

Chronic lymphocytic leukaemia (CLL) is characterised by clonal expansion of small relatively monomorphic B lymphocytes in peripheral blood, bone marrow and other secondary lymphoid organs.^{1,2} CLL represents a rare haematological malignancy in Asians (<5% of all leukaemia), whereas it is 10-fold more common in the Western hemisphere, accounting for >30% of all leukaemia cases.³

The clinical course of CLL patients is heterogeneous in nature. Some patients experience an aggressive disease

progression, while others have an indolent course of disease. Clinico-haematological, biochemical and genetic features play a significant role in determining the clinical course and in predicting the treatment response and prognosis. Studies have shown that the standard Rai⁴ and Binet⁵ clinical staging systems are not very efficient in predicting prognosis of patients, especially in the early stage of the disease. Therefore, there is a need to identify more markers that may improve the existing staging systems for better prediction and risk stratification of CLL patients at diagnosis.^{2,6}

Numerous studies have reported that the variety of molecular abnormalities seen in CLL patients, which are ultimately responsible for the development and progression of this illness, reflect clinical heterogeneity.^{7,8} However, for cytogenetic and molecular analysis of CLL, high degree of technical and medical expertise is required for each and every case, and the process is laborious and costly. To overcome these difficulties, many different biological laboratory markers such as beta-2 (β^2) microglobulin, serum soluble cluster of differentiation CD23, CD38 and zeta-chain-associated protein kinase (ZAP70), have been evaluated as prognostic markers in

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previous studies. Currently, these biomarkers are seldom used in the standard staging and follow-up of CLL patients.⁹

Among biological parameters, serum protein electrophoresis (SPE) has been studied and recommended for the initial staging of CLL patients. Different patterns on SPE can be detected that include either hypogammaglobulinaemia or hypergammaglobulinaemia, and immunoglobulin peaks, that are all considered abnormal SPE. Hypogammaglobulinaemia, the most prevalent among all abnormal SPE patterns, is assumed to be a predictor of infection and is correlated to tumour burden.¹⁰ Different prevalence rates of Hypogammaglobulinaemia, and monoclonal paraprotein have been reported.^{11,12} Similarly, the prognostic significance of abnormal SPE has been studied in CLL, but has shown contradictory findings regarding infection risk, treatment-free survival (TFS) and overall survival (OS).^{10,13,14}

Since there is a lack of data regarding frequency of SPE abnormalities and its prognostic significance in local CLL patients, the current study was planned to fill the gap by assessing the association of SPE abnormalities with clinicopathological characteristics, and its impact on OS in CLL patients.

Patients and Methods

The prospective study was conducted at the Haematology and Immunology departments of the University of Health Sciences (UHS), Lahore, Pakistan, from 2019 to 2022. The sample was raised using convenience sampling technique from among CLL patients presenting at the Institute of Nuclear Medicine & Oncology Lahore (INMOL) Cancer Hospital, Lahore, Pakistan. Those included were diagnosed CLL cases with at-least 2-year follow-up. The CLL diagnosis was done according to the International Workshop on Chronic Lymphocytic Leukaemia (IWCLL) criteria that is based on persistent lymphocytosis, typical lymphocyte morphology on peripheral blood smears and immunophenotyping results.²

Patients with relapsed/refractory CLL, and acute or chronic lympho-proliferative disease were excluded, and so were those with a history of previous treatment.

The sample size was calculated as per the guidelines of the World Health Organisation (WHO) using the formula:

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d^2}$$

In the formula, $Z_{1-\alpha/2}$ was 95% confidence level 1.96, P was the anticipated frequency of abnormal SPE in CLL = 7%¹⁵,

d was the margin of error = 7%, and n was the required sample size = 51.

After approval from the UHS ethics review committee, and informed consent from all the patients, baseline clinical and laboratory characteristics were retrieved from the patients' charts. Clinical stage of the patients was determined using the Binet staging system⁵. History and physical examination was conducted with help of clinical haematologist of INMOL Hospital, and data was noted on a predesigned proforma. Patients were followed up from the time of diagnosis to death or last follow-up, which was up to a maximum of 2 years, and was termed OS. Treatment indications and response assessments were done in line with IWCLL guidelines.²

Peripheral blood samples were collected at baseline before the initiation of treatment. Of the 5ml venous blood sample collected by a trained phlebotomist using aseptic technique, 3ml was dispersed in ethylenediaminetetraacetic (EDTA) acid vacutainer for complete blood count (CBC) and peripheral smear examination, while 2ml was kept in plain vacutainers for serum separation.

Lactate dehydrogenase (LDH) and β 2-microglobulin levels were measured using spectrophotometric principle/enzyme-linked immunosorbent assay (ELISA) through a commercially available kit (Randox, and R&D Systems, United States, respectively).

SPE was determined using commercially available Capillarys Protein (E) 6 in vitro diagnostic kit (Sebia Inc., US) on Capillarys2 flex piercing system (Sebia Inc., US). Normal concentration of gammaglobulin ranged 0.7-1.5g/dl, according to the manufacturer's direction.

Patients were divided into normal SPE group, and with abnormal SPE group with Hypogammaglobulinaemia, and hypergammaglobulinaemia patterns.

Data was analysed using SPSS 21 and GraphPad Prism 8.0. Data was expressed as frequencies and percentages, or mean \pm standard deviation, or median with interquartile range (IQR), as appropriate. Chi-square, Fisher's exact, student's t-test and Mann-Whitney U tests were used, as appropriate. Cox proportional hazard model was used to assess the association between 2-year OS and predictor variables. Survival curves were plotted using Kaplan-Meier estimates, and log-rank statistics were used to evaluate the differences between prognostic factors and

2-year OS. Two-sided $p < 0.05$ was considered statistically significant.

Results

Of the 50 patients, 40(80%) were males and 10(20%) were females. The overall mean age was 60 ± 11 years. Clinical and demographic data was collected in detail (Table 1).

Table-1: Demographic and clinical characteristics of the patients (n=50).

Characteristics	CLL patients
Demographic	
Mean age (\pm SD)	60 (\pm 11) years
Age \leq 55 years	20 (40%)
Gender, n (%)	
Female	10 (20%)
Male	40 (80%)
Clinical	
B symptoms, n (%)	35 (70%)
Fever, n (%)	20 (40%)
Weight loss, n (%)	22 (44%)
Night sweat, n (%)	03 (6%)
Family history, n (%)	03 (6%)
Lymphadenopathy, n (%)	27 (54%)
Organomegaly, n (%)	22 (44%)
Binet stage, n (%)	
A	14 (28%)
B	11 (22%)
C	25 (50%)
Bone marrow Infiltration, n = 27 (%)	
Non-Diffuse	11 (40.7%)
Diffuse	16 (59.3%)
Typical/atypical morphology, n (%)	
Typical	45 (90%)
Atypical	05 (10%)

CLL: Chronic lymphocytic leukaemia, SD: Standard deviation.

Table-2: Laboratory parameters.

Laboratory	
Median TLC (IQR)	102.35 (184.78) \times 10 ⁹ /L
Median ALC (IQR)	89.30 (162) \times 10 ⁹ /L
Lymphocytosis \geq 30 \times 10 ⁹ /L	37 (74%)
Median ANC (IQR)	05 (4.96) \times 10 ⁹ /L
Mean Hb (\pm SD)	10.92 (\pm 2.63) g/DI
Anaemia (Hb \leq 10g/dL)	22 (44%)
Mean platelet count (\pm SD)	148.84 (\pm 73.42) \times 10 ⁹ /L
Thrombocytopenia (Platelets \leq 100 \times 10 ⁹ /L)	13 (26%)
Median β 2 microglobulin (IQR)	3.16 (2.20) mg/L
Elevated β 2 microglobulin \geq 3.5 mg/L	18 (36%)
Median LDH (IQR)	334 (221.25) U/L
SPE, n= 40 (%)	
Normal SPE	28 (70%)

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Abnormal SPE	12 (30%)
Del 17p, n=17 (%)	
Not detected	15 (88.2%)
Detected	02 (11.8%)

ALC: Absolute lymphocyte count, ANC: Absolute neutrophil count, CLL: Chronic lymphocytic leukaemia, DI 17p: Deletion 17p, Hb: Haemoglobin, IQR: Inter-quartile range, LDH: Lactate dehydrogenase, SD: Standard deviation, TLC: Total leukocyte count, SPE: Serum protein electrophoresis.

SPE values were available for 40(80%) patients, and, among them, 12(30%) had abnormal levels (Table 2). Treatment was required in 29(72.5%) cases, while overall response rate was 25(86.2%), with 4(13.8%) failures (Table 3). Median 2-year OS was 16.5 months (95% confidence interval [CI]: 10-20 months).

Table-3: Treatment regimen and treatment response (n=40).

Therapeutic	
Treatment status	
Untreated	11 (27.5%)
Treated	29 (72.5%)
Treatment regime, n = 29 (%)	
Intensive chemoimmunotherapy (Fludarabine, Cyclophosphamide and Rituximab (FCR), Bendamustine and Rituximab (BR), Fludarabine and Cyclophosphamide (FC)/Fludarabine (F)	11 (38%)
Non-intensive chemoimmunotherapy (Chlorambucil with Deltacortil or Rituximab)	09 (31%)
Others (CVP/CHOP with or without R and Ibrutinib)	09 (31%)
Treatment response at 6 months, n = 29 (%)	
Complete response	14 (48.3%)
Partial response	11 (37.9%)
Treatment failure	04 (13.8%)

CVP: Cyclophosphamide, vincristine and prednisolone, CHOP: Cyclophosphamide, doxorubicin, vincristine and prednisolone.

Table-4: Baseline clinical prognostic factors stratified by SPE status in CLL patients (n=40).

Characteristics	Normal SPE (n = 28)	Abnormal SPE (n = 12)	p-value ^a
Males (n, %)	22 (78%)	11 (91%)	0.65
Presence of B symptoms, n (%)	18 (64%)	10 (83.8%)	0.28
Lymphadenopathy, n (%)	14 (50%)	08 (66.6%)	0.49
Organomegaly, n (%)	12 (42.8%)	07 (58.3%)	0.36
Binet stage, n (%)			
A	10 (35.7%)	00 (0%)	0.01
B	06 (21.4%)	01 (14.3%)	
C	12 (42.9%)	11 (91.7%)	
Bone marrow	09 (69.2%)	06 (66%)	0.24

ap-value from Chi-square test or Fisher's exact test as appropriate, p-value in bold indicates statistical significance

SPE: Serum protein electrophoresis, CLL: Chronic lymphocytic leukaemia.

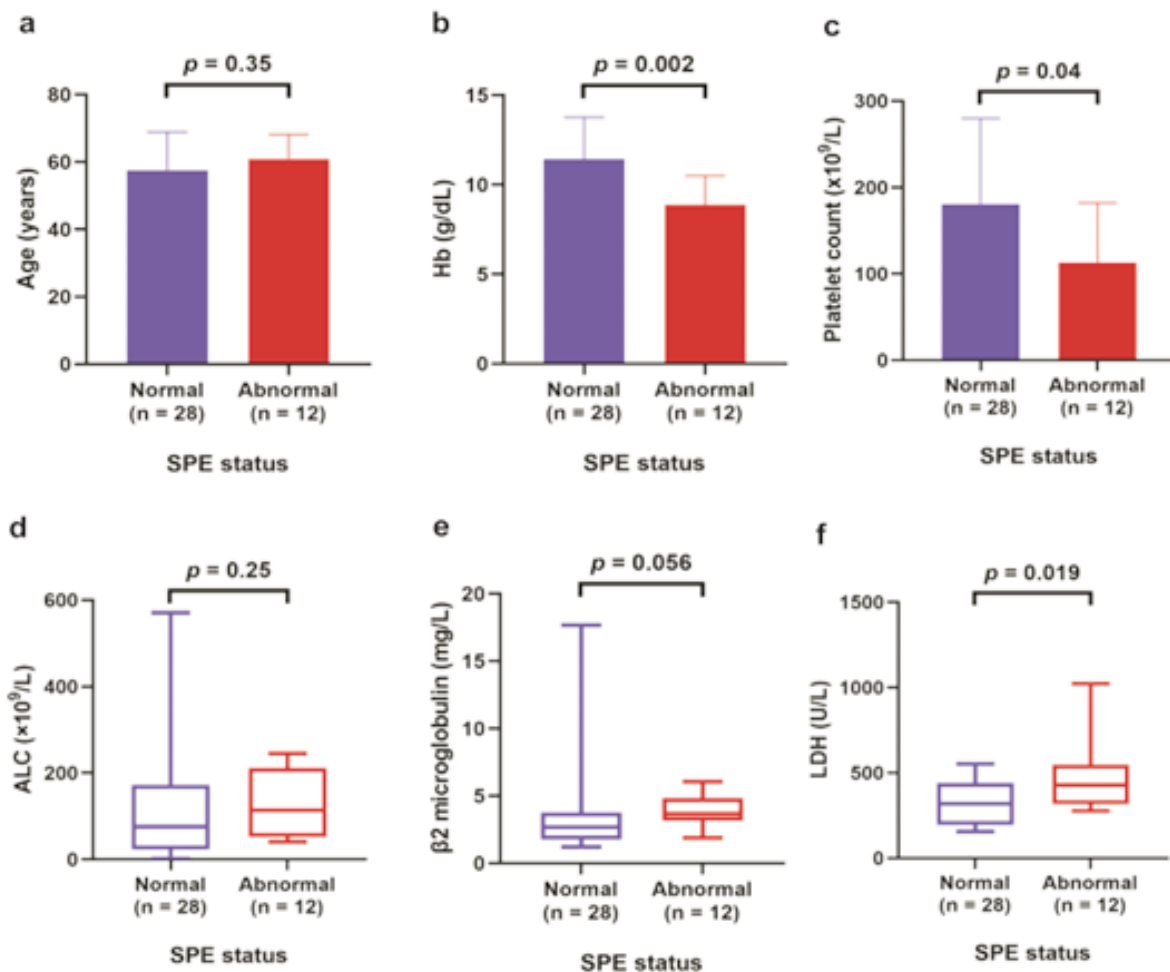


Figure-1: Patient characteristics stratified by serum protein electrophoresis (SPE) status in chronic lymphocytic leukaemia (CLL) patients. These include age (a), haemoglobin (Hb) (b), platelet count (c), absolute lymphocyte count (ALC) (d), beta-2 ($\beta 2$) microglobulin (e) and lactate dehydrogenase (LDH) levels (f). The X-axis displays the SPE status as normal or abnormal, while the Y-axis represents different variables in question. The number of samples analysed in both groups is shown below the SPE status. In a-c, the columns represent mean values with standard deviation (SD) bars, while for d-f, each group tested is represented as a box and whisker plot covering the interquartile range (IQR), median values as horizontal lines within the box, and error bars representing minimum and maximum values.

Abnormal SPE was not significantly associated with gender, presence of B symptoms, lymphadenopathy, organomegaly and bone marrow (BM) infiltration ($p > 0.05$). A significant association was observed for Binet stage C compared to patients with normal SPE (Table 4).

Among laboratory parameters, patients with abnormal SPE were found to have reduced mean Hb levels (8.88 ± 1.62 vs 11.41 ± 2.35 , $p = 0.002$) and reduced mean platelet count (112 ± 69.8 vs 180 ± 99.4 , $p = 0.04$). Moreover, elevated median LDH levels (428 [IQR: 747] vs 318 [IQR: 394], $p = 0.01$) and marginally elevated $\beta 2$ -microglobulin levels (3.67 [IQR: 4.19] vs 2.68 [IQR: 16.42], $p = 0.056$) were observed in CLL patients with abnormal SPE (Figure 1).

In univariate analysis, significant or near-significant risk factors for poor OS were elevated $\beta 2$ -microglobulin levels

(hazard ratio [HR]: 3.49, 95% CI: 1.05-11.60, median OS: 22 months, $p = 0.029$), anaemia with Hb levels ≤ 10 g/dl (HR: 3.15, 95% CI: 0.95-10.49, median OS: 22 months, $p = 0.048$) and thrombocytopenia with platelet count $\leq 100 \times 10^9/L$ (HR: 2.77, 95% CI: 0.89-8.60, median OS: 22 months, $p = 0.07$) (Figure 2).

Moreover, when considering abnormal SPE as a risk factor, univariate analysis showed a trend of significant association of SPE status with survival outcomes (HR: 3.15, 95% CI: 0.95-10.42, $p = 0.06$).

All the identified significant risk variables in univariate analysis were subjected to multivariate analysis and none of them could retain the status as significant independent predictor of poor OS in CLL patients (Table 5).

Table-5: Cox univariate and multivariate analysis of 2-year overall survival (OS) in CLL patients (n=40).

Study variables	HR	95% CI	p-value ^a
Cox univariate analysis			
Age			
>55 years (n = 24)	1.00	-	0.77
≤55 years (n = 16)	1.18	0.37-3.80	
Gender			
Female (n = 08)	1.00	-	0.29
Male (n = 32)	3.01	0.39-23.32	
Binet stage			
A (n = 10)	1.00	-	
B (n = 08)	2.30	0.21-25.50	0.50
C (n = 22)	5.25	0.66-41.60	0.12
Bone marrow infiltration			
Non-diffuse (n = 08)	1.00	-	0.55
Diffuse (n = 15)	1.67	0.32-8.73	
Typical/Atypical morphology			
Typical (n = 35)	1.00	-	0.15
Atypical (n = 05)	2.61	0.71-9.63	
ALC			
<30×10 ⁹ /L (n = 09)	1.00	-	0.59
≥30×10 ⁹ /L (n = 31)	1.51	0.33-6.91	
Hb			
>10 g/dl (n = 22)	1.00	-	0.06
≤10 g/dl (n = 18)	3.15	0.95-10.49	
Platelet count			
>100×10 ⁹ /L (n = 28)	1.00	-	0.08
≤100×10 ⁹ /L (n = 12)	2.77	0.89-8.60	
β2 microglobulin			
<3.5 mg/L (n = 25)	1.00	-	0.04
≥3.5 mg/L (n = 15)	3.49	1.05-11.60	
Del 17p			
Not detected (n = 15)	1.00	-	0.62
Detected (n = 02)	0.04	0.00-12793.41	
SPE status			
Normal (n=24)	1.00	-	
Abnormal (n=12)	3.15	0.95-10.42	0.06
Cox multivariate analysis (including all independent variables with p < 0.1 in Cox univariate analysis)			
Haemoglobin			
>10 g/dl (n = 19)	1.00	-	0.26
≤10 g/dl (n = 17)	2.38	0.51-11.06	
Platelet count			
>100×10 ⁹ /L (n = 24)	1.00	-	0.59
≤100×10 ⁹ /L (n = 12)	1.48	0.35-6.27	
β2-microglobulin			
<3.5 mg/L (n = 22)	1.00	-	0.48
≥3.5 mg/L (n = 14)	1.69	0.39-7.27	
SPE status			
Normal (n=24)	1.00	-	
Abnormal (n=12)	1.71	0.45-6.5	0.42

CLL: Chronic lymphocytic leukaemia, CI: Confidence interval, ALC: Absolute lymphocyte count, Del 17p: Deletion 17p, Hb: Haemoglobin, HR: Hazard ratio, SPE: Serum protein electrophoresis. ap-value, probability that the hazard ratio = 01 (null hypothesis). p-value in bold indicates statistical significance.

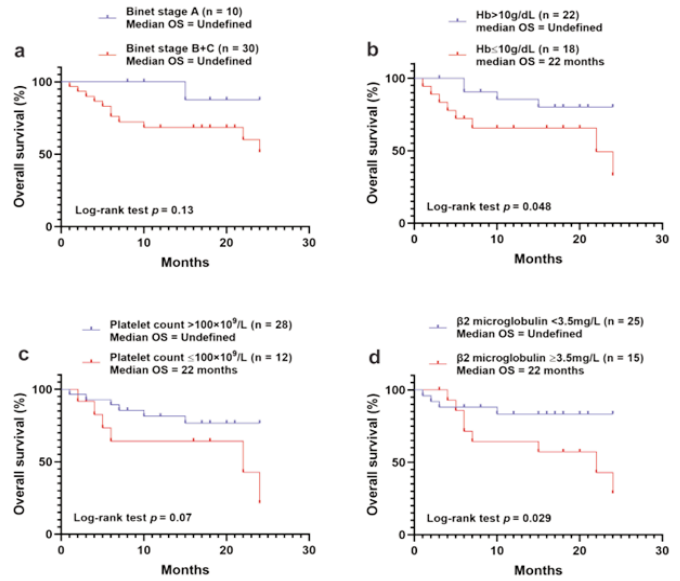


Figure-2: Kaplan-Meier estimates for 2-year overall survival (OS) in chronic lymphocytic leukaemia (CLL) patients according to Binet stage (a), haemoglobin (Hb) (b), platelet count (c), and beta-2 (β2) microglobulin (d).

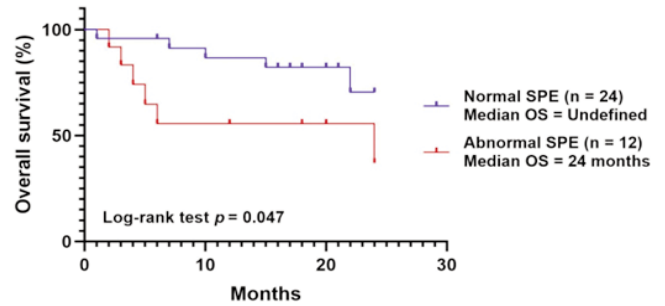


Figure-3: Kaplan-Meier estimates for 2-year overall survival (OS) in chronic lymphocytic leukaemia (CLL) patients according to serum protein electrophoresis (SPE) status.

The survival curves of CLL patients with normal SPE differed significantly from CLL patients with abnormal SPE (p=0.04) (Figure 3).

Discussion

CLL is a heterogeneous disease characterised by a variable clinical course. Some individuals present with aggressive condition that needs treatment right away, while others have a more stable, non-progressive disease that may not require treatment for years. Over the past 20 years, a number of prognostic markers have been discovered and evaluated to identify individuals with a poor prognosis in order to optimize the clinical care of CLL patients with closer follow-up periods¹⁶.

The present study evaluated the prognostic impact of abnormal SPE in a geographical novel sample set of Pakistani CLL patients. The study confirmed the previously reported significant impact of abnormal SPE as it was found associated with poor prognostic markers, such as advanced Binet stage, high LDH and high β -2microglobulin levels. Moreover, in the present CLL sample set, a significant trend of association of abnormal SPE with survival outcomes was also noted.

The present study found that frequency of abnormal SPE was 30% in the CLL cohort, out of which 10(25%) cases were with hypogammaglobulinaemia and 2(5%) had hypogammaglobulinaemia, while no patient was seen having monoclonal band. This was in agreement with a previous study,¹¹ but differed from other European and Egyptian studies.^{9,12,15,16} Overall, 56% cases in a study⁹ had abnormal SPE, while in another study, the corresponding value was 28%.¹⁵ So wide variation in overall frequency of abnormal SPE has been reported in the literature ranging from as low as 7-13% to as high as 30-80%.¹⁵

The mean age at diagnosis in the current CLL cohort was 60 years, with 40% of patients presenting at a younger (≤ 55 years) age. This age distribution in CLL was consistent with other Pakistani and Indian studies.^{17,18} In comparison, median age at diagnosis was slightly higher in Chinese CLL patients (64 years) and Caucasian CLL patients (70-72 years), with $<10\%$ patients being diagnosed at age <55 years.¹⁹⁻²¹ This may reflect referral bias, but may also be suggestive of a genetic predisposition for Indo-Pak CLL patients to present at a younger age, especially considering higher rates of consanguinity in this ethnic group, and can be of significance as this earlier-age CLL may represent a severe disease with worse prognosis and inferior survival outcomes.²² Regarding gender distribution, male predominance seemed to be a universal characteristic of almost all CLL cohorts.¹⁷⁻²⁴

CLL has a highly variable natural course with OS ranging from a few years to several decades. The Binet and Rai staging systems still provide a simplified way based on a few simple clinical and laboratory variables, especially in resource-limited healthcare systems, to predict CLL disease progression at diagnosis despite being almost half-a-century old.^{4,5} On the contrary, cytogenetic and complex molecular testing has been recommended by the IWCLL guidelines for CLL prognostication.² Among all genetic biomarkers, only immunoglobulin heavy variable (IGHV) gene mutation status and deletion 17p (Del 17p), which is a form of tumour protein 53 (TP53) genetic aberration, are recommended at present to predict CLL

progression and chemo-resistance.²³

In the current study, 72% of the CLL patients presented with advanced Binet stages B and C that seems to be a hallmark of the disease in under-developed regions of the world.^{17,18,24} The representation of advanced Binet stages among CLL patients decreases sharply for Chinese (43.9%) and European (18.8%) patients.^{9,19}

In the present study, CLL cases with abnormal SPE were significantly associated with Binet stage C which was in line with previous studies.^{9,16,25} While considering other laboratory parameters, statistically significant lower mean Hb levels and low platelet count were found in present CLL sample with abnormal SPE, which was contrary to a study.⁹ Moreover, high β 2-microglobulin and higher LDH levels were present in the current CLL patients with abnormal SPE, as suggested by previous reports^{16,25}.

In the current study, higher β 2-microglobulin levels appeared to be a prognostic clinicopathological marker of poor 2-year OS, which has already been reported.^{26,27} The relative percentage of CLL patients with elevated β 2-microglobulin was 10.7% in Europeans, 36% in a local study²⁸ and 70-75.4% in Indians.^{9,18}

In the present study, while considering the impact of abnormal SPE on 2-year OS, significant association was observed ($p=0.04$). This was in contrast to earlier findings showing no significant impact on OS in CLL patients with any kind of immunoglobulin (Ig) abnormality on SPE¹⁵. Rather patients with abnormal SPE/Ig aberrations had significantly reduced TFS, which was not measured in the current study in which only first-line treatment outcomes were measured.

Moreover, multivariate analysis of the risk variables, including elevated β 2-microglobulin, anaemia, thrombocytopenia and abnormal SPE showed that no factor retained the status of being a significant independent predictor of poor OS in the current CLL patients. The reason for this might be the small sample size with shorter follow-up.

Among genetic features, only Del 17p data was available for less than half of the current patients, and only 2(11.8%) harboured it, which is concordant with detection rates of 11-18.5% in other studies^{18,28}, while European and Chinese studies displayed lower frequencies of 5-7.8%.^{19,20}

Some of these studies^{19,20,28} also suggested significant prognostic relevance of Del 17p positivity, which the current study did not determine because of limited Del 17p testing facilities.

A vast majority (64.9-80%) of Indo-Pak CLL patients needed treatment at the time of diagnosis as reflected in the present and other such studies from the region.^{17, 18} On the contrary, only about half or a quarter of CLL cases necessitated therapeutic interventions among Chinese and Western cohorts.^{9,19} These observations in Indo-Pak CLL patients were consistent with published literature^{17,18,28} showing higher proportions of younger patients in the region with aggressive disease behaviour and shorter time to first treatment. As suggested earlier, another reason for this could be delay in making proper diagnosis and referring patients to appropriate cancer care centres.²²

The current study has limitations as it had a small sample size, comprised single-centre data and lacked molecular analysis. Despite the limitations, however, the current study, to the best of our knowledge, is the first to report the prognostic significance of SPE and its associations with clinicopathological parameters in Pakistani CLL patients.

Conclusion

Abnormal SPE could be used as surrogate biomarker of advanced disease as CLL cases with abnormal SPE presented with enrichment of poor prognostic markers, such as Binet stage C, lower mean Hb levels, higher median LDH and β 2-microglobulin levels. Abnormal SPE could be a significant predictor of poor OS in CLL patients.

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References

1. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127:2375-90. doi:10.1182/blood-2016-01-643569.
2. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131:2745-60. doi:10.1182/blood-2017-09-806398
3. Yang S, Varghese AM, Sood N, Chiattono C, Akinola NO, Huang X, et al. Ethnic and geographic diversity of chronic lymphocytic leukaemia. *Leukemia*. 2021;35:433-9. doi:10.1038/s41375-020-01057-5.
4. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. *Blood*. 1975;46:219-34. PMID: 1139039.
5. Binet J, Auquier A, Dighiero G, Chastang C, Piguët H, Goasguen J, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. *Cancer*. 1981;48:198-206. doi: 10.1002/1097-0142(19810701)48:1<198::aid-cncr2820480131>3.0.co;2-v
6. Szymczyk A, Nowaczyńska A, Korpysz M, Donica H, Bojarska-Junak A, Długosz-Danecka M, et al. Evaluation of the prognostic and predictive value of free light chains in patients with chronic lymphocytic leukemia—preliminary results. *Acta Haematol Pol*. 2019;50:15-20. doi: 10.2478/ahp-2019-0003
7. Sutton L-A, Ljungström V, Enjuanes A, Cortese D, Skaftason A, Tausch E, et al. Comparative analysis of targeted next-generation sequencing panels for the detection of gene mutations in chronic lymphocytic leukemia: an ERIC multi-center study. *Haematologica*. 2021;106:682-691. doi:10.3324/haematol.2019.234716
8. Rani L, Gogia A, Singh V, Kumar L, Sharma A, Kaur G, et al. Comparative assessment of prognostic models in chronic lymphocytic leukemia: evaluation in Indian cohort. *Ann Hematol*. 2019;98:437-43. doi:10.1007/s00277-018-3525-0.
9. Chauzeix J, Laforêt MP, Deveza M, Crowther L, Marcellaud E, Derouault P, et al. Normal serum protein electrophoresis and mutated IGHV genes detect very slowly evolving chronic lymphocytic leukemia patients. *Cancer Med*. 2018;7:2621-8. doi:10.1002/cam4.1510.
10. Rizzo D, Chauzeix J, Trimoreau F, Woillard J, Genevieve F, Bouvier A, et al. IgM peak independently predicts treatment-free survival in chronic lymphocytic leukemia and correlates with accumulation of adverse oncogenetic events. *Leukemia*. 2015;29:337-45. doi: 10.1038/leu.2014.198.
11. Parikh SA, Leis JF, Chaffee KG, Call TG, Hanson CA, Ding W, et al. Hypogammaglobulinemia in newly diagnosed chronic lymphocytic leukemia: Natural history, clinical correlates, and outcomes. *Cancer*. 2015;121:2883-91. doi: 10.1002/cncr.29438.
12. Asmaa MZ, Hosny BH, Rashed FE-ZA, Mohamed Z. Serum Immunoglobulin Paraprotein in Patients with Chronic Lymphocytic Leukemia. *Med J Cairo Univ*. 2018;86:389-94.
13. Andersen MA, Vojdeman FJ, Andersen MK, Brown PdN, Geisler CH, Weis Bjerrum O, et al. Hypogammaglobulinemia in newly diagnosed chronic lymphocytic leukemia is a predictor of early death. *Leuk Lymphoma*. 2016;57:1592-9. doi: 10.3109/10428194.2016.1142082.
14. Matutes E, Polliack A. A matter of life or early death in CLL: do serum immunoglobulin levels really count? *Leuk Lymphoma*. 2016;57:1501-2. doi: 10.3109/10428194.2016.1170830
15. Corbingi A, Innocenti I, Tomasso A, Pasquale R, Visentin A, Varettoni M, et al. Monoclonal gammopathy and serum immunoglobulin levels as prognostic factors in chronic lymphocytic leukaemia. *Br J Haematol*. 2020;190:901-8. doi: 10.1111/bjh.16975
16. El-Hussiny MA, Ibrahim L, Azmy E, Shamaa S. Prognostic significance of serum monoclonal immunoglobulin in B-chronic lymphocytic leukemia. *Egypt J Haematol*. 2012;37:240-245. doi: 10.7123/01.EJH.0000419280.71909.65
17. Nazir A, Ali S, Badar F, Siddique N, Hameed A. Outcomes of first line chemotherapy in patients with chronic lymphocytic leukemia. *Pak J Med Sci*. 2016;32:1213-1217. doi:10.12669/pjms.325.10480.
18. Gogia A, Gupta R, Sharma A, Kumar L, Raina V, Rani L. Chronic lymphocytic leukemia: An Indian experience. *JCO*. 2019; 37:e19007-e19007, doi:10.1200/JCO.2019.37.15_suppl.e19007.
19. Chan TS-Y, Lee Y-S, Del Giudice I, Marinelli M, Ilari C, Cafforio L, et al. Clinicopathological features and outcome of chronic lymphocytic leukaemia in Chinese patients. *Oncotarget*.

- 2017;8:25455-25468. doi:10.18632/oncotarget.16037
20. Rotbain EC, Frederiksen H, Hjalgrim H, Rostgaard K, Egholm GJ, Zahedi B, et al. IGHV mutational status and outcome for patients with chronic lymphocytic leukemia upon treatment: a Danish nationwide population-based study. *Haematologica*. 2020;105:1621-9. doi:10.3324/haematol.2019.220194
 21. Tian Z, Liu M, Fang X, Zhou X, Li P, Li Y, et al. Distinct age-related clinical features and risk assessment in Chinese with chronic lymphocytic leukemia. *Front Oncol*. 2022;12:885150. doi:10.3389/fonc.2022.885150.
 22. Tejaswi V, Lad DP, Jindal N, Prakash G, Malhotra P, Khadwal A, et al. Chronic lymphocytic leukemia: real-world data from India. *JCO Glob Oncol*. 2020;6:866-72. doi:10.1200/GO.20.00032.
 23. Korubo KI, Okite UP, Ezeugwu SI. Chronic Lymphocytic Leukemia: Prognostic Factors at Presentation in a Resource-Limited Center. *JCO Glob Oncol*. 2021;7:56-62. doi:10.1200/GO.20.00276.
 24. Basabaen AA, Abdelgader EA, Babekir EA, Eltayeb NH, Altayeb OA, Fadul EA, et al. Clinical presentation and hematological profile among young and old chronic lymphocytic leukemia patients in Sudan. *BMC Res Notes*. 2019;12:1-6. doi:10.1186/s13104-019-4239-7.
 25. Mozas P, Piñeyro JA, Nadeu F, Magnano L, Rivero A, Rivas-Delgado A, et al. Serum monoclonal component in chronic lymphocytic leukemia: baseline correlations and prognostic impact. *Haematologica*. 2021;106:1754-1757
 26. Yun X, Zhang Y, Wang X. Recent progress of prognostic biomarkers and risk scoring systems in chronic lymphocytic leukemia. *Biomark Res*. 2020;8:1-11. doi.org/10.1186/s40364-020-00222-3
 27. Al-Rekabi AN, Alwan AF, Alobaidi NK. Assessment of beta-2 microglobulin and CD49d in patients with chronic lymphocytic leukemia pre-and posttherapy. *Iraqi J Hematol*. 2020;9:155-9. doi:10.4103/ijh.ijh_44_20.
 28. Mahmood R, Khan SA, Altaf C, Malik HS, Khadim MT. Clinicohematological parameters and outcomes in a cohort of chronic lymphocytic leukemia patients with Deletion 17p from Pakistan. *Blood Res*. 2018;53:276-80. doi:10.5045/br.2018.53.4.276.

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AH: Study concept, design, preparation, data collection, analysis, writing, methodology.

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RBJ: Study concept, design, data collection, methodology.

NA: Study conception, design, final approval.