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Outcome of Post Induction Therapy for Acute Myeloid Leukemia in Nanakaly Hospital-Erbil

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Abstract

Background: Acute myeloid leukaemia in adult constitutes 80% of whole acute leukaemia cases; its frequency progressively increases with age.
Objective: To evaluation the parameters of AML patients clinically and haematologically in Erbil City.
Patients and Methods: A particular analysis of hospital records retrospective study of 29 patients with AML was taken on. The cases were analyzed and achieved at Nanakaly hospital in Erbil city during the years 20021-2022. Diagnosis was established on peripheral blood and bone marrow reports. The myeloid origin confirmation was concerned by cytochemistry, morphological subtyping was concerned according to the (FAB) criteria, biochemical tests, and cluster CDs was done by flowcytometery. Microsoft excel version 2010 and (GraphPad Prism 9.0.) was in employment for carrying out statistical analysis.
Results: This study included 18 males and 11 females. Their ages ranged from 5 and 80 years with a mean age of 38.4 years. CD13 and CD33 are most expressed CD markers (75% and 70% respectively). CD22 and TdT lowest expressed CDs (10% and 5% respectively). Depending on the complete remission/Partial remission association, the p-value of platelets was significant (0.0207), CD64 and CD117 showed greater significant (<0.0001, <0.0001 respectively), BM hypercellularity fragments (P=0.0068), trials (P<0.0001), and blast percentage (P=0.0365).
Conclusion: CDs and BM results are essential tools in the identification of AML. CD13 and CD33 are the most frequent CDs in this study. Morphologic valuation of BM was statistically significant, cellularity of BM and blast percentage was significantly correlated with post induction response in patients with AML.
Keywords: Acute Myeloblastic Leukemia, Immunophenotyping, bone marrow reports, Flow Cytometry and CD Markers

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Introduction

Acute myeloid leukemia(AML) is a class of neoplastic blood illnesses distinguished by the proliferation and accumulation of immature haematopoietic cells in the bone

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marrow and blood. AML accounts for roughly 20% of childhood acute leukemias and 80% of adult acute leukemias. The incidence of AML rises with age; in individuals over the age of 65, the incidence is roughly 30 times that of AML in children [1,2]. AML is a clonal hematopoietic neoplasm with a high prognosis (the chance of recovery or recurrence) [3,4]. Characterized by immature myeloid cell proliferation, reduced apoptosis, and genetic instability. These genetic changes may include the improper production of oncogenes or the loss of functionality of the tumor suppressor genes [5]. AML has a yearly, age-adjusted incidence of 3.5 cases per 100,000 people, increasing to 15-20 occurrences per 100,000 beyond the age of 60. As a result, it significantly contributes to the morbidity and death of the elders [6]. In the Western population, the median age of AML patients at the time of diagnosis was observed to be about 70 years, and the prevalence rate of AML is directly related to age [7]. The clinical and biochemical features of AML patients are intimately connected to the process of aging, and the patient's age has a substantial impact on management and treatment results [8]. AML pathogenesis is a multistep process that includes mutagenesis, epigenetic instability, and the creation of copy number abnormalities. During leukemogenesis, initiating mutations impact hematopoietic stem and progenitor cells, resulting in preleukemic/leukemic stem cells and, eventually, frank leukemia [6-9].

The lacks of a basic definition for "refractory" AML affects how patients are handled in clinical practice as well as the eligibility of individuals with resistant illness for clinical studies. Failure to achieve complete remission (CR) following one cycle of moderate or high-dose cytarabine-based induction has been linked to a poor prognosis in AML[10]. Achieving CR with 5% BM blasts and indications of haematopoietic recovery is seen as an essential pre-requisite for long-term survival post AML induction treatment. The Internacional Working Group (IWG) and European Leukaemia Net (ELN) responses criteria contain a criterion for partial remission (PR) 5-25 percent [BM] blasts with a 50percentage drop in blasts & a recovery in peripheral blood count [11, 12]. The most prevalent acute leukemia in adults is AML, which is defined by the clonal proliferation of myeloid blasts caused by somatic mutations in primitive multipotential hematopoietic cells [13, 14]. The detection of the fundamental processes of AML pathogenesis provides the foundation for determining prognosis and risk classification of patients, which are critical in selecting effective therapy methods [15,16]. Consequently our study was planned on the full of initial data including clinical and hematological parameters of AML patients that are received chemotherapy according to Nanakali Hospital guidelines in Erbil City.

Patients and Methods

Patients and sample

Twrnty nine cases of AML was directed as a retrospective study from July 1, 2021, to March 11, 2022. The Complete blood count (CBC), bone marrow aspiration reports and Immunophenotyping (CDs) results were obtained from the database kept in Nanakaly hospital for Hematology and Oncology in Erbil city, Kurdistan region-Iraq.

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Complete blood count was done by an automated cell counter (Medonic) and biochemical tests achieved for all the patients before receiving chemotherapy medications.

Direct immunological fluorescence labeling was performed on bone marrow aspiration using unique direct conjugated flourochrome-labeling monoclonal mice anti-human antibodies according to the manufacturer's instructions. To diagnose acute leukemia, immunofluorescence was examined using a Becton Dickinson FACS Caliber flow cytometer coupled with BD Cell Quest pro software (BD Biosciences, San Jose, CA, USA)[16].

This panel includes:

A primary panels used to differentiate AML from ALL: CD13, CD33, CD117, MPO, CD19, CD79a, & cCD3.

CD34 and CD38 were also measured, with CD34 being a non-lineage specific marker expressing in hematopoietic progenitor cells.

If monocytic AML was predicted, CD64 was performed.

The expression percent of CDs from gated blast cells was recorded and the expression was measured positive when ≥ 20 percentage of the gated cells expressed it at the detection time [17, 18].

Statistical Analysis

Our study data were arrived into an Excel master sheet and arranged for analysis statistically by using (GraphPad Prism 9.0.). Some of the data were presented as percentage values. A Mann Whitney U Test was applied for comparing assessment between 2 independent samples as regards of the rate of recurrence of positive cells and the data were expressed as Median and 75th percentile values. Correlation between CD markers with other independent variables were performed using Spearman's rank correlation test, while the comparison of dichotomous or categorical variables was performed using Pearson's chi-squared test or Fisher's exact test.

Results

Over one year, we are collected 29 diagnosed AML patients, 18 (62.07%) were males and 11 (37.93%) were females, their ages was ranged from 5 to 80 years old, and overall mean age of the patients was 38.47 years old, as shown in Figure (1).

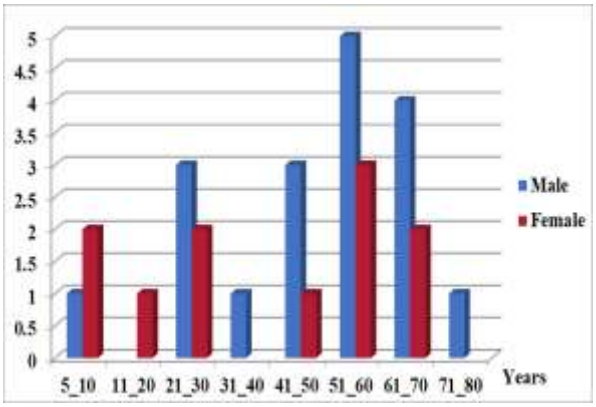

Figure (1): Age and Gender distribution of study patients

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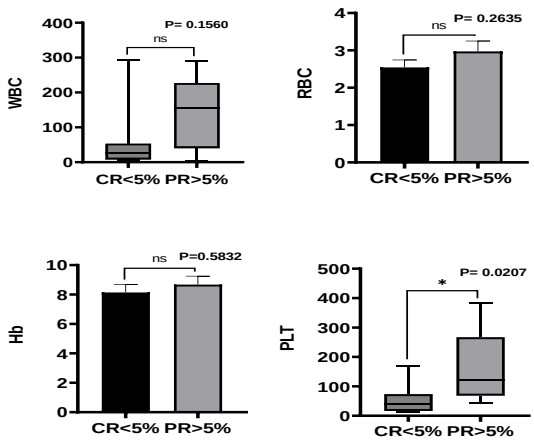
The morphological classification was concluded depending on the FAB criteria. The patients were classified as stated by FAB/WHO classification as follows: 5 cases are morphologically undifferentiated, 4 cases of M0, 9 cases of M2, 6 cases of M3, four cases of M5 and one case of M6, and there were no cases of M1, M4 and M7 was detected. The clinical and laboratory parameters of studied patients were addressed before starting of chemotherapy and the cases are classified depending on post induction bone marrow evaluation to two groups; patients who achieved complete remission (CR) which includes 21(72.4%) patients and those with partial remission (PR) 8 (27.5%) of patients. CBC result of mean ±SE of platelet count was 51.69±12.38 in the CR group and in PR group was 158.6±58.71, and the P values of platelet count showed significant (P = 0.0207) Figure (1). The results of other parameters which includes Hb, RBC and WBC were showed non-significant, Table (1).



Figure(2). Percentage distribution of RBC, WBC, Hb(Hemoglobin) and platelets depending on CR/PR association

The mean results of immunophenotyping CD markers which are summarized in figure2, and P-value of CD expressions depend on the correlations between the achievement of CR and PR, Table (1).

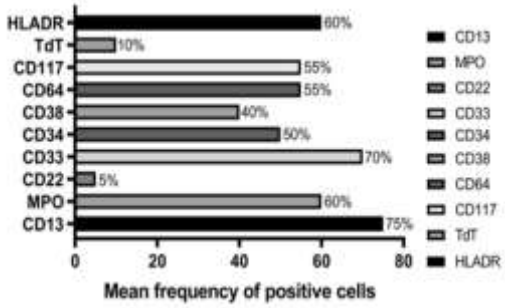


Figure (3): Expression of positive CD markers according to AML patients

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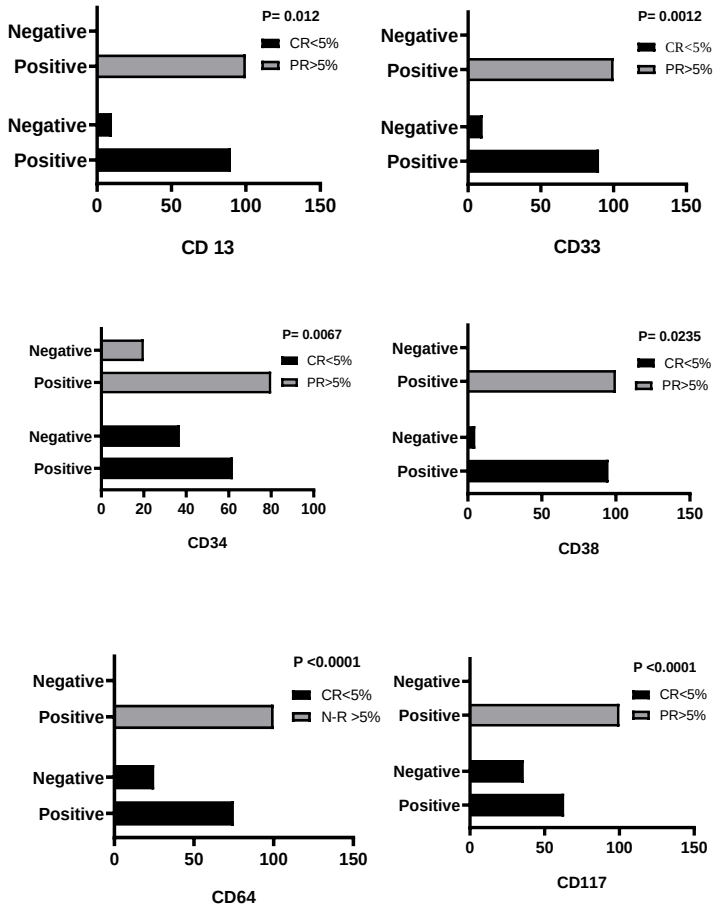
CD13 and CD33 were the most frequently expressed CD markers in this study (75% and 70% respectively) and they were the most diagnostic myeloid markers. This study result of CD13 showed a significant association between CR/PR ratio (P= 0.0012), also the result of CD33 indicated significant (P=0.0012) for the same association (table1). Both of MPO and HLADR are detected as second most frequent CDs in 60% of cases, and the CR/PR association p value of MPO showed significant (P=0.0103), but HLADR was reported non-significant(P=0.2272) depending on the correlation of CR with PR ratio in this study. CD64 and CD117 were expressed in 55% of cases, and the association of CR/PR p value of both showed strongly.



Significant (P<0.0001)

Figure (4): The expression of various CD markers(CD13, CD33, CD34, CD38, CD64 and CD117) in AML patients which categorized by CR/PR association

The result of CD34 was 50% and CD38 was 40%, and the P value of CR and PR association of both was significant, for CD34 (P=0.0067) and CD38 (P=0.0235). Less frequent CDs was expressed in our cases was CD22 and TdT (10% and 5% respectively), as showed in figure2. Other CDs were reported as negative in this study were including (CD2, Cytoplasmic CD3, CD7, CD10, CD19 and Cytoplasmic CD79 A).

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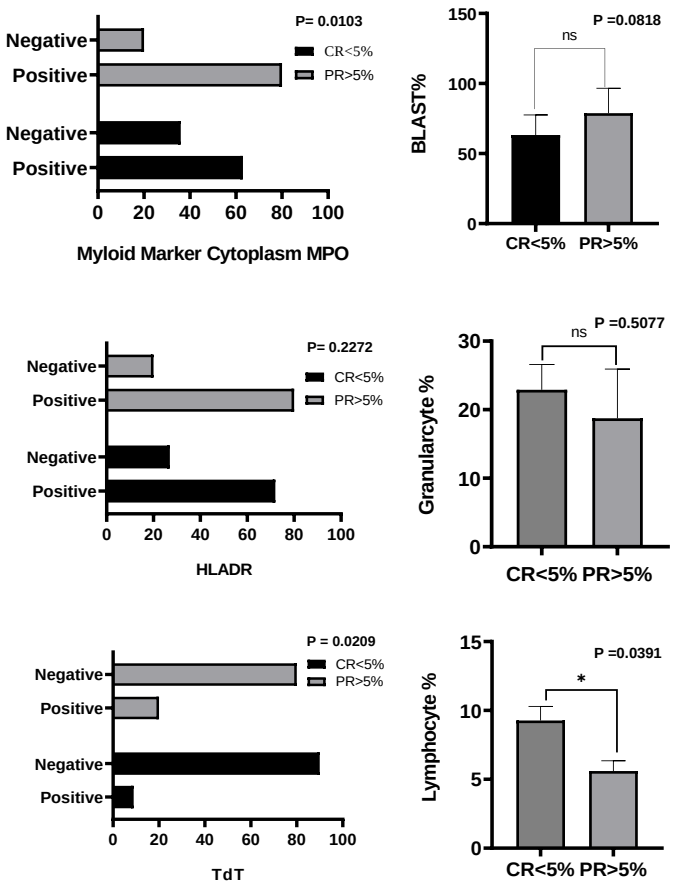

Figure (5): The expression of various antibody markers(Myloid Marker Cytoplasm (MPO), Human Leukocyte Antigen Receptor (HLADR) and Terminal Deoxynucleotidyl Transferase (TdT)), Blast%, Lymphocyte% and Granulocyte% in AML patients categorized by CR/PR association

The morphologic assessment of the bone marrow biopsy performed before therapy induction depending on CR/PR association. In our analysis, we found differences in the outcomes between the patients with blast%, trials and fragment cellularity changes. The blast percentages median was 55.50 of CR group and the median was 2.000 of PR group and the range between them was 84.00-88.00, also p-value showed significant (0.0365), as showed in table1. The hypercelularity of CR group is 90% but for PR group was %100, also the percentage of fragments in CR group was 10% but no fragments were seen in the PR group, p value of the CR/PR ratio indicated significant (0.0068). Changes were happened in the composition of trials in the bone marrow results makes the data to be strongly significant (P<0.0001), the CR group hypercellularity percentage was 80% and the marrow showed 20% of cellular marrow smear, but PR group hypercullarity was 100% and the marrow smear were showed clear, as reported in Table (1) and Figure (5).

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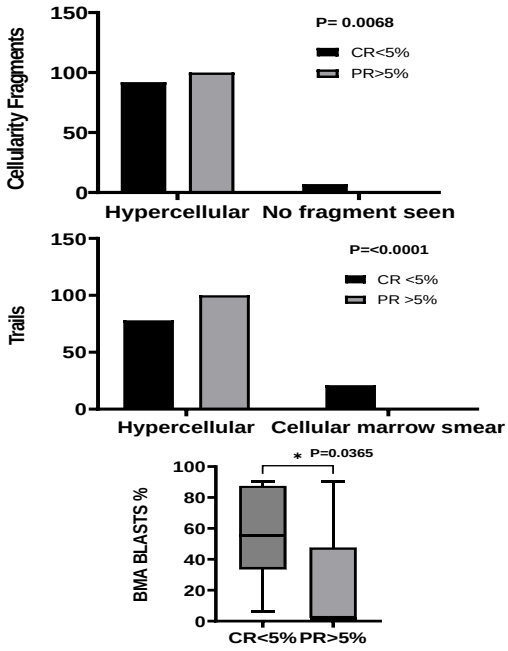

Figure (6): Considerable difference of Blast percentage, Trials and Cellularity fragments composition, depending on CR/PR association

Table (1): Hematological Parameters and Immuonphenotyping(CD) expression depends on CR/PR association

CBC	Mean		SE		Median		Range		P Value
	CR	PR	CR	PR	CR	PR	CR	PR	
WBC (10^9/1)					25.60	155.5	288.8	287.9	0.1560
RBC	2.542	2.972	0.2021	0.2801					0.2635
Hb (g/dl)	8.142	8.680	0.5483	0.5704					0.5832
Platelet 10^9/1	51.69	158.6	12.38	58.71					**0.0207***
FlowCytometry									
BLAST %	63.18	78.80	4.338	7.965					0.0818
Lymphocyte %	9.273	5.600	1.019	0.748					**0.0391***
Granulocyte %					21.00	12.50	36.00	30.00	0.5077
CD13									**0.0012****
CD33									**0.0012****
CD34									**0.0067****
CD38									**0.0235***
CD64									**<0.0001*****

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CD117									**<0.0001****
HLADR									0.2272
MPO									**0.0103***
BMA									
Cellularity Fragments									**0.0068***
Trails									**<0.0001****
BLASTS %					55.50	2.000	84.00	88.00	**0.0365***

Discussion

AML is a heterogeneous group of illnesses which can show with different morphologic, immunophenotypic and cytogenetic characteristics. The discovery of these patterns might be important for a better prognosis assessment and a suitable treatment strategy [19]. The age and gender distribution of the cases in this research are quite. Comparable to previous reports from Iraq [20, 21].

This study was incorporated 29 of AML patients, 18 males and 11 females overall mean age of the patients was 38.47 years old. According to (Pouls RK *et al*, 2012), who carried out a study in Erbil city, 94 adult patients were diagnosed as AML, 58 of them were males and 36 were females; studied patients ranged between 16 and 75 years with a mean age (±SD) of 33.8±21.3 years. Nevertheless (Ahmadzadeh A, et al, 2018) reported that male patients were more than female patients (45 male and 37 female)[22] Most of prior research have revealed a Greater frequency of AML in men but the male predominance is not as evident as in ALL [8, 23], the median age of patients in this study was 36 years old.

The commonest subtype in our series was AML-M2, were accounted for 9 cases (31% of subtypes), this was slightly higher than the frequency of 27-29% was reported in other studies (20, 24). Second common subtype is AML-M3 was 20%, which are similar to results were reported by (Pouls RK et al, 2012) in Erbil and by (SALIM BW et al, 2018) in Baghdad. The proportion of AML-M5 was 4(13.7%), it is higher than the results which reported by (25). Four cases of AML-M0 (13.7%) and only one case of AML-M6 were reported in our study, similar to results reported for AML-M6 in (Pouls RK et al,2012) and smaller results was reported by (Al Allawi et al, 1990) for AML-M0.

The mean ±SE of platelet count was 51.69±12.38 in the CR group and in PR group was 158.6±58.71, and the P values of platelet count showed significant (P = 0.0207). Sadek NA, *et al*, in 2020 reported the level of platelets which measured the level of Mean±SE was59.31±8.07 and the P values were strongly significant (P=0.000) (26). The results of other parameters which including: Hb, RBC and WBC were showed non-significant.

The mean results of CD13 and CD33 were the most frequently expressed CD markers in this study (75% and 70% respectively) and they were the most diagnostic myeloid markers.

According to Bradstock K, *et al*, the American society of hematology in 1994, the

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myeloid markers CD13 & CD33 were the most effective diagnostically (71% & 79%t of patients positive, respectively) [27]. Salim BW, Jalal SD, *et al*. (2018) revealed greater CD expression values in Duhok city, with CD13 and CD33 being the most commonly expressed markers (92.6% and 85.2%, respectively) [25]. In our study the result of CD13 showed a significant association between CR/PR ratio (P= 0.0012), also the result of CD33 indicated significant (P=0.0012) for the same association Table [1]. Both of MPO and HLADR are detected as second most frequent CDs in 60% of cases, and the CR/PR association p value of MPO showed significant (P=0.0103), but HLADR was reported non-significant (P=0.2272) depending on the correlation of CR with PR ratio in this study. When compared to data gathered from the literature, the frequency of the typically expressed myeloid related antigens in AML patients was within the ranges for CD13 (92.6& vs 60-90%), CD33 (85.2% vs 70-90%), and MPO (73.2%t vs 0-75%), but somewhat greater for CD117 (92.6% vs 60-70%) [28, 29]. Bain BJ. et al, in 2010 indicates greater than our result in UK, The sensitivity of flow-cytometry in the identification of MPO can be improved when using the 3% cut-off instead of the 10 percent cut-off in this investigation, where MPO was expressed in 73.2% of AML patients (since the enzymatically inactive proenzyme can be also detected) [29]. Bradstock K, *et al*, reported that HLA-DR was present in 70% of cases [27]. CD64 and CD117 were expressed in 55% of cases, and the association of CR/PR p value of both showed strongly significant (P<0.0001). Sadek AN, *et al*, reported smaller results for CD64 which expressed in only 4 cases (11.8%)[26]. CD117 has a higher specificity for myeloid lineage than CD13 or CD33 and CD13 is more specific than CD33 [26]. Bain BJ, *et al*, in 2010 indicated that the myeloid marker CD117 were the most frequent expressed antigens in the study reported in UK [25]. The result of CD34 was 50% and CD38 was 40%, and the P value of CR and PR association of both was significant, for CD34 (P=0.0067) and CD38 (P=0.0235). Sadek AN, et al, in 2020 reported that CD34 in almost all cases are positive [26]. Bradstock K, et al, reported the hemopoietic progenitor cell markers CD34 were detected in 42% cases [27]. Geller RB, Zahurak M, Hurwitz CA, et al, in 1990 reported that p value of the expression of CD34 (P = 0.008) was significant in predicting response to therapy; patients with leukaemic cells expressing CD34 had a complete remission (CR) rate of 59%[30]. Keyhani A, *et al*, in 2000 investigated CD38 expression in 304 AML and its strongly significant (P<0.001)[31]. Less frequent CDs was expressed in our cases was CD22 and TdT (10% and 5% respectively), as showed in Figure (3). Salim BW, Jalal SD, *et al*, in 2018 reported a slightly smaller percentage (<5%) for TdT marker expression in Duhok city [25]. Other CDs were reported as negative in this study was including (CD2, Cytoplasmic CD3, CD7, CD10, CD19 and Cytoplasmic CD79 A).

The blast percentages median was 55.50 of CR group and the median was 2.000 of PR group and the range between them was 84.00-88.00, p value showed significant (0.0365). The hypercelularity of CR group is

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90% but for PR group was %100, the percentage of fragments in CR group was 10% but no fragments were seen in the PR group, where the p value of the CR/PR ratio indicated significant (0.0068). Changes were happened in the composition of trials in the bone marrow results makes the data to be strongly significant (P<0.0001), the CR group hypercellularity percentage was 80% and the marrow showed 20% of cellular marrow smear, but PR group hypercellarity was 100% and the marrow smear were showed clear.

Conclusions

In conclusion, immunophenotyping study and bone marrow examinations are essential tools in the identification of AML. CD markers like CD13 and CD33 are the most frequent expressed markers, MPO and HLADR markers are the second most frequent CD markers in this study. Morphologic assessment of the bone marrow was statistically significant to identify AML patients. Cellularity of bone marrow and percentage of the blasts were significantly correlated with post induction response in patients with Acute Myeloid Leukemia.

Recommendations

We recommend the greater quantity of participants sharing the next studies, measuring and evaluation the positive samples cytogenetically and ensuring and support the results by sequencing methods.

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Source of funding: The current study was funded by our charges with no any other funding sources elsewhere.

Ethical clearance: The current study was accepted by the ethics committee of the Erbil polytechnic university college of Health and Medical. Participation in this study was optional, and the collected information and samples would be used only for research purposes.

Conflict of interest: Nil

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نتيجة العلاج التحريضي لسرطان الدم النخاعي الحاد في مستشفى ناناكالي - أربيل

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الملخص

خلفية الدراسة: ابيضاض الدم النخاعي الحاد عند البالغين يشكل ٨٠٪ من حالات ابيضاض الدم الحاد. يزداد تواترها تدريجياً مع تقدم العمر.

اهداف الدراسة: لتقييم معاملات مرضى ابيضاض الدم النقوي الحاد سريرياً وأمراض الدم في مدينة أربيل.

المرضى والطرائق: تم إجراء تحليل خاص لسجلات المستشفى دراسة بأثر رجعي لـ ٢٩ مريضًا مصابًا بابيضاض الدم النقوي الحاد (AML). وقد تم تحليل الحالات وتم الحصول عليها في مستشفى ناناكالي في مدينة أربيل خلال الأعوام ٢٠٢١ - ٢٠٢٢. تم إجراء التشخيص على تقارير الدم المحيطي ونخاع العظام. كان تأكيد الأصل النخاعي قلقًا بشأن الكيمياء الخلوية ، وكان التصنيف الفرعي المورفولوجي مهتمًا وفقًا لمعايير (FAB) ، والاختبارات الكيميائية الحيوية ، وتم إجراء الأقراص المضغوطة العنقودية عن طريق قياس التدفق الخلوي. (Microsoft Excel) إصدار ٢٠١٠ و (GraphPad Prism 9.0) كانا في العمل لإجراء التحليل الإحصائي.

النتائج: شملت هذه الدراسة ١٨ ذكراً و ١١ أنثى. تراوحت اعمارهم بين ٥ و ٨٠ سنة بمتوسط اعمار ٣٨,٤ سنة. CD13 و CD33 هما أكثر علامات CD تم التعبير عنها (٧٥٪ و ٧٠٪ على التوالي). CD22 و TdT أقل الأقراص المدمجة المعبر عنها (١٠٪ و ٥٪ على التوالي). اعتمادًا على الارتباط الكامل للمغفرة / المغفرة الجزئية ، كانت القيمة p للصفائح الدموية كبيرة (٠,٠٢٠٧) ، وأظهرت CD64 و CD117 أهمية أكبر (<٠,٠٠٠١ ، <٠,٠٠٠١ على التوالي) ، شظايا فرط الخلايا في نخاع العظم (P = 0.0068) ، التجارب (P < 0.0001) ، ونسبة الانفجار (P = 0.0365).

الاستنتاجات: تعد علامات القرص المضغوط وتقارير نقي العظم أدوات أساسية في تحديد ابيضاض الدم النخاعي الحاد. CD13 و CD33 هما أكثر الأقراص المضغوطة شيوعًا في هذه الدراسة. كان التقييم المورفولوجي لنخاع العظام ذا دلالة إحصائية ، وارتبطت خلوية نخاع العظم ونسبة الانفجار بشكل كبير مع استجابة ما بعد الحث في المرضى الذين يعانون من ابيضاض الدم النخاعي الحاد.

الكلمات المفتاحية: ابيضاض الدم النقوي الحاد ، التنميط المناعي ، تقارير نخاع العظم ، قياس التدفق الخلوي وعلامات القرص المضغوط

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