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Research Article
Empirical Study on Exploring the Role of CD180 and MD-1 Prognostic Indicators for the Chronic Lymphocytic Leukaemia (CLL) Disease

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Abstract

Background and Objective: Chronic Lymphocytic Leukaemia (CLL) is a frequent type of leukaemia disease. This study was focused on investigating the role of prognostic indicators, such as CD180 and MD-1 for Chronic Lymphocytic Leukaemia (CLL) pathogenesis because they involve cell signalling and proliferation. **Materials and Methods:** A total of 12 normal controls and 52 patients were taken to determine the expressions of CD180 and MD-1 with different variations in comparison with the IgVH (Immunoglobulin Heavy Chain variable region gene) mutational status, FISH (fluorescence *in situ* hybridization) and Rai staging. **Results:** The quantitative data findings were evident that CD180 and MD-1 expressions showed insignificant differences among CLL patients at the protein level based on SPSS results. On the contrary, they resulted in significant differences for subgroups of established biomarkers like Rai staging (stages 0, I, II and III), FISH (13q and non-13q deletions) and IgVH (mutated and unmutated). **Conclusion:** The CD180 and MD-1 have been used as prognostic indicators to evaluate the outcomes relevant to the cell cycle and survival rate of CLL cells.

Key words: Chronic Lymphocytic Leukaemia (CLL), patients, prognostic indicators, pathogenesis, fluorescence *in situ* hybridization (FISH), Rai staging

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The most frequent type of Leukaemia disease is Chronic Lymphocytic Leukaemia (CLL) caused in adults[1]. The disease heterogeneity of CLL is due to molecular underpinnings such as the functional status of signalling molecules, different phenotype profiles in malignant B cells and diverse genetic and epigenetic lesions[2]. The pathogenesis of CLL included contributors, such as genetic and epigenetic aberrations, different receptors for activation of cell interactions with the microenvironment, mutation status of Immunoglobulin Heavy chain (IgVH) genes to determine the reactions of malignant B cells for stimulation of antigens and antigenic B cell receptor (BCR) stimulation. In spite of the signalling of B cell receptor (BCR) is a critical portion of the CLL disease pathogenesis. Earlier studies demonstrated that the targeted therapy against the BCR signalling involvement resulted in objective responses for over 70% of CLL patients[3]. Furthermore, CLL B-cells are influenced by the genome positions relevant to the chromosomal locations of microRNAs[4]. Therefore, the study focuses on reflecting the malignant proliferation of B-cells mechanism by microRNA profiling.

The microRNA (miRNA) profiles can be utilized to detect the malignant CLL from normal B cells based on progression, prognosis and drug resistance. The aggressive and indolent CLL cases were differentiated based on the signature profile of 13 microRNAs. Recent studies illustrated the interplay role of BCR signalling and microenvironmental stimuli with the involvement of miRNAs[5]. Indeed, the regulation of BCR signalling and production of immunoglobulin has been carried out based on miRNAs. The BCR activation in patients with shorter survival can reduce the expression levels of some microRNAs like miR-223, miR-29c and miR-150. Packham *et al.*[6] revealed that miRNAs are released by donor cells with the microvesicles (MVs) circulation. However, the plasma features are not affected while continuing the MVs to induce the abnormal gene profiles like the malignance cell group that could be reduced through the therapy. Thus, the profiling of microRNAs could reflect the B-cells with malignant proliferation. Doghish *et al.*[7] discussed the significant development of CLL cells with their genome positions regarding the chromosomal locations of miRNAs. These reports did not discuss the significance of biomarkers, such as CD180 and MD-1 mRNAs as prognostic indicators for CLL disease course.

The prognostic markers like CD38, Zap70 and CD49d may provide unfavorable results in the pathogenesis of CLL as they are involved with the enhanced signalling of BCR directly or indirectly, allowing B cells to survive among CLL patients[8]. To overcome these problems, CD180 and MD-1 receptors are considered potential biomarkers as they provide a favourable prognosis of CLL through the regulation of Toll-like Receptors (TCR) and the proliferation of normal B cells[9]. However, no studies provided detailed information about the miRNA expression levels of MD-1 and CD180 in the pathology of CLL. The present study includes the analysis of colocalization and expression of MD-1 and CD180 on the B cell surface in patients who suffered from CLL disease. Moreover, the biological significance of pathobiology in CLL B cells is demonstrated under the colocalization of CD180 and MD-1 on the cell surface.

MATERIALS AND METHODS

Study area: The samples from 52 previously untreated patients and 12 age-matched control subjects with verified CLL diagnosis were obtained from the Department of Oncohematology of King Saud University, Saudi Arabia. The study was conducted on 10th April, 2023.

Sample size/data collection (inclusion and exclusion criteria): The data of 12-age matched normal control and 52 untreated CLL patients were collected from the King Saud University, Saudi Arabia. To evaluate the expression levels of CD180 and MD-1 under different conditions like FISH and IgVH, the isolation of peripheral blood B cells was considered from 52 untreated patients of CLL. Whereas, Rai staging included 51 untreated patients to predict the CD180/MD-1 expressions. This collected data was analysed using IBM® SPSS® (version 26.0) software to understand the pathogenesis of CLL in terms of molecular mechanisms.

Research methodology: To perform the empirical study for prognostic biomarkers of CD180 and MD-1 in CLL disease pathobiology, the positivism research philosophy and analytical research design were used to understand their role in the diagnosis process of CLL disease in comparison with the established prognosis indicators like IgVH, Rai staging and FISH tests based on quantitative investigation. This research philosophy helps to get insights into the analysis under various conditions because this research includes the comparison of CD180 and MD-1 prognostic indicators for Rai staging, IgVH and FISH. This research study includes an analysis based on a quantitative method using collected primary data and a deductive research approach to determine the study objectives significantly.

Most initial CLL cases did not show symptoms like unintentional weight loss, anaemia or infection, tiredness and night sweats. Accordingly, the diagnosis is performed based on the routine blood test, including a high count of white

Table 1: Rai staging system for CLL patients

Stage	Risk	Median survival (Yr)	Clinical features
IV	Very high	0	Thrombocytopenia and lymphocytosis (platelets<100*10^9 L^{-1})
III	High	2	Anaemia and lymphocytosis (Hgb<11 g dL^{-1})
II	Intermediate	6	Enlarged liver of spleen based on lymphocytosis
I	Intermediate	6	Enlarged lymph nodes based on lymphocytosis
0	Low	>10	Lymphocytosis presence in peripheral blood (PB) and bone barrow only

blood cells (WBC)[10]. Advanced CLL cases have been identified through cervical or marginal lymphadenopathy, moderate or mild splenomegaly, thrombocytopenia and hepatomegaly. Ultimately, it will cause infection, bleeding and bone marrow failure. The diagnosis of CLL has been performed by considering a process of increasing at least or more than 50% of blood lymphocytes with 5000 lymphocytes/µL of peripheral blood (PB) for three months[11]. These cells were cloned by implementing flow cytometry (FC) based on the clinical and morphological evaluation of peripheral blood and patients. On the other hand, tests were performed to get insights about the prognostication and tumour[12]. However, those tests included cellular and serum biomarkers, fluorescence in site hybridisation (FISH), deep sequencing and disease Rai staging.

Rai staging: Rai has developed a clinical staging strategy for CLL through the staging system that determines the initial prognosis among individual patients for accurate treatments. Based on the Rai system, five stages included 0 to IV considered lymphadenopathy, lymphocytosis, anaemia, thrombocytopenia and hepatic or splenomegaly[13]. Binet *et al.*[14] opined that the median survival time for patients with low-risk of CLL (Rai stage 0-I) was 12 years while the patients who come under the higher-risk category of Rai IV stage whose median survival rate was lower than three years old. As shown in Table 1, the remaining CLL cases come under the intermediate risk (Rai I-II) category.

Deep sequencing: The IgVH gene mutation is one of the popular deep sequencing methods to test the presence of CLL among patients[15]. However, IgVH genes are categorized into two types based on their clinical shared characteristics, such as unmutated and mutated. The IgVH gene mutation correlates with a longer survival time of up to 293 months and the lethargic disease course. Moreover, it is often present with the 13q14 abnormality for a single chromosome. On the contrary, the unmutated status of IgVH is associated with a shorter survival rate and no improved prognosis. Furthermore, the patients have abnormalities relevant to cytogenetic causes like deletion of 13q and non-13q[16]. The somatic hypermutation has occurred in germinal centres (GCs) of lymph nodes, normal B cells of a patients' group and the B cells of CLL.

Fluorescence *in situ* hybridisation (FISH): The diagnostic significance of metaphase banding and DNA fluorescent probe techniques in CLL are used based on fluorescence *in situ* hybridisation (FISH) tests to analyse the chromosomes. Ghia *et al.*[17] demonstrated that genetic abnormality was presented within the malignant clone of patients (80% of CLL cases). The CLL pathology, clinical characteristics and response of therapy impact by the chromosomal defects. Parikh and Shanafelt[18] illustrated the most common stratified aberrations of CLL disease courses with the use of FISH tests. Therefore, non-13q and 13q deletion are the most related karyotypes to perform the overall prediction and patient survival rate associated with the chemotherapy.

Chronic Lymphocytic Leukaemia (CLL) results in heterogeneity based on clinical outcomes. This is caused due to factors like phenotypic profile, the status of signalling molecules and genetic and epigenetic lesions. The genetic aberrations, antigenic B cell receptor (BCR) stimulation, cell activation and interaction and mutation status of IgVH play a key role in the pathogenesis of CLL disease[19]. The studies showed that over 70% of CLL cases provided objective responses with the targeted therapy against the BCR signalling kinases. Potential receptors like CD180 and MD-1 would be considered in this study for a favourable prognosis of CLL as they regulate the Toll-like receptors and activate the normal B cell proliferation[20]. Therefore, the data findings of this study will include the colocalization of CD180 and MD-1 receptors relevant to the pathology of CLL B cells based on the established prognostic methods such as Rai staging, IgVH genes mutation and FISH (13q and non-13q deletion) in CLL patients.

Studied parameters: The research study includes the comparison of differences between expression levels of CD180 and MD-1 for CLL patients against different Rai staging (I, II and III-IV). In addition, the differences between IgVH mutation status (mutated and unmutated) and FISH deletion (13q and non-13q deletions) were analyzed to compare the effects of MD-1 and CD180 in the population of CLL patients.

Ethical consideration: As the collection of CLL patients' data involves individual clinical decisions, the research study should incorporate ethical considerations for all activities, including data collection and analysis. However, the written informed consent was taken from the Institutional Review Board and Research Ethics Committees of the University when collecting the data. Moreover, it is vital to protect the rights of patients with research access and consider all steps regarding the data retrieval consent for clinical data usage, limitation of access to confidential data and minimization of disclosure risks. Therefore, the research study used the effective strategy of preventing the disclosure of confidential data while informing the patients about the purpose of data collection relevant to the research uses.

Statistical analysis: Based on the hypothesis test of the ANOVA, the variance estimates are compared for two independent samples of normally distributed CLL patients' data. The assumptions of the ANOVA test included independent observations, normal distribution of data and population data with similar variances. However, a one-way ANOVA is implemented for comparing the differences among three or more groups contrary to the two-way ANOVA test and determining the effect of parameters on the other factors[21]. Thus, this study used the suitable method of one-way ANOVA to compare the MD-1 and CD180 levels for CLL patients against Rai staging as it includes three groups, such as group 1 (Rai stages 0-I), group 2 (Rai stage II) and group 3 (Rai stages III-IV).

The independent samples t-test is used for comparing two groups from the population or to determine whether the population means differ from each other. It is an appropriate method to understand the mean differences between IgVH mutation status and FISH deletion for comparing the effect of prognostic indicators MD-1 and CD180 in CLL patients. Because the FISH Test includes non-13q and 13q deletion while IgVH involves mutated and unmutated statuses. The equality of means t-test is considered to find out the mean differences between unmutated and mutated IgVH gene status for MD-1 or CD180 mRNA levels[22]. In the case of the FISH Test, Levene's Test is used to evaluate the variation of CD180 or MD-1 mRNA expressions in CLL patients.

Graphical modelling is a great method to investigate the medical data of mRNA profiles for CLL patients. The current study used the box plot as graphical modelling to visualize the strongest differences in each group based on data distribution and skewness using data quartiles and averages. The box plot includes lower and upper quartiles, median, averages and minimum scores[23]. The CD180 or MD-1 mRNA expression levels were analysed for CLL patients using a box plot against different mutational statuses (Mutated and Unmutated), Rai staging (0-I, II and III-IV) and FISH (13q and non-13q).

The SPSS Version 26.0, IBM Corp was used to analyse the differences of proposed prognostic indicators such as MD-1 or CD180 mRNA in CLL patients against FISH, Rai and IgVH tests. However, the statistical significance was considered as $p<0.05$.

RESULTS

The disease predictions are required to be performed for each patient to propose the personalised treatment procedure. Medical practices like chromosomal aberrations, IgVH mutational status and genetic mutations have been incorporated with some biomarkers or prognostic indicators to be exploited in predicting favourable conditions for disease prognosis. However, recent studies have been related to the correlation of IgVH with the 13q deletion and non-13q deletion that are associated with mutated IgVH CLL cells and unmutated IgVH cells for the prognosis of disease course. Due to the limited usage of clinical practice, it is necessary to develop high-priced molecular techniques for determining the IgVH mutational status. The study revealed that CD38 overexpression on CLL is related to advanced disease stage, short-term survival rate and unfavourable prognosis. In the U-CLL, chemotherapy or chemoimmunotherapy is required for elevated expression of CD38. Moreover, chromosomal aberrations like chromosome 11 (del 11q), chromosome 6 (del 61), del 13q and del 17q included in CLL that encountered the ataxia telangiectasia mutated (ATM) locus.

CD180 or MD-1 mRNA expressions for Rai staging: The SPSS results for CD180 and MD-1 expressions are analysed for three different groups of Rai staging, including stages 0-I, II and III-IV. However, the total number of patients is 51 for Rai staging ($n = 51$) and the ANOVA tests to understand the expression of CD180 and MD-1 differed for different Rai stages. The retrieved p-values were 0.899 and 0.774 for CD180 and MD-1, respectively. These p-values do not indicate significant results as they are greater than 0.05 and accept the null hypothesis. Therefore, CD180 and MD-1 do not result in significant expression among patients of Rai staging. This section included data analysis results for investigating the expression of CD180 and MD-1 biomarkers in CLL patients using IBM® SPSS® (version 26.0) software tool.

Figure 1 shows the results for CD180 expression among CLL patients with different Rai staging. However, the results indicated that CD180 expression differed for three patient groups owing to the unequal median lines. However, it differed largely for both group 2 and 3 patients than the

group 1 patients. The data distribution is normal for group 3 patients while it is positively skewed for both group 1 and group 2 patients.

Figure 2 depicted the box plot results for MD-1 mRNA expression for three different Rai staging groups among CLL patients. The MD-1 expression differs for all three groups as their median lines are contradictory to each other. There is a large difference in MD-1 expression among group 3 patients than the group 1 and 2 patients. The data distribution is positively or right skewed for groups 1 and 3 while it is negatively skewed for group 2 patients.

CD180 or MD-1 mRNA expressions for IgVH: The independent samples t-tests resulted in the p-values of 0.634 for CD180 and 0.336 for MD-1 expressions for 52 patients. The p-values resulted in higher values than significance value of 0.05. Thus, there is an insignificant difference in CD180 and MD-1 expressions for CLL patients with unmutated (n = 28) and mutated (n = 24) statuses because of the rejected alternative hypothesis.

Figure 3, CD180 mRNA expression results are analysed for IgVH mutational status with unmutated and mutated cases. The box plot showed that mutated and unmutated statuses of IgVH are differentiated for CD180 expression as the median lines are not equal. The scattering or dispersion of data is equal for both mutated and unmutated statuses which indicates CD180 expression differs similarly for both plots. The data distribution is not similar for these plots, such as left or negatively skewed and positively skewed for unmutated and mutated IgVH statuses, respectively.

Figure 4 shows the box plot graph for MD-1 mRNA expression of CLL patients with unmutated and mutated IgVH statuses. As there are unequal median lines, MD-1 expression differs for both mutated and unmutated groups of patients. However, the dispersion of MD-1 expression is higher for the unmutated patients' group compared to the mutated patients. Contrary to CD180 expression, the unmutated group has positively skewed data while the mutated group has negatively or left skewed data. Thus, the MD-1 mRNA expression is differentiated for IgVH mutation status under unmutated and mutated conditions.

CD180 or MD-1 mRNA expressions for FISH: The total number of patients is 52 taken to analyse the relationship between CD180 or MD-1 and FISH. Accordingly, the independent samples t-tests are performed for CD180 and MD-1 in comparison with FISH, including 13q deletion (n = 22) and non-13q deletion (n = 30) for CLL patients. However, the p-values were obtained as 0.674 for CD180 and 0.439 for MD-1 (i.e. p>0.05). Therefore, an insignificant difference existed for CD180 and MD-1 expressions in CLL patients regarding non-13q and 13q deletions.

Based on Fig. 5 (Box-plot graphs), a significant difference resulted between 13q and non-13q deletions for CD180 expression of CLL patients due to the median line of non-13q being outside of the 13q deletion type. The CD180 expression does not differ largely for both deletion types due to the equal scattering of data. The significant differences resulted in data distributions of 13q and non-13q deletions, such as positively skewed and normally distributed, respectively.

Figure 6 illustrated the box-plot graphs for MD-1 expression in comparison with FISH, a significant difference was observed between 13q and non-13q deletions due to the non-alignment of median lines. The equal data scattering was reported for 13q and non-13q deletions which means MD-1 expression does not differ. Both box plots showed different data distributions, such as 13q deletion type data is negatively skewed while non-13q has positively or right skewed data in CLL patients.

The ANOVA tests disclosed that insignificant expressions of CD180 (p = 0.899>0.05) and MD-1 (p = 0.774>0.05) were reported for CLL patients under Rai staging (stages 0-I, II and III-IV) as they accepted the null hypothesis. Moreover, the box plot results for three groups of patients with Rai staging showed a difference in both MD-1 and CD180 expression levels. The prognostic indicators CD180 and MD-1 expressions are demonstrated at the protein level in CLL patients in comparison with the established prognosis methods like FISH, Rai staging and IgVH mutational status of genes. The ANOVA tests disclosed that insignificant expressions of CD180 (p = 0.899>0.05) and MD-1 (p = 0.774>0.05) were reported for CLL patients under Rai staging (stages 0-I, II and III-IV) as they accepted the null hypothesis. Moreover, the box plot results for three groups of patients with Rai staging showed a difference in both MD-1 and CD180 expression levels. Based on the independent samples t-test of IgVH for CD180 and MD-1, there were insignificant differences due to the acceptance of the null hypothesis. However, they resulted in p-values of 0.634 and 0.336 for CD180 and MD-1 prognostic indicators. Furthermore, box plots showed a significant difference between CD180 and MD-1 expressions for both unmutated and mutated status of IgVH because of the unequal median lines. Similarly, independent t-tests for FISH showed that CD180 (p = 0.674>0.05) and MD-1 (p = 0.439>0.05) expressions were not significant for CLL patients with non-13q and 13q deletion types at mRNA levels. The box plot for the FISH test determined that CD180 and MD-1 expressions differed for CLL patients with 13q and non-13q deletions because of the non-alignment of median lines.

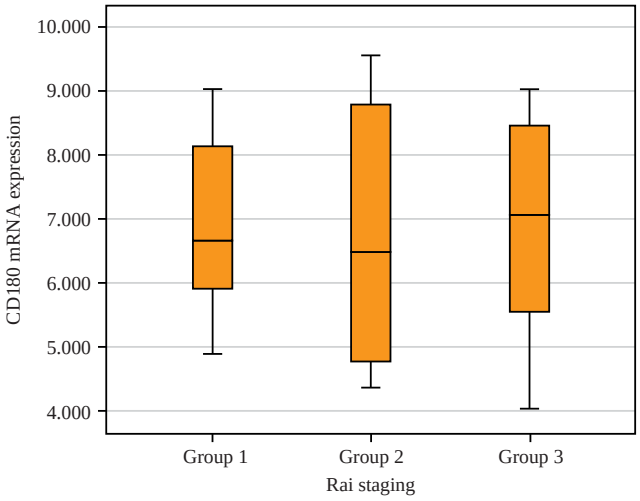


Fig. 1: CD180 mRNA expression with different Rai staging (group 1, 2 and 3) in CLL patients

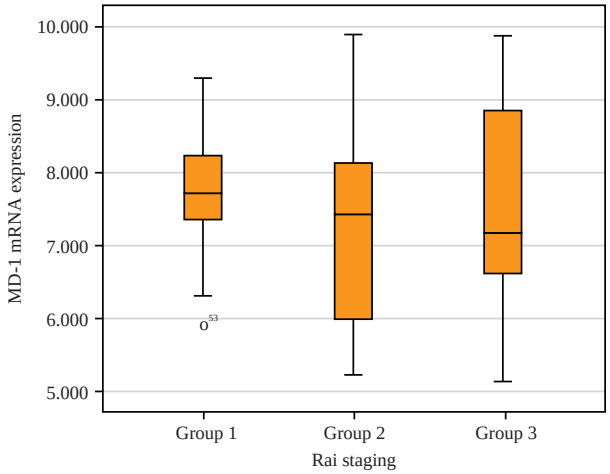


Fig. 2: MD-1 mRNA expression vs Rai staging for CLL patients

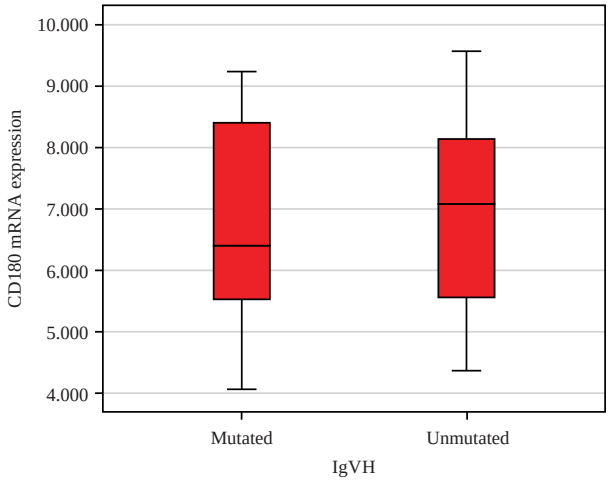


Fig. 3: CD180 mRNA expression for CLL patients with different mutational status (i.e. unmutated and mutated) of IgVH

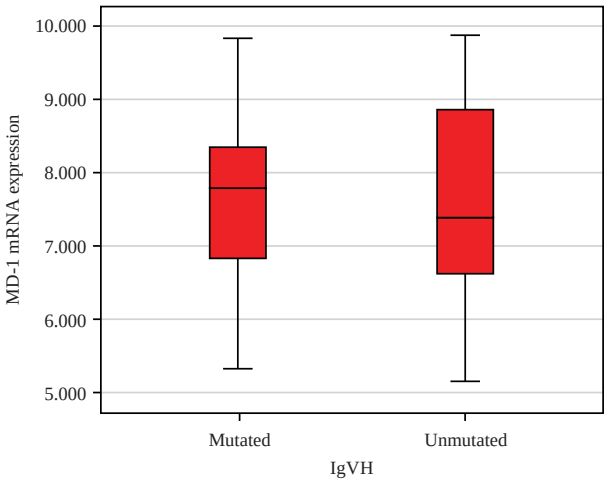
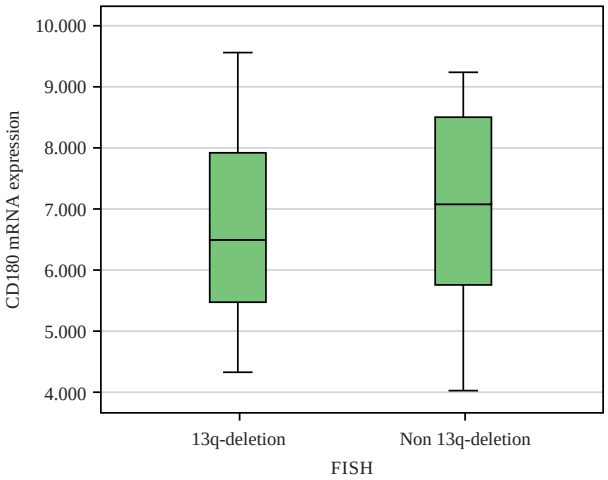


Fig. 4: MD-1 mRNA expression for CLL patients with different mutational statuses of IgVH (unmutated and mutated)



mRNA expression results for different FISH deletion karyotypes among CLL patients (13q and non-13q deletions)

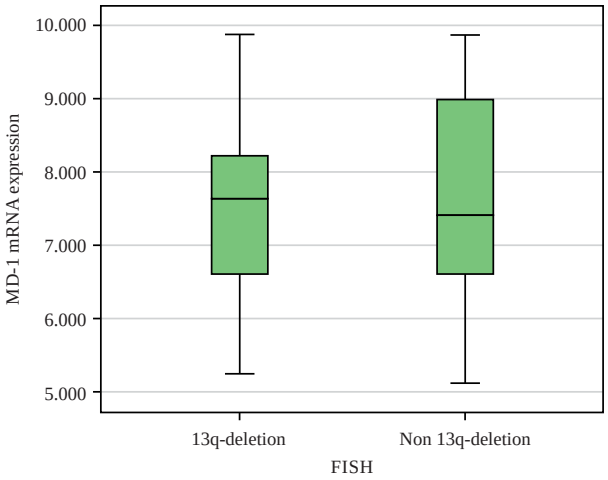


Fig. 6: MD-1 mRNA expression for CLL patients with different karyotype deletions of FISH (13q and non-13q deletions)

DISCUSSION

Different clinical courses resulted in different mutations at the chromosomal level, such as a favourable prognosis for CLL cases for a longer survival period over 10 years with normal karyotype or del13q and an unfavourable prognosis for CLL cases for a shorter survival period up to 32 months based on TP53 local removal that associated with del17q[24]. Even though there are various medical practices available to treat the individual heterogeneity of CLL disease course, it is a challenge to propose the optimal prognosis and treatment regimen to carry out the stratification for individual patients. However, different prognostic models have been improved for the consolidation of prognostic indicators to predict clinical results. Accordingly, one of the receptors is CD180 which performs prognostication and stratification of CLL cases for individual patients. Porakishvili *et al.*[25] opined that CD180 receptors with expression of heterogeneity are associated with M-CLL. Another study demonstrated that CD180 ligation resulted in the upregulation of the phosphorylation of ZAP70[26].

The CD180 expression has been varied in lymph nodes of B-CLL cells due to the heterogeneity and the binet staging of the disease course. On the contrary, CD180 expression is not correlated with the PB of CLL cells. The studies showed that the median expression of CD180 was reduced in stage B of Binet than in stage A under the cohort stages of treated and untreated conditions. The reduced expression of CD180 is correlated with the morbidity that progresses the CLL in LNS for untreated patient cases. The reduced density of CD180 expression could proliferate the CLL cells and increase their activation. On the other hand, the activated B cells have been down-regulated with the CD180 expression based on the study by Meyer-Bahlburg and Rawlings[27].

Edwards *et al.*[28] discussed that the increased CD86 expression and proliferation marker of Ki67 leads to the ligation of CD180 in the PB of CLL cells for 60% of patients. The data analyses are evident that the survival rate improved with the increased expression levels of CD180 among CLL patients who are in the early disease stages. Another study revealed that CD180 and H-score expression patterns were similar in the LNs of both treated and untreated patients which means CD180 expression is not impacted by the treatment method for LNs[28]. It indicated that CD180 represented as the prognostic indicator independently to determine the CLL progression using medical treatment methods. Furthermore, the heterogeneity of CD180 expression across all treatments resulted from the data analysis for the treated cohort. The current study analysed the CD180 or MD-1 expression for CLL patients under different treatment conditions but no results were included for molecular inhibitors like ibrutinib or idelalisib. Future research is required to evaluate the impact of molecular therapies on the LNs or PBs of CLL cells for CD180 or MD-1 expressions.

The CLL cells could enable the signalling for survival among patients with the expression of CD180. However, the signalling could happen for CLL cell survival within the LNs and the synergy between CD180 and BCR could promote CLL cell proliferation. The favourable disease outcome resulted in some CLL cases based on signalling apoptosis with p38MAPK[29]. Thus, it is essential to consider investigating the clinical heterogeneity of CD180 signalling in the LNs and PB in CLL patients. In this study, CD180 expression with heterogeneity is demonstrated for CLL patients. Based on the flow cytometry, CD180 was shown to be expressed as low or negative, higher and intermediate in PB of CLL cells. The lower level of heterogeneity in expression patterns of CD180 and higher H-score resulted in tonsillar control samples for CLL patients. In this section, the collected data was analysed and the results were to determine the mRNA expression levels of CD180 or MD-1 with the implementation of Rai stage, FISH and IgVH in CLL patients.

The ANOVA Test results for CD180 or MD-1 mRNA expressions did not show significant differences for different groups of Rai staging, such as group 1, 2 and 3 while the box plots depicted that they differed for sub-groups of Rai staging (stage 0-I, stage II and stage III-IV) that supported the studies of Edwards *et al.*[28]. The median line is higher for group 3 patients of CD180 expression level which means it is reduced for group 3 than remaining group 1 and 2 patients. Group 1 patients have greater median lines than group 2 and 3 patients for the MD-1 expression so it is reduced for Group 1 patients. Therefore, CD180 and MD-1 expressions were reduced for Rai staging III-IV and Rai staging 0-I, respectively. In the case of IgVH, CD180 or MD-1 mRNA levels did not result in significant differences for CLL patients based on the analysis of independent samples t-tests. However, t-tests for CD180 and MD-1 did not reject the null hypotheses as their $p>0.05$. The box plot results disclosed the reduced expression for the unmutated case because of the greater median line in comparison with the mutated case. On the contrary, MD-1 mRNA expression was reduced for mutated cases regarding the IgVH mutational status. The current studies showed that CD180 expression has no significant difference for unmutated and mutated IgVH. Thus, CD180 expression was reduced for CLL patients under unmutated conditions while MD-1 mRNA expression was reduced for mutated genes IgVH of CLL patients. The independent t-test results for MD-1 and CD180

showed insignificant differences among CLL patients under FISH, including 13q and non-13q karyotype deletions. The expression of CD180 was reduced for non-13q deletion while MD-1 expression at the protein level was reduced for 13q deletion in the FISH deletion karyotypes for CLL patients.

These data findings suggest that CD180 and MD-1 biomarkers can be used to predict the heterogeneity in CLL disease course, including progression, responsiveness of therapy and tumour aggressiveness. The research study of these prognostic biomarkers provides insights into the behaviour of CLL cells by evaluating the expression of CD180 and MD-1 with the surface receptors. Moreover, CD180 and MD-1 have been truly utilized as biomarkers by relating the signalling activity with clinical data on therapy responses, survival rate and disease progression. These data could also be established in the investigations of other haematological malignancies. Therefore, it would become possible that CD180 and MD-1 prognostic biomarkers can predict the outcomes of CLL disease progression. These studies are limited to the investigation of CD180 and MD-1 expressions against established biomarkers, like FISH, IgVH and Rai staging. The research study can be extended to explore the expressional and functional studies of CD180 and MD-1 with the highlights of potential therapeutic avenues as prognostic biomarkers in CLL pathology.

CONCLUSION

In summary, the role of prognostic indicators like CD180 or MD-1 has been evaluated in the CLL disease course. A better understanding of predictive biomarkers can be used to predict the survival rate of patients with CLL. The well-established biomarkers, such as Rai staging, FISH and IgVH in comparison with the CD180 and MD-1 will be utilized to improve risk stratification and guide treatment decisions. The prognostic value was reported for subgroups of Rai staging (0, I, II and III), FISH with 13q and non-13q deletions and IgVH with mutated and unmutated statuses. Further studies need to evaluate the potential benefits of CD180 and MD-1 biomarkers in the CLL pathology and study the prognostic value of other biomarkers in the CLL pathogenesis.

SIGNIFICANCE STATEMENT

Chronic Lymphocytic Leukaemia is a heterogeneous disease that poses the need for identifying the molecular predictors for treatment. Since CLL therapy transitioned to target therapies from genotoxic combination therapies, it remains important to identify the predictive biomarkers to improve the survival rate of CLL patients. The study aims to investigate the importance of prognostication of CLL to categorize patients into different risk categories for making clinical management decisions efficiently. It focuses on comparing the emerging prognostic molecular biomarkers in CLL with the well-established prognostic markers. Special emphasis will be considered to evaluate the role of predictive biomarkers in relevant to newer targeted therapies. Therefore, this research study will be useful as a reference for clinicians, professionals, trainees and laboratory investigators of molecular pathology practice.

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