

Challenging Cases in Hematology
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Objectives

Correlate peripheral blood smear findings with hematologic data
Identify diagnostic criteria used in characterizing leukemias, myelodysplastic disorders and anemias
Discuss the FAB and WHO criteria for classification of leukemia

Case 1

HISTORY

A 6 year old male is admitted to the hospital for a work up for juvenile - on - set diabetes mellitus.

He also was being treated for a macrocytic anemia.

Laboratory Results

WBC 2.9
RBC 2.53
HGB 10.2
HCT 29.3
MCV 116.0
MCH 40.6
MCHC 34.2
RDW 18.1
PLT 76,000
MPV 6.9
Retic 0.4%

Additional Laboratory Tests

Bone Marrow:
Decreased M:E ratio
Erythroid Hyperplasia and Dysplasia
3% Blasts

Evaluation of Anemia

Red Cell Count
Hemoglobin and Hematocrit
Reticulocyte Count
Red Cell Indices
MCV: HCT/RBC (microcytic, macrocytic)
MCH: HGB/RBC
MCHC: HGB/HCT (hypochromic)
RDW: SD/Mean (anisocytosis)

Causes of Anemia

Acute or chronic blood loss
Nutritional deficiency

Increased RBC destruction
Hematopoietic stem cell damage
Acquired or hereditary factors
Bone marrow replacement by malignant cells
Infection or toxicity

Classification of Anemia

Morphologic

Size and hemoglobin concentration of the red blood cells

MCV

MCHC

Pathophysiologic

Impaired Production

Survival

Synthesis

Megaloblastic Anemia (Macrocytic)

Result of abnormal DNA synthesis. They are named after the giant abnormal erythroid precursor cell found in the bone marrow

TYPES:

Vitamin B12

Folic Acid

Pernicious Anemia : secondary to an absence of an intrinsic factor necessary for absorption of B12

Megaloblastic Anemia

Lab Findings

Macrocytic- Normochromic Anemia

Neutropenia with hypersegmentation

MCH increased

Anis and Poik with Oval Macrocytes

Howell Jolly Bodies

Thrombocytopenia with giant platelets

Bone Marrow: hypercellular with increased giant erythroid precursors.

Myelodysplastic Disorders MDS

Dysplastic changes in one or more cell lines

Can occur as a primary or secondary

< 30% Blasts in the Bone Marrow (FAB)

< 20% Blasts in the Bone Marrow (WHO)

Peripheral blood cytopeinas

Macrocytic Anemia

Thrombocytopenia with giant forms

Can progress to acute leukemia

Presenting Symptoms

Bone marrow failure

Fatigue due to the anemia

Anemia does not respond to therapy

Increased infection due to decreased white count

Easy bruising and bleeding due to decreased platelet count

FAB Classification of 1982

Refractory Anemia

RA

Refractory Anemia with Ringed Sideroblast

RARS

Refractory Anemia with excess Blasts

RAEB

Refractory Anemia in Transformation

RAEB-t

Chronic Myelomonocytic

CMML

WHO Classification 2001

de novo Myelodysplastic Syndromes

Refractory Anemia

Refractory Anemia with Ringed Sideroblasts (RARS)

RARS with unilineage dysplasia

RARS with multilineage dysplasia

Refractory cytopenia with multilineage dysplasia (RCMD)

Refractory anemia with excess blasts (RAEB)

Therapy related MDS (TR-MDS)

WHO Classification 2008

Refractory cytopenias with unilineage dysplasia (RCUD)

Refractory Anemia with Ringed Sideroblasts (RARS)

Refractory Cytopenia with Multileage Dysplasia (RCMD)

Refractory Anemia with Excess Blasts -1 (RAEB-1)

Refractory Anemia with Excess Blasts -2 (RAEB-2)

Myelodysplastic Syndrome – unclassified (MDS-U)

MDS associated with isolated del(5q)

Refractory Anemia

Least severe

Ineffective Erythropoiesis

Hypercellular marrow with mild dyserythropoiesis

Less than 5% blasts in the marrow

Oval macrocytes

Cytopenias

15% transform into AML

Patient Outcome

Best prognosis who have

a normal karyotype

No neutropenia

No thrombocytopenia

Median length of survival 70 months

Management

Supportive therapy

Chemotherapy

Bone Marrow Transplant

Case 2

HISTORY

A 44 year old male was first seen in the VA hospital and then transferred to University for re-evaluation. He was not responding to treatment that was prescribed.

Laboratory Results

WBC 122.2
RBC 2.48
HGB 8.2
HCT 26.0
MCV 90.7
MCH 32.9
MCHC 36.3
RDW 14.3
PLT 27,000
MPV 9.0

Additional Laboratory Results

Fibrinogen 67 mg/dl
PT 19.3 sec INR 2.0
aPTT 39.5 sec
FSP >40 ug/dl
D-Dimer 4250 NG/ML

Flow Cytometry

CD Markers

Cluster of Differentiation

A system of nomenclature developed in order to label membrane proteins or complexes of proteins in which physical properties or interactions with monoclonal antibodies are known

CD designations are most commonly seen on hematopoietic cells

Flow Cytometry

Yields information regarding the biophysical and biochemical nature of a cell

Analysis is by light scatter and fluorescence dyes that have been tagged with monoclonal antibodies

Light Scatter-Dot Plot

SSC vs FS

Granulocytes

Monocytes

Lymphocytes,

nucleated RBC's

RBC's, platelets,

Fluorescence Analysis

Contour plots

Topographical map

Color Analysis

Colors, Fluorescent intensity

Cell phenotype by quadrant

Contours

Contour Plot Examples

Cluster of Differentiation

Cluster of antibodies recognizing the same antigen

CD Markers

Antigens define characteristics

Function

Lineage

Developmental stage

CD Markers

Immature

CD 34 Stem Cell

CD 38 Hematopoietic

CD 117 Stem cells ,Mast

TdT Precursor Lymph

HLA-DR Hematopoietic

B Cell

CD 10 Precursor B

CD 19 Precursor and mature B

CD 20 B cell activation

CD 22 B cell activation cytoplasmic

Kappa B cell with Fc receptor

Lambda

FMC7 B cell

CD Markers

T Cell

CD 2 Early

CD 3 Pan -T cell

CD 4 T cell subset

CD 5 T cell

CD 7 Early

CD 8 T cell

CD 56 T - cell subset

Erythroid

CD 36

CD 71

Glycophorin A

Megakaryocyte

CD 31

CD 41 GP IIb/IIIa

CD 42

CD 61 GP IIb/IIIa

CD Markers

Granulocytic

CD 13 Pan Myeloid

CD 33 Pan Myeloid

CD 15 Promyelocyte

CD 11b Myelocyte

MPO Myeloid

Monocytic

CD 11b Granulocytic and monocytic

CD 13 Early monocyte

CD 14 Mature monocytes

CD 33 Granulocytic and monocytic

HLA-DR Monocytic maturation

Acute Leukemia

Characterized by having symptoms of short duration, onset sudden, many immature cells in bone marrow and peripheral

> 30% blasts in B.M. (FAB)

> 20 blasts in B.M. (WHO)

Platelet Count usually decreased

WBC count Variable

N/N Anemia

Acute Myelocytic Leukemia

FAB Classification

M0- Undifferentiated maturation

M1- AML with minimal maturation

M2- With maturation (50% other forms)

M3- Promyelocytic M3v

M4- Myelomonocytic M4E- Eosinophil

M5- Monocytic

M6- Erythrocytic

M7- Megakaryocytic

WHO Classification

Classification based on:

Histopathological Features

Genetic Features

Pathogenesis of the neoplasms

Standardization of nomenclature

Advances in therapy

WHO Classification

Acute Myeloid Leukemia 2001

Acute myeloid leukemia with recurrent cytoplasmic abnormalities

AML with t(8:21)(q22;q22)

AMI inv(16) p13q22) or t(16;16)(p13;q22)

Acute myeloid leukemia with multilineage dysplasia
Acute myeloid leukemia and myelodysplastic syndrome
Acute myeloid leukemia not otherwise categorized
AML: minimally differentiated
(FAB Classification)
Acute leukemia of ambiguous lineage

***WHO Classification 2008**

for Myeloid Neoplasms

1. Acute Myeloid Leukemia and Related Precursor Neoplasms
2. Acute Leukemias of Ambiguous Lineage
3. Myelodysplastic Syndromes (MDS)
4. Myeloproliferative Neoplasms (MPN)
CML, ET, CNL, PV, Primary Myelofibrosis
5. MDS/MPN Neoplasms
CMML, JMML, Atypical CML, MDS/MPN unclassified
6. Myeloid neoplasms associated with eosinophilia and Abnormalities of PDGFRA, PDGFRB, FGFR1

* World Health Organization of Tumors of Haematopoietic and Lymphoid Tissue 2008

M 3 - Promyelocytic

More common in young adults (males)

Often massive bleeding due to thrombocytopenia and DIC

Bi-lobed promyelocytes containing primary granules (heavy), Auer rods, Faggot cells

Chromosome abnormality 15:17

Frequency of occurrence 10%

M3v

Very tiny or non visible granulation

Bi-lobed nucleus resembling a monocyte

Often confused with monocytic leukemia

Higher WBC count than M3

Frequency 20 - 30% of cases

Abnormal karyotype t (15;17)

DIC

Patient Diagnosis

Flow Cytometry

Positive:

CD 33, CD 13, Myeloperoxidase, CD 45

Negative:

CD14, CD 11b, CD34, DR

DR, CD 14 and CD 11b is positive in M4 and M5

Final Diagnosis M3v

Case 3

History

A 49 year old male visited the ER of a local hospital with complaints of a severe headache for several days.

He was transferred to UH and upon arrival he was in a coma and had bleeding into the brain.

Laboratory Results

WBC 207.4
RBC 3.00
HGB 9.4
HCT 27.0
MCV 86.2
MCH 29.0
MCHC 34.0
RDW 15.0
PLT 119
MPV 8.7
PT 18.5
INR 1.7
APTT 33.5

Flow Cytometry

Myeloid Markers

CD 13 and CD 33

Monocytic Markers

CD 14 and CD 11b

Final Diagnosis

Myeloid Monocytic

M 4 Myelomonocytic

Predominant cells: myeloblast and monoblast

Round to oval nucleus can be notched or indented

Chromatin is delicate and reticular

Visible Nucleoli

More common in males >50 or occurring in the first few months of life

Soft tissue infiltrates (gums)

Highest of white counts

Frequency of occurrence 20%

Case 5

HISTORY

A 4 year old male presents to his local pediatrician with irritability, loss of appetite and a distended abdomen.

On physical exam the patient was febrile and irritable. The spleen was massively enlarged, liver was normal size and there was no lymphadenopathy.

Laboratory Results

WBC 298.9
RBC 3.38
HGB 11.0
HCT 33.0
MCV 85.6
MCH 30.6
MCHC 33.2
RDW 17.4

PLT 366,000
MPV 9.5
Retic 1.4%

Additional Laboratory Tests

Bone Marrow
Hypercellular marrow
M:E Ratio: 25: 1
LAP SCORE: 6
Philadelphia Chromosome: Negative

Myeloproliferative Disorders MPS

Increase in RBC's, WBC's and platelets
Tend to transform into an Acute Leukemia
Characterized by excess proliferation of one or more normal cells
Difficult classifying due to overlap
Bone marrow can be hypercellular and often becoming fibrotic
Includes CML, PM, PV, and ET

MPS

General Characteristics:

Patients usually over the age of 40
Disease advances slower
Extramedullary hematopoiesis
Bizarre RBC morphology
Platelets elevated

Chronic Myelocytic Leukemia CML

Clonal stem cell disorder
Marked leukocytosis with all stages of granulocytic maturation
LAP <10
Ph⁺ positive in 90% of the cases
BCR/ABL
Hepatosplenomegaly
Chronic phase
Accelerated / Blast phase

Sub Groups of CML

CML Common:

Ph +, < 2% blasts

Juvenile:

< 5 yrs old, > 10% blasts, resistant to therapy, very large spleen, PH -

Chronic Neutrophilic:

Ph absent, LAP inc, rare, more bands than other

Atypical CML:

Features of CML and MDS
Ph absent, no basophilia, less favorable prognosis

CML

Laboratory Findings

WBC count ranges from 100,000 - 200,000

N/N Anemia

Increased platelets, giant and fragments

Differential: complete spectrum

Increase Eos and Baso

Bone Marrow: hypercellular 25:1

LAP <10 (CML neutrophils don't produce)

Philadelphia Chromosome (arm of 22 is translocated to 9) positive in 90% of the cases

BCR/ABL Mutation

Three Phases of CML

Initial Phase or Chronic: 2 - 3 years, highly treatable

Accelerated or transitional: rising peripheral WBC count with increase of the blast, 6 - 18 months, resistant to treatment

Blast Crisis: about 3/4 of the patients enter this phase, 1- 20% blasts, 3 - 4 months, unresponsive to treatment

Looks like M2

2 - 6 years from diagnosis

CML

Classified as a myeloproliferative disorder which include:

WHO 2008 Myeloproliferative Neoplasms

Chronic Neutrophilic Leukemia

Primary myeloidfibrosis

Polycythemia Vera

Essential Thrombocythemia

Chronic Eosinophilic Leukemia

Mastocytosis

Myeloproliferative Neoplasm - unclassifiable

BCR – ABL Gene

BCR: Breakpoint Cluster Region

DNA fragment localized on chromosome 22

Defines the chromosomal break

ABL: protein possessing increased tyrosine kinase activity

BCR – ABL Fusion Gene

The gene arrangement of the bcr region of chromosome 22 is detected by DNA technology in Southern blots

FISH is used to detect the bcr-abl gene

Used for the effectiveness of therapy

Apoptosis

Programmed cell death

Case 6

HISTORY

A 10 year old male presents to the E.R. with swollen glands. The glands were so enlarged that he had trouble swallowing.

On physical observation there was a large mass found in the neck area.

Hematology Results

WBC 234.9

RBC 4.59
HGB 14.8
HCT 42.2
MCV 87.3
MCH 30.7
MCHC 35.1
RDW 13.4
PLT 72,000
MPV 7.0

Case 6 Flow Markers

CD 14 Negative
CD 20 Negative
CD 19 Negative
Kappa Negative
CD 34 Negative

CD 5 Positive
CD 2 Positive
CD 7 Positive
CD 10 Positive
TdT Positive
CD 38 Positive
* CD4/CD8 Positive

Acute Lymphoblastic Leukemia

ALL

Accounts for 80% of all leukemia in children.

Peak age 3 - 5 years

Second peak the elderly (adult form)

Not commonly seen in adults, which have a poor prognosis

CNS involvement is common and a major complication

Enlarged spleen, liver and lymph nodes

Laboratory Findings

60% of the cases have elevated WBC count

Thrombocytopenia, N/N Anemia

Auer rods not present

Immunologic Markers: CALLA or CD 10 is positive in 70% - 80% of ALL cases

Normal lymphocytes are negative

FAB Classification

L1- Lymphocytic, childhood

Small uniform lymphoblasts

L2- Lymphocytic Adult

Large pleomorphic lymphoblasts

L3- Burkitts, poor prognosis

Deeply basophilic and vacuolated

WHO Classification

ALL 2001

Precursor B – lymphoblastic Leukemia/Lymphoma

Precursor T-Lymphoblastic Leukemia/Lymphoma

When the leukemic process is limited to a mass lesion and at least 25% lymphoblasts are seen in the bone marrow, the designation lymphoma is used

***WHO Classification 2008**

for Lymphoid Neoplasms

1. Precursor Lymphoid Neoplasms

B lymphoblastic leukemia/lymphoma

T lymphoblastic leukemia/lymphoma

2. Mature B-Cell Neoplasms

3. Mature T-Cell and NK cell Neoplasms

4. Hodgkin Lymphoma

5. Histiocytic and Dendritic Cell Neoplasm

6. Post – Transplant Lymphoproliferative Disorders PTLN

* World Health Organization of Tumors of Haematopoietic and Lymphoid Tissue 2008

Immunophenotyping

Immature B Cell ALL: CALLA (CD10) pos

Most common: 75% children, 40% adult

Pre B Cell: 2 nd most common

CALLA (CD 10) pos

B Cell ALL: uncommon

Corresponds to L3

Pre -T Cell ALL: Teenage males, T markers

15-20% of childhood

25% of adult

Null: no lymphocytic surface antigens, TdT+

T - Cell and B - Cell Distribution

Null cell 2%

Early pre B 65%

Pre B 20%

B - cell 1%

T - cell 15%

T - Cell

Most often seen in teenage males that present with tissue masses

Elevated peripheral blasts

High Risk Disease

15 – 20% of ALL cases

TdT , CD 2, CD 4, CD8, CD1a, CD10, CD7, CD5, CD3 Positive

CD 34, CD 19, SIg, Negative

Thank You