CD9 is a Very Helpful Marker for Discriminating Between AML-M3 from HLA-DR Negative Non-M3 AML

Esmaeil Shahabi Satlsar¹,², Mohammad Mosleh³, Mahdieh Mehrpouri⁴

Esmaeil Shahabi Satlsar PhD, Clinical Laboratory Sciences Department, School of Paramedical Sciences, Guilan University of Medical Sciences, Rasht, Iran
esmaeilshahabi@yahoo.com
+989112443060

Submitted: 11 March 2020
Accepted: 8 June 2020

¹Clinical Laboratory Sciences Department, School of Paramedical Sciences, Guilan University of Medical Sciences, Rasht, Iran
²Thakhte Tavous Pathobiology Laboratory, Flow Cytometry Department, Tehran, Iran
³Hematology and Blood Banking department, school of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
⁴Clinical Laboratory Sciences Department, School of paramedical Sciences, Alborz University of Medical Sciences, Karaj, Iran

Short Title: CD9 to differentiate APL from HLA-DR negative AML
Key Words: AML-M3, CD9, Flow cytometry, HLA-DR negative AML
Conflict of interest: The authors declare no conflict of interest

To The Editor,
CD9 is a cell-surface marker whose its carcinogenic properties have been proven in several solid tumors. Previous studies reported that the blasts cells either B-Cell acute lymphoblastic leukemia (ALL) or acute myeloid leukemia (AML), as well as normal B-cell precursors (Hematogones) express CD9 (1,2,3). Acute promyelocytic leukemia (APL), a highly aggressive type of AML with an increased risk of death due to hemorrhage; therefore flow cytometry providing an accessible and useful tool for rapid diagnostic of the APL. Promyelocytes in APL are characterized by negative expression for HLA-DR, CD11b, CD34, and are often positive for cMPO (bright), CD13, CD33, CD34 (Dim), CD64 and CD117 (Dim to moderate). Although, another subtype of AML that entitled HLA-DR negative non-M3 AML also lacks the expression of HLA-DR, and CD34 (4,5). Differential diagnosis of APL from HLA-DR negative non-M3 AML cannot be based on morphology and lack of HLA-DR antigen expression; rather, it requires molecular confirmation of PML-RARA using cytogenetic, which leading to delayed diagnosis. In our
previous study, evaluating the expression of CD9 in AML cases (6), showed differences in expression of CD9 between blasts of APL and HLA-DR negative non-M3 AML. With respect to the interesting results of the previous study, it was tempting to continue evaluating this marker in patients with APL and HLA-DR negative non-M3 AML. Given this, we evaluated CD9 expression in 101 patients with APL and 94 patients with HLA-DR negative non-APL using flow cytometry; moreover, molecular evaluation for PML-RARA was performed in all studied patients for confirming a suspected diagnosis of APL.

Flow cytometric analysis was performed using Beckman Coulter Cytomics FC 500 Flow Cytometer with MXP software. To this end, bone marrow (BM) and/or peripheral blood (PB) samples were taken from the patients between March 2015 and January 2020. The following conjugated-monoclonal antibody were used; CD9 (FITC-Coulter), HLA-DR (PE-Dako), HLA-DR (PE-Immunostep), CD13 (Percp/Cy5-BD), CD33 (APC-Coulter), CD64 (PE-Dako), CD117 (PE-Coulter), CD34 (PE-BD), CD45 (Percy-Immunostep) in four-color combinations. Resulting data showed that both APL patients and HLA-DR negative non-APL patients expressed CD9 marker; although, each have a distinct pattern of expression. Consistent with these, our data delineate that all APL patients (100%) showed homogeneous and moderate or bright expression of CD9, which is similar to the pattern of CD33 expression. Conversely, we found that in HLA-DR negative non-APL patients, CD9 expression was detected in 59 of 94 (62.7%) of patients, with a heterogeneous pattern and dim to moderate expression, which is unique and completely different pattern from those with APL (Figure 1). The timely diagnosis of APL continues to be challenging despite advancements in medical diagnostics in many countries of the world. Furthermore, the abnormal morphology of promyelocytes, especially in the hypogranular variants, (7) and expression of CD11b, can lead to a diagnostic error in some patients.

The most straightforward explanation of our results is that expression patterns of CD9, along with other myeloid markers such as CD13, CD33, and CD64 can be helpful in precise differential diagnosis of patients with APL from patients with HLA-DR negative non-APL.

References
Figure 1. Differences in CD9 expression patterns in APL and HLA-DR negative non-APL.  
A: APL blasts shows homogenous and mod to bright expression, B: HLA-DR negative non-APL have dim to mod and heterogeneous expression.