Bone Marrow Lymphocytes: can they be an alternative for bone marrow lymphoblasts as early predictors of relapse and remission in all at the post-induction chemotherapy phase?

Linfócitos da Medula Óssea: podem ser uma alternativa para os linfoblastos da medula óssea como preditores precoces de recidiva e remissão na fase de quimioterapia pós-indução?

Linfocitos de Médula Ósea: ¿pueden ser una alternativa a los linfoblastos de médula ósea como predictores precoces de recaída y remisión en la fase posterior a la quimioterapia de inducción?

ABSTRACT
Background: Acute Lymphoblastic Leukemia (ALL) is a hematological malignancy that arises due to unusual proliferation of lymphoid precursor cells in bone marrow. Induction chemotherapy is vital for achieving complete remission in ALL, but accurately predicting relapse risk and identifying patients likely to sustain remission remains challenging.

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Conventionally, bone marrow lymphoblasts have been used as the gold standard for assessing treatment response and predicting relapse in ALL. However, emerging evidence suggests that bone marrow lymphocytes may serve as alternative predictive biomarkers.

Aim: This study aims to review the utility of bone marrow lymphocytes as early predictors of relapse and remission in ALL during the post-induction chemotherapy phase. It will explore the advantages and limitations of assessing lymphocytes compared to lymphoblasts and discuss the potential mechanisms underlying their prognostic significance. Patients and Methods: Total of 105 newly diagnosed ALL patients; 75 with B ALL and 30 with T ALL was selected to the study. Laboratory investigations included Complete Blood Count (CBC) with the manual differential count analysis and obtaining bone marrow parameters from myelogram reports. The patients were followed until the end of the induction phase and, the laboratory parameters were taken. Correlation bivariate analysis, Paired sample t-Test was performed to establish the associations between peripheral blood parameters with the bone marrow parameters. Receiver operating characteristic (ROC) curve analysis was performed to establish cutoff values for peripheral blood parameters and the bone marrow lymphocytes with aiming to investigate bone marrow response. All the analysis was performed using Microsoft Excel 2013.12 SPSS version 26.

Results: In B ALL at Day 8, Lymphocytes% in Bone Marrow (L%_BM) showed a significant strong negative correlation (r = -0.357, p<0.05) with NLR. ROC curve analysis showed cut off values for Lymphocytes; NLR =<0.400 indicates the presence of L%_BM >10% in B ALL. When considering T ALL D8, the ALC>=1756/mm3 suggests the presence of >10% L%_BM in D8. Conclusion: Our research demonstrated strong correlations to predict the Lymphoblasts% in Bone Marrow (BL%_BM) & L%_BM by using peripheral blood parameters which is straight forward and easily obtainable. Moreover, we were successfully able to set up cut off peripheral blood parameter values to monitor the response of the Lymphocytes of the bone marrow. Before implementing the results, further studies could be carried out by increasing the sample size for both B & T ALL with the accurate monitoring of the patients.

Keywords: Acute Lymphoblastic Leukemia, complete blood count, induction chemotherapy, bone marrow lymphoblast, bone marrow lymphocytes.

RESUMO

Contexto: A Leucemia Linfoblástica Aguda (LLA) é uma neoplasia hematológica maligna que surge devido à proliferação incomum de células precursoras linfóides na medula óssea. A quimioterapia de indução é vital para alcançar a remissão completa na LLA, mas a previsão precisa do risco de recidiva e a identificação de pacientes com probabilidade de manter a remissão continuam sendo um desafio. Convencionalmente, os linfoblastos da medula óssea têm sido usados como padrão ouro para avaliar a resposta ao tratamento e prever a recidiva na LLA. Entretanto, evidências emergentes sugerem que os linfócitos da medula óssea podem servir como biomarcadores preditivos alternativos. Objetivo: Este estudo tem como objetivo analisar a utilidade dos linfócitos da medula óssea como preditores precoces de recidiva e remissão na LLA durante a fase de quimioterapia pós-indução. Ele explorará as vantagens e limitações da avaliação dos linfócitos em comparação com os linfoblastos e discutirá os possíveis mecanismos subjacentes à sua importância prognóstica. Pacientes e métodos: Um total de 105 pacientes recém-diagnosticados com LLA, 75 com LLA B e 30 com LLA T, foi selecionado para o estudo. As investigações laboratoriais incluíram hemograma completo (CBC) com análise manual da contagem diferencial e obtenção de parâmetros da medula óssea a partir de relatórios de mielograma. Os pacientes foram acompanhados até o final
da fase de indução e os parâmetros laboratoriais foram registrados. A análise bivariada de correlação e o teste t de amostras pareadas foram realizados para estabelecer as associações entre os parâmetros do sangue periférico e os parâmetros da medula óssea. A análise da curva ROC (Receiver Operating Characteristic) foi realizada para estabelecer valores de corte para os parâmetros do sangue periférico e os linfócitos da medula óssea com o objetivo de investigar a resposta da medula óssea. Todas as análises foram realizadas com o Microsoft Excel 2013.12 SPSS versão 26. Resultados: Na LLA B, no Dia 8, Linfócitos% na medula óssea (L%_BM) mostrou uma correlação negativa forte e significativa (r = -0,357, p<0,05) com a NLR. A análise da curva ROC mostrou valores de corte para linfócitos; NLR =<0,400 indica a presença de L%_BM >10% na LLA B. Ao considerar a LLA T D8, a ALC>=1756/mm3 sugere a presença de >10% de L%_BM em D8. Conclusões: Nossa pesquisa demonstrou fortes correlações para prever a% de linfoblastos na medula óssea (BL%_BM) e L%_BM usando parâmetros do sangue periférico, que são simples e fáceis de obter. Além disso, conseguimos estabelecer com sucesso valores de parâmetros de sangue periférico de corte para monitorar a resposta dos linfócitos da medula óssea. Antes de implementar os resultados, outros estudos poderiam ser realizados com o aumento do tamanho da amostra para LLA B e T com o monitoramento preciso dos pacientes.


RESUMEN
Antecedentes: La Leucemia Linfoblástica Aguda (LLA) es una neoplasia hematológica maligna que surge debido a la proliferación inusual de células precursoras linfoides en la médula ósea. La quimioterapia de inducción es vital para lograr la remisión completa de la LLA, pero sigue siendo difícil predecir con exactitud el riesgo de recaída e identificar a los pacientes con probabilidades de mantener la remisión. Convencionalmente, los linfoblastos de la médula ósea se han utilizado como patrón oro para evaluar la respuesta al tratamiento y predecir la recaída en la LLA. Sin embargo, la evidencia emergente sugiere que los linfocitos de la médula ósea pueden servir como biomarcadores predictivos alternativos. Objetivo: Este estudio pretende revisar la utilidad de los linfocitos de médula ósea como predictores tempranos de recaída y remisión en LLA durante la fase de quimioterapia post-inducción. Se explorarán las ventajas y limitaciones de evaluar los linfocitos en comparación con los linfoblastos y se discutirán los mecanismos potenciales subyacentes a su importancia pronóstica. Pacientes y métodos: Se seleccionó para el estudio a un total de 105 pacientes con LLA de diagnóstico reciente, 75 con LLA B y 30 con LLA T. Las pruebas de laboratorio incluyeron recuento sanguíneo completo (RSC), análisis de sangre y análisis de sangre. Las investigaciones de laboratorio incluyeron el recuento sanguíneo completo (CBC) con el análisis manual de recuento diferencial y la obtención de parámetros de médula ósea a partir de informes de mielograma. Los pacientes fueron seguidos hasta el final de la fase de inducción y, se tomaron los parámetros de laboratorio. Se realizó un análisis bivariante de correlación y una prueba t de muestras apareadas para establecer las asociaciones entre los parámetros de sangre periférica y los parámetros de médula ósea. Se realizó un análisis de la curva de características operativas del receptor (ROC) para establecer los valores de corte de los parámetros de sangre periférica y los linfocitos de la médula ósea con el fin de investigar la respuesta de la médula ósea. Todos los análisis se realizaron con Microsoft Excel 2013.12 SPSS versión 26. Resultados: En LLA B en Día 8, Linfocitos% en Médula Ósea (L%_BM) mostró una correlación negativa fuerte y significativa (r =-0,357, p<0,05)
con NLR. El análisis de la curva ROC mostró valores de corte para los linfocitos; NLR =<0,400 indica la presencia de L%_BM >10% en LLA B. Cuando se considera LLA T D8, el ALC>=1756/mm3 sugiere la presencia de >10% L%_BM en D8. Conclusiones: Nuestra investigación demostró fuertes correlaciones para predecir el porcentaje de linfoblastos en la médula ósea (BL%_BM) y el porcentaje de linfoblastos en la médula ósea (L%_BM) mediante el uso de parámetros de sangre periférica que son sencillos y fáciles de obtener. Además, pudimos establecer con éxito valores límite de parámetros de sangre periférica para controlar la respuesta de los linfocitos de la médula ósea. Antes de aplicar los resultados, podrían llevarse a cabo nuevos estudios aumentando el tamaño de la muestra tanto para la LLA B como para la LLA T con un seguimiento preciso de los pacientes.

Palabras clave: Leucemia Linfoblástica Aguda, hemograma completo, quimioterapia de inducción, linfoblastos de la médula ósea, linfocitos de la médula ósea.

1 INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is a hematological malignancy that results from an unusual proliferation of lymphoid precursor cells in bone marrow, blood, and extra medullary sites. This associates with poor differentiation of either B or T Lymphoblasts (Terwilliger and Abdul-Hay, 2017). In ALL, the unregulated proliferation of Lymphoblasts results in ≥20% out of all the cells in the marrow (Terwilliger and Abdul-Hay, 2017). ALL is the most common childhood cancer, accounting for roughly one-third of all pediatric malignancies. In comparison, ALL accounts only for about 20% of all adult leukemias (Hoffbrand and Moss, 2016).

ALL is occurred due to a result of the malignant transformation of a lymphoid precursor cell in the B or T cell lines. The B lineage cell is affected by the majority of the change (Gallegos-Arreola, 2013). The molecular pathology of ALL involves the translocations of chromosomes in genetic heterogeneity. The pathophysiology of ALL occurs within the bone marrow while the lymphoid progenitor cells show poor differentiation and abnormal proliferation of Lymphoblasts. According to Bhat et al. (2019), blasts are the precursor cells which are not usually found in peripheral blood. However, the increased number of Lymphoblasts takes all the space within the bone marrow while absorbing all of the nutrition. Ultimately, the whole of the bone marrow gets infiltrated with the blasts (Conter et al., 2004). As a result of that, there would be a decreased number of erythrocytes in the blood, causing anemia. The decreased number of neutrophils results in an increased risk of infection. And also, a reduced number of
platelets can cause hemorrhages (Conter et al., 2004). According to the recently updated classification of WHO in 2016, ALL is categorized into several groups B cell ALL, T cell ALL, and Common ALL. The T & B cell ALLs are subcategorized according to recurrent genetic abnormalities (Terwilliger and Abdul-Hay, 2017; Chiaretti et al., 2014).

Laboratory diagnosis is a crucial part of both screening and confirmation of ALL. Anemia, abnormal elevated counts of leukocytes, and thrombocytopenia are characteristics of the peripheral blood smear (Chiaretti et al, 2014). Definitive diagnosis requires an inspection of a bone marrow smear and the presence of ≥ 20% of lymphoblasts is suggestive of ALL. Confirmation of diagnosis and risk stratification can be achieved by using flow cytometry, immune phenotyping, and cytogenetic testing (Cancer.Net, 2017). The involvement of the CNS can be examined by performing a CSF analysis followed by an inspection by brain MRI if the evidence is present. (Coustan-Smith and Jose, 2002).

Peripheral blood parameters provide vital information in the diagnosis of patients with hematological disorders (Huang et al., 2021; Rabizadeh et al., 2014; Dai et al., 2020). Absolute Neutrophil Count (ANC) and Absolute Lymphocyte Count (ALC) are two essential indicators of immune system behavior. Both ANC and ALC are important diagnostic markers used in a variety of clinical settings, and variations in both indicators can provide crucial insights into a person's health status (Jang et al, 2019; Stefaniuk et al, 2020). The total WBC count is multiplied by the percentage of segmented neutrophils and band forms to obtain the ANC (Nelson textbook of pediatrics, 2020). The reference range of ANC of an adult is about 2500-7500/microL (Hoffbrand and Moss, 2016). Similarly, ALC can be derived by multiplying the total WBC count by the proportion of lymphocytes. Normally the reference range of ALC of an adult is about 1500-3500/microL (Medscape, differential blood counts, 2019). NLR is a subclinical inflammation marker that is easy to assess, robust, and affordable. NLR also indicates an impaired cell mediated immunity associated with systemic inflammation (Faria et al., 2016).

Chemotherapy for ALL includes four major steps, such as induction, central nervous system (CNS) prophylaxis, consolidation, and long-term maintenance (Cancer.Net, 2017). The main aim of induction chemotherapy is to restore normal hematopoiesis while suppressing the signs and symptoms of the patient (Terwilliger and Abdul-Hay, 2017). Prophylaxis therapy may be given depending on the predetermined risk for CNS. High-risk patients may go on allo-stem cell transplantation while others
will be continued with therapy for consolidation and long-term maintenance (Terwilliger and Abdul-Hay, 2017). Treatment regimens can vary among the different treatment protocols adopted; Sri Lanka uses the UKALL 2011 protocol for the treatment of children with ALL (Gunasekera et al., 2020). After appropriate treatments, if the percentage of blasts (Lymphoblasts) in the bone marrow is less than 5%, blood counts have returned to normal, and no further leukemia is present in the body, it is considered complete remission (Pui and Campana, 2000) in current practice. When ALL returns to patients who have previously undergone treatment, this is referred to as relapsed ALL (Locatelli et al., 2012). According to Raetz and Bhatla (2018), even though this is treatable, 10%-20% of patients have been predicted to relapse. Furthermore, the relapse rate of ALL in Children below 15 years is approximately 10% while the relapse rate among adults is 50% (Pierro et al, 2016).

Currently, the medical staff often use bone marrow biopsies, and chromosomal analysis to identify a relapsed ALL. However, these investigations are not easy to conduct, time-consuming, and require experienced personnel. Therefore, if we are able to set up a way to screen and identify the relapsed conditions of ALL through peripheral blood it would reduce a significant number of challenges of early identification. According to Dai et al. (2021) peripheral blood blast count in ALL is an important predictive marker. After 7 days of therapy, a blast count of 1000/µL in peripheral blood has been correlated to a significantly better survival outcome in pediatric ALL. According to Gajjar et al. (1995) the persistence of circulating leukemic blast cells after a week of remission induction chemotherapy was an important early predictor of treatment failure. Many studies confirmed that reduction in peripheral blood blast count or recovery of normal hematopoiesis has become the most important predictor of the outcome of ALL (Dai et al, 2021; Oskarssons et al, 2016). In addition, Rabin et al. (2012) discovered that ALL patients having an Absolute Lymphocyte Count (ALC) greater than 1.5×10⁹/L experienced a significantly higher probability of a 6-year relapse-free survival and an Overall Survival (OS) than those with an ALC of less than 1.5×10⁹/L. According to Gupta et al. (2015), the ALC recovery at the completion of induction appears to be a strong predictive factor for better survival in children with ALL.

Performing Minimal residual disease (MRD) testing at the end of induction was one of the independent prognostic factors for determining the risk for relapse in ALL, which was in accordance with previous studies (Campana & Coustan-Smith, 1999). According to various studies, it has been found that quantification of residual disease in
marrow at the end of induction is strongly correlated with the risk of relapse (Wasserman et al, 1992; Brisco et al, 1994, 1996; Steenbergen et al, 1995). However, not all patients were able to undergo MRD testing due to the cost and complexity, especially in developing countries. In addition, the main drawback for detection of MRD is that it is being conducted by molecular testing rather than functional markers of the remaining leukemic cells (Dai et al, 2021).

Since, most of the patients are associated with less than 5% blast cells or zero in the bone marrow (remission) at the end of induction phase, the Lymphoblasts% in the bone marrow would not be an indicator to establish correlations with the peripheral blood parameters in this stage. As such, in the absence of Lymphoblasts (leukemic) and the presence of adequate % lymphocytes in the bone marrow after induction therapy would suggest as a better indicator for remission (Gupta et al., 2015). Since so many study groups have proven the peripheral blood lymphocytes as a prognostic marker for ALL at the post induction therapy (Rabin et al., 2012; Gupta et al., 2015), if one can set up a way to monitor the response of the bone marrow at the post induction phase of ALL by establishing associations in between peripheral blood parameters and BM lymphocytes it may provide a better outcome than the conventional examining way.

2 MATERIALS AND METHODS

The total of 105 ALL patients were selected for the study. Among them, 75 were of B ALL and 30 were of T ALL confirmed by bone marrow biopsy and flow cytometry based immuno-phenotyping. Total White Blood Cell Count (WBC), Hemoglobin (HB), Platelet count (PLT), Blast% in Peripheral Blood (BL_PB), Neutrophil to Lymphocyte Ratio (NLR), Platelet to Lymphocyte Ratio (PLR) and Blast to Lymphocyte Ratio in the Peripheral Blood (BLR_PB) were calculated using 5-part Mindray BC6800 automated hematological analyzer. PB Differential Counts were performed manually using Leishman’s stained blood smears. Blasts% in Bone Marrow (BL_BM), Lymphocytes% in Bone Marrow (L_BM) and Blast to Lymphocyte Ratio (BLR_BM) were obtained by the Bone Marrow (BM) myelogram reports of ALL patients. The whole induction therapy phase was targeted and all the aforesaid counts were used in the study for the initial (D0), induction phase I at 8th day (D8) and induction phase II at 29th day (D29). Results were tabulated in Excel sheets and were analyzed using Microsoft Excel 2013.12 SPSS version 23 (Released 2015, IBM statistics for Windows version 23, IBM Corp., Armonk, NY).
software. Initially, the data in all sub groups (D0, D8 & D29) was checked for normality. Pearson bivariate correlation analysis was used when the data showed normal distribution and Spearman bivariate correlation analysis was used when the data did not follow normal distribution. Both parametric and non-parametric correlation methods were used in the analysis in order to establish correlations among the PB and BM parameters within the same phase and in between different phases and, p<0.05 considered as significance. Then the Wilcoxon Signed Ranks Test analysis was to find out the mean significance of the PB & BM parameters in between different phases. Finally, Receiver Operating Characteristic (ROC) curves were plotted, and cutoff values for the peripheral blood parameters with respect to the lymphocytes% of the BM at the post induction phase.

3 RESULTS

First, the data of the blood parameters were tested for the normality using SPSS. This was carried out to all the parameters in different phases of B & T ALL. None of the parameter was able to distribute in the normal pattern.

3.1 CORRELATION BIVARIATE ANALYSIS

Correlation bivariate analysis (non-parametric Spearman) was performed in the Do, D8, and D29 groups to find the associations between the BM parameters (BL%_BM, L%_BM) and other PB parameters. The analysis was carried out separately for B ALL and T ALL groups. The PB parameters that associated with weak, moderate or strong significant correlations with BL%_BM & L%_BM. The data revealed that the overall Spearman correlations of the PB parameters of B ALL show a more affinity with L%_BM than BL%_BM. Among all, the D8 is prominent and the PLT_PB continuously showed a weak correlation with L%_BM in all the phases. In addition, the D8 data shows L%_PB and N%_PB have moderate statistical significance (p<0.01) correlations with L%_BM. However, BL%_BM too has significant moderate correlations with BL_PB & N%_PB in D0. In case of T ALL, it is clearly noticed that the number of PB parameters were increased to have correlations with BM parameters of BL%_BM & L%_BM. In addition, the correlations were improved in compared with the B ALL as most of them show moderate or strong correlations. In D29, the correlations with L%_BM were improved than other two phases.
3.2 FINDING THE MEAN SIGNIFICANCES OF BM AND PB PARAMETERS OF B ALL AND T ALL IN DIFFERENT PHASES

Paired sample t-Test analysis was carried out using SPSS for different phases of B ALL & T ALL to find out mean statistical difference of the PB & BM parameters in between two phases among whole phases of initial and induction (D0, D8 & D29). First, the data normality was checked. Then the parametric Paired sample t-Tests were used when the corresponding data sets followed the normal distribution and the non-parametric 2 Related Sample test (Wilcoxon Signed Ranks Test) were used when the corresponding data sets did not follow the normal distribution. P < 0.05 was considered as significance.

3.3 MEAN SIGNIFICANCE OF THE BM PARAMETERS OF B ALL IN DIFFERENT PHASES

The three prominent parameters; BL%_BM, L%_BM and BLR_BM of the BM were chosen for the analysis. The Wilcoxon Signed Ranks Test results revealed that the BL%_BM showed statistical significance (p=0.000) in between D0-D8 & D0-D29 with decreased mean values while it also showed statistical significance (p=0.026) in between D8-D29 with slightly increased mean values. The BLR_BM too comprehensively followed the same pattern with the statistical significance (p=0.000) in between all the phases. The L%_BM showed statistical significance (p=0.000) in between all the phases with increased mean values.

3.4 PB PARAMETERS IN B ALL IN DIFFERENT PHASES

The three PB parameters; NLR_PB, PLR_PB and BLR_PB were chosen for the analysis and the Wilcoxon Signed Ranks Test results revealed that the NLR_PB showed statistical significance (p=0.000) in between D0-D29 & D8-D29 with increased mean values. However, D0-D8 did not show and significance even though the mean values were increased. Both the PLR_PB and BLR_PB showed statistical significance (p=0.000) in between D0-D8 & D0-D29 with increasing mean values and decreasing mean values for PLR_PB and BLR_PB respectively.
3.5 BM PARAMETERS OF T ALL IN DIFFERENT PHASES

As aforementioned, the Wilcoxon Signed Ranks Test results of T ALL patients revealed that the BL%_BM exhibited statistical significance (p=0.000) in between D0-D8 & D0-D29. In both these periods showed decreased mean values. Conversely, in D8-D29 did not show any significance even though the mean values were slightly increased. The BLR_BM too behaved in identical pattern as in BL%_BM. The L%_BM showed statistical significance (p=0.000) in between D0-D8 & D8-D29 with increased and slightly decreased mean values, respectively. However, D0-D29 did not exhibit any significance, despite the mean values being slightly decreased.

3.6 PB PARAMETERS IN T ALL IN DIFFERENT PHASES

As mentioned in B ALL the three PB parameters; NLR_PB, PLR_PB and BLR_PB were chosen for the analysis. The Wilcoxon Signed Ranks Test results revealed that the NLR_PB showed statistical significance in between D0-D8 (p=0.008) & D0-D29 (p=0.003) with increased mean values. However, during D8-D29, although the mean values were increased, no significance was observed. BLR_PB showed statistical significance (p=0.000) in between D0-D8 & D0-D29 with decreasing mean values while D8-D29 did not exhibit any significance even though the mean values were slightly increased. Similarly, the PLR_PB values showed statistical significance in between D0-D8 (p=0.002) & D0-D29 (p=0.001) with increasing mean values. However, in between D8-D29 did not show any significance, even though the mean values being increased.

3.7 DISTRIBUTION OF LYMPHOCYTES% IN PB AND BM IN B & T ALL

Figure 1: Box plot of L%_PB and L%_BM distribution in all 3 phases of D0, D8 & D29; (a) B ALL, (b) T ALL.

Source: Prepared by the authors
Results in Figure 1 revealed that in both B & T ALL, the Lymphocyte percentage (%_L) in both PB and BM at D8 are dominant compared to other phases (D0 & D29). In addition, the %_L _BM have been decreased at D29 in both B & T ALL.

3.8 RECEIVER OPERATING CHARACTERISTIC (ROC) CURVE ANALYSIS

Initially, it was planned to set up Receiver Operating Characteristic (ROC) curves for the peripheral blood parameters using SPSS by considering, BL%_BM > 5% in a relapsed state and BL%_BM =< 5% as a remission state. However, we were unable to find acceptable data as most of BL%_BM values were confined to remission state (<5%) thus, unable to set up two groups (variables) for the BL%_BM. So, as the next attempt the L%_BM was chosen in our analysis. Two groups; variable 0 < 10% of L%_BM, variable 1 > 10% of L%_BM with peripheral blood parameters and subjected to ROC curve analysis. This was continued for all the phases in B ALL and T ALL.

The cut off values for peripheral blood parameters obtained from ROC curve analysis (Table 1 & Figure 2), for example, N%_PB >=27.5% indicates that the marrow has L%_BM >10% of in B ALL D8. Conversely, NLR =<0.400 indicates the presence of L%_BM >10%. When considering T ALL D8, the ALC>=1756/mm3 suggests the presence of >10% L%_BM. Rest of the cut off values for different PB parameters are shown in Table 1. The results presented in Table 1 display the area under curve (AUC) comparisons between T ALL and B ALL. Notably, T ALL exhibited a significantly higher AUC compared to B ALL. Consequently, T ALL demonstrated enhanced specificity and sensitivity (Figure 2).

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Table 1: Cut off values of PB parameters with the statistical parameters obtained from the ROC curve analysis in different chemotherapy phases of B ALL & T ALL for the prediction of L%_BM.
4 DISCUSSION

ALL is the most common malignancy diagnosed in children, representing nearly one-third of all pediatric cancers (Hoffbrand and Moss, 2016). ALL is occurred as a result of a process of malignant transformation of progenitor lymphoid cells in the B or T lineages in the bone marrow. The majority of the transformation affects the B lineage cell (Gallegos-Arreola, 2013). Chemotherapy, remains the preferred and most commonly used treatment for ALL. There are various chemotherapy regimens and it is typically given in cycles, with each period of treatment followed by a rest period to allow the body time to recover (Nguyen et al., 2015). Chemotherapy is mainly divided into 3 phases. In this study, we mainly focused on the induction phase I (D8) and phase II (D29) chemotherapy cycles. Induction is a short and intensive process that usually lasts for around one month. BM samples were obtained during the initial stage as well as in...
Induction phases; D8 and D29, followed by a resting period to allow the body to recover (American cancer institute, 2018). In this study, our main focus was to establish correlations between the bone marrow parameters and other peripheral blood parameters of individuals who were diagnosed with ALL as explained in materials and methods.

The statistical analysis of the results was performed using SPSS version 26 software. At the normality examining step, all the PB and BM parameters except L%_PB, did not follow normal distribution the entire analysis was confined to non-parametric. In the next step, correlation bivariate analysis was performed among the sets of parameters and also within the same phase by examining the interrelationships of PB parameters with the BL%_BM & L%_BM. In contrast, the T ALL shows strong correlations in between their PB and BM parameters while the correlations with L%_BM are always prominent. Rubnitz et al (2013) revealed that an ALC ≥ 500 cells/mm3 was significantly more prevalent among patients with B-lineage ALL and it is related to favorable presenting features and good initial treatment response at the end of the induction phase. Equally, the data of the study of Rochet et al (2012) showed that peripheral ALC ≥500 cells/mm3 at all- time points; i.e. days 15, 21, and 28 after induction chemotherapy and before first consolidation chemotherapy, had a good prognosis in acute leukemia. In our study too, most of the patients were presented with >500 cells/L in D0 (both B & T ALL) showed remission (<5% Lymphoblasts in the BM) at the end of the induction phase.

In the next step, the Wilcoxon Signed Ranks Test analysis was performed to find out the significance of mean differences from D0 to D29. The purpose of employing this statistical method was to study the chemotherapy effect across the different phases of the treatment. In addition to the BM parameters, the PB parameters; the mean values of NLR and the PLR were continuously increased from D0 to D29 while BLR was shown reciprocal of it in both B & T ALL. Youssef et al. (2023) described the prognostic role of NLR, PLR, and lymphocyte-to- monocyte ratio (LMR) in predicting post-induction treatment response in adult and pediatric ALL patients. They have found that an increase in NLR and PLR, together with a decrease in LMR upon ALL diagnosis could predict future resistance to commonly used induction procedures and also revealed that the PLR was significantly reduced in adult (P<0.01) and pediatric (P<0.01) patients who presented with high initial WBC counts. However, in our results, we too observed high NLR & PLR levels with decreased BLR & WBC values at the end of the induction phase along with the remission of the majority of patients.
All of these findings lead a pathway to establish cutoff values for the peripheral blood parameters with respect to the BL%_BM. In the extended analysis, this could be achieved by performing Receiver Operating Characteristic (ROC) curve using SPSS. Since the BL%_BM > 5% is considered as relapsed BM condition (Blood; Buchman et al, 2022) an attempt was made to establish cut off values from ROC curves for the peripheral blood parameters by considering, BL%_BM > 5% in a relapsed state and BL%_BM <= 5% as a remission state. Conter et al (2000), previously reported that a rapid drop in peripheral circulating Lymphoblasts (BL%_PB) and WBC count on D8 of induction is a favorable prognostic factor for B ALL. In this study, we too have observed this rapid drop in peripheral circulating Lymphoblasts among almost all the B ALL as well as T ALL so, we were unable to set up two groups (variables) for the BL%_BM. As the next attempt the L%_BM was chosen in our analysis. According to Whiteside et al (2022), lymphocytes are a significant predictor of tumor-infiltrating lymphocytes because they indicate the host's immune reaction against them, and they observed the same thing after induction therapy started. Generally, L%_BM > 10% in the bone marrow is considered to be significant (Krober et al, 1999).

Obtaining reliable cut off values for peripheral blood parameters associated to the L%_BM (>10% and <10%) makes it possible to predict the bone marrow by peripheral blood parameters as expected from the beginning and it enabled to predict the BL% BM at each and every phase for both B ALL & T ALL. According to Gupta et al (2015), the ALC recovery at the completion of induction appears to be a strong predictive factor in both pediatric and adult malignancies. Lymphocyte recovery has been linked to better survival in adults with acute myeloid leukemia (AML), non-Hodgkin lymphoma (NHL), Hodgkin lymphoma, sarcomas, and carcinomas, as well as children with ALL, AML, NHL, immune thrombocytopenia, osteosarcoma, and Ewing sarcoma. They further stated that the ALC cut-off points indicating the most accurate power in predicting survival at D15 and D29 induction were 500 and 1000/mm3, respectively. Moreover, De Angulo et al (2007), from MD Anderson Cancer Institute revealed that ALC D15 <350/mm3 independently predicts poor survival in ALL patients, whereas a D15 value of >350/mm3 predicts favorable outcome. Furthermore, Rabin et al (2012), found that high ALC >1500 cells/mm3 in post induction was an independent and clinically significant predictor of improved survival.

All of the previously conducted research studies have confirmed that high levels of ALC following induction therapy serve as a positive prognostic marker. However, we
aimed to determine the cutoff values for L%_BM instead of ALC in peripheral blood. Our findings indicate that for T ALL (D8), an ALC of 1756/mm3 or greater suggests an L%_BM of over 10%. Similarly, for B ALL (D8), a NLR of 0.400 or less with a significance p<0.05 indicates an L%_BM of over 10%. NLR provides an indirect measure of ALC in peripheral blood and is therefore more interesting. Likewise, we were able to predict L%_BM (<10% or >10%) based on various cutoff values in peripheral blood parameters. These results demonstrate that higher L%_BM values after induction therapy are a more favorable prognostic factor than the presence of blasts in the bone marrow.

5 CONCLUSION

This study is an attempt to find a correlation between PB parameters and BM parameters of ALL patients which may lead to finding a cost-effective, convenient method to screen the bone marrow response to induction chemotherapy of both B & T ALL patients using peripheral blood parameters. This study was carried out in three phases; D0, D8, and D29. We were successful in obtaining strong positive and negative significant correlations between L%_BM and a few of the peripheral blood parameters in the difference phases of B ALL and T ALL. In addition, mean significances of BM and PB parameters of B ALL and T ALL in different phases was performed. Finally, cut-off values for some of the peripheral blood parameters were calculated using ROC curves based on the bone marrow lymphocytes. The number of patients for both B ALL and T ALL should be widened and accurate monitoring of the patients are required to validate the initial findings before implementing them.
REFERENCES


