AML-230:Predictive Value of CD34-Positive versus CD34-Negative Leukemic Stem Cells on Survival Outcome in Acute Myeloid Leukemia

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Abstract

Context: Leukemic stem cells (LSCs) have emerged as a potential factor contributing to an overall dismal outcome in acute myeloid leukemia (AML).
Objective: In this study, using flowcytometric immunophenotyping (FCMI), we have demonstrated LSC identification and quantification in both CD34+ and CD34- compartment to find its relevance in predicting treatment outcomes. Furthermore, we correlated the impact of LSC quantification at diagnosis as a predictor of long-term survival.
Design: This is a prospective analysis of patients diagnosed with AML (per standard WHO criteria).
Setting: Tertiary care cancer center.
Patients: A total of 161 patients with a diagnosis of non–acute promyelocytic leukemia (non-APL) AML patients diagnosed over a period of two years (January 2019 to December 2020) were evaluated.
Interventions: Bone marrow aspirates collected in EDTA were processed for FCMI using a stain-lyse-wash technique with a single-tube, 10-color comprehensive antibody panel. LSCs were identified primarily in the CD34+CD38− compartment; however, in a substantial number of CD34− AML, LSCs were segregated from the CD34−CD38− compartment using CD117 as an alternate gating marker and CD123, CD33, and CD11b as LSC-specific markers in all cases.
Main Outcomes Measures: 1. CD34+ versus CD34− LSC and 2. Baseline LSC% as a predictor of survival.
Results: LSCs were identified in 82.6% (133/161) of patients. CD34+ LSCs and CD34− LSCs were isolated in 82.53% (104/126) and 82.85% (29/35) of cases, respectively. The median survival times (in weeks) in CD34− LSCs was higher than in CD34+ LSCs; EFS (54 vs. 47; *P*=0.27), RFS (79 vs. 48; *P*=0.14), and OS (121 vs. 71; *P*=0.41) were found to be comparable in both groups. Furthermore, the total study cohort was divided into three groups based on diagnostic LSC percentage, i.e., LSC^{neg} (LSC% <0.004%), LSC^{low} (LSC%=0.004%–0.892%), and LSC^{high} (LSC% >0.892%). The LSC^{high} group had significantly shorter OS, EFS, and RFS compared to the LSC^{low} and LSC^{neg} groups.
Conclusions: This study confirms the presence of LSCs beyond the usual CD34+CD38− compartment; however, there is no difference in survival outcomes in both groups. Also, LSC quantification is a powerful and independent prognostic parameter in AML predicting long-term survival and risk of relapse.

Background

Acute myeloid leukemia (AML) is an aggressive hematological malignancy with an inferior long-term outcome despite of reduction in leukemic burden in majority of patients through high intensity chemotherapy. Although initial studies showed that LSCs reside in CD34+CD38- niche of AML blasts, now there is increasing evidence of their distribution in CD34- compartment as well. In this study, using flowcytometric immunophenotyping (FCMI), we have comprehensively demonstrated LSC identification and correlated the impact of LSC quantum at diagnosis as predictor of long-term survival.

Objective

1. LSC identification and quantification in both CD34+ and CD34- compartment using FCMI to find its relevance in predicting treatment outcomes.
2. Impact of LSC quantification at diagnosis as a predictor of long-term survival.

Materials & Methods

Bone marrow aspirates collected in EDTA were processed for FCMI using a stain-lyse-wash technique with a single-tube, 10-color comprehensive antibody panel. LSCs were identified primarily in the CD34+CD38− compartment; however, in a substantial number of CD34− AML, LSCs were segregated from the CD34−CD38− compartment using CD117 as an alternate gating marker and CD123, CD33, and CD11b as LSC-specific markers in all cases. Figure 1 & 2 shows the gating strategy for CD34+ and CD34- LSC.

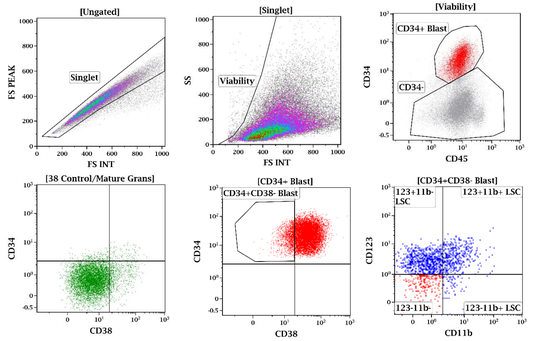


Figure 1: Flow cytometry immunophenotyping gating strategy through dot plots demonstrating leukemic stem cell identification. CD34 positive blasts (red dots) are sequentially segregated into CD38- and CD38+ component using mature granulocytes (green dots) as negative internal control. Leukemic stem cells (blue dots) were further isolated from CD34+CD38- component using lymphocytes (orange dots) as internal control for CD123 and CD11b.

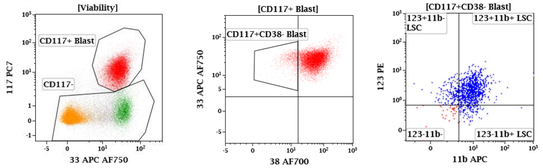


Figure 2: Flow cytometry dot plots showing leukemic stem cell identification in a case of CD34- acute myeloid leukemia. Blasts (red dots) were identified using CD117 and CD33 followed by CD38 negative and CD38 positive subset using monocytes (green dots) as internal control. Leukemic stem cells (blue dots) were further isolated and quantified from CD38-component of blasts using lymphocytes (orange dots) as internal control for CD123 and CD11b.

Results

A total of 161 patients with a diagnosis of non–-APL AML over a period of two years (January 2019 to December 2020) were evaluated. LSCs were identified in 82.6% (133/161) of patients. CD34+ LSCs and CD34− LSCs were isolated in 82.53% (104/126) and 82.85% (29/35) of cases, respectively. Though the median survival times (in weeks) in CD34− LSCs was higher than in CD34+ LSCs; EFS (54 vs. 47; *P*=0.27), RFS (79 vs. 48; *P*=0.14), and OS (121 vs. 71; *P*=0.41) were found to be comparable in both groups.

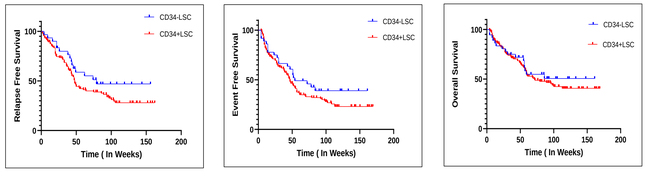


Figure 3: Survival analysis represented by Kaplan Meier plots showing comparable event free survival, overall survival and relapse free survival in CD34+ LSC and CD34-LSC patients

The total study cohort was divided into three groups based on diagnostic LSC percentage, i.e., LSC^{neg} (LSC% <0.004%), LSC^{low} (LSC%=0.004%–0.892%), and LSC^{high} (LSC% >0.892%). The LSC^{high} group had significantly shorter OS, EFS, and RFS compared to the LSC^{low} and LSC^{neg} groups.

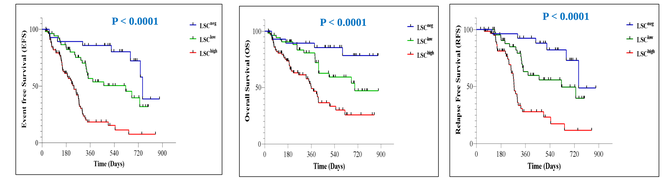


Figure 4: Survival analysis represented by Kaplan Meier plots showing shorter event free survival, overall survival and relapse free survival in LSC^{high} cohort in contrast to LSC^{low} and LSC^{neg} patients

Conclusion

This study confirms the presence of LSCs beyond the usual CD34+CD38− compartment; however, there is no difference in survival outcomes in both groups. Also, LSC quantification is a powerful and independent prognostic parameter in AML predicting long-term survival and risk of relapse.

References

1. Terwijn M, Zeijlemaker W, Kelder A, et al. Leukemic stem cell frequency: a strong biomarker for clinical outcome in acute myeloid leukemia. PLoS One. 2014 Sep 22;9(9):e107587. doi: 10.1371/journal.pone.0107587.
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