International Journal of Scientific Research in Dental and Medical Sciences

www.ijsrdms.com

Flow cytometric Analysis of Acute Promyelocytic Leukemia: A Series of Four Cases with Review of Literature

Swati Agarwal *, Gunja Dwivedi, Vijay Rajak, Rajani Rajkumari

Department of Pathology, RNT Medical College, Udaipur, Rajasthan, India

ARTICLE INFO

Article history:
Received 07 May 2024
Received in revised form 02 June 2024
Accepted 06 June 2024
Available online 09 June 2024

Keywords:
Acute Myelogenous Leukemia
Acute Promyelocytic Leukemia
Retinoic Acid Receptor Alpha

ABSTRACT

Acute Promyelocytic Leukemia (APL) is an aggressive form of Acute Myeloid Leukemia (AML), a cancer of the bone marrow. APL causes accumulation of promyelocytes in the peripheral blood and bone marrow and is associated with translocation t(15; 17). The diagnosis of APL can be made with the help of a complete blood count, peripheral blood film examination, bone marrow, and flow cytometry. Immunophenotypically, APL is characterized by positivity for CD117 and cMPO, bright positivity for CD33 and CD13, and negative for CD34 and HLA-DR. Here, we present four cases suspected of acute leukemia on peripheral blood film and bone marrow examination and diagnosed as APL in flow cytometric analysis.

1. Introduction

APL is a subtype of AML-M3 in which abnormal promyelocytes are predominantly present. The disease is characterized by a translocation t(15; 17) (q22; q12) involving the retinoic acid receptor alpha (RARA) gene and is distinguished from other forms of AML by its responsiveness to all-trans retinoic acid (ATRA; also known as tretinoin) therapy.[1] Excellent response to ATRA in PML- RARA positive cases has been observed, while variable RARA translocation has a variable response. Clinically, the patient presents with bleeding manifestations, headache, fatigue, and weakness. The hypergranular and microgranular variants have characteristic morphology, which should be identified and communicated to the clinician immediately.[2] Our flow cytometry laboratory was established in the year 2022, and a total number of 29 cases were diagnosed as AML, out of which four cases were reported as APML. Hence, here we present the four cases with their complete blood count, peripheral blood film, bone marrow findings, and flow cytometric analysis.

2. Case presentation

Case 1

A 16-year-old female patient was admitted to the medicine department with complaints of fever for six days, bleeding from nose and mouth for four days, petechiae, and purpura all over the body for two days. The complete blood count results were as follows: RBC- 1.83×10^6/uL; Hemoglobin- 6.1g/dL; Platelet- 15×10^3/uL; WBC- 26.28×10^3/uL. On peripheral blood film examination- hypochromic red cells, hyperleukocytosis with ~95% blasts, and blast equivalent were seen. Blasts were moderate to large with a high N: C ratio, irregular nuclear membrane, cleaved and bilobed nuclei, fine chromatin, inconspicuous nucleoli, and a moderate amount of cytoplasm. A few cells showed cytoplasmic granules and auer rods. Few faggot cells were also seen. The bone marrow aspiration- slides were diluted entirely with blood. No bone marrow particles or megakaryocytes were seen in the submitted smear. Thus, diagnosis of Acute leukemia was suggested on peripheral blood film. Immunophenotypic analysis of CD45 vs. Side Scatter revealed ~86% blast population, which showed dim CD45 expression and moderate to high side scatter. The blasts were moderate positive for CD33 and cMPO, dim to moderate positive for CD13, CD64, dim positive for CD2 and negative for CD34, CD117, CD8, CD7, CD4, CD56, CD5, CD3, HLA-DR, CD11b, CD14, CD15, cCD3, cCD79a and all B-cell lineage markers. Findings were suggestive of APML.

Case 2

A 13-year-old male patient had visited the outpatient department (OPD) with complaints of fever, headache, and vomiting for 10 days. The results of the complete blood count were as follows:-RBC- 3.81×10^6/uL, Hemoglobin- 10.9 g/dL; Platelet- 47×10^3/uL; WBC- 23.48×10^3/uL. On peripheral blood film examination- hypochromic red cell, hyperleukocytosis, blast cells~ 95% atypical promyelocyte cells with increased N: C ratio, irregular nuclear membrane, folded buttock-shaped nuclei, fine chromatin, 0-

* *Corresponding author. Swati Agarwal*
E-mail address: *swati.agwl07@gmail.com*
Department of Pathology, RNT Medical College, Udaipur, Rajasthan, India
https://doi.org/10.30485/IJSRDMS.2024.457050.1580

1 nucleoli, a moderate amount of cytoplasm showing granules and auer rods were observed. No bone marrow aspiration smears were recovered. Thus, diagnosis of Acute leukemia was suggested on peripheral blood film. Immunophenotypic analysis of CD45 vs. Side Scatter revealed that ~ 84% of the population showed CD45 dim expression and moderate to high side scatter. The cells were bright positive for cMPO, moderately positive for CD33, and dim to moderate positive for CD13, CD15, CD56, and CD64. The cells were negative for CD34, CD3, CD79a, CD117, HLA-DR, CD14, CD11b, CD7, CD4, CD8, CD2, CD5 and all B cell lineage markers. Findings were suggestive of APML.

Case 3

A 51-year-old female patient was admitted to the medicine department with complaints of fever, mouth sore from 20 to 25 days, and headache. The complete blood count results were as follows: RBC- 2.04×10^6/uL, Hemoglobin- 6.6 g/dL, Platelets- 15×10^3/uL, WBC- 4.15×10^3/uL. On peripheral blood examination- hypochromic red cells, TLC - 4.15× 10^3/uL, blast cells ~ 87% blast/blast equivalent were observed. These cells had a high N: C ratio, an irregular nuclear membrane with many cells showing bilobed nuclei, fine chromatin, inconspicuous to prominent nucleoli, and a moderate granular cytoplasm. Many cells showed auer rods. On bone marrow aspiration- >80% blast and blast equivalent cells were observed. Cells were medium to large in size, having a high N: C ratio, a moderate amount of granular cytoplasm, an irregular nuclear membrane with open chromatin, and 1 to 2 nucleoli; the occasional number of blasts showing irregular nucleoli were also seen. Thus, diagnosis of Acute leukemia was suggested on peripheral blood film and bone marrow. Immunophenotypic analysis of CD45 vs Side Scatter revealed ~ 87% of blasts show CD45 dim expression and moderate to high side scatter. The blasts were moderate to bright positive for cMPO, moderate positive for CD33, CD64, and CD38, dim to moderate positive for CD13, and dim positive for CD117. The blasts were negative for CD34, HLA-DR, CD19, CD10, CD15, CD14, CD11b, and all T cell lineage markers. Findings were suggestive of APML.

Case 4

A 55-year-old female patient was admitted to the Medicine department with complaints of high-grade fever with chills for seven days, cough without expectoration, and easy fatigability. The complete blood count results were as follows: RBC- 1.88×10^6/uL, Hemoglobin- 5.0g/dL, Platelet- 43×10^3/uL, WBC- 2.26×10^3/uL. On peripheral blood examination- hypochromic red cells, TLC- 2.32×10^3cells/uL, Platelets- 41×10^3cells/uL, blasts equivalents: 90%, the blasts are medium to large, having high N: C ratio, irregular nuclear membrane, and fine chromatin, many cells showed nuclear folding. The cells had a moderate amount of granular cytoplasm. On bone marrow aspiration, smears showed>90% blast and blast equivalent presence. Cells were medium to large, having a high N: C ratio, a moderate amount of granular cytoplasm, an irregular nuclear membrane with open chromatin and 1 to 2 nucleoli, and an occasional number of blasts showing folded and irregular nucleoli were also seen. A diagnosis of Acute leukemia was suggested on peripheral blood film and bone marrow. Immunophenotypic analysis of CD45 vs Side Scatter revealed that 93.7% of blasts showed CD45 dim expression and moderate to high side scatter. The blasts were bright positive for cMPO, moderate to bright for CD64, moderate positive for CD13, CD33, and CD14, and dim to moderate positive for CD38 and CD11b. The blasts are negative for HLA-DR, CD34, CD15, CD36, CD117, and all B and T cell lineage markers. Findings were suggestive of APML.

Table 1. analysis of the CBC parameters of all 4 cases.

	Hemoglobin	Platelets	WBC Count	PBF-blast Cells in %(Approx)
Case 1	Low	Low	Increased	95
Case 2	Low	Low	Increased	95
Case 3	Low	Low	Normal	87
Case 4	Low	Low	Low	90

Table 2. The initial laboratory test data of all 4 cases.

	Hemoglobin (g/dL)	WBC (Per uL)	RBC (Per uL)	Platelets (Per uL)	PBF	Bone Marrow
Case 1	6.1	$26.28x10^3$	$1.83x10^6$	$15x10^3$	~95%blasts	Diluted
Case 2	10.9	$23.48x10^3$	$3.81x10^6$	$47x10^3$	~95% atypical promyelocyte	Not received
Case 3	6.6	$4.15x10^3$	$2.04x10^6$	$15x10^3$	~87% blasts	>80% blasts
Case 4	5.0	$2.26x10^3$	$1.88x10^6$	$43x10^3$	~91%blasts	>90% blasts

Table 3. analysis of the intensity of immunophenotypic results of all four cases.

Markers	Bright Positive	Moderate Positive	Dim to Moderate Positive	Dim Positive	Negative	Moderate to Bright Positive
cMPO	Case 2, 4	Case 1	-----	-----	-----	Case 3
CD13	-----	Case 4	Case 1,2,3	-----	-----	-----
CD33	-----	Case1,2,3,4	-----	-----	-----	-----
CD56	-----	-----	Case 2	-----	Case 1, 3, 4	-----
CD64	-----	Case 3	Case 1,2	-----	-----	Case 4
CD2	-----	-----	-----	Case 1	Case 2, 3, 4	-----
CD117	-----	-----	-----	Case 3	Case 1, 4, 2	-----
CD8	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD7	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD4	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD5	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD3	-----	-----	-----	-----	Case 1,2,3,4	-----
CD11b	-----	-----	Case 4	-----	Case 1, 2, 3	-----
CD14	-----	Case 4	-----	-----	Case 1, 2, 3	-----
CD15	-----	-----	Case 2	-----	Case 1, 3, 4	-----
CD38	-----	Case 3	Case 4	-----	Case 1, 2	-----
CD19	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD10	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD34	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD36	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
HLA-DR	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
cCD79a	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
cCD3	-----	-----	-----	-----	Case 1, 2, 3, 4	-----
CD20	-----	-----	-----	-----	Case 1, 2, 3, 4	-----

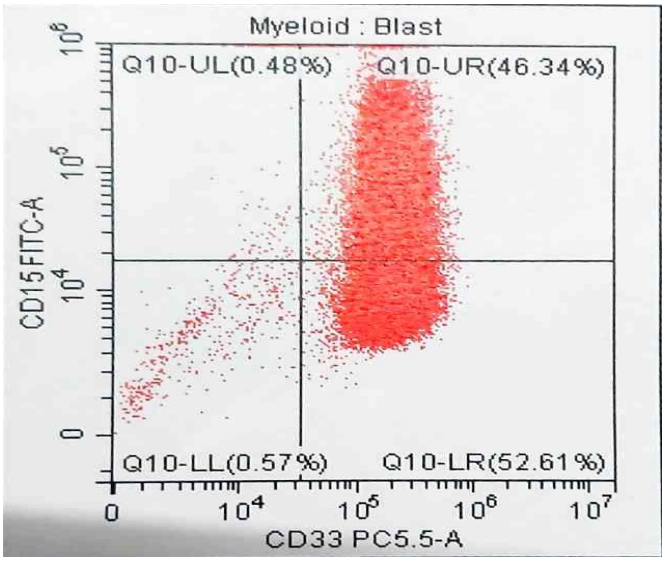

Fig. A. Blasts show moderate positive for CD33.

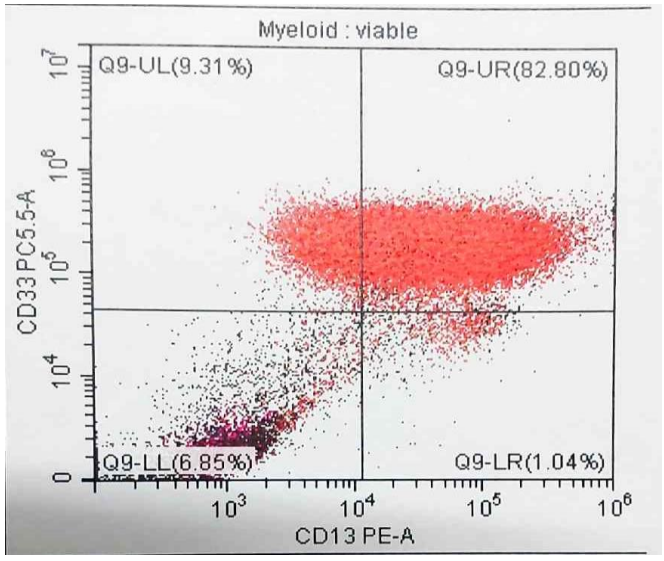

Fig. B. Blasts shows dim to moderate positive for CD13.

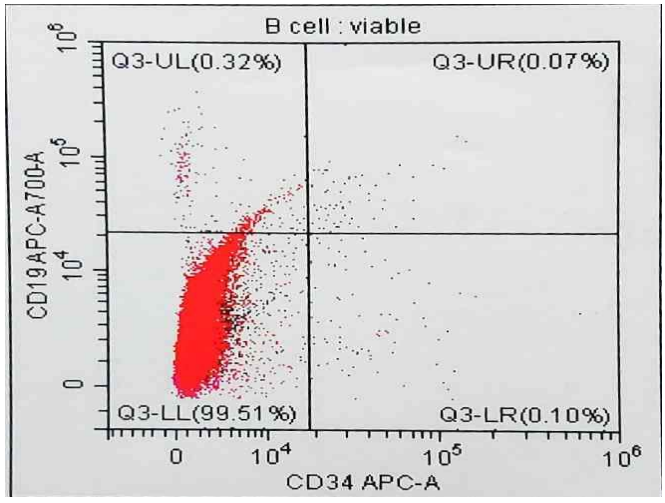

Fig. C. Blasts show negative for CD34.

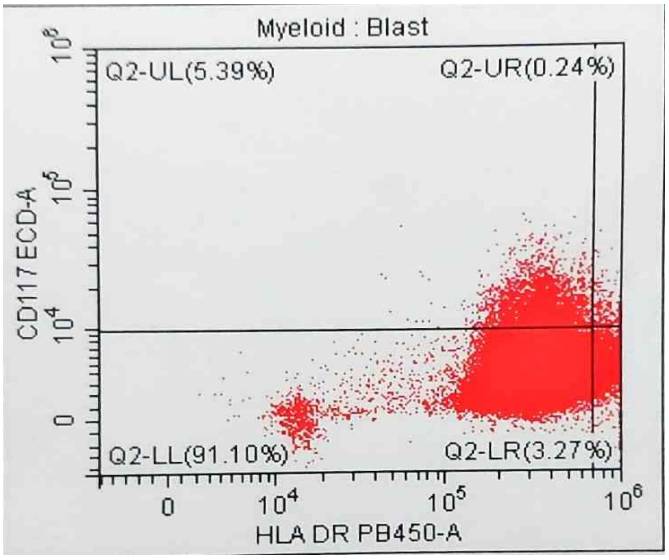

Fig D: Blasts shows negative for HLA DR.

Fig A-D. Case 2: Flowcytometry pictures.

3. Discussion

APL is illustrated with complaints of fever, headache, and bleeding tendency, and abnormal promyelocytes in peripheral blood smear and bone marrow are present. APL has undergone a remarkable transformation in its prognosis over the years. With the introduction of targeted therapies such as ATRA and arsenic trioxide, along with advances in supportive care, the survival rates for APL have significantly improved.[3] What was once a highly fatal disease has become highly curable, especially when diagnosed early and treated promptly with appropriate therapies. In a study conducted by Sravani et al., from January 2010 to June 2015, 133 cases of APL were diagnosed initially based on morphology, of which 125 cases were included.[2] A higher number of patients (80%) presented with fever. 37(29.6%) patients presented with signs and symptoms of anaemia, like generalized weakness, fatigue, and giddiness. Gastrointestinal symptoms were seen in 11 (8.8%) patients. One patient presented with lower limb pain and deep vein thrombosis. One unusual feature of gum hypertrophy was seen in four patients (3.2%). There was only one case, each with hepatomegaly and splenomegaly.[4, 5] Three patients (2.4%) were found to have cervical lymphadenopathy. 94 (75.2%) presented with moderate anaemia and 18 (14.4%) patients had severe anaemia. Only 13 patients had normal values of Hemoglobin. The majority of patients, 56(44.8%) patients, presented with leucopenia, while 44 (35.2%) patients presented with leucocytosis, and 25(20%) patients had normal leucocyte count. Thrombocytopenia: twenty-five patients had a normal range of TLC. Most of the patients, 59 (47.2%), presented with severe thrombocytopenia, 56 (44.8%) had moderate thrombocytopenia, 8(6.4%) patients had mild thrombocytopenia, and only 2 had normal platelet counts. Based on immunophenotyping, Cy MPO, CD117, CD13, and CD33 were positive in 100% of cases.[5-7]

4. Conclusion

APL is indeed a medical emergency due to its potential for rapid progression and life-threatening complications. Pathologists play a crucial role in determining the diagnosis based on the morphological evaluation of peripheral blood or bone marrow smears, especially considering characteristic features like abnormal promyelocytes with auer rods. Early suspicion and

prompt diagnosis are essential for initiating appropriate treatment, typically with ATRA and arsenic trioxide, which has drastically improved the prognosis of APL, transforming it from a highly fatal condition to one that is highly curable when treated promptly and effectively.

Conflict of Interest

The authors declared that there is no conflict of interest.

Acknowledgments

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

[1] Misawa S, Lee E, Schiffer CA, Liu Z, Testa JR. Association of the translocation (15; 17) with malignant proliferation of promyelocytes in acute leukemia and chronic myelogenous leukemia at blastic crisis. 1986;67(2):270-4. https://doi.org/10.1182/blood.V67.2.270.270.

[2] T Sravani PP, Tandon A, Uppin MS, Uppin SG, Narendra A, Gundeti Sadashivudu TR. Acute Promyelocytic Leukaemia: A Large case series from Southern India based on clinical, pathological, immunophenotypic, cytogenetic and molecular studies. National Journal of Laboratory Medicine. 2019;8(4):8-12. https://doi.org/10.7860/NJLM/2019/42483:2365.

[3] Weis K, Rambaud S, Lavau C, Jansen J, Carvalho T, Carmo-Fonseca M, et al. Retinoic acid regulates aberrant nuclear localization of PML-RARα in acute promyelocytic leukemia cells. Cell. 1994;76(2):345-56. https://doi.org/10.1016/0092-8674(94)90341-7.

[4] Sterrenberg L, Haak HL, Brommer EJ, Nieuwenhuizen W. Evidence of fibrinogen breakdown by leukocyte enzymes in a patient with acute promyelocytic leukemia. Haemostasis. 1985;15(2):126-33. https://doi.org/10.1159/000215133.

[5] RD B. Acute myeloid leukaemia with recurrent genetic abnormalities. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 2008:81-9.

[6] Akhtar K, Ahmad S, Sherwani RK. Acute promyelocytic leukemia, hypogranular variant: a rare presentation. Clinics and Practice. 2011;1(1):e11. https://doi.org/10.4081/cp.2011.e11.

[7] Nowak D, Stewart D, Koeffler HP. Differentiation therapy of leukemia: 3 decades of development. Blood, The Journal of the American Society of Hematology. 2009;113(16):3655-65. https://doi.org/10.1182/blood-2009-01-198911.

How to Cite this Article: Agarwal S, Dwivedi G, Rajak V, Rajkumari R. Flow cytometric Analysis of Acute Promyelocytic Leukemia: A Series of Four Cases with Review of Literature. International Journal of Scientific Research in Dental and Medical Sciences. 2024;6(2):94-98. https://doi.org/10.30485/IJSRDMS.2024.457050.1580.