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Case Report

Pathology Section

Paediatric Acute Promyelocytic Leukaemia: A Rare Case Report

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ABSTRACT

Acute Myeloid Leukaemia (AML) is a malignant disorder of the bone marrow in which there is maturational arrest in blood cell progenitors, resulting in the failure of normal haematopoiesis. Acute Promyelocytic Leukaemia (APML) is a subtype of AML with a defined clinical course and a biology that is distinct from other forms of AML. Morphologically, the most common form of APML shows the presence of heavily granulated cells with folded and twisted nuclei in the bone marrow. Biologically, the cytogenetic changes define the syndrome, and molecular changes in the chromosomes play a critical role in leukaemogenesis. The occurrence of APML in the paediatric population is very rare, accounting for <5%. Here, a case is presented of a four-year-old child who with fever, one episode of non projectile vomiting, and two episodes of loose watery stools. Upon further investigation, the child had immature myeloid series cells in the peripheral blood smear, which on Bone Marrow Aspiration (BMA), flow cytometry, and Fluorescent In Situ Hybridisation (FISH) confirmed a case of APML. A major manifestation of this chimeric Promyelocytic Leukaemia (PML)-Retinoic Acid Receptor Alpha (RARA) protein is a maturation block at the promyelocyte stage of myeloid differentiation, leading to the accumulation of blasts and promyelocytes. Both Fluorescence In-Situ Hybridization (FISH) and Polymerase Chain Reaction (PCR) methods can detect the fusion gene, with PCR having the advantage of detecting the three major fusion transcripts and rare submicroscopic complex translocations. Additionally, quantitative PCR can be used to monitor minimal residual disease in APML following treatment. In this case, the patient survived her first episode of disease emergence, but during her relapse, she could not survive as she developed Disseminated Intravascular Coagulation (DIC), possibly due to chemotherapeutic agents. The patient might have developed differentiation syndrome, in which there is a large and rapid release of cytokines from leukaemic cells affected by chemotherapy agents. The challenge in treating such cases is to overcome differentiation syndrome and find a new therapy options.

Keywords: Differentiation, Flow cytometry, Platelets, Promyelocytes

CASE REPORT

A four-year-old female presented with complaints of low-grade fever, breathlessness, and other constitutional symptoms such as upper respiratory tract infection and easy fatiguability since one week. Routine laboratory investigations revealed low haemoglobin, a high total leukocyte count, and severely reduced platelets [Table/Fig-1]. Further peripheral smear examination showed an increased number of immature myeloid series cells, with the maximum being promyelocytes followed by myeloblasts [Table/Fig-2]. Simultaneous clinical and radiological evaluation revealed splenomegaly in the patient. A Bone Marrow Aspiration (BMA) was performed and reported as [Table/Fig-3] with Myeloperoxidase positivity [Table/Fig-4]. The patient was advised to undergo flow cytometric analysis [Table/Fig-5], as this analysis is an essential component in diagnosing APML. The flow cytometric charts are enclosed [Table/Fig-6].

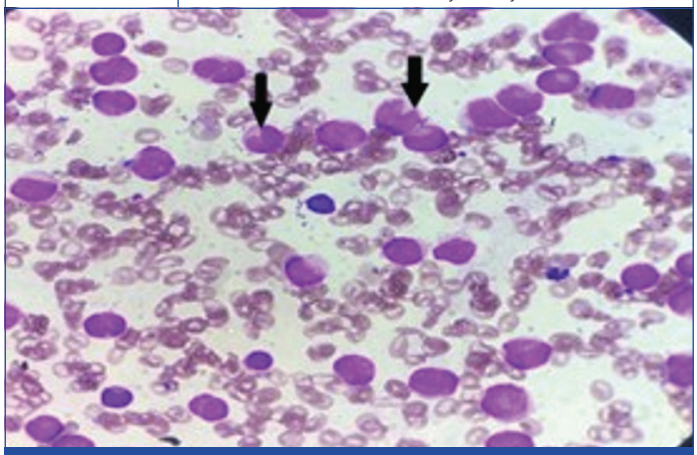
Tests	Observed value	Reference range
Red blood cell count (millions/(mm)^3)	2.58	4.5-2.0
Haemoglobin (gm/dL)	8.1	11-14
Mean corpuscular volume (fl/dL)	93	75-87
Mean corpuscular haemoglobin (pg/dL)	31	24-30
Mean corpuscular haemoconcentration (%)	32	31-37
Red cell distribution width (%)	18	11-14
Total leukocyte count (/(mm)^3)	80,000	5000-15000
Platelet count (lacs/(mm)^3)	14,000	2,00,000-4,90,000
Differential leukocyte count		
Myeloblast (%)	18	00
Promyelocyte (%)	28	00
Myelocyte (%)	19	00
Metamyelocyte (%)	16	00
Band forms (%)	05	00
Segmented neutrophil (%)	04	15-80
Lymphocyte (%)	10	60-90
Monocyte (%)	00	2-10
Eosinophil (%)	02	1-10
Basophil (%)	00	00

[Table/Fig-1]: Complete blood count.

The child received four cycles of chemotherapy along with all-trans retinoic acid, followed by which she entered the phase of remission, experiencing complete symptom-free survival for one year. After one year, the patient developed symptoms of anaemia. As a previously diagnosed case of APML-M3, she underwent further evaluation for laboratory investigations. The complete blood count [Table/Fig-7] revealed a raised total leukocyte count.

Further peripheral smear examination revealed increased immature myeloid series cells, with a higher number of promyelocytes and severely reduced platelet count [Table/Fig-8]. Due to thrombocytopenia, the patient could not undergo a Bone Marrow Aspiration (BMA) procedure. Simultaneously, she was started on chemotherapy with all-trans retinoic acid based on the peripheral smear findings, laboratory investigations, clinical examination, and her previous disease course. She was considered to be in the relapse phase of APML. During the first cycle of chemotherapy in her relapse phase, the patient presented with sudden onset pulmonary haemorrhage, easy bruising, and multiple petechiae over her extremities and dependent parts of the body. Laboratory investigations

Peripheral smear	
Red blood cells	Mild anisopoikilocytosis showing predominantly normocytic normochromic red blood cells with occasional pencil cells and few nucleated red blood cells
Total leukocyte count	The total count raised on smear with the highest percentage of promyelocytes is 28%, having bilobed/folded buttock-shaped nuclei with moderate to abundant cytoplasm and granular to sparsely granular.
Platelets	Severely reduced on smear.
Absolute platelet count	14,000 cells/ $(mm)^3$ as per cell counter.
Haemoparasite	Not seen
Opinion	Peripheral smear findings suggestive of Acute Myeloid Leukaemia AML-M3
Advice	Bone marrow studies and flow cytometry

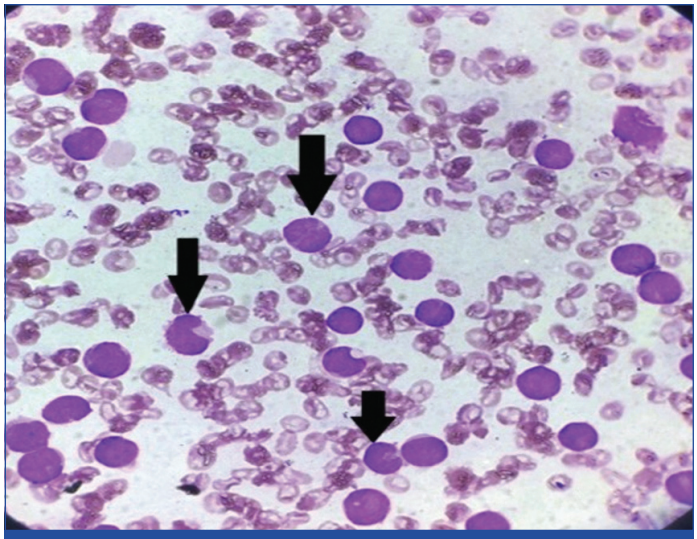


[Table/Fig-2]: Peripheral blood film demonstrates leukaemic promyelocytes having typical bilobed/folded buttock-shaped nuclei. The cytoplasm is moderate to abundant and is granular to sparsely granular (Leishman stain100x).

Bone marrow smear	Satisfactory
Cellularity	Hypercellular
Erythroid cells	Within normal limits
Myeloid cells	
Myeloblast	29%
Promyelocyte	35%
Myelocyte	05%
Metamyelocyte	05%
Band forms	14%
Segmented neutrophils	04%
Lymphocytes	07%
Monocytes	01%
Eosinophils	00%
Basophils	00%
M:E Ratio	5:1
Haemoparasite	Not seen
Bone marrow Impression	Hypercellular with evidence of Acute Myeloid Leukaemia AML-M3 with myeloperoxidase positivity.
Advice	Flow cytometric analysis FISH analysis

[Table/Fig-3]: Bone Marrow Aspirate (BMA) findings.

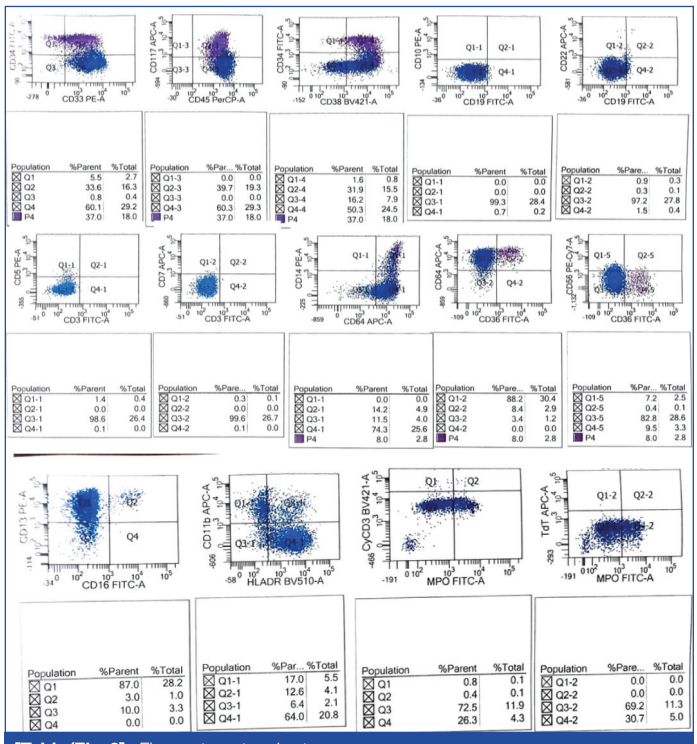
showed a sudden fall in platelets. Despite being managed with repeated transfusions of random donor platelets and single donor platelets within acceptable limits for her age and body surface area, her condition worsened, leading to Disseminated Intravascular Coagulation (DIC). Unfortunately, she could not be saved. The clinical scenario was further correlated with her laboratory investigation results, showing serially declining platelets followed by a sudden fall (when she developed DIC), a deranged coagulation profile, and raised D-dimers levels. She could not withstand the chemotherapy due to bleeding manifestations and succumbed to death, which is



[Table/Fig-4]: Bone Marrow Aspirate (BMA) demonstrates cells with moderate to abundant dense granular cytoplasm. The nuclei are oval to reniform (kidney-shaped), often bilobated, and eccentric (Plasmacytoid appearance). The nuclear chromatin is fine with prominent nuclei. (myeloperoxidase stain, 100x)

CD13	Positive
CD33	Heterogenous intensity
CD117	Moderately positive
CD34	Moderately positive
HLA-DR	Low expression
CD16	Negative

[Table/Fig-5]: Positive markers for promyelocytic expression.



[Table/Fig-6]: Flow cytometry charts.

one of the most dreaded complications in APML (Therapy-induced-a new challenge in treating paediatric cases of APML).

In this case, the rare finding was that APML was found in a four-year-old female, with the diagnosis confirmed through BMA, flow cytometry [Table/Fig-5], and FISH [Table/Fig-9].

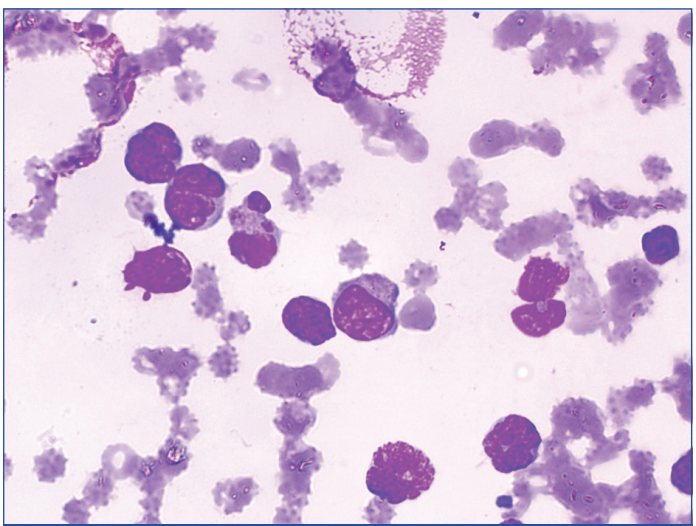
DISCUSSION

AML-M3 (APML), for the first time in the literature, was identified as a distinct type of AML. With a declining course, it leads to severe bleeding. The incidence rate of APML is 5-10% in newly diagnosed AML cases, originating from a balanced translocation t(15;17)

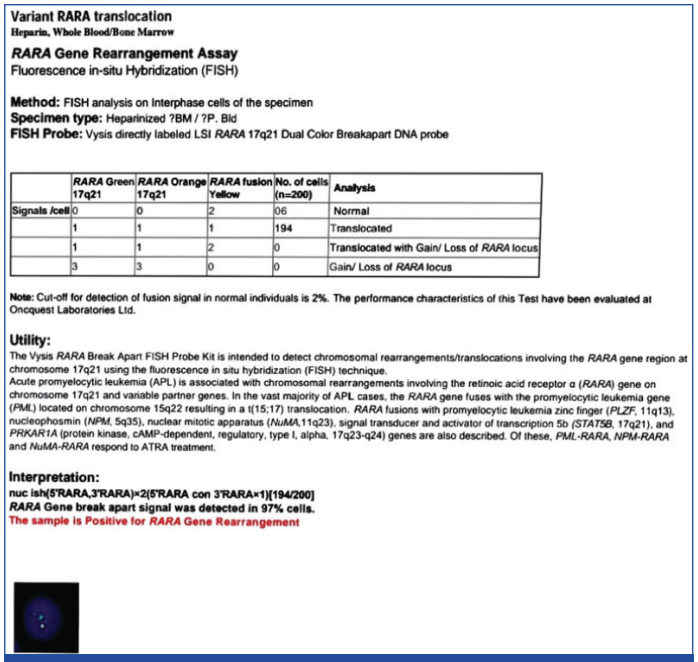
(q22.1;q12.21), accelerating the fusion process of the PML gene with the RARA gene, by clogging normal myeloid differentiation. The resulting PML-RARA fusion oncoprotein induces leukaemia

Tests	Observed value	Reference range
Red blood cell count (millions/(mm)^3)	3.57	4.5-2.0
Haemoglobin (gm/dL)	9.9	11-14
Mean corpuscular volume (fl/dL)	82	75-87
Mean corpuscular haemoglobin (pg/dL)	28	24-30
Mean corpuscular haemoconcentration (%)	34	31-37
Red cell distribution width (%)	18	11-14
Total leukocyte count (/(mm)^3)	96,500	5000-15000
Platelet count (lacs/(mm)^3)	10,000	2,00,000-4,90,000
Differential leukocyte count		
Myeloblast (%)	24	00
Promyelocyte (%)	46	00
Myelocyte (%)	10	00
Metamyelocyte (%)	05	00
Band forms (%)	05	00
Segmented neutrophil (%)	06	15-80
Lymphocyte (%)	03	60-90
Monocyte (%)	00	2-10
Eosinophil (%)	01	1-10
Basophil (%)	00	00
Peripheral smear:		
Red blood cells	Mild anisopoikilocytosis showing predominantly normocytic normochromic red blood cells with occasional pencil cells.	
Total leukocyte count	Total count raised on smear.	
Platelets	Severely reduced on smear.	
Absolute platelet count	10,000 cells/ (mm)^3 as per cell counter	
Haemoparasite	Not seen	
Opinion	Peripheral smear findings suggestive of relapse case of Acute Myeloid Leukaemia {AML-M3}	
Advice	1. Bone marrow studies	
	2. Flow cytometry	
	3. FISH	

[Table/Fig-7]: Complete blood count after relapse.



[Table/Fig-8]: Peripheral blood film demonstrating increased number of promyelocytes having characteristic bilobed/folded buttock shaped nuclei with moderate cytoplasm with hypogranularity in promyelocytes (Leishman stain, 100x).



Variant RARA translocation
Heparin, Whole Blood/Bone Marrow

***RARA* Gene Rearrangement Assay**
Fluorescence in-situ Hybridization (FISH)

Method: FISH analysis on Interphase cells of the specimen
Specimen type: Heparinized ?BM / ?P. Bld
FISH Probe: Vysis directly labeled LSI *RARA* 17q21 Dual Color Breakapart DNA probe

	RARA Green 17q21	*RARA* Orange 17q21	*RARA* fusion Yellow	No. of cells (n=200)	Analysis
Signals /cell	0	0	2	06	Normal
	1	1	1	194	Translocated
	1	1	2	0	Translocated with Gain/ Loss of *RARA* locus
	3	3	0	0	Gain/ Loss of *RARA* locus

Note: Cut-off for detection of fusion signal in normal individuals is 2%. The performance characteristics of this Test have been evaluated at Oncquest Laboratories Ltd.

Utility:
The Vysis *RARA* Break Apart FISH Probe Kit is intended to detect chromosomal rearrangements/translocations involving the *RARA* gene region at chromosome 17q21 using the fluorescence in situ hybridization (FISH) technique.
Acute promyelocytic leukemia (APL) is associated with chromosomal rearrangements involving the retinoic acid receptor α (*RARA*) gene on chromosome 17q21 and variable partner genes. In the vast majority of APL cases, the *RARA* gene fuses with the promyelocytic leukemia gene (*PML*) located on chromosome 15q22 resulting in a t(15;17) translocation. *RARA* fusions with promyelocytic leukemia zinc finger (*PLZF*, 11q13), nucleophosmin (*NPM*, 5q35), nuclear mitotic apparatus (*NuMA*,11q23), signal transducer and activator of transcription 5b (*STAT5B*, 17q21), and *PRKAR1A* (protein kinase, cAMP-dependent, regulatory, type I, alpha, 17q23-q24) genes are also described. Of these, *PML-RARA*, *NPM-RARA* and *NuMA-RARA* respond to ATRA treatment.

Interpretation:
nuc ish(5'RARA,3'RARA)×2(5'RARA con 3'RARA×1)[194/200]
***RARA* Gene break apart signal was detected in 97% cells.**
The sample is Positive for *RARA* Gene Rearrangement

[Table/Fig-9]: FISH report suggesting positivity for RARA (Retinoic Acid receptor Alfa) gene rearrangements.

[1]. Describing the myeloperoxidase-positive blast cells within the blast cytoplasm, there is a common presence of linear azurophilic granules and sometimes Auer rods, although this finding could be found in other AML subtypes [2]. The level of PML-RARA transcript at the end of transcription therapy holds high predictive value for relapse [3]. The laboratory features of APML are associated with a hyperfibrinolytic state leading to bleeding as the clinical manifestation, often resulting in DIC. DIC is more common in the hypergranular promyelocytic subtype, accounting for 60% to 100% of all APML cases in adults. Just 5-10% of paediatric APML cases are caused by translocation t(15;17)(q24.1;q21.2), with its incidence rising with advancing age [4]. The coagulopathy in APML has a multifactorial cause; tissue factor is present in cytoplasmic granules of promyelocytes alongside leukocyte proteases and elastase, acting as procoagulants that stimulate clotting factor consumption and DIC development. Enhanced fibrinolysis results from increased promyelocyte expression of Annexin II, a receptor for plasminogen and tissue plasminogen activator, with malignant promyelocytes containing plasminogen activator. In some cases, acute fulminant DIC is triggered by the administration of thromboplastin contents from promyelocytes [5]. Depletion of alpha-2 antiplasmin (which normally has three-fold function including plasmin proteolysis, inhibition of plasminogen binding to fibrin, and cross-linking fibrin) occurs during chemotherapy induction, a finding that is more predictive of bleeding complications due to unregulated fibrinolysis, leading to the destruction of functional haemostatic plugs and depletion of fibrinolysis. Immunophenotyping of APML cells demonstrates positivity for CD117, CD13, CD33, and myeloperoxidase with a high side scatter.

The balanced translocation t(15;17)(q24.1;q21.2) serves as the cytogenetic hallmark of APML and is the most common mutation driving APML development, described in 95% of APML cases [1]. This translocation involves the fusion of the PML gene on chromosome 15 with the RARA gene on chromosome 17, leading to the PML-RARA fusion gene and the PML-RAR protein [6]. The mechanism through which PML-RARA leads to APML development has been extensively described over several decades. PML-RAR retains the ability of RAR to bind retinoic acid-responsive elements and dimerise with the retinoid X receptor protein but inhibits the normal gene transcription regulated by these elements, ultimately leading to the suppression of RAR target genes and a blockage in differentiation at the promyelocyte stage [2]. In this translocation, the PML gene on chromosome 15 joins with the RARA gene on

chromosome 17, leading to formation of the PML-RAR alpha fusion gene and the development of the PML-RAR alpha protein. This translocation blocks the differentiation of the myeloid series at the promyelocytic stage. By administering all-trans-retinoic acid in combination with chemotherapy, this differentiation is promoted towards the terminal stage of myeloid series cells.

CONCLUSION(S)

APML typically occurs in individuals between the second and fifth decades of life, which translates to the age range of 22 to 44 years. This case presents a rare occurrence of a four-year-old who developed DIC during her relapse. As previously discussed, the pathogenesis of APML highlights that the balanced translocation t(15;17)(q24.1;q21.2) serves as the cytogenetic hallmark of APML. All Trans Retinoic Acid (ATRA) functions by relocating PML, restoring the normal structure of nuclear bodies, and degradation of PML-RAR alpha protein through caspase-mediated cleavage and proteasome degradation. Additionally, under therapeutic concentrations of ATRA, PML-RAR alpha is converted from a transcription corepressor to a transcription activator. In this particular case, the patient survived the initial emergence of the disease but unfortunately succumbed during the relapse, likely due to DIC. The proposed cause of DIC could be attributed to the chemotherapeutic agents administered to the patient. It is possible that the patient developed differentiation syndrome, in which there is large and rapid release of cytokines from leukaemic cells affected by chemotherapy agents. The challenge for the treatment of such cases is to overcome the differentiation syndrome and find a new therapy for these patients.

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