CASE REPORT

Morphological Diagnosis and Immunophenotypic Characterization of Onco-hematologic Malignancy (Acute Myelogenous Leukemia-M3 and M4): Two Pediatric Case Reports and Literature Review

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ABSTRACT

Background: Acute myelogenous leukemia (AML) is an ensemble of malignant myeloid precursor bone marrow neoplasm. Acute promyelocytic leukemia (APL-M3) and acute myelomonocytic leukemia (AML-M4) are common sorts of pediatric AML. Even though these onco-hematologic malignancies are uncommon and sound for about 15% of childhood and adolescent leukemia. **Case Report:** We have reported two Sudanese female patients, a 9-years-old girl with APL-M3 and an 11-years-old with AML-M4. Interestingly, the patient with APL manifested bruises and coagulation disorders. Autoimmune hemolytic anemia (AIHA) and tonsillitis were associated with the AML-M4 patient. **Conclusion:** It is pertinent to conclude that the combined interpreted morphology and immunophenotyping are fundamental tools in the determination of acute leukemias. APL represents an emergency and the planning of diagnosis and executive management ought to be very short. AIHA is one of the possible causes of anemia in AML-M4 cases, if a blood transfusion is ordered, ought to be given consciously in such cases to remain away from unsafe hemolysis.

Key words: Acute myeloid leukemia, acute non-lymphoblastic leukemia, acute myelogenous leukemia-M4, APL, Sudan

INTRODUCTION

Acute myelogenous leukemia (AML) is the most common family of leukemia. It is recognized as a clonal hemopoietic stem cell disorder and proclaimed by repression of differentiation resulting in the gathering of cells at various stages of deficient maturation. Furthermore, diminished production of mature hemopoietic ingredient occurs.[1] AML is rare in children and adolescents, but most common in adults. It comprises 80% of acute leukemias in adults and 15–20% in children.[2] Acute promyelocytic leukemia (APL) and acute myelomonocytic leukemia are prevalent types of pediatric AML. The frequency of APL among pediatric AML is less than 10%. APL is a disease characterized by a discrimination block at the promyelocytic stage. These unusual promyelocytes are viewed as practically identical to blasts for the goal of diagnosis. APL is linked to specific chromosomal abnormalities (15;17) translocation, this translocation is appropriate for diagnosis instead of blast count.[3] AML-M4 is uncommon and appears approximately 3% of pediatric AML. It is realized by >20% (WHO classification) or >30% (French-American-British [FAB] classification) of myeloid and monocytoid cells in the peripheral blood and bone marrow.[4] Both APL-M3 and AML-M4 may be associated with coagulation abnormalities.[3,5] Diagnostic techniques for both leukemias incorporate immature cell count, morphology, cytochemistry, immunophenotyping, cytogenetics, and histochemistry in connection with clinical highlights. All these diagnostic techniques are integral. Cell morphology remains the fundamental indicative tool to survey the number and morphology of blast cells. Immunophenotyping by flow cytometry is a strength

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adjuvant tool in portraying cell surface and cytoplasmic markers in AML-M3 and AML-M4. The exhibition of characteristic myeloid descent markers CD34, CD13, CD33, CD117, CD11b, CD11c, CD14, and human leukocyte antigen–D-related (HLA-DR) permits the distinction of both leukemias to form other sorts of AML.[6]

CASE REPORT

Case 1

A 9-years-old Sudanese girl presented with fever, generalized weakness, easy fatigability, extensive bruising, petechiae located in upper and lower limbs, and occasional epistaxis. She was in a generally unsatisfactory condition. She had not had hepatosplenomegaly and lymphadenopathy. The vital signs were seen normal, heart rate 132/bm, blood pressure 105/73 mmHg, and oxygen saturation 96%. The complete blood count revealed slight normocytic normochromic anemia, marked leukocytosis, and extreme thrombocytopenia. The peripheral blood film indicated that 88% of leukocytes are neoplastic promyelocytes (faggot cells) [Figure 1]. Her coagulogram manifested prothrombin time 20 s, control 13.5 s, international normalization ratio 1.4, partial thromboplastin time 43 s, control 25–40 s, fibrinogen was normal, and elevated d-dimer [Table 1].

Bone marrow aspiration indicated hypercellular marrow appearing propagation of blasts. The blasts were medium to huge in size with high N:C proportion, moderate quantities of cytoplasm, fine nuclear chromatin, and 1–2 noticeable nucleoli. A few of the blasts were binucleated. Megakaryocytes were slightly reduced in number, but typical in morphology, erythroid-myeloid ratio diminish, and some mitotic shapes are frequently detected. Cytochemical myeloperoxidase and Sudan Black B were positive and periodic acid-Schiff was negative. The flow cytometry examination for bone marrow aspiration demonstrated blasts/immature cells positive for CD45, CD13, CD33, and CD117 in the blasts, and negative for CD11b, CD34, and HLA-DR as well as increased side scattered [Figure 2 and Table 1]. The final determination in view of morphological and immunophenotypic findings was indicated acute promyelocytic leukemia (APL), FAB-M3 hypergranular was resolved. Unfortunately, the patient died before therapy initiation.

Case 2

An 11-years-old Sudanese girl presented with a history of marked fatigue, fever, and severe pain in the throat. There was no other contributory history. She did not take explicit prescriptions or received blood items before investigation. On examination, she had a high-grade fever and congested both tonsils. The soft palate was also inflamed. The vital signs reveal heart rate 126/bm, blood pressure 100/70, body mass index 18.6, and O_2 saturation 99%. She had no previous clinical proof of infection. A throat swab for culture and sensitivity was ordered and *Streptococcus* sensitive to Amoxyclav was reported. Complete blood check at presentation demonstrated normocytic normochromic anemia, elevated leukocyte count, and severe thrombocytopenia. The peripheral blood smear indicated some spherocytes. The differential leukocyte count exhibited 40.3% blasts and 35% monocytic series [Figure 3]. Cytochemical periodic acid-Schiff was negative, myeloperoxidase and non-specific esterase were positive. The presentation of spherocytes is evidence of autoimmune; therefore, a direct anti-human globulin test was obtained positive. The sort of antibody could be recognized as immunoglobulin G with monospecific antibodies. The reticulocyte check was just 0.7%. Nonappearance of reticulocytosis was clarified by bone marrow invasion by neoplastic blast cells. Virology screening for hepatitis C virus, hepatitis B virus, and HIV was negative. The finding of acute myelomonocytic leukemia M4 was affirmed by immunophenotyping on bone marrow aspirate which uncovered 45% myeloblast population positive for CD34, CD13, CD33, CD45, CD117, and HLA-DR and the monocytoid series were positive for CD14, CD64, CD11c, CD11b, and HLA-DR [Figure 4 and Table 2].

The patient began dexamethasone 10 mg each 12 h with continuous improvement of hemoglobin level as it arrived at 10.3 g/dl when the chemotherapy began once more. The patient finished the primary cycle and begun steroid withdrawal with a stable hemoglobin level. The hemoglobin dropped again consequently to chemotherapy triggered myelosuppression. The assessment of the primary pattern of chemotherapy with bone marrow aspiration appeared complete recovery. The Coombs' test was remained positive after the first dose and reported negative after the second

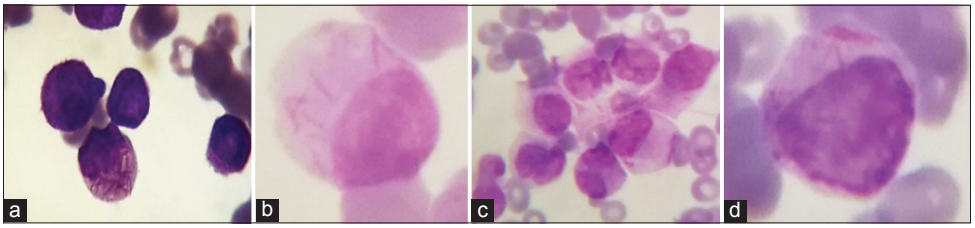

Figure 1: Peripheral blood picture; (a) Faggot cell and blast with blebs; (b and d) blast cells with many Auer rods; (c) Blast with intracytoplasmic microgranules, Auer rod, and internuclear bridge

Table 1: Laboratory findings for patient with AML-M3

Test	Patient result	Reference interval	Interpretation
Hematology			
WBC	36.20	4.0–10.0 × 10^9/l	Critical
RBC	3.33	3.8–5.5 × 10^{12}/l	L
Hemoglobin	10.3	12–15 g/dl	L
Hematocrit	27	35–45%	L
MCV	81	78–98 fl	N
MCH	31	26–33	N
MCHC	38	30–36	N
RDW	13	11.5–15%	N
Platelet count	21	150–400 × 10^9/l	L
MPV	10.3	9–13 fl	N
PDW	17.1	13–17 fl	Critical
P-LCR	24.6	13–43%	N
Absolute differential counts			
Neutrophils	1.2	1.5-6.0 × 10^9/l	L
Lymphocytes	0.4	6.0-8.3 × 10^9/l	L
Monocytes	0	0.1-1.3 × 10^9/l	L
Eosinophils	0	0-0.5 × 10^9/l	N
Blasts	1.0	0 × 10^9/l	Critical
Promyelocytes	31.9	0 × 10^9/l	Critical
Myelocytes	0.7	0 × 10^9/l	Critical
Metamyelocytes	1.0	0 × 10^9/l	Critical
Frequency differential counts			
Band neutrophils	0	0–5%	N
Segmented neutrophils	3	20–40%	L
Lymphocytes	1	40–70%	L
Atypical lymphocytes	0	0–6%	N
Monocytes	0	0–10%	N
Eosinophils	0	0–6%	N
Basophils	0	0–1%	N
Metamyelocytes	3	0%	Critical
Myelocytes	2	0%	Critical
Promyelocytes	88	0%	Critical
Blasts	3	0%	Critical
Normoblasts	2	100	N
Coagulation			
PT	20	11–16 s	H
INR	1.4		
PTT	43	25–40 s	H
Fibrinogen	3.63	2–4 g/dl	N
D-Dimer	7.0	< 0.3 mg/l	Critical

(*Contd...*)

Table 1:(*Continued*)

Test	Patient result	Reference interval	Interpretation
Immunophenotyping			
Variables	**Approximately positive (%)**		**Interpretation**
CD45	85.0		Positive
CD13	55.4		Positive
CD33	78.5		Positive
CD117	82.0		Positive
CD34	11.5		Negative
HLA-DR	16.25		Negative
CD11b	22.1		Negative

WBC: White blood cells, L: Low, RBC: Red blood cells, MCV: Mean corpuscular volume, N: Normal, MCH: Mean corpuscular hemoglobin, MCHC: Mean cell hemoglobin concentration, RDW: Red blood cell distribution width, H: High, MPV: Mean platelet volume, PT: Prothrombin time, INR: International Normalized Ratio, PTT: Partial thromboplastin time, CD: Cluster of differentiation, HLA-DR: Human leukocyte antigen–D-related. AML: Acute myelogenous leukemia

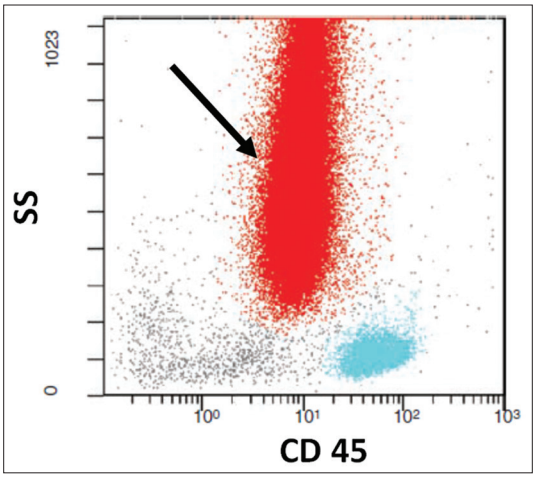

Figure 2: Scattergram of CD45 versus side scatter (SS) indicate the granularity of leukemic cells (black arrow)

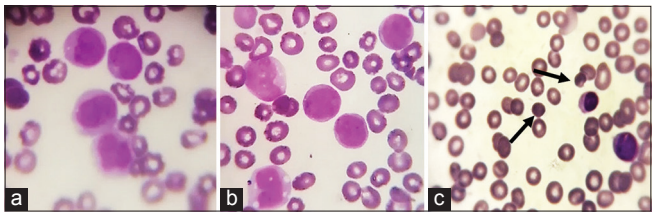

Figure 3: Blood smear; (a) monocytic series (monoblast, promonocytes, and monocyte); (b) blasts with prominent nucleoli and monocytic series; (c) spherocytes noted in a different area of the blood film (arrow)

short course of dexamethasone. At present, the patient is steady in remission with acceptable hemoglobin levels with no proof of hemolysis.

Regrettably, clonal chromosomal variations could not be investigated in either patient because short of facilities.

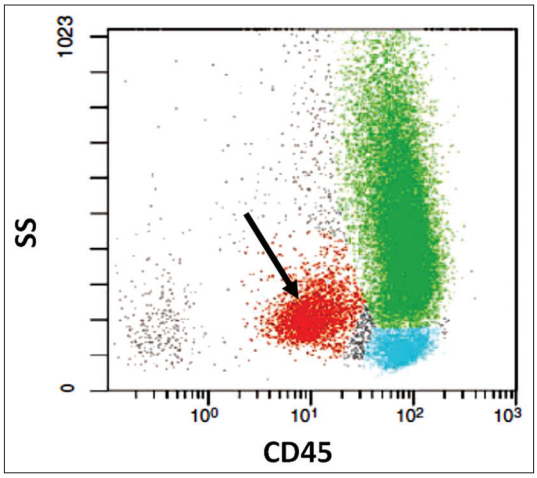

Figure 4: Scattergram of CD45 versus side scatter (SS) indicates the myeloid population in (black arrow) and monocytic series in (green)

DISCUSSION

AML is an onco-hematologic neoplastic disease characterized by the proliferation of myeloid cell antecedents with or without maturation. The underlying symptoms of AML are connected to anemia, neutropenia, and thrombocytopenia, which promote due to bone marrow infiltration of neoplastic blasts.[7] AML can detect at any age category, but the incidence increases with age.[2] The AML in childhood comprised 20% up to 27% compared to adult AML comprised of 76% up to 86%.[8] For over 20 years, the FAB order for intense leukemia has been a significant framework of classification. This order empowered the diagnosis of an assortment of morphologic and cytochemical subtypes of intense leukemia through an organized rule. Morphological studies alongside with cytochemical stains consolidated the diagnosis of over

Table 2: Laboratory outcomes for a patient with AML-M4

Test	Patient result	Reference interval	Interpretation
Hematology			
WBC	84.0	4.0–10.0 × 10^9/l	Critical
RBC	2.44	3.8–5.5 × 10^{12}/l	L
Hemoglobin	9.6	12–15 g/dl	L
Hematocrit	24	35–45%	L
MCV	99	78–98 fl	N
MCH	40	26–33	N
MCHC	39	30–36	H
RDW	16.4	11.5–15%	H
Platelet count	42	150–400 × 10^9/l	L
MPV	10.3	9–13 fl	N
PDW	17.1	13–17 fl	H
P-LCR	24.6	13–43%	N
Absolute differential counts			
Neutrophils	1	1.5–6.0 × 10^9/l	L
Lymphocytes	6.8	6.0–8.3 × 10^9/l	N
Monocytes	10	0.1–1.3 × 10^9/l	H
Eosinophils	0.7	0–0.5 × 10^9/l	H
Blasts	40.3	0 × 10^9/l	Critical
Promonocytes	25.2	0 × 10^9/l	Critical
Myelocytes	0	0 × 10^9/l	Critical
Metamyelocytes	0	0 × 10^9/l	Critical
Frequency differential counts			
Band neutrophils	0	0–5%	N
Segmented neutrophils	1.2	20–40%	L
Lymphocytes	8	40–70%	L
Atypical lymphocytes	0	0–6%	N
Monocytes	12	0–10%	N
Eosinophils	0.8	0–6%	N
Basophils	0	0–1%	N
Metamyelocytes	0	0%	Critical
Myelocytes	0	0%	Critical
Promonocytes	30	0%	Critical
Blasts	48	0%	Critical
Normoblasts	0	100	N
Coagulation			
PT	15.5	11–16 s	N
INR	1.0		
PTT	36	25–40 s	N
Fibrinogen	2.9	2–4 g/dl	N
D-Dimer	0.2	<0.3 mg/l	N

(*Contd...*)

Table 2: (*Continued*)

Test	Patient result	Reference interval	Interpretation
Immunophenotyping			
Variables	**Approximately positive (%)**		**Interpretation**
CD45	62.0		Positive
CD13	70.4		Positive
CD33	87.6		Positive
CD117	82.0		Positive
CD34	49.5		Positive
HLA-DR	75.8		Positive
CH11c	76.4		Positive
CD11b	58.0		Positive
CD14	94.0		Positive
CD64	79.1		Positive

WBC: White blood cells, L: Low, RBC: Red blood cells, MCV: Mean corpuscular volume, N: Normal, MCH: Mean corpuscular hemoglobin, MCHC: Mean cell hemoglobin concentration, RDW: Red blood cell distribution width, H: High, MPV: Mean platelet volume, PT: Prothrombin time, INR: International Normalized Ratio, PTT: Partial thromboplastin time, CD: Cluster of differentiation, HLA-DR: Human leukocyte antigen–D-related. AML: Acute myelogenous leukemia

80% of AML cases.[9] To the best of our knowledge, pediatric AML has been reported in the literature.

Both of our patients fulfilled the criteria of the presence of 40% blasts in the peripheral blood smear in two cases. In both of our patients, practically the entirety of the cell could be recognized as blast cell, which made up over half of the nucleated marrow cells. We herein, reported both of the cases as FAB M3 and M4 subtypes, as there was more than 40% differentiation in the myeloid lineage as well as the erythroid antecedents were scant.

In the APL-M3, the blast demonstrated abnormal intracytoplasmic granules, the nuclei are bilobed or have a monocytoid view. APL with t(15;17) (q22;q12) is an AML in which abnormal promyelocytes predominate. Both hypergranular ("typical" APL) and hypogranular (microgranular) presentations can be observed. Three other morphological subcategories of APL-M3 were accounted for: APL-M3 with basophilic morphology, APL-M3 with M2-like blast morphology, and APL-M3 with M1-like blast morphology (early promyelocytic).[3] Morphologically, most pediatrics APL saw as hypergranular APL, along with significant coagulopathy with serious bleeding episodes that enhanced outcomes.[10] In case 1, the coagulopathy findings, morphological diagnosis, and cytochemical were clearly observed the AML-M3. Immunophenotypically, the triple-negative of (CD34-, HLA-DR-, CD11b-) alongside with positive (CD33+, CD13+, CD117+) was proof of the diagnosis of APL-M3.

Acute myelomonocytic leukemia represents 5–10% of the entirety of AML. It is characterized by a significantly augmented leukocyte along with the presence of myeloid and monocytoid series in peripheral blood and/or bone marrow.[11] Monocytic series (monoblasts and promonocytes) comprise at least 20% of all marrow blood cells. The monoblasts are vast with plentiful cytoplasm containing few granules and pseudopodia. The nucleus is vast and immature and may have multiple nucleoli. Promonocytes also are present and may have contorted nuclei resembling (embryo shape). Acute myelomonocytic leukemia with abnormal eosinophil (AMML Eo) has also been recognized in the medical literature.[11] In case 2, present a patient of *de novo* AML-M4 associated with autoimmune hemolytic anemia (AIHA). The case is managed with antibiotics, steroids, and chemotherapy. The patient highlighted remarkable improvement for anemia after the steroid doses with declines in all clinical and laboratory findings of hemolysis when the patient went into abatement. A similar picture of AML-M4 with autoantibodies against erythrocytes with positive anti-human globulin test with the clue of hemolysis has been reported by Essa *et al.*[12] The method of advancement of AIHA in *de novo* AML-M4 is muddled. A few reports hypothesize that an immune response was delivered against a tumor antigen expressed on malignant erythroblast derived erythrocytes. Nonetheless, this was precluded in some cases because antibodies were likewise directed toward the intact erythrocytes. Another clarification might be identified with the advancement of immunologic regulatory cells as suppressor T cells, which permitted the advancement of autoimmunity.[12] Eventually, morphological performance, positive myeloperoxidase, and alpha naphthyl acetate (non-specific esterase) indicated for AML-M4 and confirmed by positive immunophenotyping CD34, CD13, CD33, CD45, CD117, and HLA-DR for myeloid series and CD14, CD64, CD11c, CD11b, and HLA-DR for monocytoid series.

The limitation of this report is that molecular analysis and cytogenetics were not used parallel with the cases.

CONCLUSION

Our discoveries propose integral utilization of immunophenotyping with morphology improves outcomes in the determination of acute leukemias. Further examination with the utilization of the expanded panel of monoclonal antibodies is suggested. Moreover, the advancement of reciprocal diagnostic methods such as cytogenetics and molecular studies ought to be produced for the additional exact conclusion of AML and its subtypes. APL represents an emergency and the planning of diagnosis and executive management ought to be very short. AIHA is one of the possible causes of anemia in AML-M4 cases, if a blood transfusion is ordered, ought to be given consciously in such cases to remain away from unsafe hemolysis.

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