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Utilization of Chronic Lymphocytic Leukaemia International Prognostic Index (CLL-IPI) for Prognostic Stratification in Newly Diagnosed Chronic Lymphocytic Leukaemia Patients

Noor Ul Huda Alhadi, Ayesha Khurshid, Manzar Bozdar, Rafia Mahmood, Aysha Khan, Samia Shafaat

Armed Forces Institute of Pathology, Rawalpindi, Pakistan

Abstract

Objective: To score and risk stratify newly diagnosed CLL patients according to CLL-IPI.

Methodology: This cross sectional study CLL-IPI prognostic scoring system was applied on one hundred and twenty-four newly diagnosed Chronic Lymphocytic Leukemia patients in the Molecular haematology department at Armed Forces Institute of Pathology, Rawalpindi from Dec 2021-2023. Five variables of 124 patients [serum β2- microglobulin levels, IGHV region gene rearrangement status, del[17p] by FISH, clinical stage, age] were noted. The sum of these scores identified patients into very high risk group (7-10), high (4–6), intermediate (2-3) and low risk groups (0-1 points).

Results: Mean patients age was 61 years (Range 27-86). Twenty(16%) were females and 104 (84%) were males. On clinical staging, 36 (29.0%) in Rai stage 0, and 24 (19.3%) in Rai stage I, 04 (3.2%) patients in Rai stage II, 44 (35.5%) in stage III, 8 (17.3%) in Rai stage IV. Del[17p] was positive in 16 (14%) and 44 (35.5%) patients had un-mutated IGHV gene rearrangement. The CLL-IPI score identified 32 (26%) patients having high risk and 8 (6%) with very high risk, 28 (23%) patients with low risk, 56 (45%) with intermediate risk respectively.

Conclusion: Most patients were found to have intermediate risk, while the very high-risk subgroup had the fewest. Each subgroup necessitates distinct management strategies, which are associated with different overall survival rates over a five-year period. The application of CLL-IPI will enhance prognostic stratification and aid tailored management in clinical settings.

Keywords: Chronic Lymphocytic Leukaemia, International Prognostic Index (CLL-IPI) Stratification.

Address for Correspondence
Dr Noor Ul Huda Alhadi
Armed Forces Institute of Pathology, Rawalpindi, Pakistan
hudahadi156@gmail.com

Introduction

Rai and Binet introduced the original Chronic Lymphocytic Leukaemia clinical staging systems laying down the foundation of CLL prognostic factors.[1,2] Emerging data regarding the molecular and genetic basis of this disease has contributed to the discovery of numerous markers related with disease durability as well as patient endurance, facilitating the diagnostic information that is harmonious to the classical staging systems.[3] One of those markers is immunoglobulin heavy chain *IGHV* gene rearrangement ,patients having un-mutated *IGHV* genes bear an aggressive illness compared with patients having mutated *IGHV* genes.[4,5]

Additional specifications include biochemical markers (LDH and β_2-microglobulin)[6] and defects of TP53 gene. The loss of 17p13 locus as well as mutation within gene TP53 is related with chemotherapy resistance and mainly an adverse clinical outcome.[7] Furthermore, next-generation sequencing has revealed a plethora of mutations/ deletions in the *SF3B1*and*NOTCH1*[8] genes which might be related to poor prognosis and significantly reduced survival. This abundance of newer markers with little research into their independent prognostic value greatly delayed their translation into clinical fields. To deal with this problem, chronic lymphocytic leukaemia's international group developed a prognostic index on the bases of most widely utilized clinical, genetic and biological prognostic parameters.[9] CLL-IPI is a breakthrough index integrating major parameters of prognostic importance with analysis of IGHV mutation and FISH for del[17p] and/or TP53 mutation.

Methodology

In this cross sectional study CLL-IPI prognostic scoring system was applied on one hundred and twenty-four newly diagnosed Chronic Lymphocytic Leukemia patients in the Molecular haematology department at

Authorship Contribution: [1] Conceived and planned the idea of the study, [6]final approval of the version to be published, [2]Collecting the data, [3]Manuscript drafting, analysis, [3]drafting the work or revising it critically for important intellectual content.

Funding Source: none
Conflict of Interest: none
Received: Nov 22, 2024
Accepted: April 10, 2025

Armed Forces Institute of Pathology, Rawalpindi, from Dec 2021 -2023.

Pakistan. The Chronic Lymphocytic Leukaemia diagnosis was made in accordance with guidelines of National Cancer Institute Working Group.[10] Written informed consent was taken from all the patients according to the Declaration of Helsinki. Newly diagnosed CLL patients, of all ages, both genders were included in my study, However Patients on treatment were excluded. Sampling technique employed was non probability convenient sampling.

Firstly, in order to complete all the development steps for construction of the prognostic index, Consent was taken, detailed clinical history was jotted down and physical examination outcome of lymphadenopathy or hepatosplenomegaly was noted for each patient. Age, sex, Presence or absence of related B-symptoms, incidental finding of CLL on routine full blood counts, performance status of patients as per the Eastern Cooperative Oncology Group (ECOG), haematological framework comprising of Hemoglobin level, Total white cell count, platelet count was documented. Patient's clinical stage (Binet and Rai) as well as biochemical profile comprising of serum LDH levels and serum β2 - microglobulin levels were noted. Each patient's blood sample was run for PCR for IGHV gene rearrangement / mutation and each patient's blood sample was tested for any cytogenetic irregularities in accordance with fluorescence in – situ hybridization techniques (FISH; del[13q], del[17p], del[11q22] and trisomy 12).

Secondly, cytogenetic testing for del[17p]/TP53 status was carried out. Under aseptic conditions, 3ml blood was collected into sodium heparin anticoagulant tube, cell harvesting was done, slides were prepared, 10ul of meta system XL ATM/TP53 deletion/ enumeration probes were applied, under fluorescent microscope having green/orange/aqua filter, 500 interphase nuclei were observed.

Thirdly, PCR for IGHV gene rearrangement was also carried out, forward and reverse primers designed from eurofins genomics were used. DNA Extraction followed by Conventional polyacrylamide gel electrophoresis was performed. Patients sample were run in duplicate along with positive and negative controls.

Lastly, five independent prognostic factors were compared and scored for each patient: TP53 status (presence of del[17p] vs no abnormalities), IGHV genere-arrangement standing (unmutated vs mutated), concentration of serum β2-microglobulin (>3·5 mg/L vs ≤3·5 mg/L), the stage of disease (Binet B-C or Rai I-IV vs Binet A or Rai 0), and ages of patients of CLL (>65 years vs ≤65 years). Sum of these parameters revealed patients in four subgroups: very high risk (7–10), high risk (4–6), intermediate risk (2-3) &low risk (0-1 points).

The statistical analysis was carried out utilizing IBM SPSS software (Statistical Package for Social Sciences, version 23). The baseline characteristics and prognostic factors of patients were evaluated utilizing the test of chi-square. All p-values were 2-sided and differences were statistically significant at p-value of <0.05. Univariate & multivariate analyses was done using 12 baseline elements. Risk score assigned for each factor was in corporate in analysis of final assessment.

Results

A total of 124 newly diagnosed patients were included in the final analysis. The age of patients ranged from 27 to 86 years, with a median age of 62 years. Of these, 52 (42%) were older than 65 years. There was a male predominance, with 104 (84%) of the patients being male. An incidental diagnosis of chronic lymphocytic leukemia (CLL) on routine complete blood counts (CBC) was observed in 32 (25.8%) patients. Splenomegaly was present in 32 (25.8%), while lymphadenopathy was noted in 72 (58%) patients (Table I).

Most patients presented with advanced Rai clinical stages: Rai 0 – 36 (29.0%), Rai I – 24 (19.3%), Rai II – 4 (3.2%), Rai III – 44 (35.5%), and Rai IV – 16 (12.9%). According to the Binet staging system, 36 (29.0%) were in stage A, 28 (22.6%) in stage B, and 60 (48.4%) in stage C. Beta-2 microglobulin levels exceeded the cutoff value of 3.5 g/dL in 52 (42%) patients.

Fluorescence in situ hybridization (FISH) analysis revealed del(17p) in 16 (14%), del(13q) in 32 (25%), del(11q) in 20 (16%), trisomy 12 in 2 (2%), while 54 (43%) showed no cytogenetic abnormality (Figure 1). Molecular analysis for IGHV gene mutation status showed that 44 (35.5%) patients had unmutated IGHV genes (Table II).

Based on the CLL International Prognostic Index (CLL-IPI), patients were stratified into different risk categories:

Table I: Clnico-Haematological Parameters.				
Category	LOW (N=28)	INTER-MEDIATE (N=56)	HIGH (N=32)	VERY HIGH (N=8)
Age > 65year	0	0	16	36
Male sex	28	40	32	4
ECOG Performance of 2-4	4	36	20	8
Palpable lymph nodes	16	28	20	8
Palpable spleen	8	12	8	4
Binet stage A	8	20	8	0
Binet stage B	4	20	4	0
Binet stage C	16	16	20	8
Haemoglobin < 10g/dl	20	48	44	12
Platelets < 150x10^9/L	20	32	34	38
Lymphocytes > 15x10^9/L	22	36	34	32

Table II: Data of patients according to CLL- IPI Prognostic Factors.			
PROGNOSTIC FACTOR		Percentage	N
Age	> 65 years	41.90%	52
Clinical stage	Rai I-IV / Binet B-C	71.0%	88
IGHV mutational status	Unmutated	35.5%	44
Serum β_2-microglobulin	>3.5mg/L	42%	64
TP53 mutational status/del 17p status	Presence of del17p and/or TP53 deleted and/or mutated	14%	16

28 (23%) were in the low-risk group, 56 (45%) in the intermediate-risk group, 32 (26%) in the high-risk group, and 8 (6%) in the very high-risk group (Figure 2). The

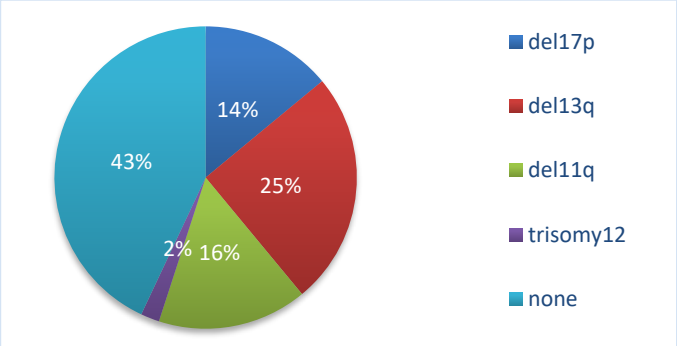

Figure 1. The frequency of cytogenetic abnormalities detected in CLL patients in our study.

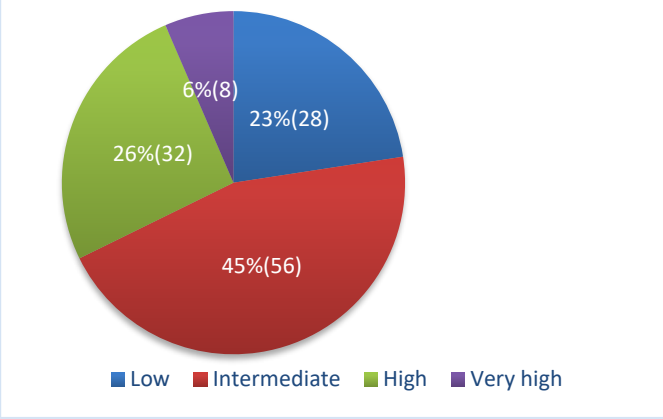

Figure 2. CLL patients segregated into various risk groups as per CLL-IPI

majority of newly diagnosed patients in our study belonged to the intermediate-risk group, while the fewest were categorized as very high-risk. Each risk subgroup requires a tailored management approach, which significantly influences the predicted five-year overall survival outcomes.

Discussion

Based on our knowledge, this is the first breakdown of data that refers to a significant treatment-free population of patients having Chronic Lymphocytic Leukaemia in Pakistan scanned for factors of prognostic value.

CLL science has evolved much since Rai[1]&Binet[2]staging systems were introduced a long time ago, before current genetic and biological information came into light. The original clinical stage based systems have now become the cornerstone of modern clinical management for patients with CLL. With the arrival of new automated haematology analyzers, the high Total Leukocyte Counts of undiagnosed CLL patients are easily flagged, immediately raising alarm which results in peripheral blood film examination by hematologists and early stage diagnosis.

Additionally, advances in the chemotherapeutic regimens and discovery of monoclonal antibodies have significantly bettered the results of patients with Chronic Lymphocytic Leukaemia, partly invalidating the preliminary relationship

between overall survival & clinical stage based prognostic index.[11,12]

Although many new markers of prognostic significance have been discovered, the standard approach is not unanimous for assimilating the parameters in a prognostic system for the purpose of reporting them in clinical trials.

After an in depth exploration of CLL's pathology, along with recognition of multiple newer prognostic markers, the question arose regarding which ones could be reinforced and implemented in clinical practice. This required the prognostic parameters to follow a number of restrictions. First one being easy availability in majority of clinical laboratories, second being their cost effectiveness. Lastly the ability of prognostic marker to furnish the clinicians with the most applicable and clinically relevant information. Thus quite a few prognostic models were proposed and enforced one after the other.

The age of patient, at first presentation of disease proved to be of value in this regard. The median age of presentation in previous studies was 62 years with greater number pf patients being male. The risk was slightly higher in men than in women, and the average age at the time of diagnosis was 70 years.[13] In our study, 41.9% patients had age greater than 65 years. Approximately 5 to 10% of patients presented with typical B-symptoms.[14] Clinical stage was advance with patients; already having developed lymphadenopathy and enlarged liver and / or spleen. The results of FISH of our study matched a previous study done on local population of patients with CLL.[15]

The IGHV gene rearrangement, to our surprise, revealed more patients with mutated status which is associated with a good prognosis; 59.5% of the patients had mutated IGHV in other studies which is comparable to our analysis of 64.5% patients with mutated IGHV.[16] In our population, we discovered that majority of patients were in intermediate and high risk groups. Each subgroup requiring specific management.

Prognostic system introduced by Wierda & colleagues[17] did not include inheritable factors as well as those that directly prognosticated the outcomes of singular cases. The scoring system formulated by Pflug &colleagues[19] was identical to CLL-IPI, reflecting the potential of utilizing weighted combination of biological, clinical, &genetic markers for precise prognostication. However, the utilization of this score[18] appear limited by the addition of serum thymidine kinase, mainly due to this factor not being routinely available in laboratories across the world.[19] Rossi &colleagues[20] developed a score that entirely depended on cytogenetics and novel gene mutations without considering the clinical or biochemical characteristics for each case.

The CLL-IPI markers are of individual prognostic value and therefore permit for elimination of the redundant prognostic tests (e.g. flow cytometry for CD38 and ZAP-70). If largely recognized, CLL-IPI can aid in concentrating valuable resources on prognostic tests of high clinical worth. Keeping with these lines, presently all parameters of the CLL-IPI are extensively accessible and routinely accepted, which should allow the broad utilization of CLL-IPI in health-care systems at least, with access to molecular / cytogenetic testing (e.g. PCR for IGHV or FISH testing) in Chronic Lymphocytic Leukaemia. Likewise, the simple characterization of CLL-IPI will help future inclusion of new markers with independent prognostic value.

This CLL-IPI score recognized 4 groups at very high risk, high, intermediate & low risk, giving prognostically relevant information regarding general survival in comparison with clinical staging systems being employed conventionally. Additionally, 4 risk groups could be replicated within the Rai and Binet clinical stage model with a significant replication to the overall model. Likewise, our data analysis confirmed that all cases with the presence del(17p) on FISH are not equally having poorer prognosis as CLL-IPI discriminated these patients into very high risk and high risk subgroups. Intermediate risk patients might not be treated except if the disease comes with severe symptoms at first presentation. High risk patients should commence treatment except those with no symptoms at all. Ultimately, patients considered at a very high risk should be provided with treatment.

Conclusion

The CLL-IPI combines biochemical, clinical, cytogenetic as well as molecular parameters in prognostic model, establishing 4 simple prognostic subgroups. Based on our knowledge, this is first of its kind study on treatment free, newly diagnosed CLL patients in Pakistan analyzing

all variables of CLL-IPI. This study will enable larger targeted management of patients in clinical practice as well as within trials testing of novel drugs in the country.

Limitations: Subject study was conducted in Northern Pakistan. The CLL gene pool of our Southern counterpart may vary. Taking into consideration the very good outcome of low risk patients for 5-year overall survival in published studies, an expectant wait – and – watch management approach appears appropriate. Comprehensive evaluation based on composite character of CLL-IPI is required for a longer time period on a greater number of patients. Further studies are required to test this dataset for overall survival after an even longer period.

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