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THEROLEOFNEWPROGNOSTICMARKERSANDCOMORBIDITIESONTHEOUTCOMEOFPATIENTSWITHCHRONICLYMPHOCYTICLEUKEMIAINAMALAYSIANREFERRALCENTRE

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The Role of new Prognostic Markers and Comorbidities on the Outcome of Patients with Chronic Lymphocytic Leukemia in a Malaysian Referral Centre

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I. Introduction

Chronic lymphocytic leukemia (CLL) is a clonal lymphoid neoplasm characterized by proliferation and accumulation of neoplastic B lymphocytes in the blood, bone marrow, lymph nodes, and/or spleen. In Western countries, CLL is the most common leukemia in adults, accounting for 5% to 11% of lymphoproliferative disorders (LPD). The incidence rate is between 2 to 6 cases per 100 000 with an increasing trend as people get older.[1] The incidence of CLL is lower in Asian subjects, including Malaysians. In Asian countries, CLL accounts for only 1% to 3% of LPD in most series.[2] Asian CLL has been reported to have different biological characteristics with a more aggressive clinical course and treatment outcomes when compared with those of Western CLL. The reasons for these differences in incidences and clinical behaviour between geographic regions are unclear but are of considerable interest. Data on CLL in Asian countries including Malaysia is also very limited because of the disease rarity.

Chronic lymphocytic leukemia has a heterogenous clinical course, ranging from relatively indolent to aggressive. At diagnosis, staging of disease can be made based on the Rai clinical staging system[3] and the Binet staging system[4]. However both staging systems have limited value in determining the clinical course of the disease in individual cases and in the identification of progressive CLL, especially during the early stages of the disease. Over the past several years, new markers with significant prognostic values have been identified. Unlike the "old" staging systems, the "newer" markers such as immunoglobulin heavy-chain variable region (IgVH) mutation status, fluorescence in-situ hybridization (FISH) cytogenetics, and CD38 and zeta-associated protein (ZAP)-70 expressions may reveal an underlying biological connection with the disease.[5] Furthermore various investigators have reported the importance of these prognostic markers not only useful to address disease progression and overall survival, but also to predict response to therapy.[6]

A set of specific chromosomal abnormalities has been reported to have predictive value for disease course and outcome. Listed in order of increasing disease severity, these include 13q14 deletion (13q-), trisomy 12, 11q22-23 deletion (11q-), and 17p deletion (17p-).[5] In the late 1990s, a novel technique looking at chromosomal abnormalities was developed which was called inter phase FISH, and this method was well suited for use in CLL, given its low mitotic rate.[7] With FISH, the number of chromosomal abnormalities seen in CLL increased from 51% to 82%.[7] At present FISH has become the standard method to detect chromosomal abnormalities in the clinical care of CLL patients. Detection of these cytogenetic abnormalities has apparent prognostic value and may influence therapeutic decisions. Additional genetic defects may be acquired during the course of the disease and therefore, the repetition of FISH analyses seems justified before subsequent second and third-line treatment.

Besides, expression of CD38 on and ZAP-70 in CLL cells has proven valuable in predicting outcome in CLL. CD38 expression is a measure of cell division and a reflection of growth in vivo. The percentage of cells within a CLL clone that display CD38 is an indicator of the potential and actual degree of cellular activation of the clone: those with higher numbers (than a defined percentage) are more responsive to activation signals or are activated and are therefore more often more aggressive.[5] CD38 expression can be evaluated by flow cytometry and its positivity is usually associated with a more virulent and progressive disease. Besides CD38 expression, intracellular expression of the ZAP-70 protein above a certain threshold of cells measured by

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immune fluorescence and flow cytometry has proven to be an important indicator of time-to-treatment and survival in CLL.[8] These "newer" predictive prognostic markers on CLL patients' outcomes have never been reported in Malaysia.

As CLL is a disease of the elderly with a median age of 67-72 years at diagnosis,1 the majority of patients with CLL commonly present with multiple comorbidities at presentation or when treatment is indicated. Recently, comorbidity was identified as an adverse prognostic factor in patients with untreated or treated CLL.[9] Several recent randomized trials deliberately focused on patients with comorbidities, using the cumulative illness rating scale (CIRS) as a semi-quantitative tool. The CIRS determines the burden of medical illness while taking into account the severity of each condition. However, the impact of comorbidities on CLL treatment outcomes and survival remains understudied in Malaysia. Thus, in this analysis, we aim to evaluate this impact as part of our objectives.

Although the incidence of CLL in Malaysia is lower than that in Western countries, a progressively increasing trend has been observed in recent years. No studies in Malaysia have so far reported on the relationship of these new prognostic markers and comorbidities with the outcome of CLL patients. The aim of this study is to analyze the clinical characteristics of patients with CLL in a Malaysian Hospital besides determining the relationship of cytogenetic abnormalities and CD38 expression on disease clinical course, and the interaction between comorbidity and treatment outcome. The findings of this study may help us improve the care, counseling and treatment strategy of CLL patients in Malaysia.

II. Materials and Methods

This observational study was carried out at the Hematology Department of Hospital Ampang, the national hematology referral centre in Malaysia from 1st January 2007 through 31st December 2016. The study enrolled 71 confirmed CLL patients with minimum one-year follow-up duration. The diagnosis of CLL was made according to the International Workshop of CLL (IWCLL) updated in 2008.10 Patients' data were retrospectively retrieved from the electronic hospital informative system. These include demographics, initial presenting features, laboratory results such as FISH cytogenetic profile and CD38 expression by flow cytometry, and first line chemotherapy administered. Besides, response to treatment and status of remission during follow-up period were also analyzed.

For comorbidity assessment, we captured the number of medical conditions present at baseline. Further quantification of the comorbidity burden was done using the CIRS score. Health problems resulting from the CLL itself were not recorded as comorbidity. The impact of high burden of medical illness, defined as CIRS ≥4 in this study was assessed and compared with those with CIRS 0 to 3.

The outcomes of disease were first investigated by using Kaplan-Meier (KM) product limit method and the log-rank test in accordance to sex, race, Binet staging, chromosomal abnormalities, CD38 expression, induction treatments and comorbidities burden. Two outcomes were assessed: Overall survival (OS) and progression free survival (PFS). Differences in OS and PFS with p-value less than 0.05 are considered statistically significant. The study was registered under the National Medical Research Register (NMRR), Malaysia. It was approved by the Medical Research & Ethics Committee (MREC), a centralized independent ethics committee for public hospitals in the country. The CIRS score (appendix I) was used to categorize and score each of the concomitant diseases.

III. Results

The survival analysis was performed in 71 CLL patients with minimum 12 months follow-up duration. The baseline demographic and characteristics of the patients are shown in Table 1. The median age at diagnosis was 64.0 year, ranging from 38.0 to 80.0 years. Male CLL patients accounted for 73.2% of the cohort. Malay patients accounted for 45.1% of the cohort, followed by 42.3% of Chinese patients, 11.3% of Indian patients and 1.4% of other ethnicity. According to the Binet staging system, majority of the CLL patients (45.1%) presented with Binet C, followed by Binet A (33.8%) and Binet B (21.1%).

The FISH cytogenetic profile was performed in 55 patients (77.5%). Chromosomal abnormalities in CLL were detected in 33 out of 55 patients (60.0%). Among the abnormalities, 13q- (27.3%), 17p- (14.5%), and tri12 (16.4%) had a known prognostic value and played an important role in CLL pathogenesis and disease progression, = determining the outcome and treatment strategies. The CD38 expression was documented in 61 out of 71 patients (85.9%), which included 14 (23.0%) CD38 positive and 47 (77.0%) CD38 negative patients.

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Table 1: The Characteristics of CLL patients

No	Characteristics	N	(%)
1	Number of patients	71	
2	Age at diagnosis, years		
	Median (IQR)	64.0	(16.0)
	Mean (SD)	62.4	(10.2)
	Range, min – max	38.0 – 80.0	
3	Sex		
	Male	52	(73.2)
	Female	19	(26.8)
4	Race		
	Malay	32	(45.1)
	Chinese	30	(42.3)
	Indian	8	(11.3)
	Others	1	(1.4)
5	Disease classification		
	Binet A	24	(33.8)
	Binet B	15	(21.1)
	Binet C	32	(45.1)
6	FISH cytogenetics		
	Normal	22	(40.0)
	13q-	15	(27.3)
	17p-	8	(14.5)
	Trisomy 12	9	(16.4)
	Complex karyotype	1	(1.8)
	Not done	16	-
7	CD38 expression		
	CD38+	14	(23.0)
	CD38-	47	(77.0)
8	Induction treatment		
	Alkylating agent based	31	(43.7)
	Fludarabine based	7	(9.8)
	Ibrutinib	1	(1.4)
	No treatment	32	(45.1)
9	Disease progression		
	Yes	28	(39.4)
	No	43	(60.6)
10	Disease mortality		
	Death	25	(35.2)
	Alive	46	(64.8)

Characteristics of CLL patients are described by absolute count (n) and percentage (%) unless otherwise specified.

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The patients' comorbidity burden is presented in Table 2. Among the patients population, 50.7% of them had at least one concurrent disease at diagnosis. Of the 24 patients presenting with ≥2 comorbidities, most had 2-3 co-existing diseases, while there were only 4 patients with >3 comorbidities. The two most common comorbidities were hypertension (32.9%) and diabetes (23.7%). Nineteen patients in the cohort had high burden of medical illness, defined as CIRS ≥4.

Table 2: The comorbidity burden of CLL patients

Characteristics	N (%)
Number of comorbidities	
0	35 (49.3)
1	12 (16.9)
≥2	24 (33.8)
CIRS score	
0 to 3	52 (73.2)
≥4	19 (26.8)

Decision to treat a CLL patient often relies on the clinical staging, the symptomatic presentation, and the disease activity. Patients in earlier stages (Rai 0-II, Binet A) are generally not treated but monitored with a "watch and wait" strategy. In our cohort, induction chemotherapy was started in 39 (54.9%) CLL patients with various regimes used. As first line therapy, chlorambucil and prednisolone (CP) regime was most commonly used at our setting due to its less toxicities and being a safer approach to our majority elderly patients especially those with multiple comorbidities. Other combinations of therapy used include cyclophosphamide, vincristine and prednisolone (CVP/COP) or cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) with combination of monoclonal anti-CD20 antibody rituximab in those patients with CD20 positivity, and fludarabine based regime such as fludarabine, cyclophosphamide and rituximab (FCR). Three of our patients were given obinutuzumab and chlorambucil combination and one patient with 17p- was given ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, both regimes made available under compassionate programmes.

Among the CLL patients, death was found in 25 out of 71 patients (35.2%); disease progression was found in 28 out of 71 patients (39.4%), with a minimum of 12 months follow-up duration. Survival analysis was performed for 55 patients (77.5%) who had FISH cytogenetic results. Survival curves were plotted among patients who had normal karyotypes, 13q-, 17p- and tri12. Inferior OS was found in patients with 17p- when compared to other chromosomal abnormalities, although result is not statistically significant ($p=0.0787$). According to the KM survival curve, the overall survival of patients with different karyotypes did not differ at the beginning of the disease until 50 months. At 50 months later, survival rate of patients with karyotype 17p- reduced greatly and all patients with karyotype 17p- died within 60 months. Patients with karyotype 17p- had the worse survival outcome with 62.5% mortality rate and a median OS of 48.033 months (95% CI: 5.260,58.257), as compared with other karyotype groups (Figure 1). Similar results were obtained for PFS, where inferior PFS was detected in patients with deletion of 17p ($p=0.0346$) when compared to other chromosomal abnormalities (Figure 2).

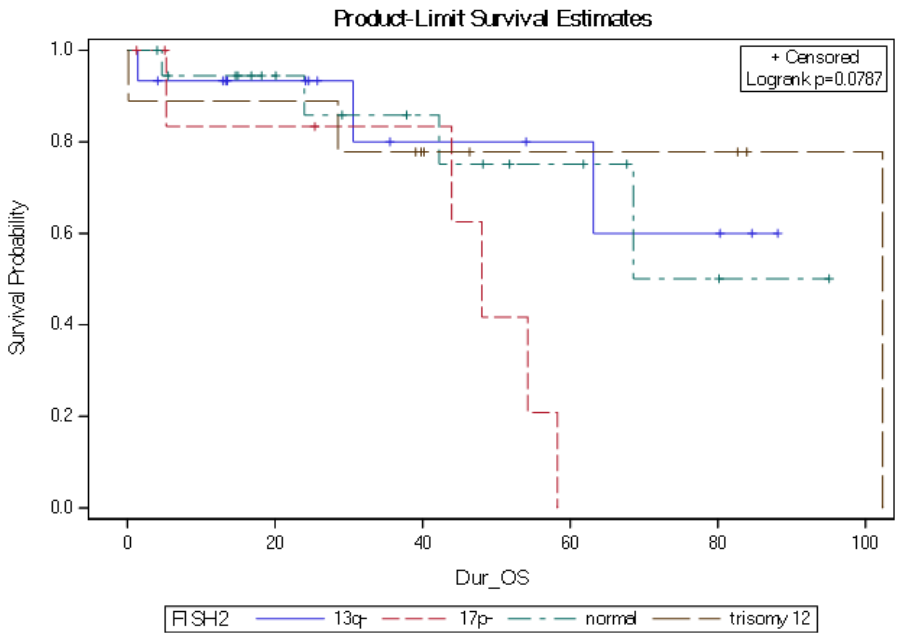


Figure 1: Overall survival of CLL patients according to FISH cytogenetics.

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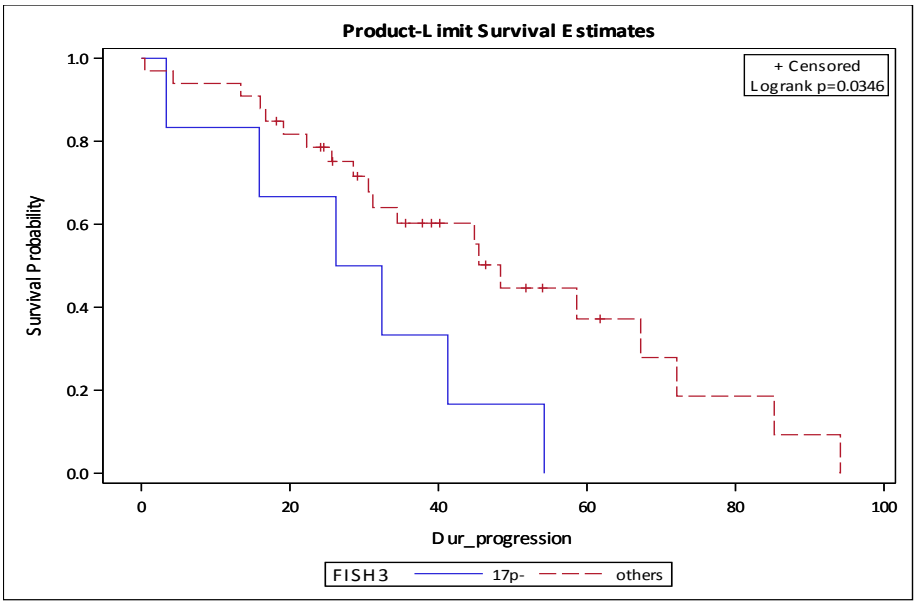


Figure 2: Progression free survival of CLL patients with 17p- compared to other chromosomal abnormalities.

From the analysis, OS of patients with different binet did not show statistically significant difference. However, patients with binet C were found to have highest mortality rate (43.8%) as compared with binet A (16.7%) and binet B (26.7%). The median OS of patients with binet C was 58.3 months (95% CI: 43.923, 102.312). The median overall survival of other binet groups cannot be estimated due to lack of events or insufficient follow-up duration (Figure 3). Patients with binet C also have inferior PFS compared to other groups, with median PFS of 32.38 (95% CI: 17.03, 48.36) months (Figure 4).

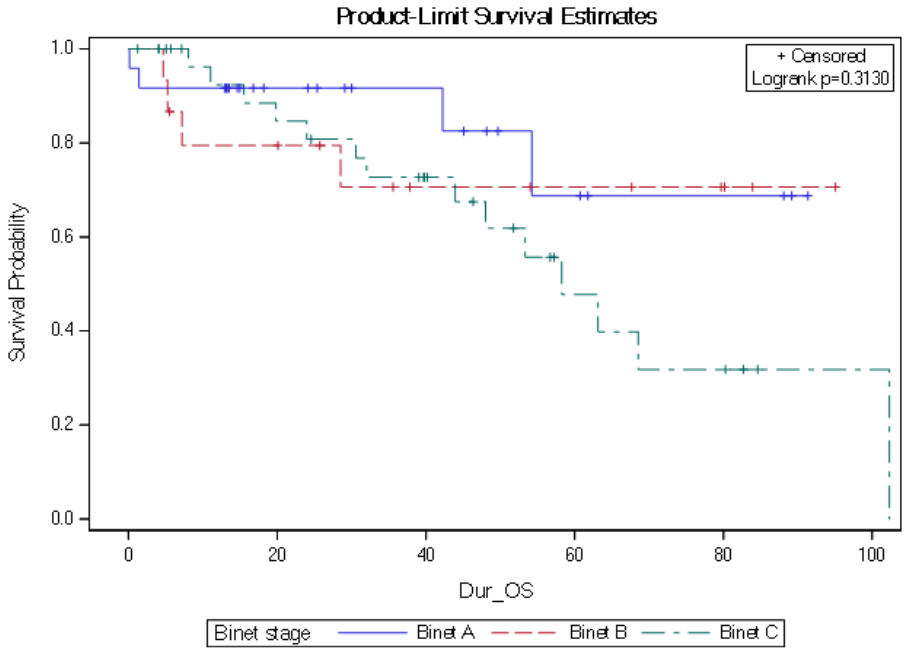


Figure 3: Overall survival of CLL patients according to binet staging.

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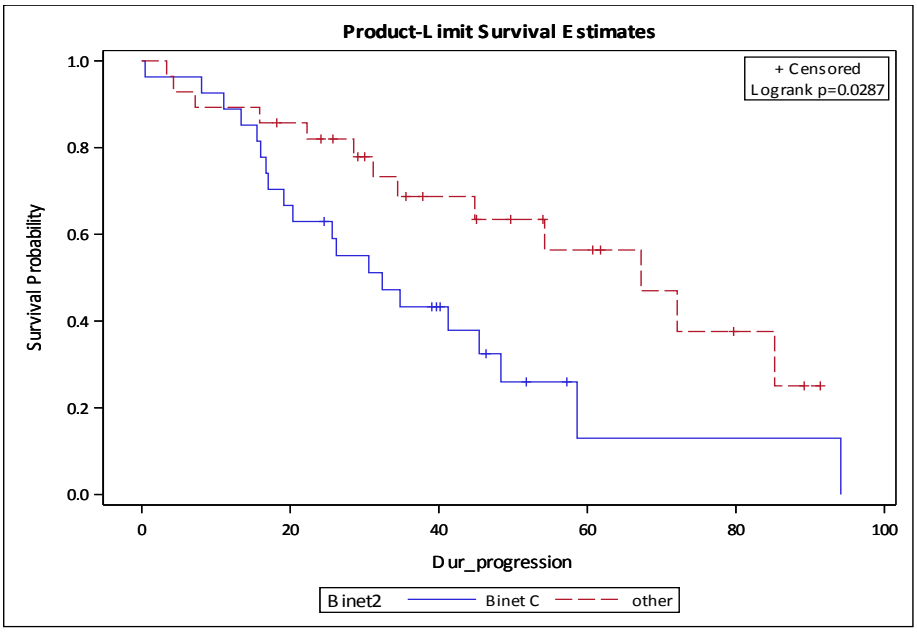


Figure 4: Progression free survival of CLL patients according to binet staging.

Overall survival of patients with CD38+ also did not show statistically significant difference from those with CD38- (p=0.1524). The median overall survival of patients with CD38+ was 53.326 months (95% CI: 28.537, 102.312) (Figure 5).

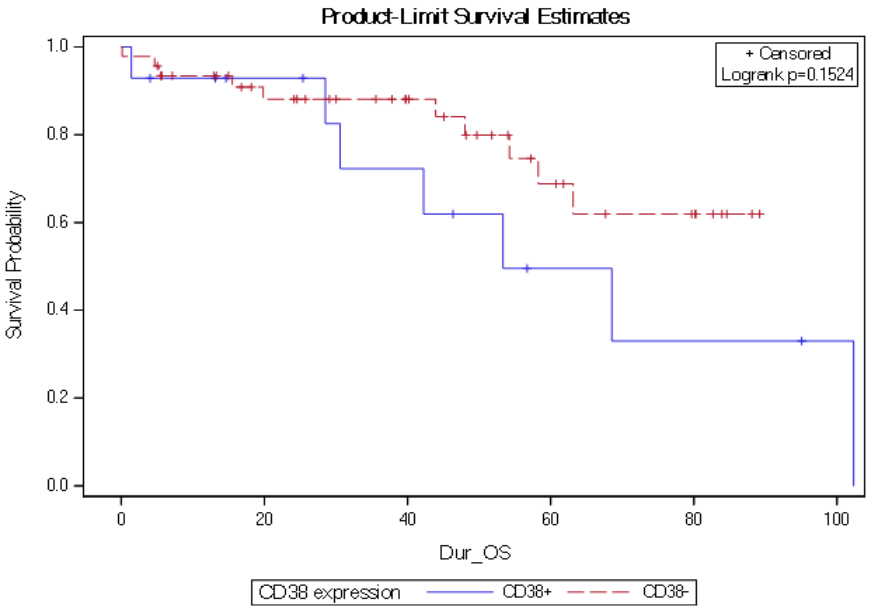


Figure 5: Overall survival of CLL patients according to CD38 positivity.

From the analysis, 39 patients who were treated for CLL presented inferior OS as compared with those who were not treated. The difference in OS is statistically significant (p=0.024) (Figure 6). Cox regression model (univariable) reveals that patients who were treated had 2.6 times higher hazards of death compared to those who were not treated (HR=2.653, p=0.042). Further analysis revealed that patients receiving fludarabine based induction protocol experienced inferior OS (median OS=48.0 months) as compared with those treated with alkylating based protocol (median OS=63.1 months). The difference in OS is statistically significant (p=0.032) (Figure 7). Cox regression model (univariable) reveals that patients receiving fludarabine based protocol had 3.15 times higher hazards of death compared to those treated with alkylating based protocol (HR=3.150, p=0.042).

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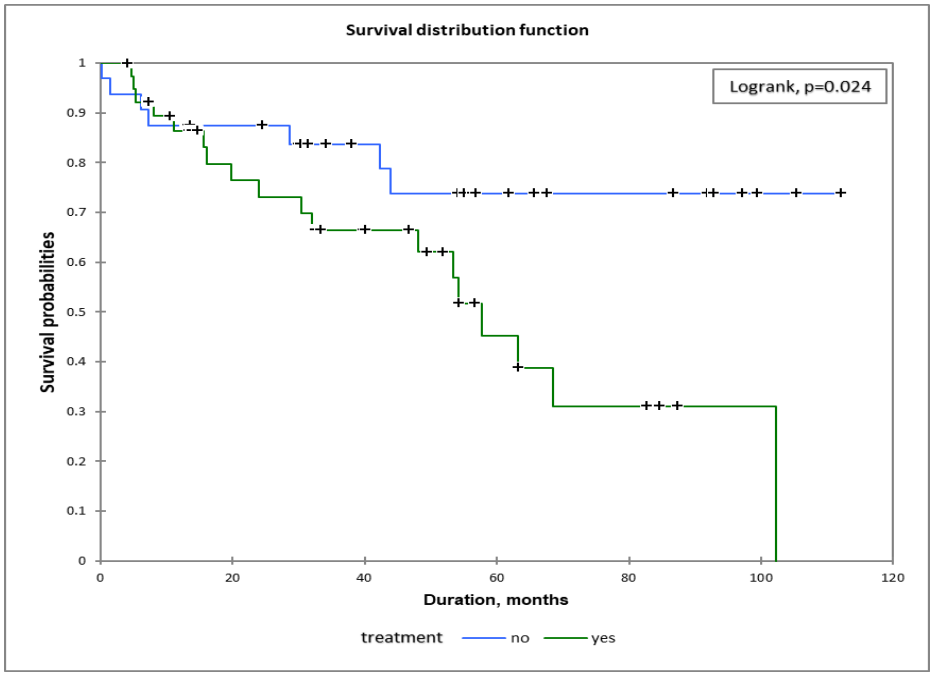


Figure 6: Overall survival of CLL patients according to treatment decision.

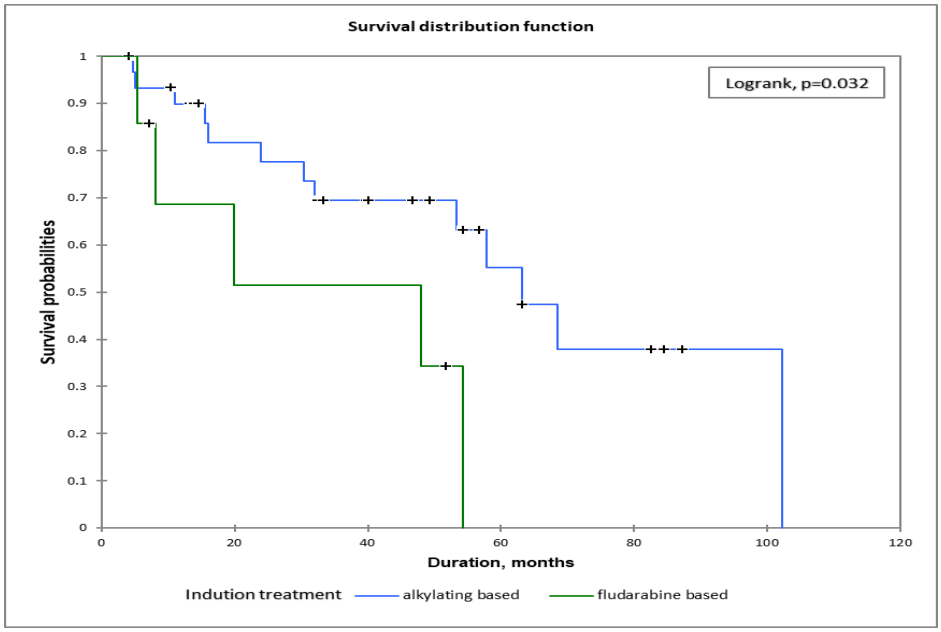


Figure 7: Overall survival of CLL patients according to induction treatment.

From our analysis, CIRS 0 to 3 has higher mortality when compared to CIRS ≥4 but survival analysis is not significant (Figures 8). This observation may be confounded by other co-variables with potential impact on OS such as age, treatment regimen, disease stage and disease risk.

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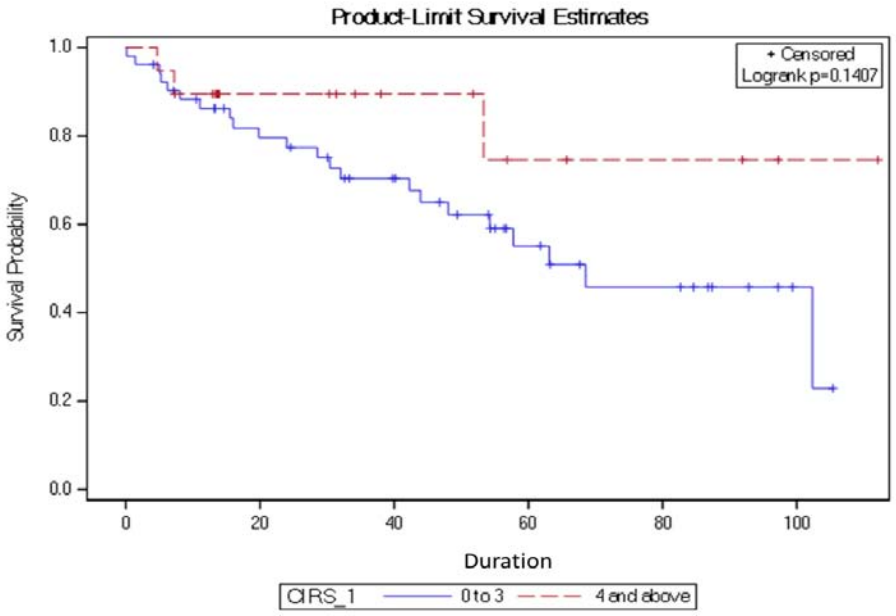


Figure 8: Overall survival of CLL patients by CIRS.

IV. Discussion

To our knowledge, this is the first study done looking at demographic data, clinical behavior as well as survival outcomes of CLL patients in Malaysia. The much lower incidence of CLL in Eastern countries, including Malaysia, is well known. Hence data on CLL in Asian countries is very limited. In Western populations, the median age at diagnosis lies between 67 and 72 years and more male than female patients (1.7:1) are affected.[11-13] In this study, the median age of our patients at diagnosis was 64 years with a male preponderance (2.7:1). The ethnic distribution for our CLL cohort was corresponding to our nation ethnic groups distribution, as majority were Malay patients.

In all cases, the diagnosis of CLL was established based on IWCLL by full blood counts, blood smears and peripheral blood for immunophenotyping. Further tests such as cytogenetic analysis by FISH and CD38 expression were analyzed as prognostic tools of the disease. In our cohort, a bone marrow was usually performed in cases with cytopenias (anaemia, thrombocytopenia) that may or may not be directly related to leukemia-cell infiltration of the marrow, or generally before initiating therapy when treatment was indicated. The diagnosis of CLL requires the presence of $\geq 5 \times 109$ B lymphocytes/L in the peripheral blood. The clonality of the circulating B-lymphocytes needs to be confirmed by flow cytometry, in which characteristically CLL cells co-express the T-cell antigen CD5 and B-cell surface antigens CD19, CD20, and CD23.

Once diagnosis of CLL is established, there are 2 widely accepted staging methods to stage the disease, the Rai clinical staging systems[3] and the Binet staging system.[4] Both systems provide not only a prognosis for a patient, but also identify when a patient is appropriate for therapy. To standardize the staging system, all the CLL patients in our cohort were staged according to the Binet system, which relied on the number of involved nodal areas and cytopenias to create a 3-group classification. Majority of our study population presented with higher risk disease, Binet stage C which is defined when there are presence of both cytopenias (hemoglobin $<$10g/dL and platelet $<$100x109/L) and any number of enlarged lymph nodes area. Binet stage C justifies treatment for all patients. We observed a poorer OS among our patients with Binet stage C with a median OS of 58.3 months, compared to other Binet stages. E. Montserrat[14] also demonstrated this observation in a study which showed patients with high-risk disease (Binet C; Rai stage III or IV) when compared to Binet stage A and B, have a worse median OS of 3-4 years.

Three of the four well-recognized cytogenetic abnormalities observed in CLL patients, including 13q-, trisomy 12, 11q- and 17p- are detected in our cohort except 11q-, with the highest frequency found in 13q- followed by trisomy 12 and 17p-. Patients with 17p- have been known to have an inferior prognosis and appear relatively resistant to standard chemotherapy regimes using alkylating drugs and/or purine analogs.[15-16] Our data demonstrated that patients with 17p- had inferior outcomes, both in OS and PFS. Based on our results, we concluded that both binet C and 17p- status may be able to predict disease progression among our patients. In addition, further analysis revealed that cohort with disease progression had inferior overall survival compared to those without disease progression (median OS=68.5 months, p=0.609).

Our analysis also showed that those who received treatment had inferior OS than those who were

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not treated, with worse outcome in those who received fludarabinebased regime. Further analysis of the five deaths that occurred in the fludarabinebased group (71.4%) showed that three of them had 17p-, one had complex cytogenetic abnormalities and one presented w Binet stage C with no cytogenetic performed. Disease-related, instead of treatment-related toxicity was the major cause of death amongst the five patients. This observation showed that fludarabine-based regime was commonly selected as treatment choice in our cohort for majority of patients who presented with a higher risk disease, in which we have earlier demonstrated that this group had an inferior survival outcome.

CD38 expression on leukemic lymphocytes was found to correlate with IgVH mutations and predicted clinical outcome. Subsequent research confirmed the prognostic value of CD38 expression, but has questioned its ability to predict IgVH mutational status.17-18 However, the most appropriate threshold to define CD38- positivity is controversial. It has been suggested that rather than a fixed, arbitrarily predetermined cut-off level, CD38 should be evaluated by its modal expression in flow cytometry or by the antigen density as measured by the antibody-binding capacity.[19] Our study demonstrated that OS of patients with CD38 expression did not show statistically significant difference from those with CD38- ($p=0.1524$). Clearly, CD38 analysis and the most reliable method for using it to determine CLL prognosis requires standardization and additional, prospective studies.

Two retrospective studies recently reported on comorbidity as a prognostic factor in CLL.20-21 In subjects with cancers others than CLL, comorbidity is associated with shortened survival.[22] Assessment of comorbidities in CLL has not really been standardized. In our study, we assessed CIRS score at enrollment to determine the burden of comorbidities of our patients. Based on our analysis, however we demonstrated that CIRS 0 to 3 has higher mortality when compared to CIRS ≥ 4 but survival analysis is not significant ($p=0.1407$). In a retrospective study, this result may be confounded by other co-variables such as age, treatment choice and disease burden. This observation may also be compounded by the lower prevalence of comorbidities (50.7%) in our study compared to that in the general CLL population (90%).[23] The lower mortality observed in patients with CIRS ≥ 4 may be related to greater chance of dose attenuations of therapy which limits therapy-related toxicity and hence mortality.

V. Conclusion

In summary, although the numbers of patients diagnosed with CLL are still small in Malaysia compared to Western countries, the incidence of CLL is definitely gradually increasing. Hence, the increasing role of response predictors in prognostication cannot be overemphasized. Nonetheless, before a good prognostication system can be implemented, the methods to determine the prognostic parameters should be fully standardized and their prognostic value should be validated in large prospective clinical trials. Our analysis is, however limited by few factors that include missing data as well as short follow-up duration of 12 months. Future prospective studies are also needed to validate CIRS effect on overall survival so that we could optimize treatment strategies in this high-risk population, including in a setting of novel "targeted" therapies. We feel that researchers should take a more profound interest in the field of CLL, especially in the era of “precision medicine” whereby true predictive markers are highly desirable.

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I acknowledge the important contributions of all the investigators who have participated in this study.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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