RESEARCH ARTICLE

REVISED Association of leukocyte nadir with complete remission in Indonesian acute myeloid leukemia patients undergoing 7+3 remission induction chemotherapy [version 2; peer review: 2 approved, 1 approved with reservations]

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Abstract

Background: The 7+3 regimen is still the main choice of remission induction chemotherapy in acute myeloid leukemia (AML). Successfully achieving complete remission (CR) and the time required to achieve it determine patient's survival. Hence, bone marrow examination on 14th day of chemotherapy is recommended to predict CR. However, the examination is invasive and still inaccurate.
Methods: A prognostic study with retrospective cohort design was conducted at two central hospitals in Indonesia based on medical record data of AML patients who underwent 7+3 induction chemotherapy from January 1st, 2015, to December 31st, 2019. The association of nadir leukocyte level and the time required to achieve it with CR occurrence was assessed.
Results: One hundred and one subjects were recruited with median age 39 years and 55% men. A total of 55.4% subjects achieved CR. Nadir leukocyte level below 200/mcl was the most optimal cut-off point and independently associated with CR (OR 2.48; 95% CI 1.03–5.97) while time required to achieve it was not.
Conclusions: The nadir leukocyte level is associated with an increase probability of CR but not for the time required to achieve it in AML patients undergoing 7+3 induction chemotherapy.

Open Peer Review

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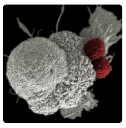
Keywords
acute myeloid leukemia, leukocyte nadir, induction chemotherapy, complete remission, association



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REVISED **Amendments from Version 1**

In this new version, we provide the revision of the "Methods" section where dose intensity of induction chemotherapy is represented by "median cumulative dose" instead of the previous "median daily dose" as one of variables considered to potentially act as confounder. Table 1 and Table 2 also have been revised to accommodate the change. Additional discussion about daunorubicin dose has been provided in "Discussion" section. The data of median daily doses of chemotherapy agents grouped by time periods of diagnosis has also been added to "Underlying Data". Overall, the revision does not change the conclusion of presented study.

Any further responses from the reviewers can be found at the end of the article

Introduction

Acute myeloid leukemia (AML) is the most common form of acute leukemia in the adult population.[1,2] AML is not a single disease entity, but rather a heterogeneous group of diseases, at least from the clinical picture of blast cell morphology and also at the genetic level which turns out to have a more pronounced prognostic impact.[3–7] This heterogeneity has implications for treatment response and prognosis.[8,9] However, the treatment principles for AML have not changed much. The first goal of curative treatment of AML is always to achieve complete remission through induction chemotherapy whose backbone regimen has not changed since it was first introduced 40 years ago.[10,11]

For the purpose of curative treatment in AML, achieving complete remission (CR) as soon as possible after induction chemotherapy is very important because it determines the patient's survival.[12] Patients with a treatment response less than CR have lower survival rate than the group of patients who achieve CR.[4,13] The time required to reach CR also carries prognostic significance. Ciftciler reported that patients who achieved CR within 30 days of the start of remission-induced chemotherapy had a better prognosis than patients who required a longer time.[14]

Therefore, both National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO) recommend bone marrow examination on the 14th day of chemotherapy to predict the occurrence of CR so that reinduction chemotherapy can be carried out earlier if the patient is predicted not to achieve CR. If the bone marrow does not reach a hypoplastic condition, defined as bone marrow cellularity <20% and residual blast cells <5%, it is recommended that the patient be given reinduction chemotherapy immediately.[9,15] However, this examination is invasive and the general condition of the patient during this period is usually very weak with severe pancytopenia which means the procedure still carries some risk for the patient.[16] Some reports also show that bone marrow examination is still less accurate in predicting CR because there are some patients who still achieve CR even when their bone marrow examination on day 14 does not show hypoplastic conditions.[15–19]

On the other hand, induction chemotherapy also causes cells in the peripheral blood to undergo a nadir and recovery cycle similar to conditions in the bone marrow, especially the leukocyte series. The understanding that the sensitivity of tumor cells to chemotherapy drugs is influenced by the genetic predisposition of the host also supports the idea that this chemotherapy sensitivity is shared by other cells in the individual's body, including leukocytes.[20] Pharmacokinetic tests of drugs used for induction chemotherapy has also shown that the level of the drug in leukocytes was directly proportional to its concentration in nucleated cells in the bone marrow.[21] This has sparked the idea of the potential use of the leukocyte nadir pattern as a predictor of CR in AML patients undergoing 7+3 remission induction chemotherapy. Examination of peripheral blood leukocyte levels also has several advantages. First, peripheral blood sampling does not have to be done by a trained specialist doctor but can also be done by nurses or laboratory personnel. Second, the examination is also widely available, and the cost is much cheaper than bone marrow examination. Third, from the patient's perspective, peripheral blood examination is more comfortable and causes less anxiety than bone marrow examination.

The aim of this study was to examine the associations of nadir leukocyte level and the time to reach it with the occurrence of CR in AML patients who underwent "7+3" remission induction chemotherapy. The hypothesis of this study was that the nadir leukocyte level and the time to reach it were associated with the occurrence of complete remission in AML patients undergoing 7+3 remission induction chemotherapy.

Methods

This was a prognostic study with a retrospective cohort design. The research sample was taken by total sampling from the medical record data of patients with a diagnosis of AML who were not acute promyelocytic leukemia (APL) or AML M3 FAB classification who underwent 7+3 induction chemotherapy at Dharmais Hospital National Cancer Center and Dr. Cipto Mangunkusumo National Central Public Hospital during the period from January 1st, 2015, to December 31st, 2019. The acceptance criteria for this study were patients aged ≥18 years, diagnosed with AML according to WHO diagnostic criteria (based on at least a bone marrow smear or biopsy and myeloid lineage confirmation from bone marrow

aspirate or peripheral blood immunophenotyping), who underwent a first-line remission induction chemotherapy 7+3 regimen, and had never undergone any remission induction chemotherapy before. The criteria for rejection were AML M3 (FAB criteria) or acute promyelocytic leukemia (APL), a myeloblastic crisis phase of chronic myeloid leukemia (CML) or when the required data were not found in the patient's medical record. By estimating the proportion of achieving CR of 60% with margin of error of 5%, a total of 103 subjects were needed for this study. The study was approved by the Universitas Indonesia Ethics Board, approval number KET-603/UN2.F1/ETIK/PPM.00.02/2021. Data were collected from June 15th to August 31st, 2021.

Research variables

The nadir leukocyte level and the time required to reach it were assessed for their associations to the occurrence of CR during the evaluation of 7+3 remission induction chemotherapy treatment. Treatment evaluations were done when the peripheral blood cells had recovered. The criteria used to define the occurrence of CR during treatment evaluation were in accordance with those established by European LeukemiaNet 2017.[22] Factors considered as potential confounders were age, gender, AML subtype, Charlson Comorbidity Index (CCI), history of myelodysplasia syndrome (MDS), history of chemotherapy/radiotherapy, prechemotherapy leukocyte level, myeloblast level at diagnosis, dose intensity of induction chemotherapy agents, occurrence of febrile neutropenia and administration of granulocyte colony-stimulating factor (GCSF). The dose intensity was represented by median cumulative dose, that was the total dose of each chemotherapy drug used in the whole process of induction chemotherapy.

Statistical analysis

Data processing was carried out using IBM SPSS Statistics version 28 (IBM SPSS Statistics, RRID:SCR_016479). Numerical data were presented as a mean with a standard deviation if the distribution was normal or as a median with a range if the distribution was not normal. Statistical significance testing was carried out according to the characteristics of the data and their objectives. Bivariate testing on nominal data was carried out by using the chi-square test or by using Fisher's exact test as an alternative if the requirements were not met. To see the difference in the mean in two groups with numerical data that had a normal distribution, an unpaired t-test was used, or the alternative Mann-Whitney U test was used instead if the distribution was not normal. The limit of significance (α) was set at 5% in the conclusion of statistical significance.

For data on nadir leukocyte levels and the time required to achieve it in the form of numerical data, the most optimal threshold was sought through the ROC (receiver operating characteristic) curve by assessing sensitivity and specificity values. The power of discrimination of the two variables was measured by the AUC (area under the curve) value. The strength of the association was expressed in terms of relative risk (RR) or odds ratio (OR) with a 95% confidence interval (CI). Variables that had the potential to become confounders were assessed for their relationship with CR occurrence through the bivariate test. When there was a variable that had p-value <0.25 in the bivariate test, this variable would be further analyzed through a multivariate logistic regression test to determine whether the variables acted as a confounder or not by looking at the changes in the OR it caused. A variable was defined as a confounder when it changed the OR (ΔOR) >10%.

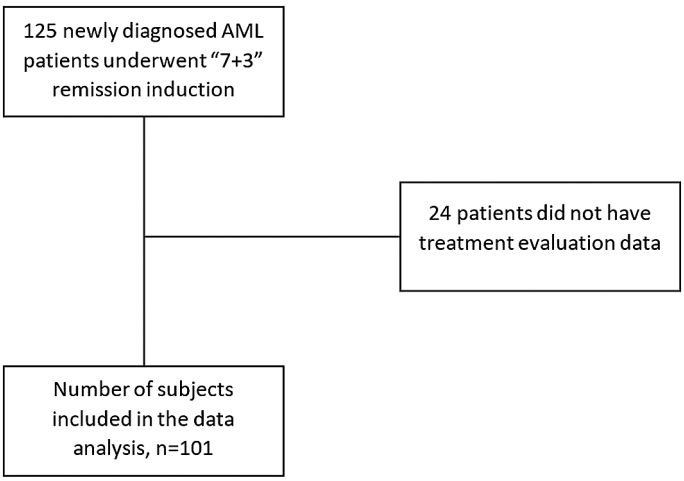

Figure 1. Research subject recruitment flow-chart.

Results

We found 125 patients with newly diagnosed AML non-APL who underwent 7+3 remission induction chemotherapy in the period from January 1st, 2015, to December 31st, 2019. Twenty-four patients were excluded because they died while undergoing the chemotherapy hence, they did not have treatment outcome data. In the end, there were 101 subjects whose data could be analyzed. The recruitment process of research subjects is shown in Figure 1.

The median age of the research subjects was 39 years (range 18–66 years). Male subjects were slightly more common than females with a ratio of 1.2, while the most common subtype was AML M2 (48.5%). The majority of patients had no comorbidities, no history of MDS, and none had a history of chemotherapy/radiotherapy. No patient had cytogenetics nor day-14 bone marrow examination data. Median daily doses of chemotherapy agents used, daunorubicin and cytarabine, in the 7+3 protocol were 50 and 102 mg/m^2/day, respectively, while the median cumulative dose of daunorubicin and cytarabine were 150 and 711 mg/m^2, respectively, as shown in Table 1. Subjects diagnosed in 2018 and thereafter had

Table 1. Characteristics of research subjects.

Characteristics	N (%) (total, n = 101)	Characteristics	N (%) (total, n = 101)
Age (year)		History of Chemo/Radiotherapy	
Median, range	39 (18–66)	No	101 (100)
		Yes	0 (0)
Sex			
Male	55 (54.5)	Daunorubicin daily dose (mg/m^2/day)	
Female	46 (45.5)	Median, range	50 (33–67)
AML Subtypes		Daunorubicin cumulative dose (mg/m^2)	
M0	-	Median, range	150 (99–202)
M1	11 (1.9)		
M2	49 (48.5)	Cytarabine daily dose (mg/m^2/day)	
M4	26 (25.7)	Median, range	102 (66–222)
M5	7 (6.9)		
M6	-	Cytarabine cumulative dose (mg/m^2)	
M7	-	Median, range	711 (460–1,544)
NOS	8 (7.9)		
		GCSF usage	
Pre-Chemo Leukocyte Level (/mcl)		Before leukocyte nadir	41 (40.6)
Median, range	13,600 (660–314,280)	After leukocyte nadir	24 (23.8)
		Did not use	36 (35.6)
Blast level at diagnosis (%)			
Mean, SD	59.32 (18.78)	Leukocyte nadir level (/mcl)	
		Median, range	230 (40–2,020)
Charlson comorbidity index			
0	91 (90.1)	Time to leukocyte nadir (days)	
≥1	10 (9.9)	Median, range	14 (4–35)
History of MDS		Complete remission	
No	93 (92.1)	Yes	56 (55.4)
Yes	8 (7.9)	No	45 (44.6)

significantly higher median daily dose of daunorubicin (58 vs 47 mg/m^2/day; p<0.01) and cytarabine (168 vs 100 mg/m^2/day; p=0.02) than those diagnosed before 2018 (See *Underlying data*).[23]

Treatment results of 7+3 remission induction chemotherapy

A total of 56 subjects (55.4%) achieved CR. There was a difference in the median nadir leukocyte level between the group of subjects who managed to achieve CR (190/mcl; range 40–940/mcl) and the group of subjects who did not (250/mcl; range 70–2,020/mcl; p = 0.02). However, the median number of days required to reach the leukocyte nadir did not differ between the groups that achieved CR (13.5 days; range 4–35 days) and those who failed (14 days; range 5–24 days; p = 0.50). Therefore, in the next analysis, only nadir leukocyte level as the independent variable was examined and its relation to CR occurrence as the dependent variable.

Determining the threshold for nadir leukocyte levels

To find the optimal threshold of the nadir leukocyte level that has a prognostic value to CR occurrence, the specificity and sensitivity of each value of nadir leukocyte level in the occurrence of CR during treatment evaluation were analyzed using the ROC (receiver operating characteristic) curve as shown in Figure 2.

From this curve, the AUC value was 0.63 (95% CI 0.52–0.74) and the most optimal cut-off point for the nadir leukocyte level was 200/mcl (73% specificity, 50% sensitivity). Based on this cut-off point, there were 40 subjects (39.6%) whose nadir leukocyte level was <200/mcl with the RR achieving complete remission of 1.52 (95% CI 1.09–2.14) compared to the group whose nadir leukocyte level was higher. In the subgroup analysis, the subject groups that reached nadir leukocytes level of <200/mcl had a higher CR proportion compared to the groups that had higher nadir leukocyte level even though not all of them reached statistical significance (see *Underlying data*).[23] In addition, along with the lower cut-off value of the nadir leukocyte level, the proportion of subjects with CR became higher (see *Underlying data*).[23]

Association between nadir leukocyte levels and complete remission occurrence during treatment evaluation

After dividing subjects into two groups based on the 200/mcl leukocyte level threshold, bivariate analysis was performed along with the other variables that had the potential to be confounders to the occurrence of CR as the dependent variable as shown in Table 2. For the GCSF administration variable, only GCSF given to subjects before the leukocyte nadir was

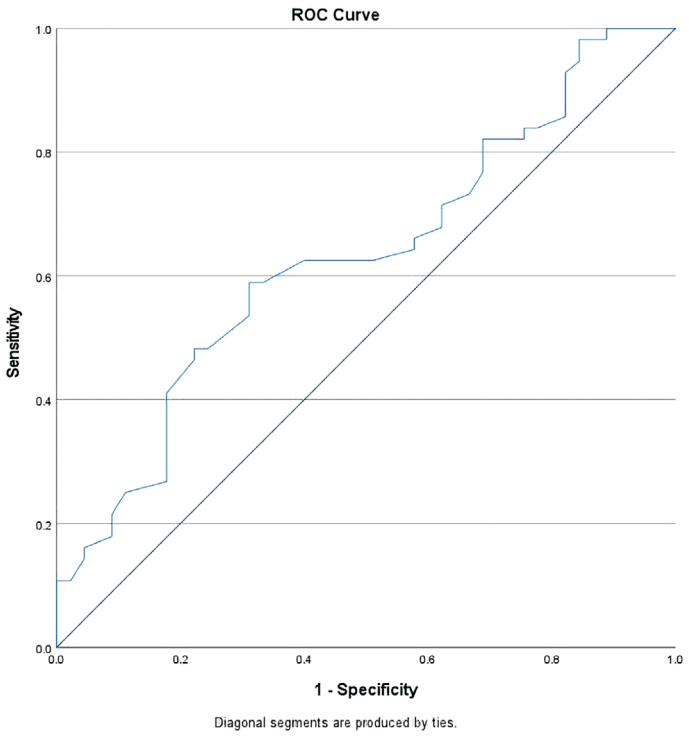

Figure 2. ROC curve of nadir leukocyte levels and CR occurrence during treatment evaluation. Diagonal segments are produced by ties.

Table 2. Bivariate test results for each independent variable with the dependent variable.

Variables	CR (n,%) (total, n = 101)**		*p-value*	Variables	CR (n,%) (total, n = 101)**		*p-value*
	Yes	No			Yes	No	
Nadir leukocyte level (/mcl)			0.02*	History of Chemo/ Radiotherapy			n.a.
<0.20	28 (70.0)	12 (30.0)		No	56 (55.4)	45 (44.6)	
≥0.20	28 (45.9)	33(54.1)		Yes	0 (0)	0 (0)	
Age (year)			0.75	History of MDS			0.75
<60	52 (55.9)	41 (44.1)		No	52 (55.9)	41 (44.1)	
≥60	4 (50.0)	4 (50.0)		Yes	4 (50)	4 (50)	
CCI			0.33	Daunorubicin cumulative dose (mg/m^2)			0.88
0	49 (53.8)	42 (46.2)		≥150	29 (54.7)	24 (45.3)	
≥1	7 (70.0)	3(30.0)		<150	27 (56.3)	21 (43.8)	
AML Subtypes			0.82	Cytarabine cumulative dose (mg/m^2)			0.10*
M1	6 (54.5)	5 (45.5)		≥711	34 (63.0)	20 (37.0)	
M2	30 (61.2)	19 (38.8)		<711	22 (46.8)	25 (53.2)	
M4	13 (50.0)	13 (50.0)					
M5	4 (57.1)	3 (42.9)		GCSF administration prior to leukocyte nadir			0.08*
				No	29 (48.3)	31 (51.7)	
Blast level at diagnosis (%)			0.17*	Yes	27 (65.9)	14 (34.1)	
Mean, SD	57.02 (±17.55)	62.20 (±20.03)					
				Febrile neutropenia			0.93
Pre-Chemo Leukocyte Level (/mcl)			0.37	No	17 (54.8)	14 (45.2)	
Median, range	11,500 (660–187,910)	21,360 (1,620–314,280)		Yes	39 (55.7)	31 (44.3)	

*variable with $p < 0.25$.
**except AML subtypes (n = 93).

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Table 3. Multivariate analysis results.

Variables	OR (95% CI)	ΔOR
Crude OR:		
Nadir leukocyte level	2.75 (1.18–6.39)	
Adjusted OR:		
+ GCSF administration	2.55 (1.09–6.00)	7.71%
+ Cytarabine cumulative dose	2.54 (1.06–6.08)	0.39%
+ Myeloblast level at diagnosis	2.48 (1.03–5.97)	2.42%

reached was considered to be a potential confounder for the relationship between the nadir leukocyte level and the occurrence of CR. A total of 41 subjects (40.6%) received GCSF before the nadir leukocyte level was reached.

From the results of the bivariate test, there were three variables that had $p < 0.25$ other than the nadir leukocyte level ($p = 0.02$), those were administration of GCSF before the leukocyte nadir ($p = 0.08$), cytarabine cumulative dose ($p = 0.10$) and myeloblast level at diagnosis ($p = 0.17$). To see the magnitude of the effect of nadir leukocyte level on the achievement of CR and to determine whether the other variables would act as confounders, a multivariate test was carried out using the logistic regression method. The crude OR was 2.75 (95% CI 1.12–6.39) while fully adjusted OR 2.48 (95% CI 1.03–5.97). None of the variables presumed as confounders changed OR more than 10% as shown in Table 3.

Discussion

Out of 125 patients who underwent "7+3" remission induction chemotherapy, 24 subjects (19.2%) did not have treatment outcome data because they died before treatment evaluation and had to be excluded. Deaths in this period were grouped into treatment related mortality (TRM). This figure is not much different from that obtained by Kayal in India (16.9%) but definitely higher than that obtained by Gbadamosi in the US (6.9%).[24,25] The results of this study indicate that there is still a gap in the quality of AML treatment between developing and developed countries.[2,5,26,27]

The median age of this study (39 years) differs from the median age of AML cases in the general population (68 years) because the subjects taken were only AML patients who underwent intensive treatment and successfully completed it. Of all subjects whose treatment results could be evaluated, 55.4% managed to achieve CR. Again, this figure is not much different from that obtained by Kayal in India (52.8%) but lower than Gbadamosi in the US (62%).[24,25] Gbadamosi's higher CR figure was probably due to the fact that the US treatment facilities are better than Indonesia and India.[2,10,28] Another factor that might play a role in this low rate was the low dose of chemotherapy drugs used in this study. Infection control barriers and TRM rates in developing countries might encourage clinicians to use lower limits of the recommended dose. Yet, the increasing median daily dose of both chemotherapy agents for subjects diagnosed in 2018 thereafter compared to those diagnosed earlier, especially for daunorubicin, might indicate that the treating physicians needed time to absorb newer guidelines which recommend higher dose of daunorubicin (60–90 mg/m^2/day) than the previous (45–60 mg/m^2/day), as more and more physicians implemented higher dose of daunorubicin with increasing year.

There was a difference in median nadir leukocyte levels in the group that managed to achieve CR compared to the group that did not, but the same could not be said on the number of days required to reach the leukocyte nadir. The data that showed a uniform median number of days needed to reach the leukocyte nadir between the two groups (i.e. 14 days) was in accordance with the day recommended by ESMO and NCCN guidelines to perform a bone marrow examination.[9] This result is similar to that reported by Marras where the time required for nadir leukocyte level to be reached was also 12 days for both *responder* and *non-responder* groups.[29] This reinforces the premise that the nadir pattern of leukocytes in the peripheral blood is similar to the pattern of hypoplasia in the bone marrow.

However, this result is different from that obtained by Han who reported that there was a difference in the proportion of subjects who achieved CR between the groups who needed more than 10 days to reach the leukocyte nadir and those who needed less.[17] These different results are probably due to differences in the characteristics of the subjects in that Han's study only recruited subjects over 55 years of age as well as differences in the AML treatment technique used. Old age is associated with a decrease in the activity of hematopoietic stem cells in the bone marrow and the mobilization of PMN cells from the bone marrow to the peripheral blood.[30,31] The most striking difference in treatment technique was that Han allowed his subjects to undergo reinduction chemotherapy if the results of the bone marrow examination on day 14 still

contained *blast* cell residues >5% while none of our subjects received reinduction chemotherapy before treatment evaluation.

This study found that the threshold for the most optimal nadir leukocyte level that had a prognostic value on the occurrence of CR during treatment evaluation was 200/mcl with an *acceptable* discriminant power (AUC 0.63) in identifying subjects who would achieve CR and those who would not ($p = 0.02$).[32] From the RR calculation, the number of subjects whose nadir leukocyte levels was <200/mcl managed to achieve CR 1.52 times more than subjects with higher nadir leukocyte levels. This association was independent based on the results of the multivariate analysis using the logistic regression method with fully adjusted OR 2.48 (95% CI 1.03–5.97). By looking at the OR changes from each step of the logistic regression analysis, none of them made OR changes >10%. Hence, it could be concluded that there were no variables acting as confounders in this study.

The association between nadir leukocyte level to the achievement of CR was then analyzed to see if it met the principles of causality by Sir Austin Bradford Hill.[33] The first principle is the temporal relationship, in which the independent variable in terms of time must precede the dependent variable. In this case, the nadir leukocyte level as an independent variable always preceded the occurrence of CR.

The second principle is an association that emphasizes the strength of the relationship between the independent variable and the dependent variable where the stronger the relationship between the variables, the more probable the concept of causality. From this study, the strength of the association was represented by a fully adjusted OR of 2.48 which is statistically significant.

The third principle is dose-dependent, if the size of the dependent variable changes along with the change in size of the independent variable, then a causal relationship becomes more likely. For the group of subjects with nadir leukocyte levels below 300/mcl, 200/mcl, and 100/mcl, the proportions of achieving CR were 58.1%, 70%, and 81.8% respectively (see *Underlying data*).[23] The proportion of the occurrence of CR was getting higher as the threshold for the nadir leukocyte level lower.

The fourth principle is consistency, the relationship between the independent variable and the dependent variable remains consistent when applied to different subjects or observations. In the group of male and female subjects or the elderly and non-elderly, the group of subjects with a nadir leukocyte level <200/mcl consistently achieved more CR than the group of subjects with a higher nadir leukocyte level, although the differences were not always statistically significant (see *Underlying data*).[23]

The fifth principle is coherence in which research results do not conflict with existing knowledge about the disease. Kinetics of leukocyte has long been used as an indicator of bone marrow recovery, which is characterized by absolute neutrophil count (ANC) levels reaching above 500/mcl after nadir.[29] This shows that the concept of low nadir leukocyte levels as a surrogate marker of the degree of bone marrow hypoplasia associated with CR does not conflict with existing knowledge about AML.

The sixth principle is biological plausibility where research results can be explained by existing theories. The degree of decrease in peripheral blood leukocyte levels due to chemotherapy exposure is considered to be comparable to what happened in blast leukemia cells, and it has been demonstrated, at least in breast cancer and lung cancer, that the degree of leukopenia is associated with response to chemotherapy.[34,35]

The seventh principle is the suitability of the results with other studies. To our knowledge, there have been no other studies reporting similar results, thus the nadir leukocyte nadir level of 200/mcl is the novelty of this study. Therefore, this principle cannot be determined at this time, and further research is needed to confirm the results found in this study. Thus, it has been shown that the results of this study are in accordance with most of the causality principles introduced by Sir Austin Bradford Hill but still require further research to confirm this relationship.

This study showed that lower nadir leukocyte levels (<200/mcl) were associated with higher proportion of subjects achieving CR than the group with higher nadir leukocyte levels. A lower level of leukocyte nadir has long been associated with the higher exposure level of chemotherapy drugs in the bone marrow and blood cells and is expected to describe drug exposure to tumor cells.[20] It is expected that the lower level of leukocyte nadir is related to the higher amount of eradicated leukemic blast cells in the patient's bone marrow. However, the role of nadir leukocyte level on CR occurrence during treatment evaluation in this study was only as strong as 63% which was symbolized by the AUC value of the ROC curve thus opening up the possibility that other variables might play roles in AML patients who achieved CR. Hence, the cut-off

point for the nadir leukocyte levels reported in this study still cannot be directly applied to daily clinical practice. A predictive model that involves other variables is needed to improve the performance of predicting CR occurrence.

Research limitations

The main limitation of this study as in other studies with retrospective design is the impossibility in controlling the variables studied. Although regimens and protocols of chemotherapy for remission induction 7+3 are standard, other treatments given to patients might vary widely between clinicians and might have prognostic impacts on patients that were not possible to include in the analysis. The sample of this study also involved only a few elderly subjects, subjects with comorbidities, or subjects with secondary AML so that the influence of these variables might be less visible in this study. In addition, the absence of cytogenetic profile data in the study sample made it impossible to assess the role of leukemia blast cell characteristics, especially in terms of the sensitivity of AML to treatment. Yet, the limited facilities for cytogenetics and molecular examinations in developing countries, including Indonesia, make simpler alternative tests (e.g., peripheral blood leukocyte levels) in providing information about disease behavior even more necessary to determine the best treatment strategy in AML patients. However, the results of this study still need to be confirmed in the patient group based on the risk of AML through cytogenetics in follow-up studies.

Conclusions

AML patients undergoing "7+3" remission induction chemotherapy who managed to achieve nadir leukocyte level <200/mcl is associated with an increased probability of CR. Time to reach the nadir leukocyte level does not have an association with the occurrence of CR.

Data availability

Underlying data

Mendeley Data: Underlying data for 'Association of leukocyte nadir with complete remission in Indonesian acute myeloid leukemia patients undergoing 7+3 remission induction chemotherapy'. http://doi.org/10.17632/xfx39znwzp.3[23]

This project contains the following underlying data:

- Data file 1: Dataset file ver 3 published.sav
- Data file 2: Supplementary Data – Changes in Proportion of CR Occurrence Along with Changes in the Nadir Leukocyte Level Threshold.docx
- Data file 3: Supplementary Data – Association of Nadir Leukocyte Level Less Than 200mcl with CR Occurrence in Several Subjects Groups.docx
- Data file 4: Supplementary Data – The Median Daily Doses of Chemotherapy Agents by Time Periods of Diagnosis.docx

Reporting guidelines

Mendeley Data: STROBE checklist for 'Association of leukocyte nadir with complete remission in Indonesian acute myeloid leukemia patients undergoing 7+3 remission induction chemotherapy'. http://doi.org/10.17632/xfx39znwzp.3[23]

Consent

Written informed consent for publication of the patients' details was obtained from the patients.

Acknowledgements

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References

1. Bray F, Ferlay J, Soerjomataram I, *et al.*: **Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries.** *CA Cancer J. Clin.* 2018; **68**: 394–424.
Publisher Full Text

2. Short NJ, Rytting ME, Cortes JE: **Acute myeloid leukaemia.** *Lancet.* 2018; **392**: 593–606.
Publisher Full Text

3. Bennett JM, Begg CB: **Eastern cooperative oncology group study of the cytochemistry of adult acute myeloid leukemia by correlation of subtypes with response and survival.** *Cancer Res.* 1981; **41**: 4833–4837.
PubMed Abstract

4. Cheson BD, Bennett JM, Kopecky KJ, *et al.*: **Revised Recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia.** *J. Clin. Oncol.* 2003; **21**: 4642–4649.

5. Estey EH: **Acute myeloid leukemia: 2019 update on risk-stratification and management.** *Am. J. Hematol.* 2018; **93**: 1267–1291.
PubMed Abstract | Publisher Full Text

6. Döhner H, Estey EH, Amadori S, *et al.*: **Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet.** *Blood.* 2010; **115**: 453–474.
PubMed Abstract | Publisher Full Text

7. Arber DA, Orazi A, Hasserjian R, *et al.*: **The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia.** *Blood.* 2016; **127**: 2391–2405.
PubMed Abstract | Publisher Full Text

8. Lichtenegger FS, Krupka C, Haubner S, *et al.*: **Recent developments in immunotherapy of acute myeloid leukemia.** *J. Hematol. Oncol.* 2017; **10**: 1–20.

9. Heuser M, Ofran Y, Boissel N, *et al.*: **Acute myeloid leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up.** *Ann. Oncol.* 2020; **31**: 697–712.
PubMed Abstract | Publisher Full Text

10. Murphy T, Yee KWL: **Cytarabine and daunorubicin for the treatment of acute myeloid leukemia.** *Expert. Opin. Pharmacother.* 2017; **18**: 1765–1780.
Publisher Full Text

11. Pophali P, Litzow M: **What is the best daunorubicin dose and schedule for acute myeloid leukemia induction?.** *Curr. Treat. Options in Oncol.* 2017 18:1 2017; **18**: 1–14.

12. Estey EH, Shen Y, Thall PF: **Effect of time to complete remission on subsequent survival and disease-free survival time in AML, RAEB-t, and RAEB.** *Blood.* 2000; **95**: 72–77.
PubMed Abstract | Publisher Full Text

13. Walter RB, Kantarjian HM, Huang X, *et al.*: **Effect of complete remission and responses less than complete remission on survival in acute myeloid leukemia: a combined Eastern Cooperative Oncology Group, Southwest Oncology Group, and M. D. Anderson Cancer Center study.** *J. Clin. Oncol.* 2010; **28**: 1766–1771.
PubMed Abstract | Publisher Full Text

14. Ciftciler R, Demiroglu H, Haznedaroglu IC, *et al.*: **Impact of time between induction chemotherapy and complete remission on survival outcomes in patients with acute myeloid leukemia.** *Clin. Lymphoma Myeloma Leuk.* 2019; **19**: 729–734.
PubMed Abstract | Publisher Full Text

15. Vainstein V, Buckley SA, Shukron O, *et al.*: **Rapid rate of peripheral blood blast clearance accurately predicts complete remission in acute myeloid leukemia.** *Leukemia.* 2014; **28**: 713–716.
PubMed Abstract | Publisher Full Text

16. Han EJ, Lee B, Hee KJA, *et al.*: **Early assessment of response to induction therapy in acute myeloid leukemia using 18F-FLT PET/CT.** *EJNMMI Res.* 2017; **7**: 1–9.
Publisher Full Text

17. Han HS, Rybicki LA, Thiel K, *et al.*: **White blood cell count nadir following remission induction chemotherapy is predictive of outcome in older adults with acute myeloid leukemia.** *Leuk. Lymphoma.* 2009; **48**: 1561–1568.
Publisher Full Text

18. Alsaleh K, Aleem A, Almomen A, *et al.*: **Impact of day 14 bone marrow biopsy on re-induction decisions and prediction of a complete response in acute myeloid leukemia cases.** *Asian Pac. J. Cancer Prev.* 2018; **19**: 421–425.
PubMed Abstract

19. Nachar VR, Perissinotti AJ, Scappaticci GB, *et al.*: **Predictors for requiring re-induction chemotherapy in acute myeloid leukemia patients with residual disease on day 14 bone marrow assessment.** *Leuk. Res.* 2017; **63**: 56–61.
PubMed Abstract | Publisher Full Text

20. Kvinnsland S: **The leucocyte nadir, a predictor of chemotherapy efficacy?.** *Br. J. Cancer.* 1999 80:11 1999; **80**: 1681–1681.
PubMed Abstract | Publisher Full Text

21. Kokenberg E, Sonneveld P, Sizoo W, *et al.*: **Cellular pharmacokinetics of daunorubicin: relationships with the response to treatment in patients with acute myeloid leukemia.** *J. Clin. Oncol.* 2016; **6**: 802–812.

22. Döhner H, Estey E, Grimwade D, *et al.*: **Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel.** *Blood.* 2017; **129**: 424–447.
PubMed Abstract | Publisher Full Text

23. Hadisantoso DW, Ranuhardy D, Rajabto W, *et al.*: **Underlying data for 'Association of leukocyte nadir with complete remission in Indonesian acute myeloid leukemia patients undergoing 7+3 remission induction chemotherapy.'.** *Mendeley Data.* 2022; v3.

24. Kayal S, Sengar M, Jain H, *et al.*: **Induction related mortality in acute myeloid leukemia: multivariate model of predictive score from the Indian Acute Leukemia Research Database (INwARD) of the Hematology Cancer Consortium (HCC).** *Blood.* 2019; **134**: 2615–2615.
Publisher Full Text

25. Gbadamosi B, Ezekwudo D, Bastola S, *et al.*: **Predictive and prognostic markers in adults with acute myeloid leukemia: a single-institution experience.** *Clin. Lymphoma Myeloma Leuk.* 2018; **18**: e287–e294.
PubMed Abstract | Publisher Full Text

26. Percival MEM, Tao L, Medeiros BC, *et al.*: **Improvements in the early death rate among 9380 patients with acute myeloid leukemia after initial therapy: A SEER database analysis.** *Cancer.* 2015; **121**: 2004–2012.
PubMed Abstract | Publisher Full Text

27. Othus M, Kantarjian H, Petersdorf S, *et al.*: **Declining rates of treatment-related mortality in patients with newly diagnosed AML given "intense" induction regimens: a report from SWOG and MD Anderson.** *Leukemia.* 2014; **28**: 289–292.
PubMed Abstract | Publisher Full Text

28. Faderl S, Kantarjian HM: **Clinical manifestations and treatment of acute myeloid leukemia**. Hoffman R, Benz E, Silbertain L, *et al.*, editors. *Hematology: Basic Principles and Practice.* 7th ed. Philadelphia: Elsevier Inc.; 2018; p. 924–943.

29. Marras T, Dettori M, Caocci G, *et al.*: **White blood cell count nadir and duration of aplasia do not associate with treatment outcome in adult patients with acute myeloid leukemia undergoing intensive chemotherapy.** *Chemotherapy.* 2020; **65**: 110–114.
Publisher Full Text

30. Gazit R, Weissman IL, Rossi DJ: **Hematopoietic stem cells and the aging hematopoietic system.** *Semin. Hematol.* 2008; **45**: 218–224.
Publisher Full Text

31. Chatta GS, Price TH, Stratton JR, *et al.*: **Aging and marrow neutrophil reserves.** *J. Am. Geriatr. Soc.* 1994; **42**: 77–81.
PubMed Abstract | Publisher Full Text

32. Mandrekar JN: **Receiver operating characteristic curve in diagnostic test assessment.** *J. Thorac. Oncol.* 2010; **5**: 1315–1316.
Publisher Full Text

33. Sastroasmoro S, Aminullah A, Rukman Y, *et al.*: **Variabel dan hubungan antar-variabel**.Sastroasmoro S, Ismael S, editors. *Dasar-Dasar Metodologi Penelitian Klinis.* 3rd ed. Jakarta: Sagung Seto; 2008; p. 255–277.

34. Poikonen-Saksela P, Lindman H, Sverrisdottir A, *et al.*: **Leukocyte nadir as a predictive factor for efficacy of adjuvant chemotherapy in breast cancer. Results from the prospective trial SBG 2000-1.** *Acta Oncol.* 2020; **59**: 825–832.
PubMed Abstract | Publisher Full Text

35. Liu W, Zhang C-C, Li K: **Prognostic value of chemotherapy-induced leukopenia in small-cell lung cancer.** *Cancer Biol. Med.* 2013; **10**: 92–98.
PubMed Abstract | Publisher Full Text

Open Peer Review

Current Peer Review Status: ✔ ? ✔

Version 2

Reviewer Report 31 August 2022

https://doi.org/10.5256/f1000research.135572.r142927

✔ **Shinta Wardani**
Division of Hematology and Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

I found the responses from the authors were sufficient to answer all of my questions. The manuscript is acceptable without any further reservation.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 13 June 2022

https://doi.org/10.5256/f1000research.121914.r137008

✔ **Smita Kayal**
Department of Medical Oncology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, Puducherry, India

This retrospective study on role of leucocyte nadir in predicting remission outcomes of intensive induction therapy in AML (acute myeloid leukemia) is an important work, especially in resource limited limited settings. It is well written with lucid discussion.

- Limitations of absence of cytogenetic data, and other aspects are mentioned. However, a comment should be added on lower average dose of daunorubicin used in the study and reason for it.
- In table 2, n to be added in column headings.
- Whether other cell components (neutrophil or lymphocyte) were also analyzed for their predictive value?

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: Our old work has been cited in this paper

Reviewer Expertise: hemato-oncology, transplant, supportive care

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 26 Jun 2022

Dwi Wahyunianto Hadisantoso, Universitas Indonesia, Central Jakarta, Indonesia

Dear Dr. Smita Kayal, we are so delighted for your valuable response and comments. Here is our response:

1. We have added some conditions that might explain the lower median daily dose of daunorubicin in this study in the last two sentences of second paragraph in "Discussion" section. We think of the higher TRM (treatment related mortality) rates in developing countries and time needed by treating clinicians to absorb newer guidelines might have significant contributions.

2. We have added "n" as total subjects in column headings of Table 2.

3. We did not analyze the counts of leukocyte differential because as the lower the level of leukocyte, the more unreliable the differential count result would become as the CV (coefficient of variance) would increase dramatically. Thus, we were afraid this could lead to a significant bias, especially for a retrospective study using secondary data. This effect also happens on leukocyte (as total white blood cells) count but far less than on its differential. The other reason is not all subjects had daily leukocyte differential count data.

Competing Interests: The authors have no competing interest to declare.

Reviewer Report 07 June 2022

https://doi.org/10.5256/f1000research.121914.r137004

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Hyewon Lee
Center for Hematologic Malignancy, National Cancer Center, Goyang, South Korea

The aim of the study is to investigate the correlation between nadir leukopenia and complete remission in patients treated with conventional induction therapy for AML (acute myeloid leukemia). It was a retrospective study, which included 101 patients who could recover from nadir and underwent response evaluation.

The binominal analyses and ROC (receiver operating characteristic) curve were adopted to explore any association between the two variables in this study. For this, the outcome should be defined as two groups, such as 'CR achieved' or 'CR not achieved'. The authors excluded 24 patients from initial cohort (n=125), because they died early before response evaluation. However, this may be a bias underestimating the proportion of CR (complete remission) group, because early death might be associated with treatment toxicity, low WBC count and prolonged nadir. This may be one of the reason why CR rate is relatively low in developing countries compared to developed countries.

In addition, nadir leukopenia is a result of intensive chemotherapy. However, the median dose of daunorubicin in this study was 50mg/m2/day, which is lower than standard dose. It should be considered when the authors analyze nadir leukocyte count and the outcome of chemotherapy. Furthermore, dose intensity should be described median cumulative dose of entire induction therapy. Because lower dose intensity is supposed to correlate to relatively higher nadir leukocyte count and subsequent induction failure, it might mislead the authors to conclude that nadir leukocyte count itself has an impact on complete remission.
To avoid this misinterpretation, multivariate analysis for achieving CR should be performed with more variables such as age, cumulative dose intensity and cytogenetic risk group of AML, in

addition to variables which were included in the presented study. Then comprehensive discussion on the results should be done, focusing on possible reasons why it was shown like that.
If there is no available data of AML biology, it should be described as a limitation of this study in discussion session.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hematooncology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 26 Jun 2022

Dwi Wahyunianto Hadisantoso, Universitas Indonesia, Central Jakarta, Indonesia

Dear Dr. Hyewon Lee, we are so delighted to have your valuable response and comments. Here is our response:

1. We agree that subjects who underwent treatment related mortality (TRM) can be a bias for the reported proportion of complete remission (CR). On the same reason, we also agree that higher rate of TRM in developing countries can be the reason why the rate of CR is lower compared to developed countries. Nevertheless, we had to exclude this group of subjects from analysis since the treatment response could not be determined. Follow up study to elucidate the characteristics of subjects who underwent TRM after induction chemotherapy might give valuable information of these premises and help to reduce its rate.

2. We agree that lower dose of chemotherapy used in this study could have an impact in

the outcome of induction chemotherapy, as we wrote in the last two sentences of the second paragraph in "Discussion" section. As this study had retrospective design that recruited subjects from January 2015 to December 2019 we think another reason that could explain the lower dose of daunorubicin is the possibility that clinicians need time to move on from the previous guidelines that recommended daunorubicin dose range between 45 – 60 mg/m2/day to the current 60 – 90 mg/m2/day as there is significant difference of median daily dose of daunorubicin for subject who were diagnosed before 2018 and 2018 afterward (47 vs 58 mg/m2/day, respectively; p=0,004). More and more clinicians were implementing higher dose of daunorubicin with increasing year to the dose range recommended by current guidelines.

3. We agree to use "median cumulative dose" as it seems more appropriate to describe the chemotherapy dose intensity (and consequently used in analysis) rather than the median of daily dose, yet we still report the median daily dose of each drug in the article as it is easier to compare them to the recommended daily doses provided by the AML guidelines. Nevertheless, these changes did not change the conclusion of the analysis, there was still no significant association between the median cumulative dose of daunorubicin nor cytarabine with the occurrence of CR (p=0.88 for daunorubicin and p=0.10 for cytarabine). There was indeed a significant association between the subjects who had higher cumulative dose of daunorubicin (≥150 mg/m2) with lower leucocyte nadir level (<200/mcl) (RR=2.11; CI 95% 1.22 – 3.67, p<0.01) but not for cytarabine cumulative dose with leukocyte nadir level (p=0.80). This interesting result may warrant follow-up study to further explore the relationship between chemotherapy dose intensity and nadir of leukocyte.

4. This study aimed to explore the relationship between leukocyte nadir with CR occurrence and some variables that were thought to potentially act as confounders were also analyzed in two steps manner of analysis as we wrote in the "Methods" section and "Statistical analysis" subsection. Those variables were age, gender, AML subtype, Charlson Comorbidity Index (CCI), history of myelodysplasia syndrome (MDS), history of chemotherapy/radiotherapy, prechemotherapy leukocyte level, bone marrow myeloblast cell level at diagnosis, dose intensity of chemotherapy agents, occurrence of febrile neutropenia and administration of granulocyte colony-stimulating factor (GCSF).
   For each of these variables we initially did bivariate analysis to identify the significancy of its relationship to the CR occurrence as the dependent variable. Only variables with p<0.25 from bivariate analysis (GCSF administration, cytarabine cumulative dose, and myeloblast level at diagnosis, as shown in Table 2) were considered "significant" then further analyzed in multivariate analysis to be determined whether they act as confounders in the relationship of leukocyte nadir level (as independent variable) and CR occurrence (as dependent variable) or not. This is to explain that the study already considered variables suggested by the reviewer as potential confounders other than cytogenetic risk group, which the latter had been written at the "Research limitations" section.

Competing Interests: The authors declare no competing interest.

Reviewer Report 27 May 2022

https://doi.org/10.5256/f1000research.121914.r137006

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Shinta Wardani
Division of Hematology and Oncology, Department of Internal Medicine, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

This research idea is very original and applicable, especially in countries with limited resources. The golden standard for assessing hypoplasia in AML (acute myeloid leukemia) patients after induction therapies bone marrow aspiration, with criteria for bone marrow cellularity <20% and residual blast <5%, which are also a criteria for complete remission. If the author wants to replace the bone marrow aspiration with the nadir leucocyte count, then my question is, has this nadir leucocyte count been compared with the hypoplastic condition through the golden standard bone marrow aspiration?

On page 8 of the last paragraph, how do the authors show that a low nadir leucocyte count is a surrogate marker of the degree of bone marrow hypoplasia?

I need your comment, the number of blast cells in the periphery is not needed in assessing bone marrow hypoplasia? Why is it only the nadir leucocyte that is used as a surrogate marker?

I want to confirm the number of subjects who took part in this study, because the abstract mentioned 101 subjects, but in the method on the full paper 103 patients.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Hematology and medical oncologist

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 05 Jun 2022

Dwi Wahyunianto Hadisantoso, Universitas Indonesia, Central Jakarta, Indonesia

Dear Dr. Shinta Wardani, we would like to thank you for your valuable comments and questions. Here is our response:

1. We need to clarify that the goal of this research is to elucidate the association between leukocyte nadir and complete remission (CR) occurrence in acute myeloid leukemia (AML) patients undergoing 7+3 remission induction based on some evidence that day-14 bone marrow examination (BME), as the gold standard for predicting CR, still has some limitations and inaccuracy.

   Hence, the result of this research is not meant to replace the BME with leukocyte nadir count, yet the latter might give some information for the treating clinicians in predicting CR occurrence irrespective of whether the day-14 BME was done or not to the patient. In this retrospective research we have stated in the article that none of our subjects underwent day-14 BME.

2. Since none of our subjects underwent day-14 BME, we could not directly analyze leukocyte nadir as a surrogate marker of bone marrow hypoplasia. Yet, the study results do not contradict the concept of the pattern of leukocyte nadir in the peripheral blood is similar to the hypoplasia pattern of nucleated cells in the bone marrow. The median time needed to achieve the nadir leukocyte level was equal for both groups (CR group and non-CR group) that were 14 days. This result is in-line with Marras et al (citation no. 29) who also found 12 days as uniform median time needed to achieve nadir leukocyte level for both responder and non-responder groups. These numbers of days are so similar to the bone marrow hypoplasia period, as the ultimate reason why the BME is recommended to be done on day-14 of induction chemotherapy. The study result that showed subjects who succeeded in achieving nadir leukocyte level less than 200/mcl had higher probability in achieving CR than who did not (RR 1.52; CI 95% 1.09 – 2.14) is analogous to the well-accepted paradigm that subjects who succeed achieving bone marrow hypoplasia in day-14 BME are predicted to achieve CR on treatment evaluation. Another concept based on association between leukocyte in peripheral blood and hematopoietic cells in bone marrow has also been used for long, as level of absolute neutrophile count (ANC),

one of the leukocyte components, rising above 500/mcl from previously nadir level marks the recovery of the bone marrow.

3. Although we are aware some previous studies have showed correlation between peripheral blast clearance with treatment response, yet we decided to not include it in analysis. Not all AML patients have peripheral blast when they are diagnosed. In this study, there were 8 subjects (7.9%) whose peripheral blast level was 0% and up to 21% of the sample had very few peripheral blast count (less than 5%) before treatment. This condition would make analysis of peripheral blast clearance to treatment response for this group of subjects become impossible and they were always excluded in such studies. Thus, we choose leukocyte nadir as independent variable since it is a universal event that can be analyzed in *all* AML patients undergoing 7+3 induction chemotherapy.

4. From statistic sample size calculation 103 subjects were needed (as we wrote in Method section), yet the study found 101 subjects eligible for analysis (as we wrote in Results section), thus this study sample comprised of 101 subjects.

Competing Interests: The authors have no competing interest to declare.