Mass General Brigham

In the Beginning there was Blood: Importance of Peripheral Blood Smears

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Disclosures

Scopio:

- Consultant

Sysmex:

- Speaker

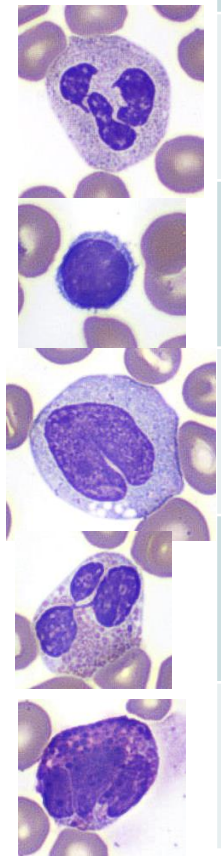
Objectives

Correctly identify white blood cells morphologic abnormalities in peripheral blood smears.

Formulate an appropriate and comprehensive differential diagnosis for abnormal morphologic findings that includes neoplastic and reactive processes.

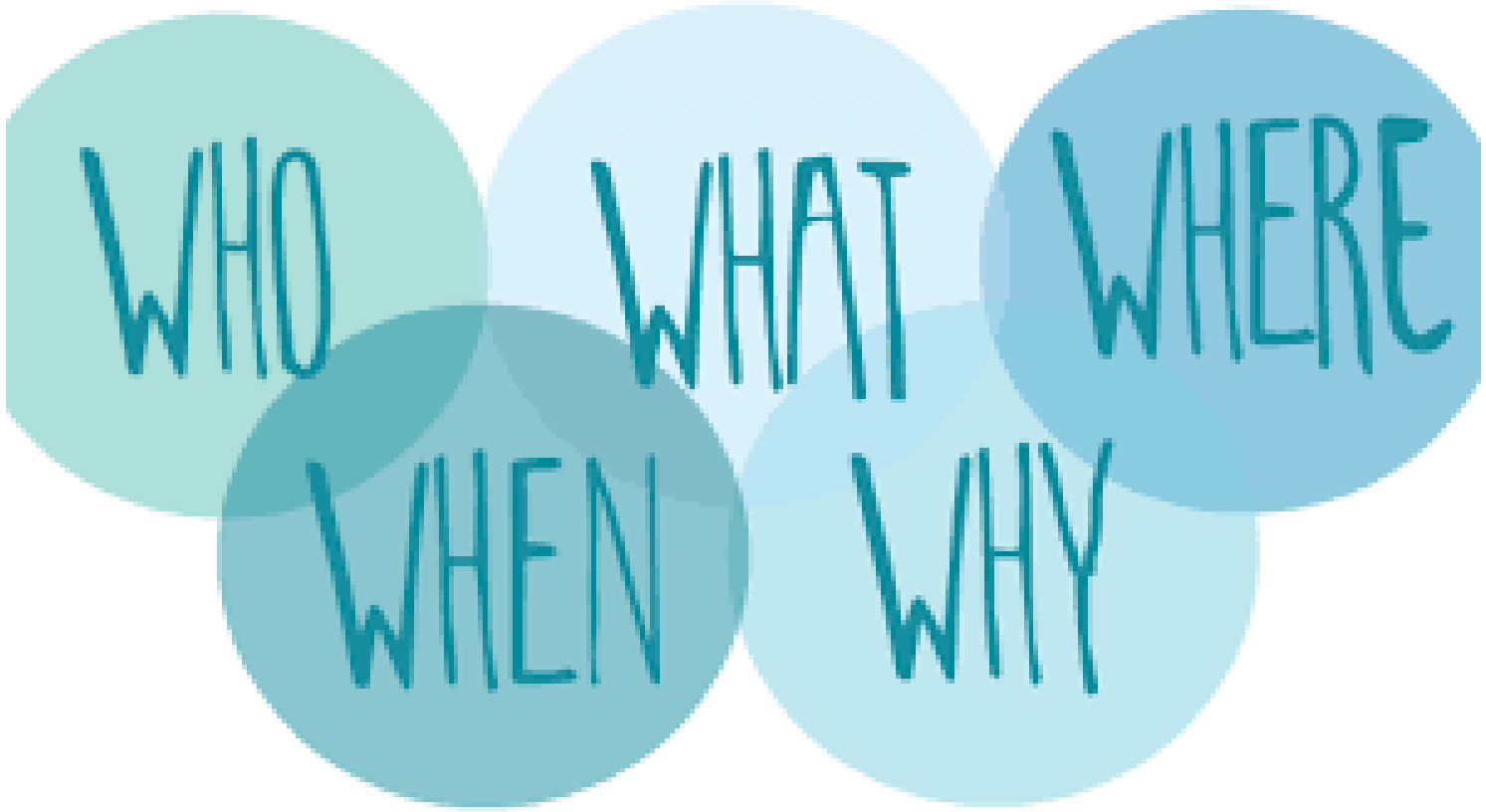
Understand the role of ancillary testing, especially flow cytometry, in a work-up of patients presenting with abnormal peripheral blood morphologic findings.

Components of a normal WBC differential



	Absolute count, cell/µL	Percent, %
WBC	4,000 – 11,000	
Neutrophils	1,920 – 7,700	48 – 76
Lymphocytes	720 – 4,100	18 – 41
Monocytes	160 – 1,100	4 – 11
Eosinophils	0 – 500	0 – 5
Basophils	0 – 150	0 – 1.5

Remember these?



What defines an abnormal WBC differential?

Abnormal morphology

- Reactive atypia
- Neoplastic atypia
- Dysplastic changes
- Inclusions; organisms
- Inclusions; others

Presence of unusual cell types

- Blasts/Equivalents
- Immature granulocytes
- Plasma cells
- Mast cells
- Nucleated RBCs

When PB smear is reviewed?

Abnormal counts (WBC, RBC, PLT)

Abrupt change in counts

Instrument “suspect” flags:

- Interfering particles are present at the lower WBC counting threshold, or lowest forward/side light scatter region for lymphocytes. Typical resulting flags include: nRBC (nucleated red blood cell), CLUMP (platelet), GIANT (platelet).
- Large mononuclear cells are present at the monocyte/neutrophil interface or with high values for high angle (90 degree) light scattering. Typical resulting instrument flags: BLAST.
- Large cells are present in the lymphoid region or at the interface between lymphoid and monocyte regions. Typical instrument flags include: ATYPICAL LYMPH, BLAST.
- There is a shifted position in the neutrophil cluster, with a large amount of forward or side light scatter. Typical instrument flags include: IMMATURE NEUTROPHILS, BANDS.

Where? In Health Records

History

- Prior malignancy
- Traveling

Test/imaging results

- Infectious work-up
- Presence of lymphadenopathy

Orders

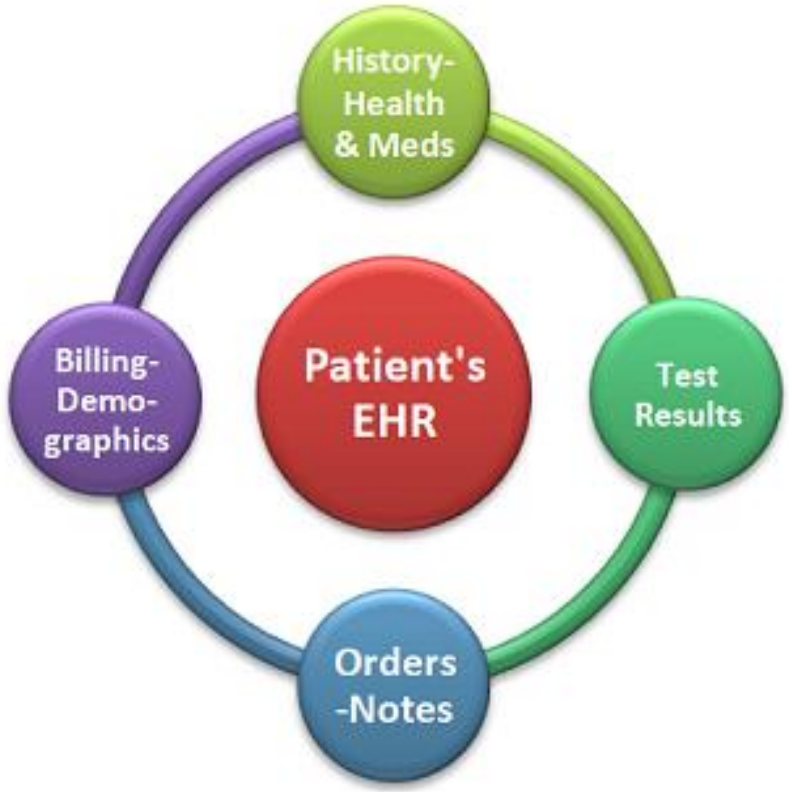
- Recent growth factors administration

Notes

- Any symptoms (fever, rash, sore throat)

Demographics

- Age
- Ethnicity



To guide further steps and/or testing!!!

The patient is presenting with neutrophilia …

Neutrophilia important facts:

>7.7 x 10^9/L or 2SD above mean

Is there left-shift?

- Mature neutrophils (ANC = segmented neutrophils + bands)
  - 10-15 μm; pale pink granular cytoplasm with segmented (3-5 lobes) nucleus with clumped chromatin; lobes connected by thin filaments*
- Left-shift (IG = metamyelocytes, myelocytes +/- promyelocytes)
- Blasts

Other cytoses or cytopenia(s)?

Morphologic changes:

- Reactive/toxic changes
- Dysplastic changes
- Inclusions

Symptomatic or incidental? Persistent? Medications?

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Causes of neutrophilia

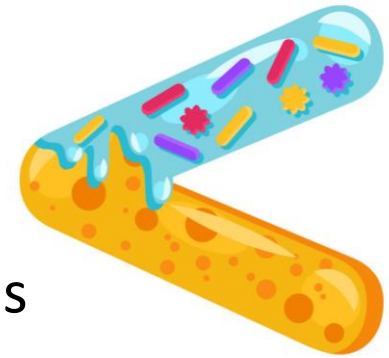
Primary

Constitutional

- Leukocyte adhesion deficiency
- Familial MPN
- Down syndrome

Acquired/Myeloproliferative neoplasms

- CML, *BCR-ABL1*+
- CNL
- aCML or other MDS/MPN
- Ph- MPN



Secondary

Infection/Inflammation

Smoking

Drugs

- Corticosteroids
- G-CSF

Stress

Paraneoplastic syndrome

Asplenism

Neutrophil reactive changes

36-year-old woman with primary CNS lymphoma on therapy with high fevers; colitis on abdomen CT; resolved after antibiotics

CBC results

WBC 23.60 x 10^9/L (H)

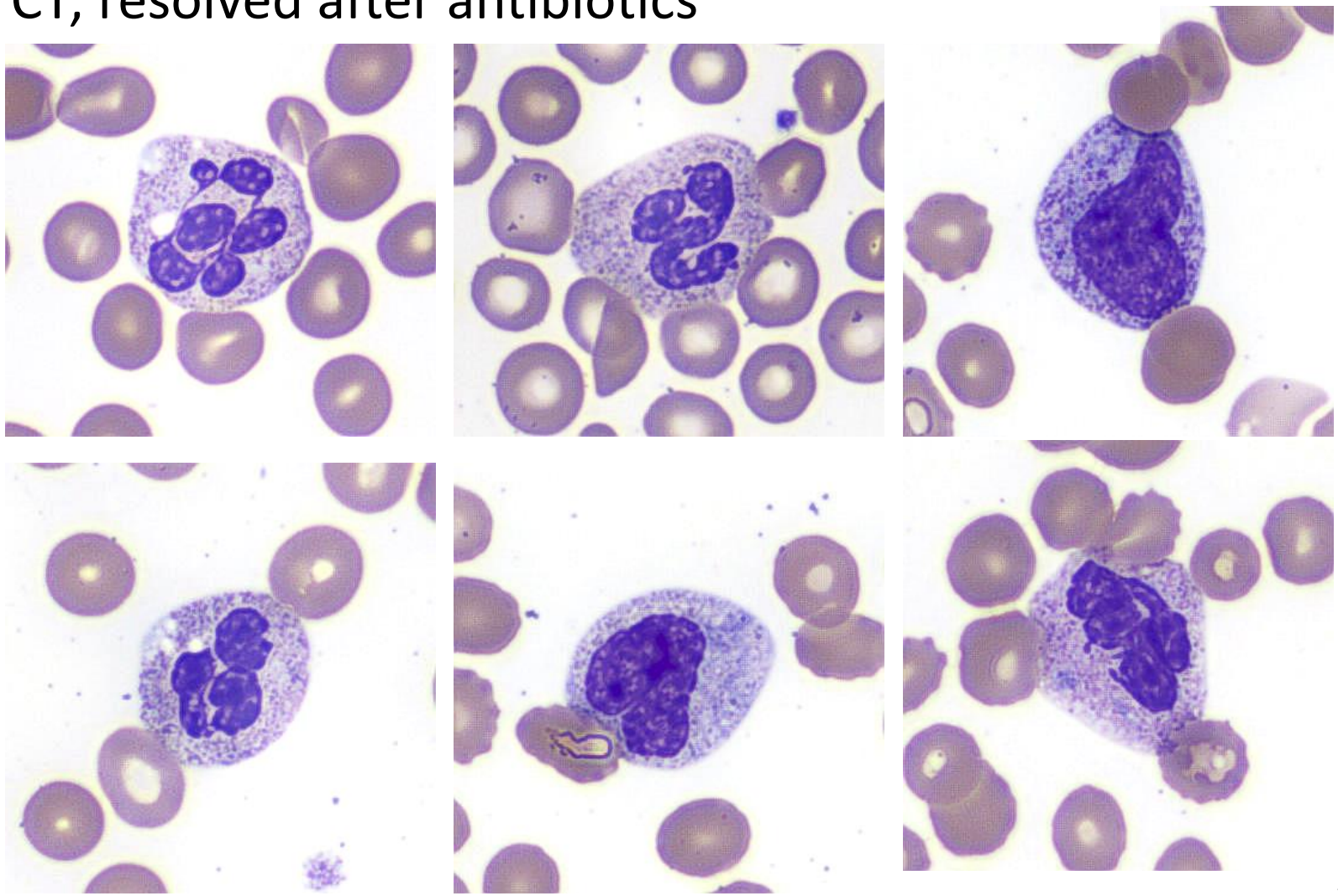
- 63% Neut (ANC 14.9)
- 6% Bands
- 6% Meta
- 14% Myelo
- 3% Pro
- 8% Lymph

HGB 9.3 g/dL (L)

HCT 26.8% (L)

MCV 90.5 fL

PLT 49 x 10^9/L (L)



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Dohle inclusions?

79-year-old woman presenting with traumatic fall with C5 fracture requiring operative management

CBC results

WBC 11.20 x 10^9/L (H)

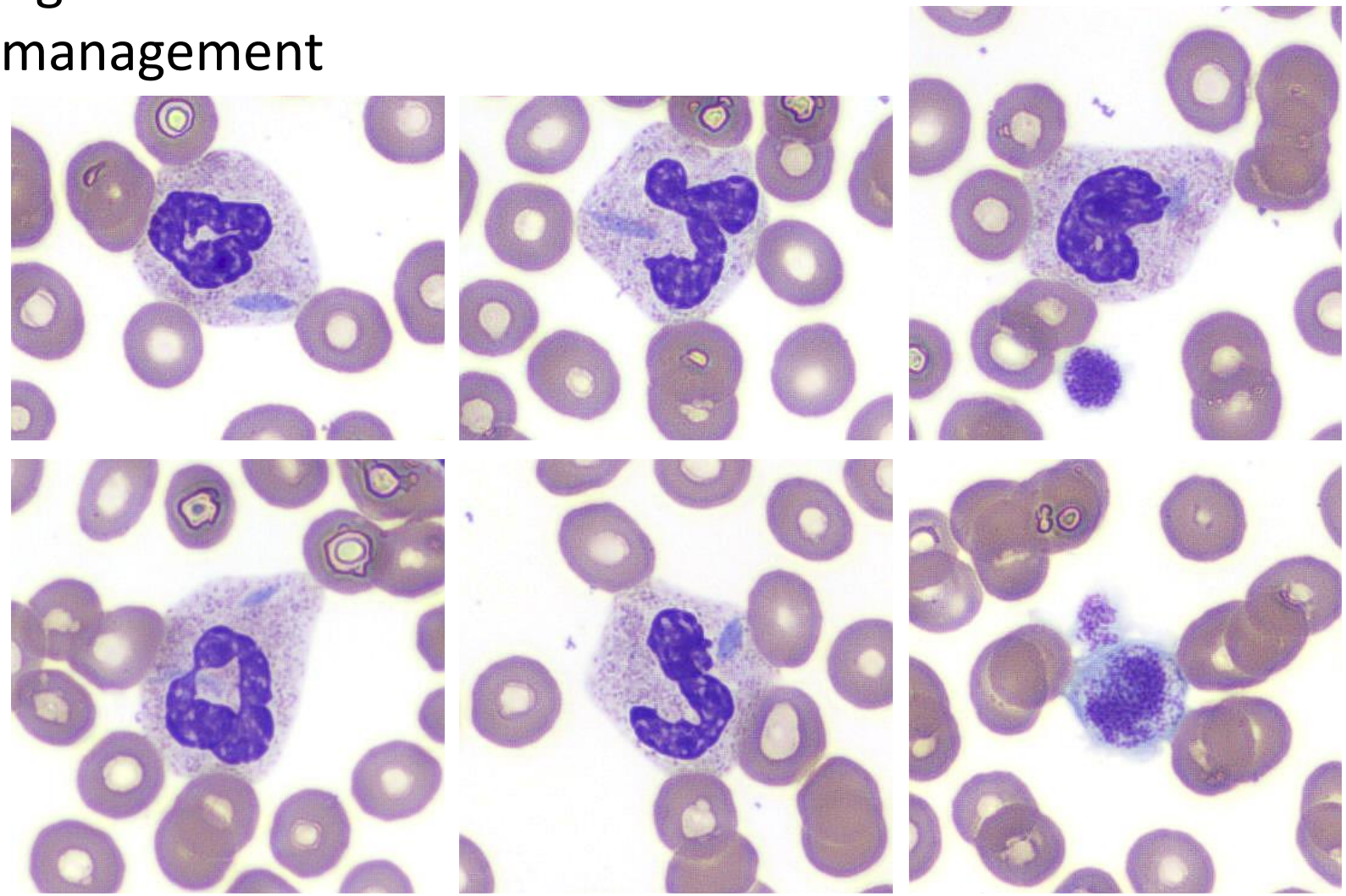
- 64% Neut (ANC 11.2)
- 20% Lymph
- 12% Mono
- 4% Eos

HGB 12.2 g/dL

HCT 36.9%

MCV 87.2 fL

PLT 60 x 10^9/L (L)



Blue-green inclusions

59-year-old man presenting with cough, treated for presumed pneumonia; CT bilateral lung nodules → metastatic adrenocortical carcinoma

CBC results

WBC 24.82 x 10^9/L (H)

- 23% Bands
- 68% Neut
- 0% Lymph
- 5% Mono
- 4% Eos

HGB 7.8 g/dL (L)

HCT 22.7% (L)

MCV 83.5 fL

PLT 164 x 10^9/L

Chemistry results

Total Protein 5.5 g/dL (L)

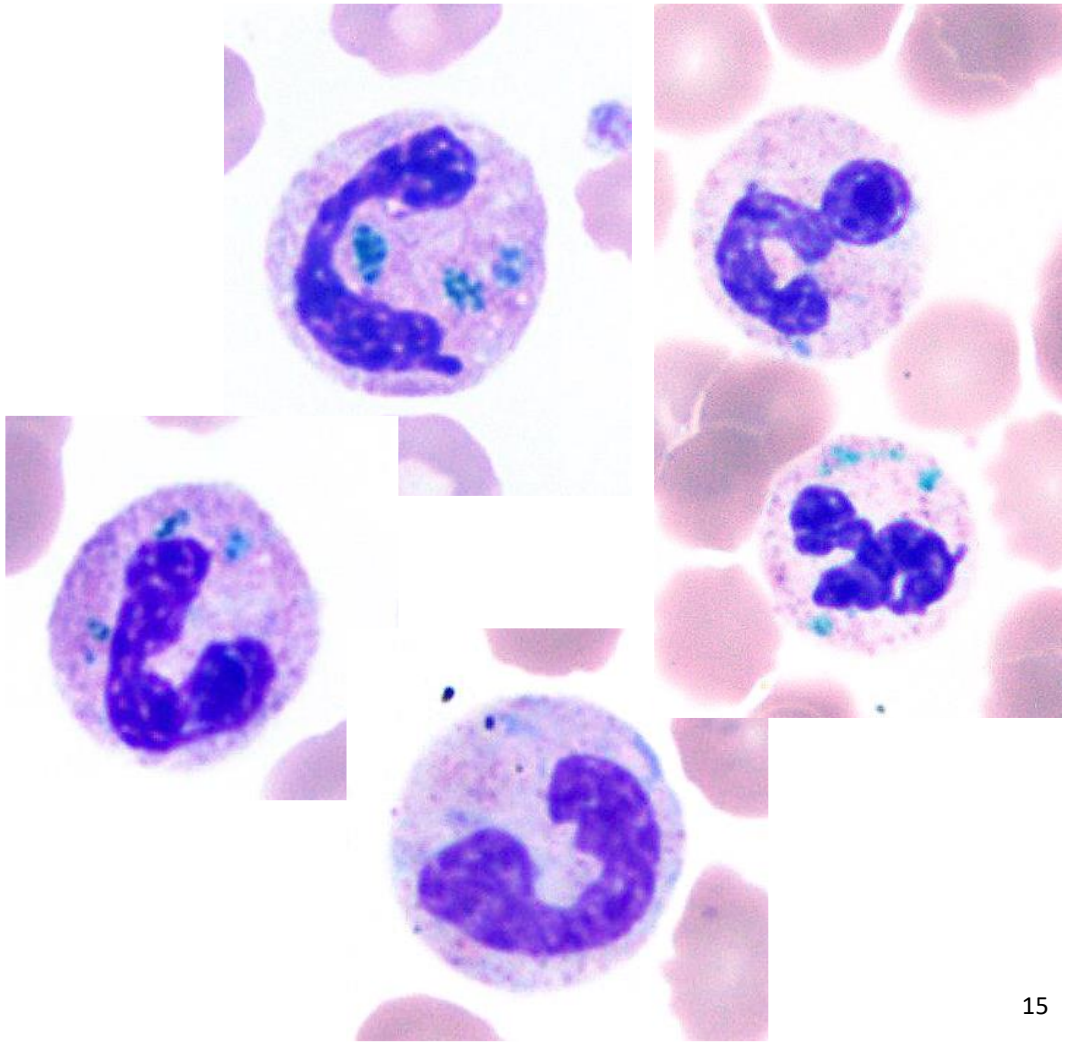
ALT 302 U/L (H)

AST 1155 U/L (HH)

Alk Phos 209 U/L (H)

BUN 51 mg/dL (H)

Creatinine 2.62 mg/dL (H)



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Human granulocytic anaplasmosis

64-year-old man s/p SCT for B-ALL admitted with low-grade fevers, cough, sweats in the setting of tick bites

CBC results

WBC 19.27 x 10^9/L (H)

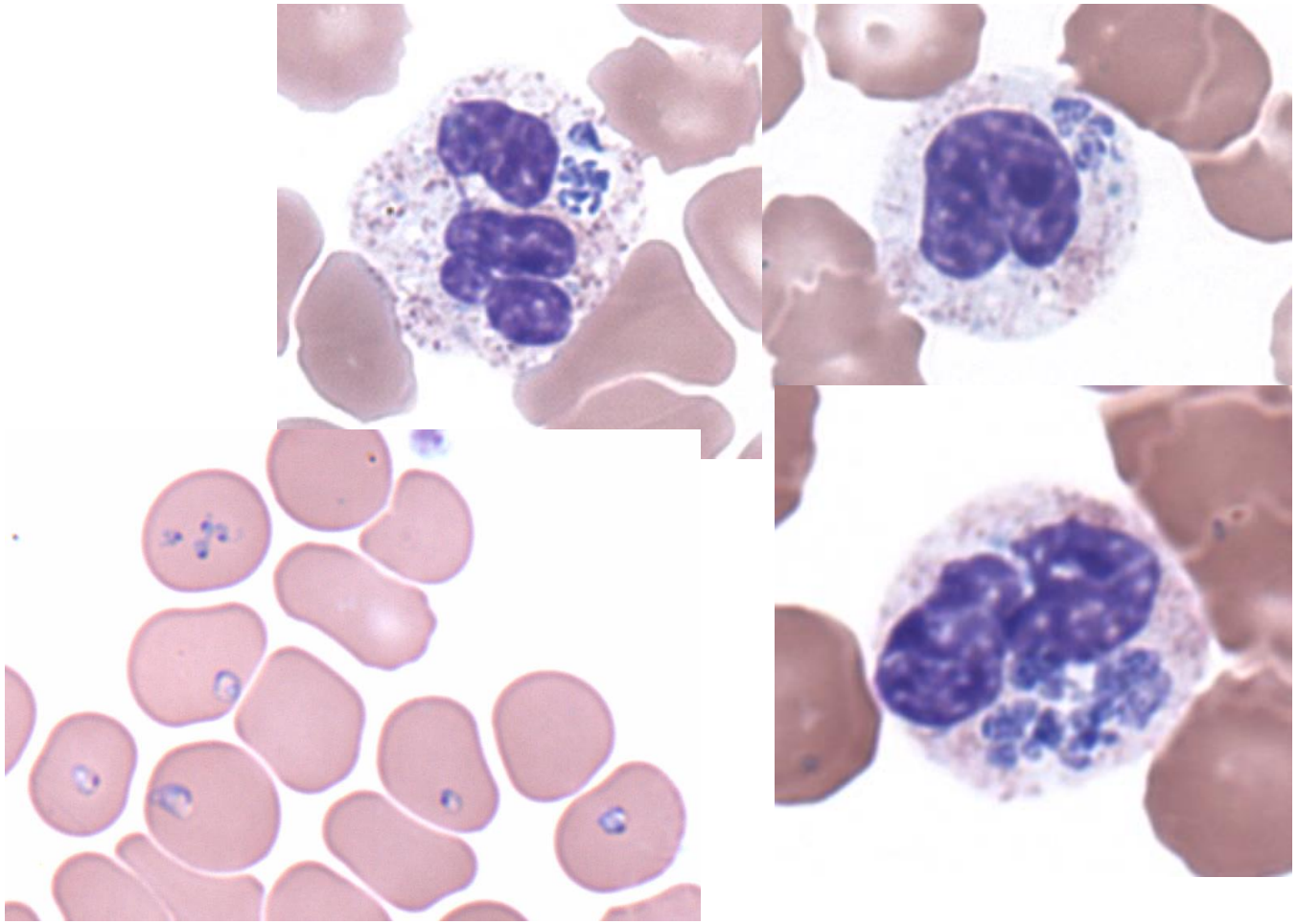
- 84% Neut
- 14% Bands
- 1% Lymph
- 1% Mono

HGB 12.4 g/dL

HCT 36.5%

MCV 100.0 fL

PLT 99 x 10^9/L



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Howell-Jolly body-like inclusions

68-year-old woman with ESRD s/p kidney transplant, admitted with cough, fever and SOB in the setting of suspected pneumonia

CBC results

WBC 11.81 x 10^9/L (H)

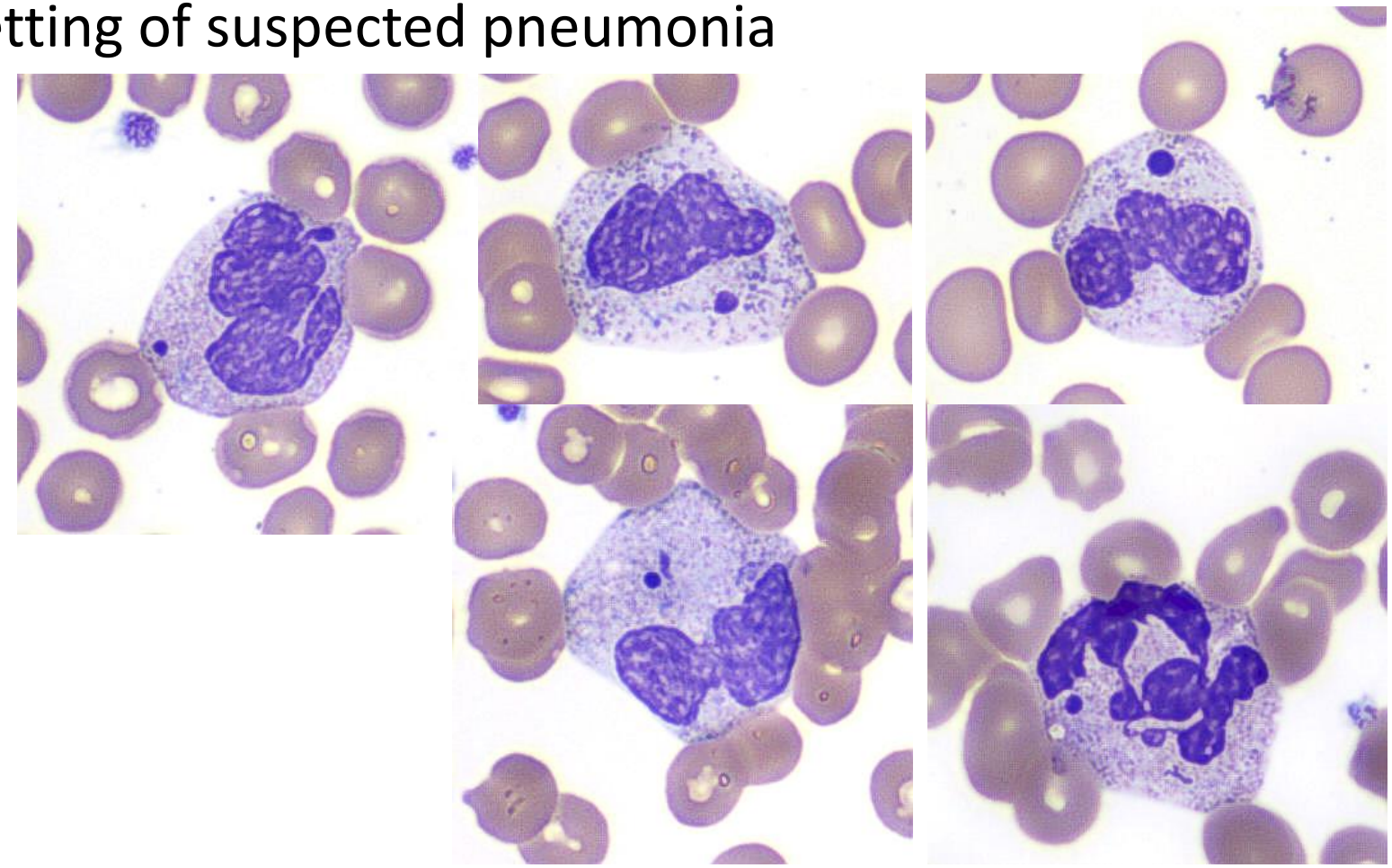
- 55% Neut
- 30% Lymph
- 15% Mono

HGB 12.9 g/dL

HCT 36.9%

MCV 90.0 fL

PLT 163 x 10^9/L



Dysplastic changes

62-year-old woman with relapsed DLBCL with progressive disease, s/p multiple therapies; presenting with fevers

CBC results

WBC 13.12 x 10^9/L (H)

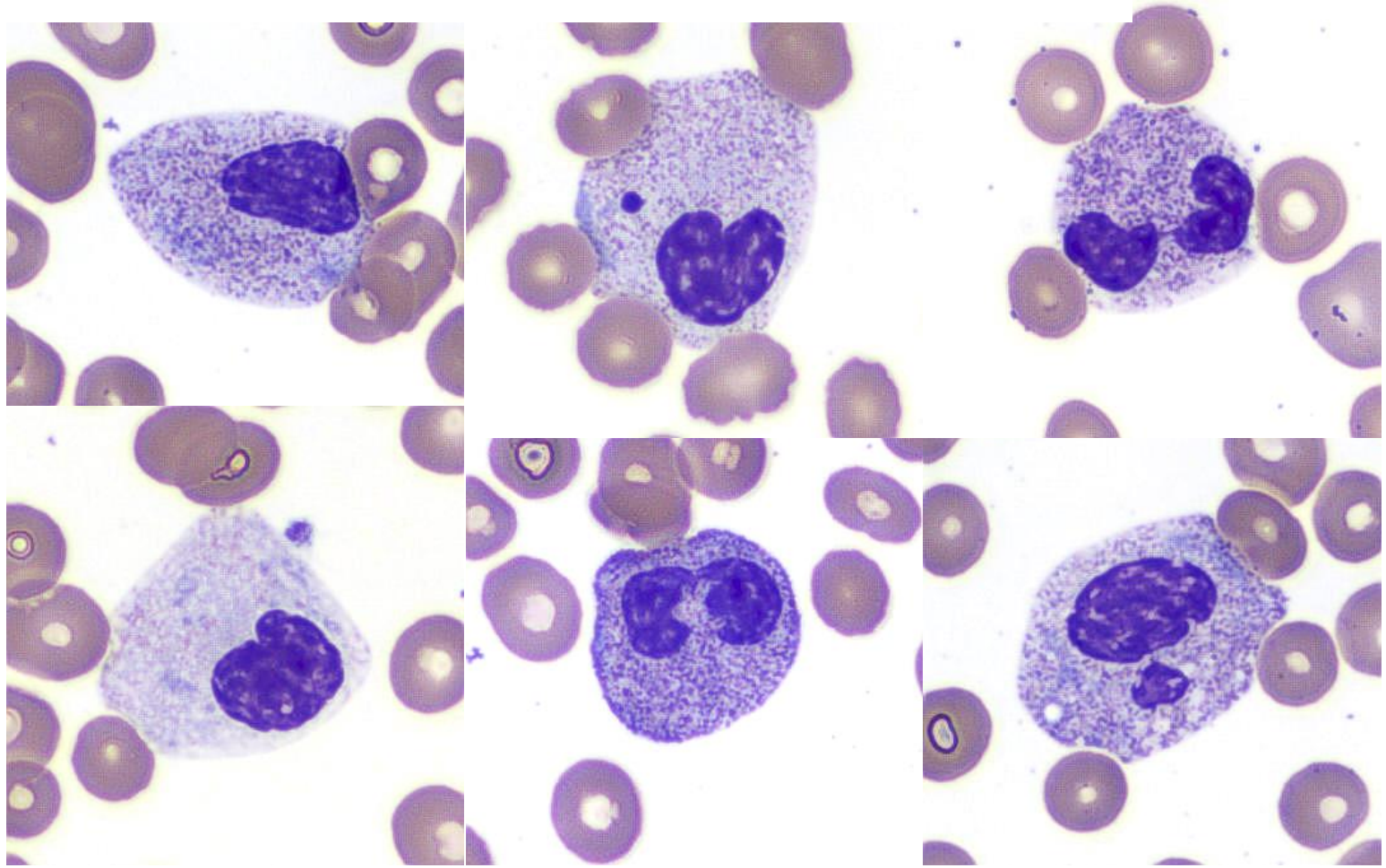
- 67% Neut
- 6% Myelo
- 12% Lymph
- 7% Mono
- 8% Eos

HGB 8.1 g/dL (L)

HCT 24.5% (L)

MCV 102 fL (H)

PLT 22 x 10^9/L (L)



What is it?

72-year-old woman with clonal hematopoiesis, progressive leukocytosis, lung opacities and clinical suspicion for disseminated fungal infection (+ glucan)

CBC results

WBC 32.86 x 10^9/L (HH)

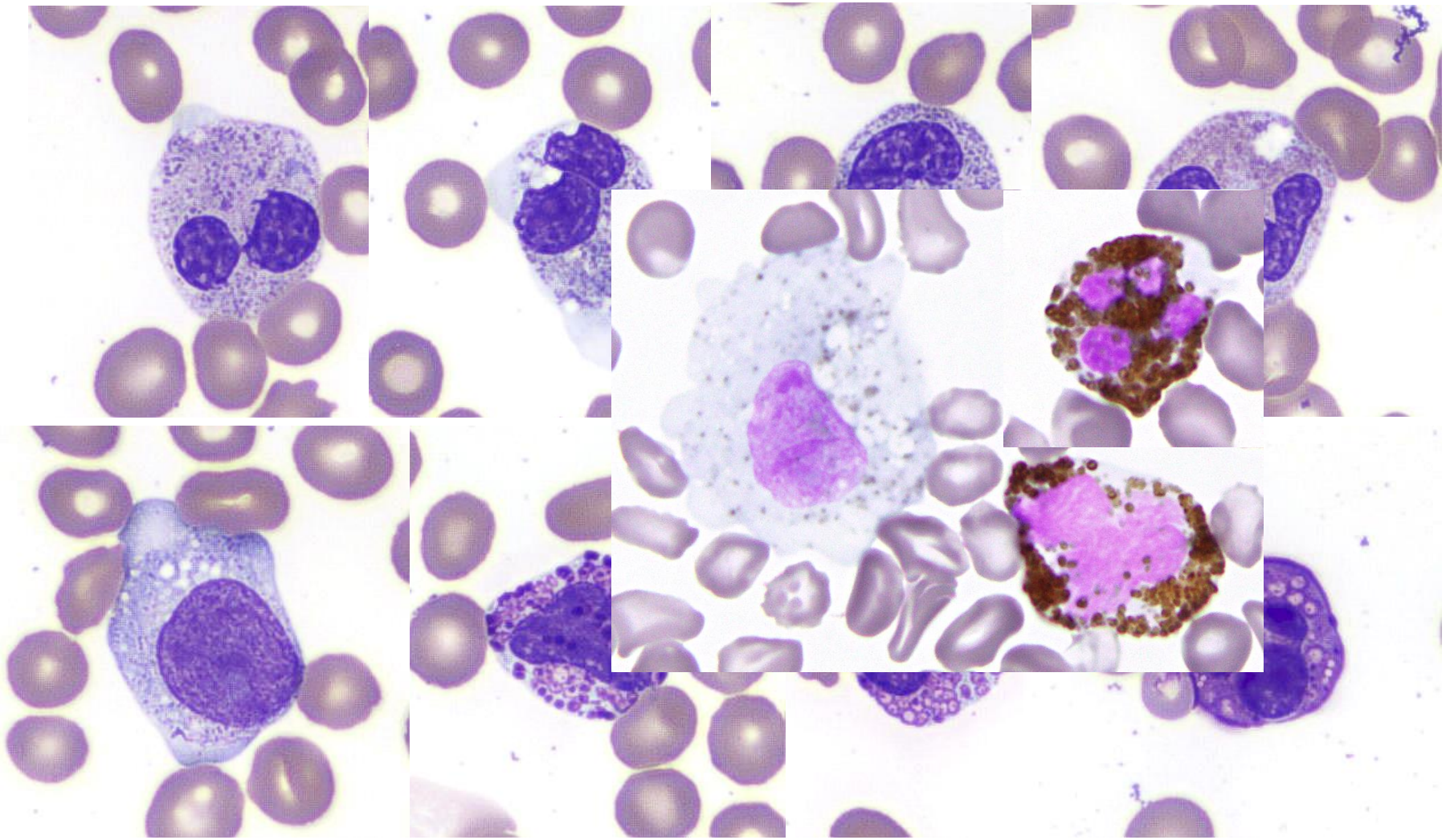
- 4% Bands
- 47% Neut
- 13% Lymph
- 16% Mono
- 10% Eos
- 10% Baso (???)

HGB 7.8 g/dL (L)

HCT 24.1% (L)

MCV 103.8 fL

PLT 101 x 10^9/L (L)



G-CSF

56-year-old man undergoing chemotherapy for Burkitt lymphoma; day 4 of granix treatment due to marked neutropenia

CBC results

WBC 31.51 x 10^9/L (H)

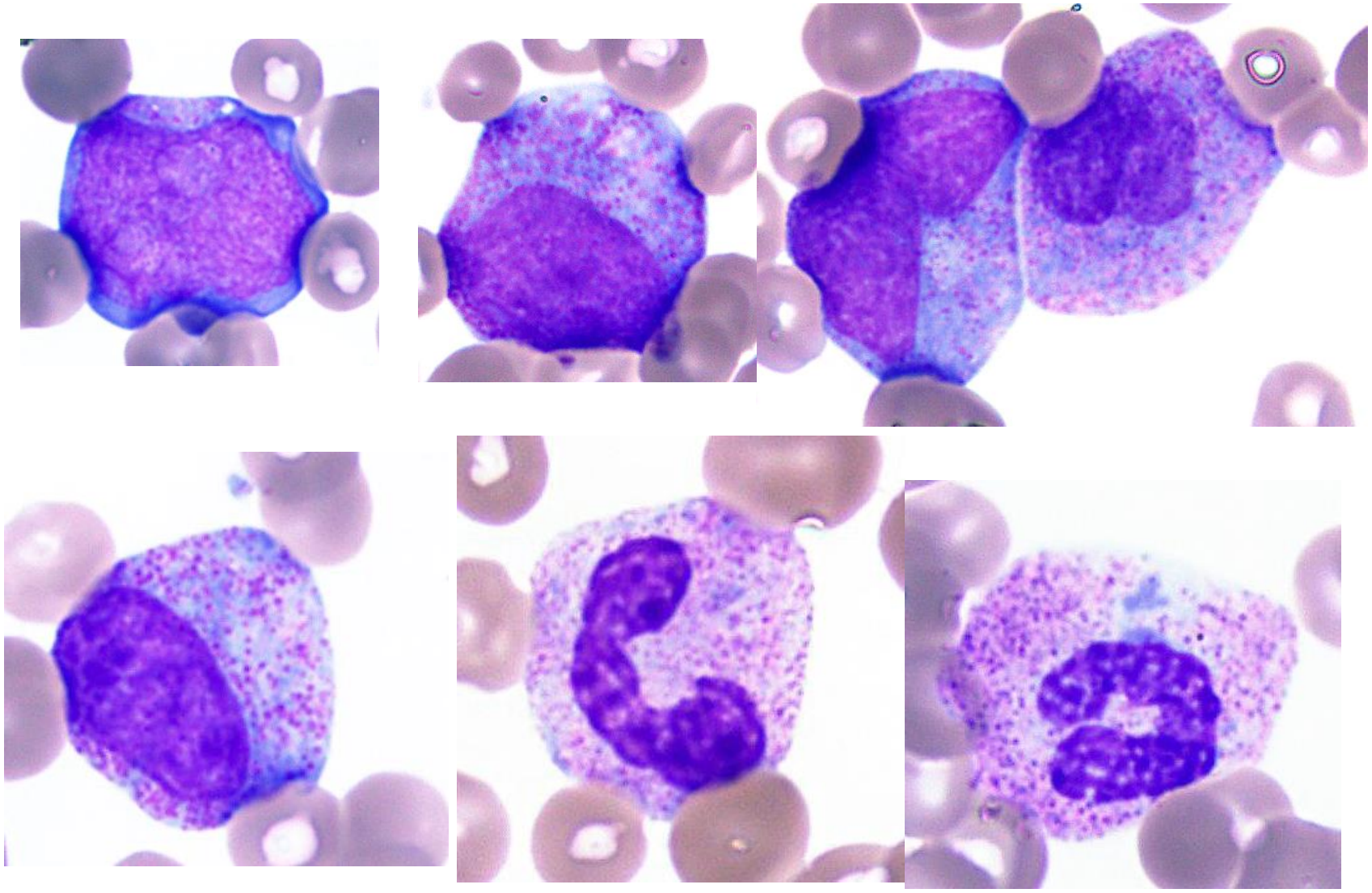
- 8% Bands
- 62% Neut
- 4% Meta
- 10% Myelo
- 5% Promyelo
- 2% Blasts
- 3% Lymph
- 6% Mono

HGB 9.5 g/dL (L)

HCT 30.0% (L)

MCV 94.6 fL

PLT 121 x 10^9/L (L)



Differential diagnosis for “chronic” non-congenital neutrophilia

Chronic myeloid leukemia, *BCR::ABL1*-positive

Chronic neutrophilic leukemia

Ph-negative myeloproliferative neoplasms

- Polycythemia vera
- Essential thrombocythemia
- Primary myelofibrosis, pre-fibrotic stage

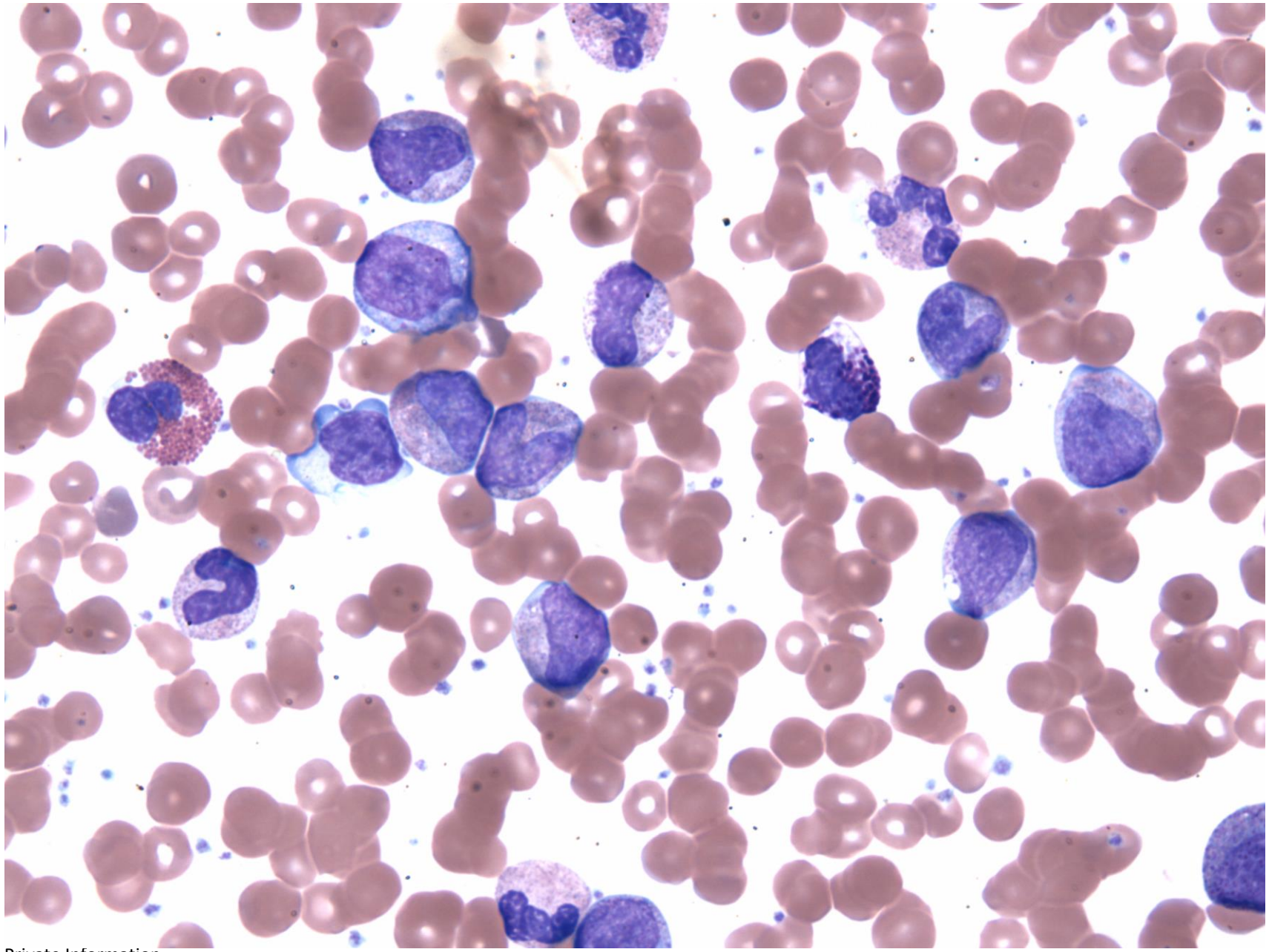
Atypical chronic myeloid leukemia

Chronic myelomonocytic leukemia, proliferative type

Acute myeloid leukemia

- Especially with *NPM1* or *FLT3* mutations

CML, *BCR::ABL1*+, p210



76yo woman

CBC results

WBC 87. 7 x 10^9/L (H)

- 37% Neutrophils
- 7% Bands
- 21% Metamyelocytes
- 8% Myelocytes
- 5% Promyelocytes
- 2% Myeloblasts
- 4% Lymphocytes
- 8% Monocytes
- 1% Eosinophils
- 7% Basophils

HCT 33.9%

MCV 92.9 fL

PLT 511 x 10^9/L (H)

PEARLS: Neutrophilia

- Careful review of granulocyte morphology may contain clues to neutrophilia etiology
- Reactive changes can mimic myeloproliferative neoplasm and clinical context is very important
- Always perform *BCR-ABL1* testing (by different methods) to exclude CML in persistent neutrophilia
- NGS analysis can help exclude a clonal process or,
- … confirm the presence of MPN and/or MDS/MPN

The patient is presenting with monocytosis …

Monocytosis important facts:

>0.8 x 10^9/L*

Largest circulating mononuclear cells (12-20 μm):

- abundant blue-gray cytoplasm often with small vacuoles and fine azurophilic granules
- folded, lobated, indented or oval nucleus without nucleolus
- nuclear chromatin is not as clumped as in neutrophils and lymphocytes

Morphologic changes:

- Reactive changes
- Abnormal monocytes
- Immature monocytes (monoblasts and promonocytes) *

Other cytoses or cytopenia(s)?

Symptomatic or incidental? Persistent? Medications?

Causes of monocytosis

Primary (>3 months)

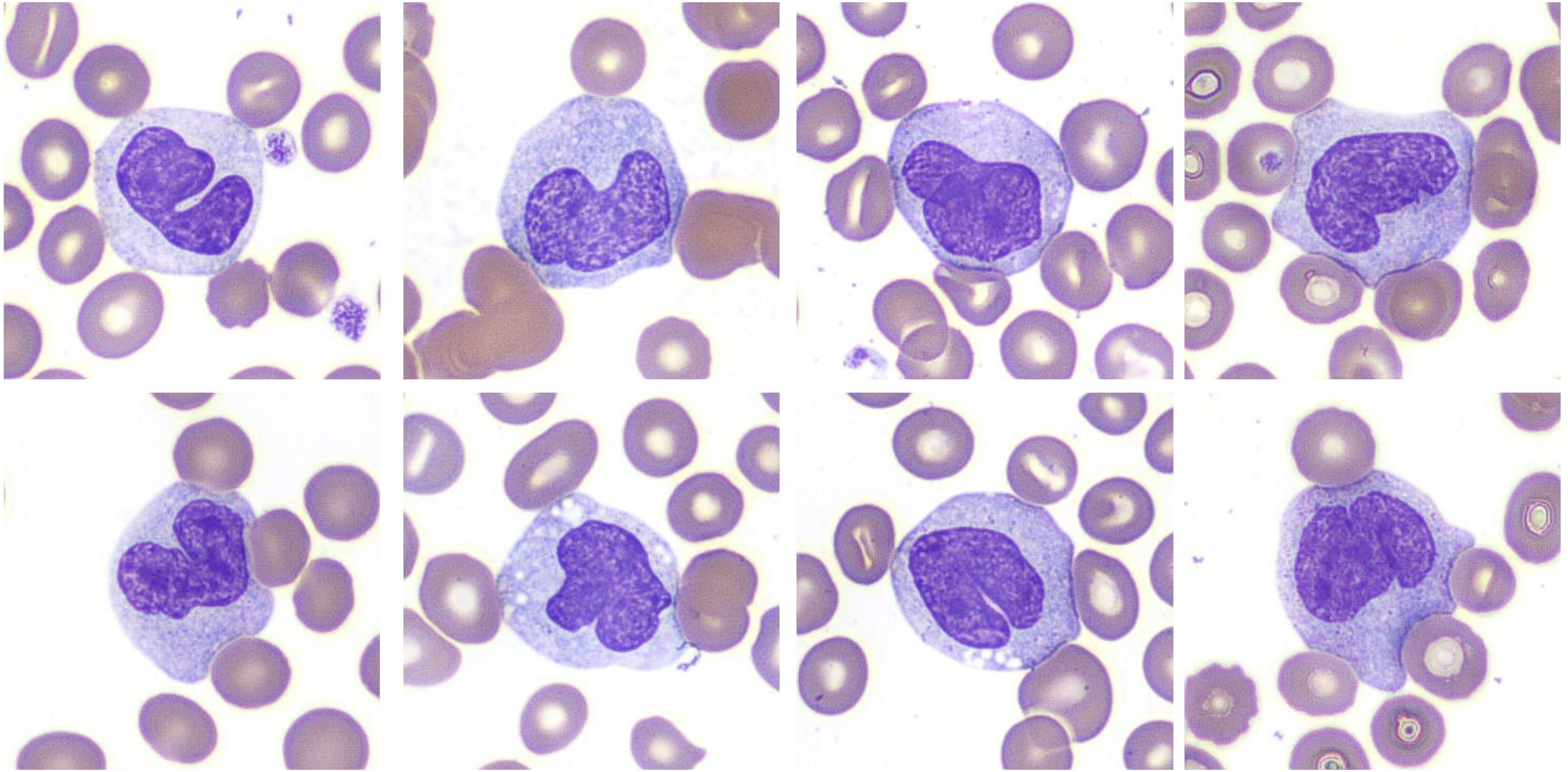
Chronic myelomonocytic leukemia
Juvenile myelomonocytic leukemia
Acute monocytic leukemia
CML with p190
PDGFRA, *PDGFRB*, *FGFR1*, *PCM-JAK2* rearranged neoplasms
Progression of Ph- myeloproliferative neoplasms

Secondary

Infection/Inflammation
Autoimmune disorders
Drugs
- Corticosteroids
- G-CSF

Associated with:
- Hematologic malignancy
  – CHL, lymphoma, PCN
- Non-hematologic malignancy
  – Breast, colorectal

Spectrum of monocyte morphology (mature)



Atypical monocytes (reactive) in the setting of COVID19 infection

54 yo man with HTN

CBC results

WBC 10.31 x 10^9/L (H)

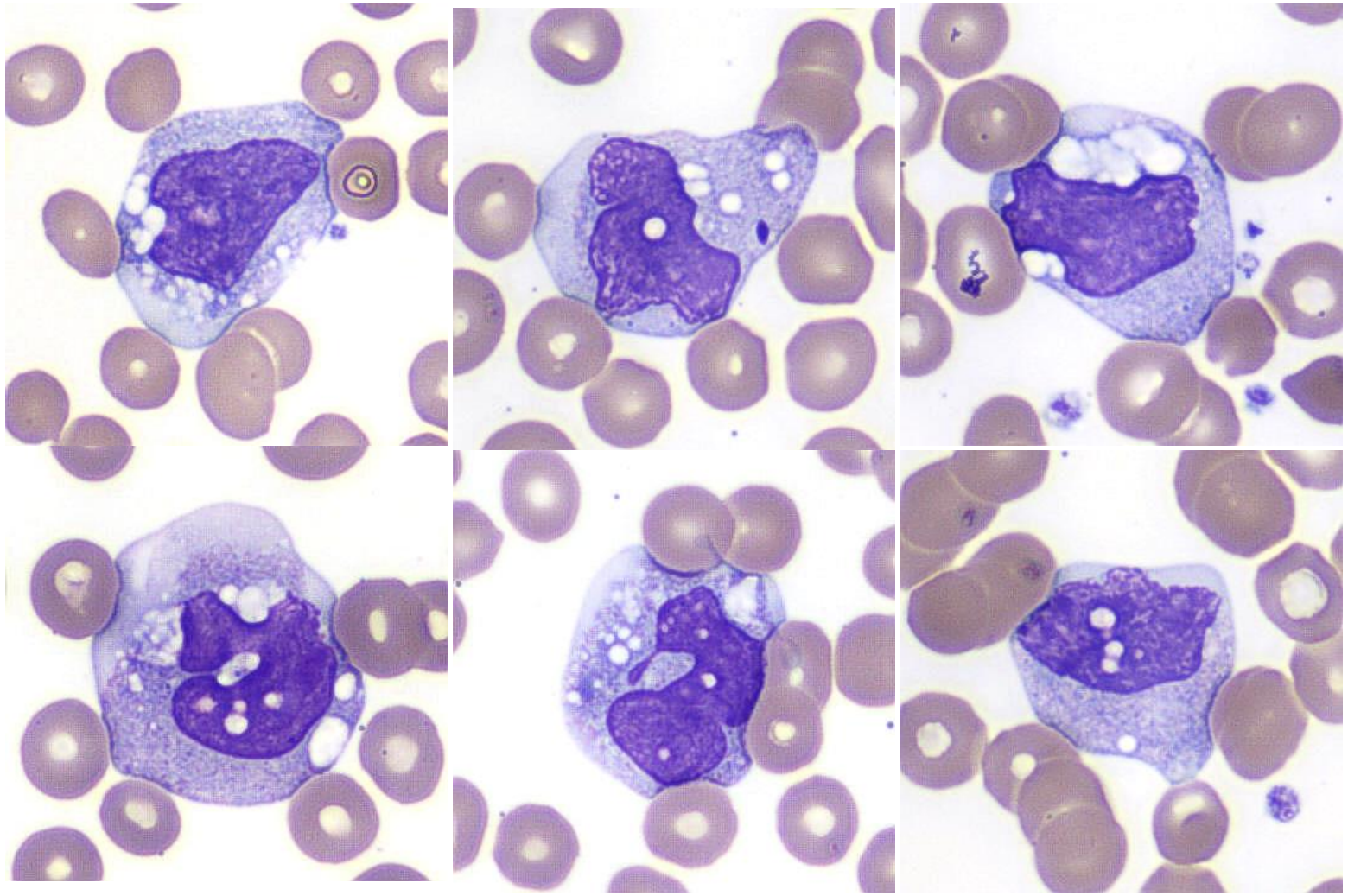
- 3% Bands
- 3% Meta
- 75% Neut
- 8% Lymph
- 11% Mono

HGB 12.6 g/dL

HCT 37.5% (L)

MCV 92.2 fL

PLT 241 x 10^9/L



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63-year-old man is presenting with leukocytosis and monocytosis

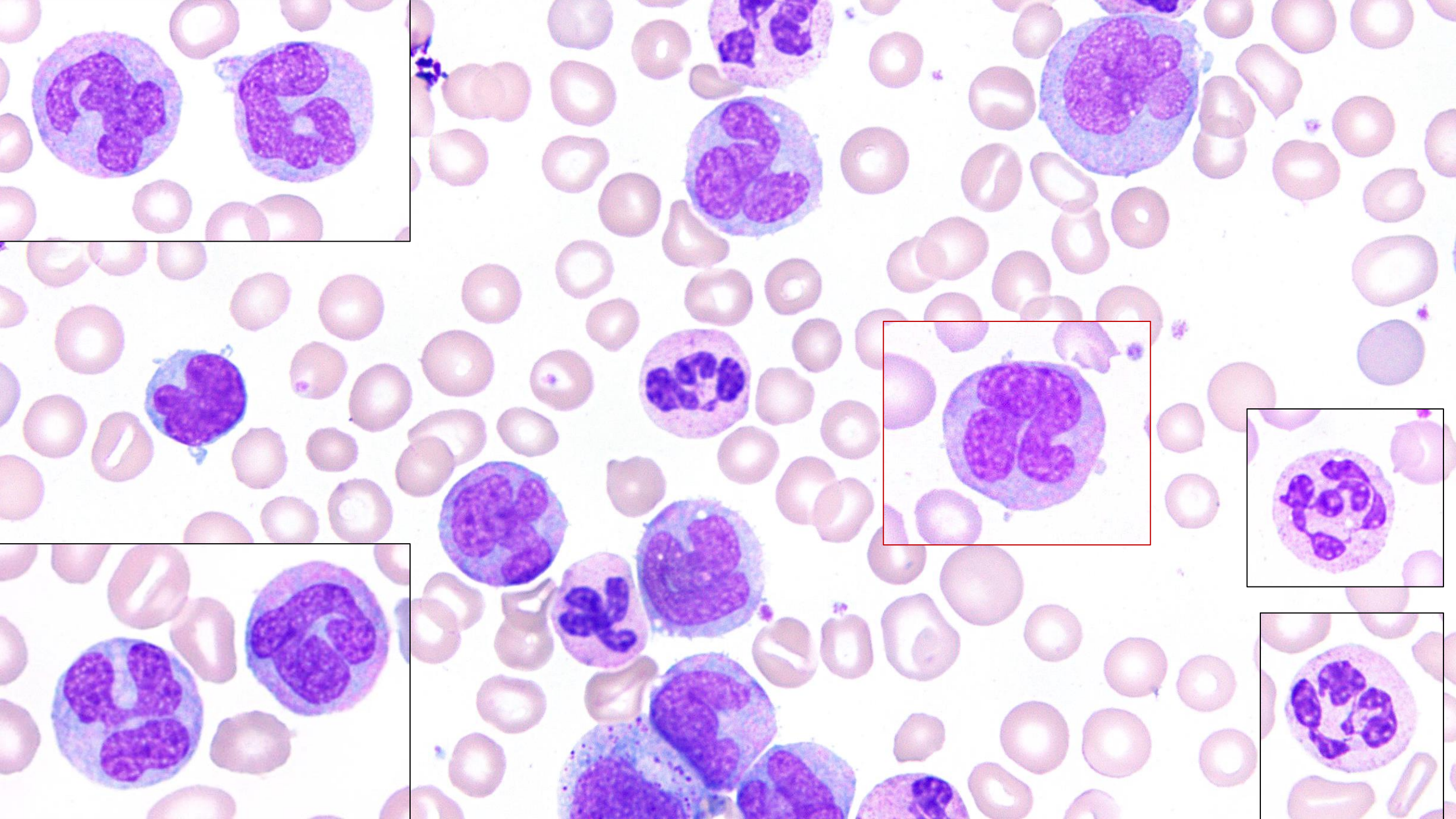
Parameters	Result	Reference range
WBC	24.09 (H)	3.81 – 8.94 K/µL
- Neutrophils	13.49 (H)	1.92 - 7.60 K/µL
- Lymphocytes	1.45	0.72 - 4.10 K/µL
- Monocytes	6.99 (HH)	0.16 - 1.10 K/µL
Hgb	10.2 (L)	12.5 – 16.3 g/dL
HCT	34.4 (L)	37.1 – 49.5%
MCV	81.9	77.6 - 97.0 fL
PLT	100 (L)	152 - 440 K/µL

PMH
- Substance abuse
- Untreated HCV
- COPD

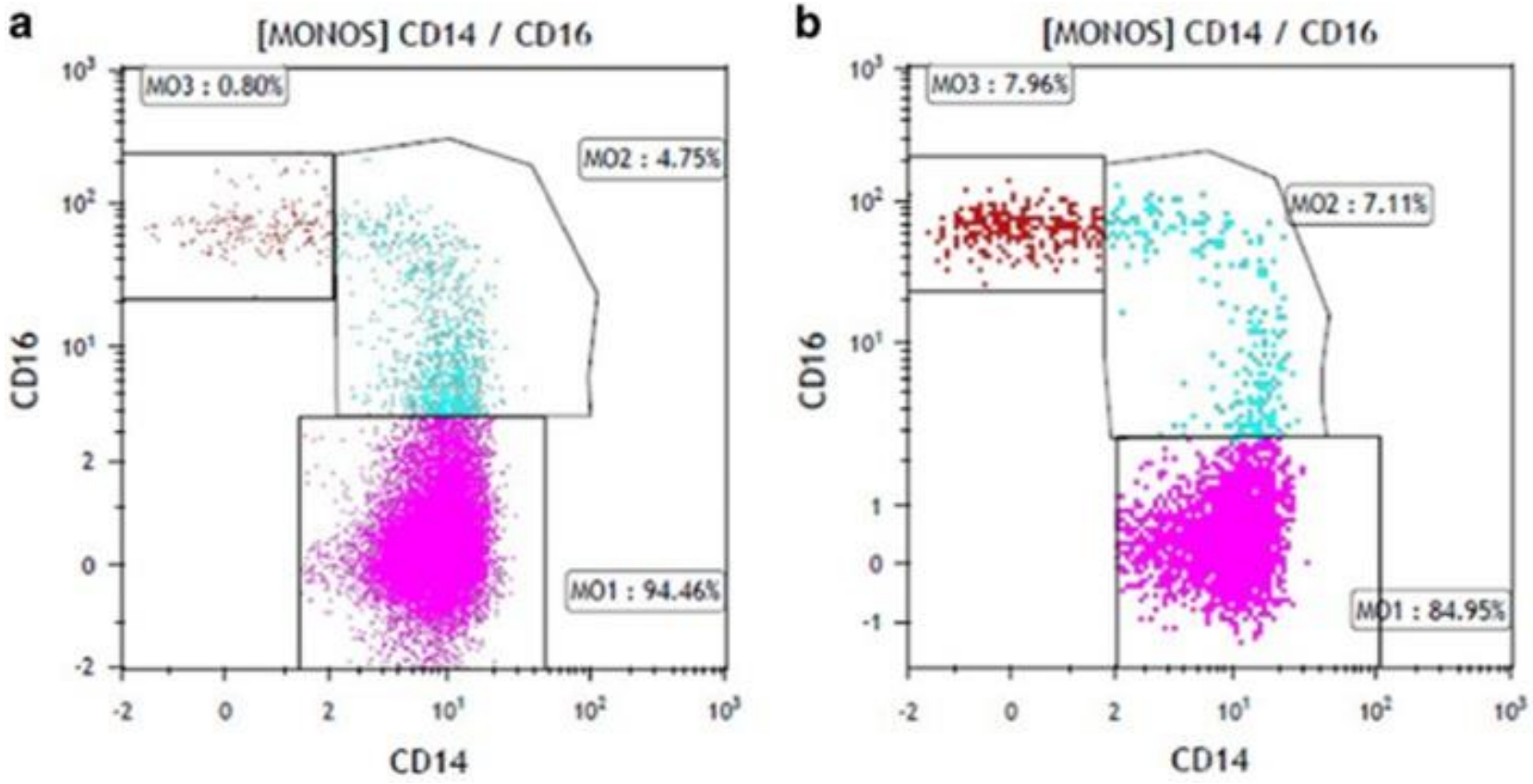
Family history
- Unremarkable

ROS
- Poor appetite
- Night sweats
- Fatigue
- Abdominal distention
- Splenomegaly

Absolute monocyte count > 5 K/µL for the past 4-5 months



Increase in MO1 fraction of >94% in a patient with chronic myelomonocytic leukemia (a) vs reactive monocytosis (b)



Patnaik MM, Timm MM, Vallapureddy R, Lasho TL, Ketterling RP, Gangat N, Shi M, Tefferi A, Solary E, Reichard KK, Jevremovic D. Flow cytometry based monocyte subset analysis accurately distinguishes chronic myelomonocytic leukemia from myeloproliferative neoplasms with associated monocytosis. Blood Cancer J. 2017 Jul 21;7(7):e584. doi: 10.1038/bcj.2017.66. PMID: 28731458; PMCID: PMC5549258.

Genetic Results:

Normal Karyotype:

- 46,XY[20]
  - Excludes
    - Chronic myeloid leukemia – t(9;22)
    - *PDGFRB*-rearranged neoplasm – t(5;12); 5q31-32

NGS:

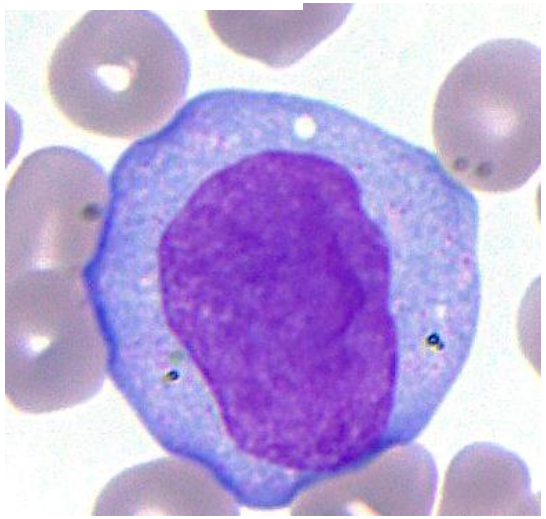
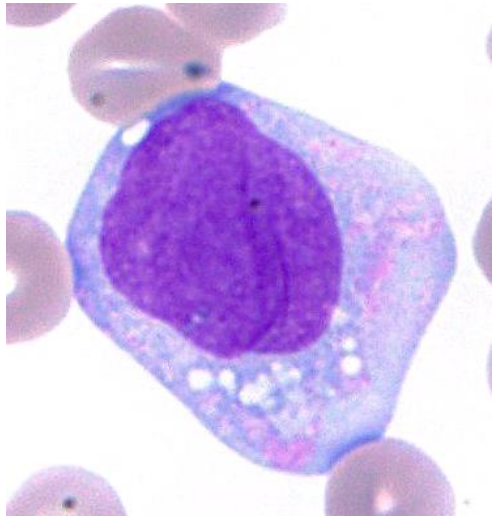
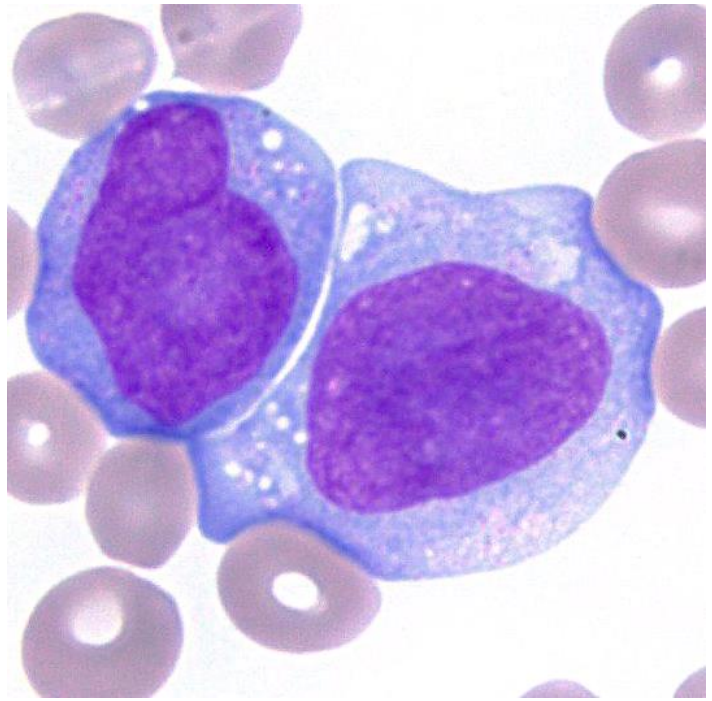
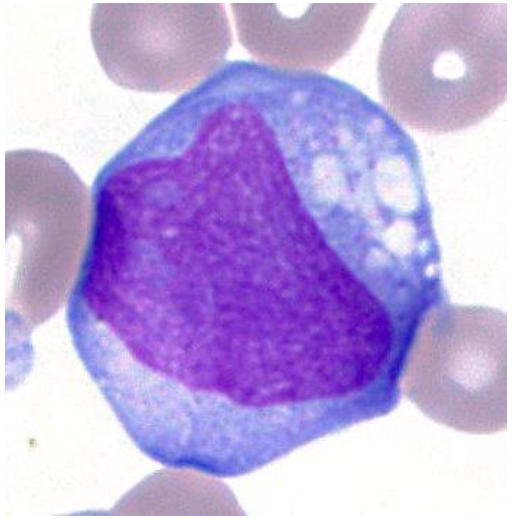
- *TET2 G1275E* VAF 49.7%
- *ASXL1 C856fs** VAF 43.9%
- *CBL C404F* VAF 19.6%
- *KRAS T58I* VAF 15.2%

<u>Same mutations are seen in:</u>

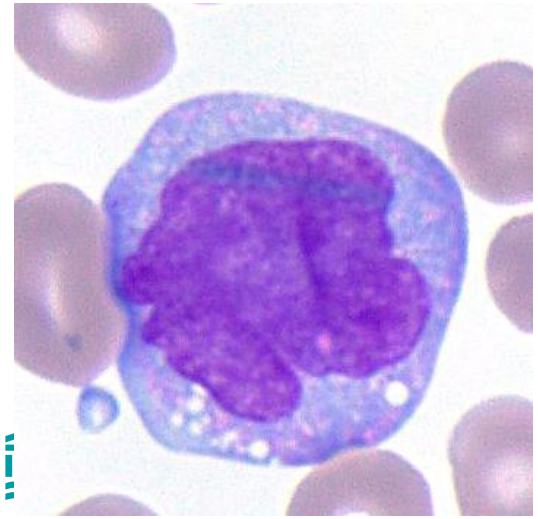
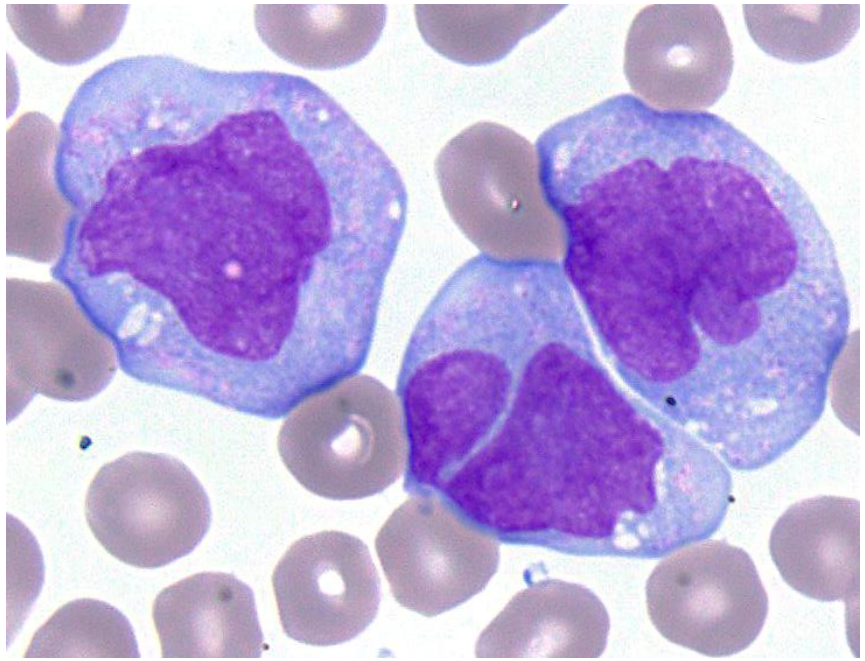
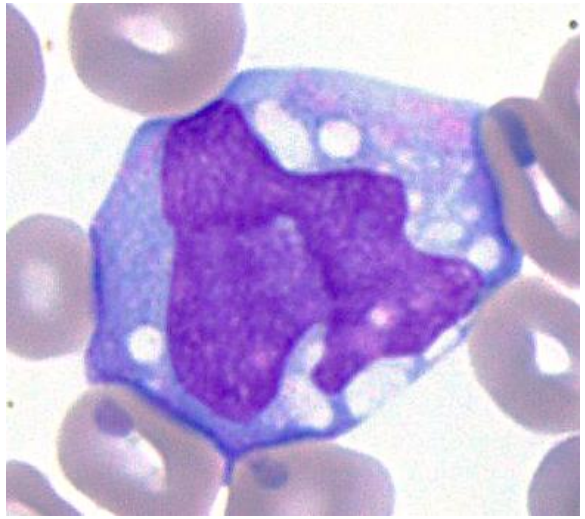
- CMML-1 (<5% PB and <10% BM)
- CMML-2 (5-19% PB and 10-19% BM)
- AML with mono differentiation (>20% PB or BM)

Distinction relies on **<u>MORPHOLOGY</u>**

Monoblasts

Promonocytes

PDGFRB-rearranged myeloid neoplasm presenting as CMML with eosinophilia

CBC results

WBC 18.27 x 10^9/L (H)

- 3% Bands
- 23% Neut
- 12% Lymph
- 13% Mono (2.37) (H)
- 49% Eos (8.95) (HH)

HGB 12.3 g/dL (L)

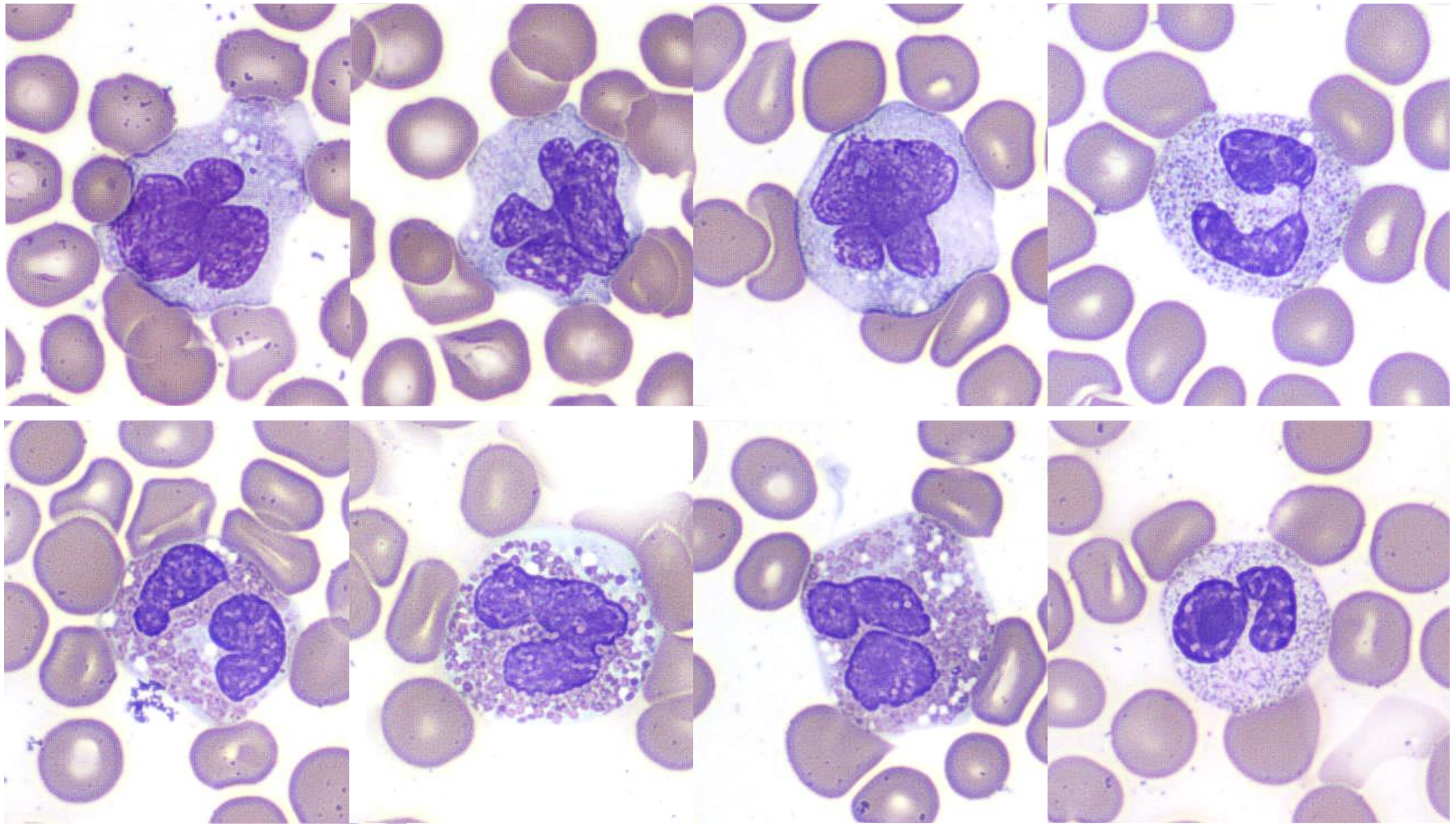
HCT 39.6% (L)

MCV 82.8 fL

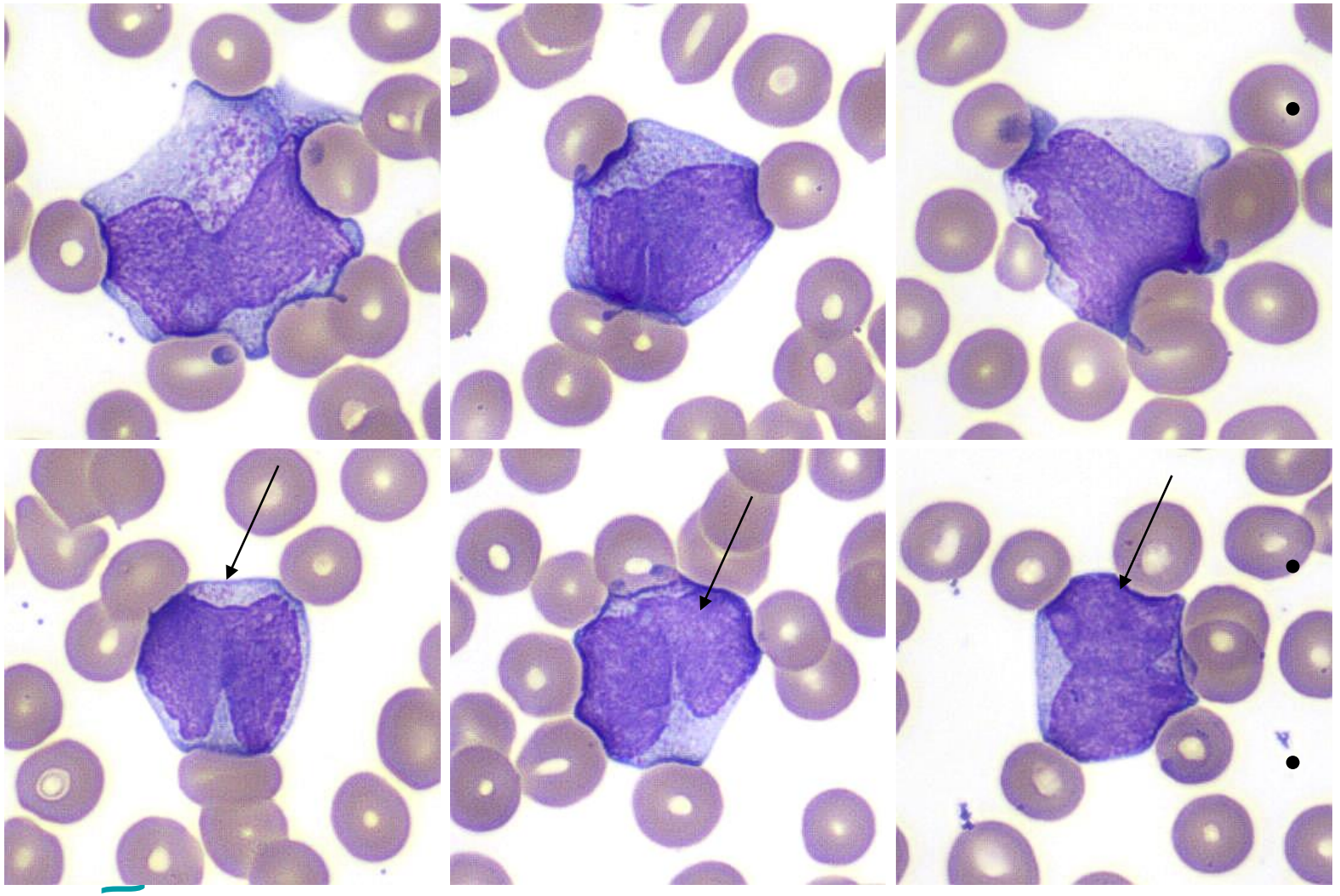
PLT 73 x 10^9/L (L)

Cytogenetics

46,XY,t(5;12)



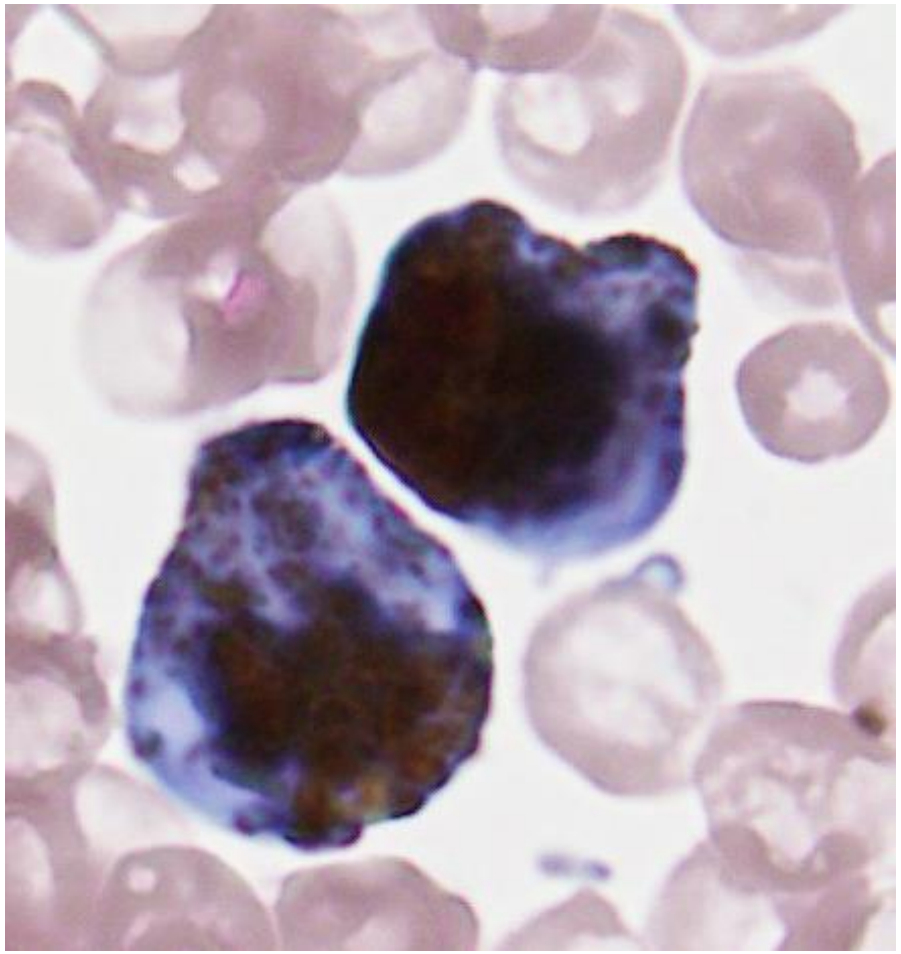
Look alike: Acute promyelocytic leukemia, microgranular variant



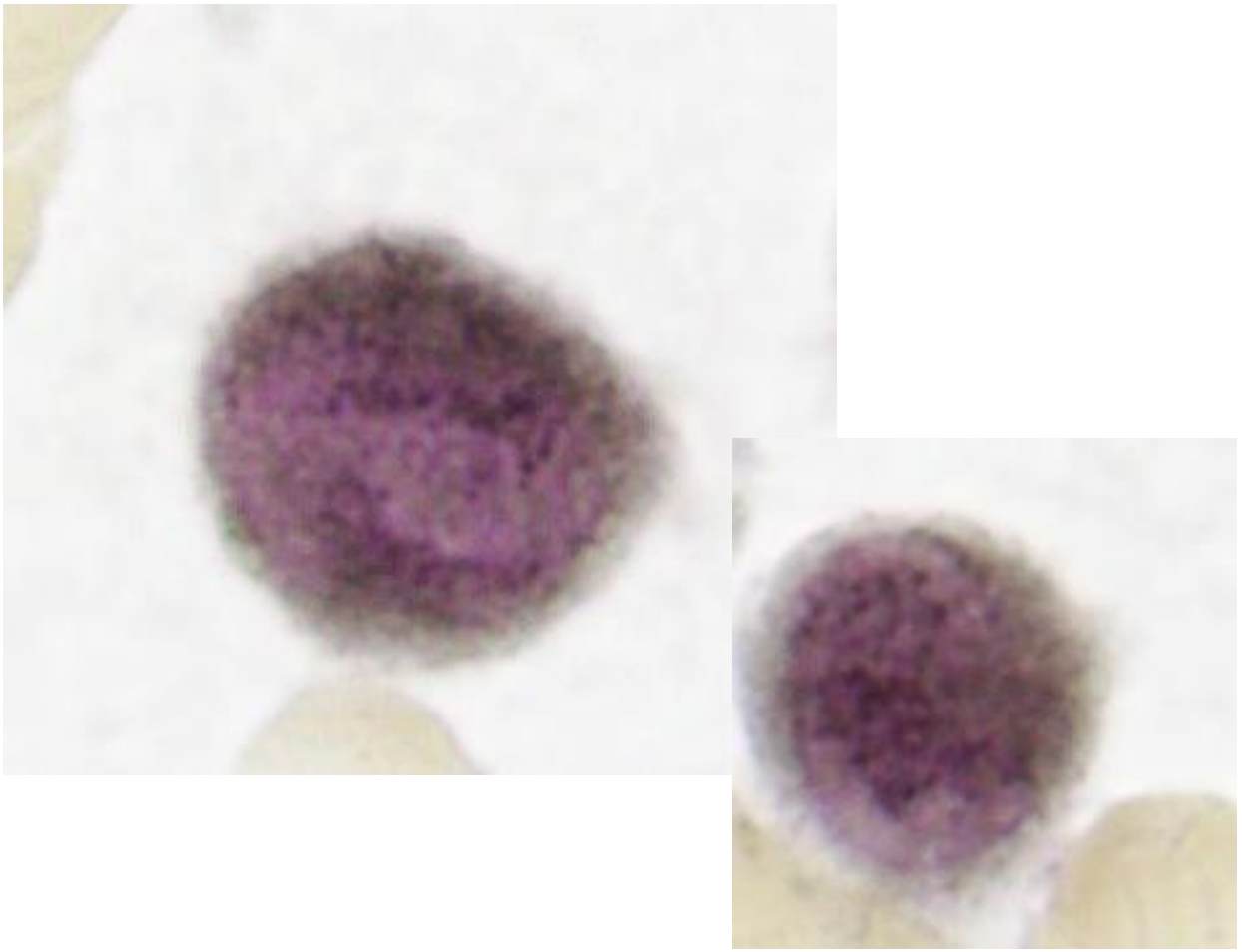
- Imperative to recognize
  - ATRA treatment
  - DIC
- Overlap in morphology
  - Large cells with moderate to abundant cytoplasm with or without fine granules
  - Folded or bi-lobed nuclei
  - More typical granular promyelocytes can be seen
- Phenotype
  - Cytochemistry
  - Flow cytometry
- Karyotype/FISH
  - t(15;17)

Cytochemistry – rapid TAT

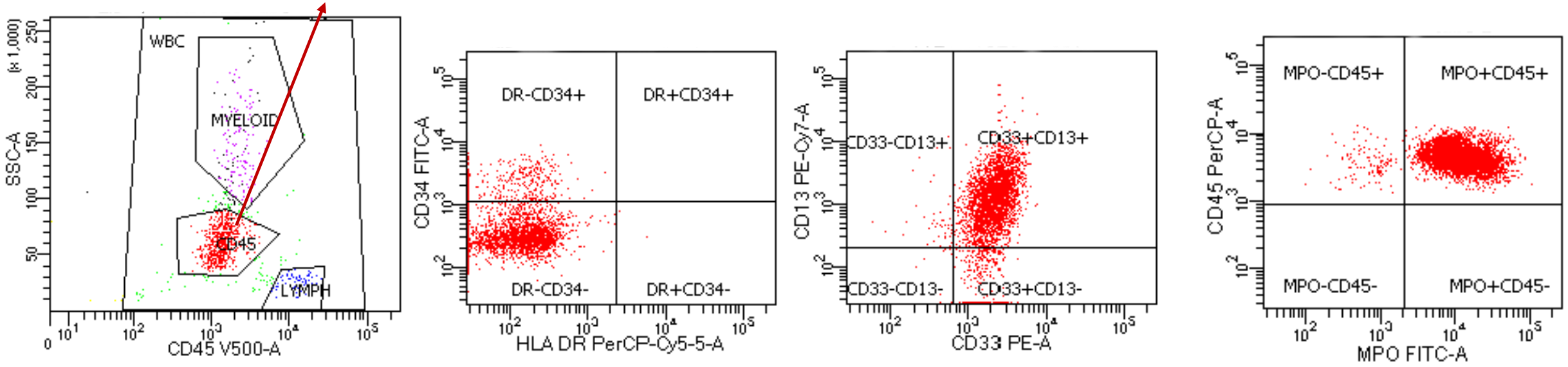
APL:
Strong MPO
Often obscures nucleus



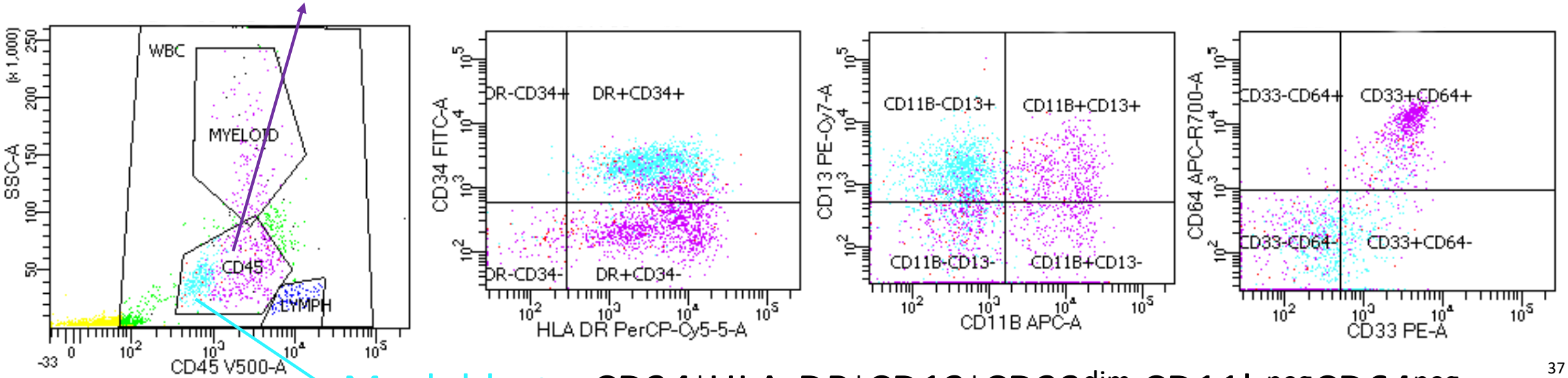
AMoL:
NSE
Variable intensity



Atypical promyelocytes: $CD13^{+}CD33^{+}MPO^{+}$ $CD34^{neg}HLA\text{-}DR^{neg}$



Immature monocytes: $HLA\text{-}DR^{+}CD13^{+}CD33^{+}CD11b^{s+}$ $CD34^{neg}CD14^{neg}$



Myeloblasts: $CD34^{+}HLA\text{-}DR^{+}CD13^{+}CD33^{dim}$ $CD11b^{neg}CD64^{neg}$

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What defines monocytic differentiation?

Morphology:

- Large cells with abundant cytoplasm +/- fine granules
- Absence of Auer rods

Cytochemistry

- Non-specific esterase

Immunophenotype (shared between mature and immature monocytes)

- **CD11b, CD14, CD64, CD68,** CD4, CD13, CD33, CD36, HLA-DR
- Aberrancies: CD34, CD117, CD56 or ↑ or ↓ of normal Ag

Back to our patient

Diagnosis – CMML1

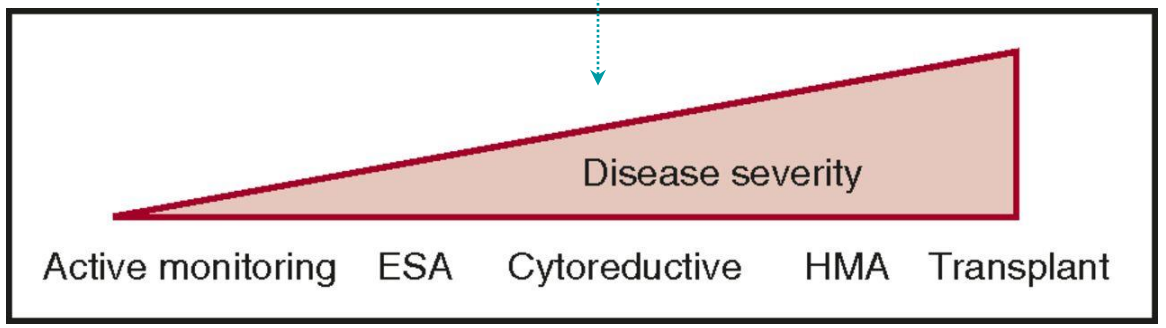
- Promonocyte/monoblast: 0% in PB / 2% in BMA

Higher risk disease/worse prognosis:

- Symptomatic
- High WBC, anemia and low PLT
- *ASXL1/KRAS*

Hydroxyurea treatment

Current therapeutic strategies in CMML



Eric Solary, Raphael Itzykson, How I treat CMML, Blood, 2017

PEARLS: Monocytosis

Persistent (>3 months) monocytosis >0.5 x 10^9/L or >10%, especially when accompanied by anemia and/or thrombocytopenia, is almost always neoplastic

and % have been lowered (5th WHO and ICCS)

Always exclude CML and *PDGFRA/B*, *FGFR1* and *PCM-JAK2*-associated neoplasms (usually, but not always, present with eosinophilia)

karyotype/FISH are essential

Recognize atypical monocytes and assess dysplasia in granulocytes

Remember immature forms/blasts equivalents include monoblasts and promonocytes and ….

Know their morphology well!

Flow cytometry is not helpful

NGS is always needed, when neoplastic monocytosis is suspected to confirm clonality and assess prognosis

Emerging role of flow cytometry to assess monocyte subsets?

The patient is presenting with lymphocytosis …

Lymphocytosis important facts:

>4.0 x 10^9/L or 2SD above mean

Smallest circulating mononuclear cells (7-15 μm):

- High N:C ratio, round or slightly undented nuclei, coarse nuclear chromatin without nucleolus
- Large granular lymphocyte (**normal** constituent; ~5% of all lymphs)
  - 15-20 μm (same size as monocyte), low N:C ratio, round to irregular nuclei, chromatin is less coarse than small lymphocyte, cytoplasm is pale blue with coarse azurophilic granules

Morphologic changes:

- Reactive changes – wide spectrum of morphologies with various shapes, sizes
- Neoplastic changes – monotonous population

Recent viral infection? Symptomatic? Incidental? Lymphadenopathy?

Other CBC changes?

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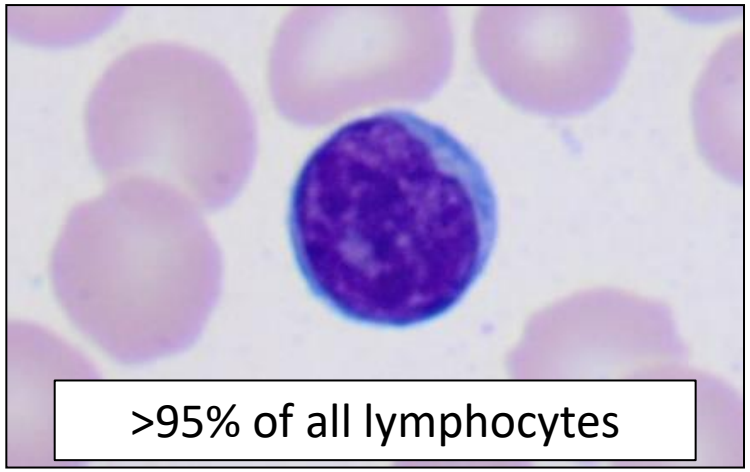
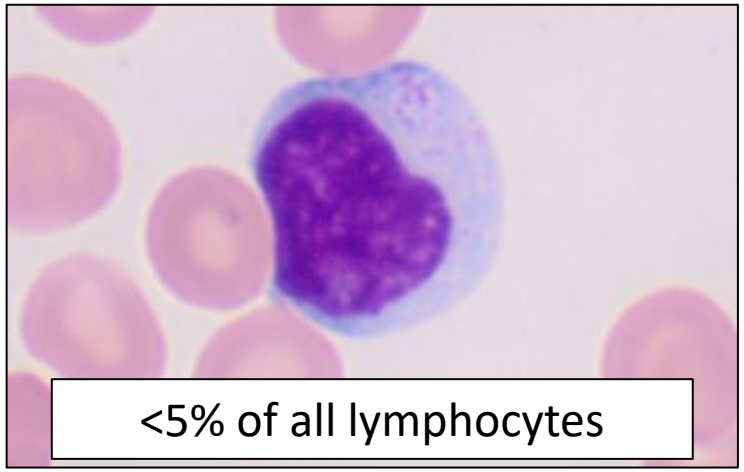
Normal Lymphocytes





	Lymphocyte	Large Granular Lymphocyte
Size	7-15 μm	15-20 μm
N:C Ratio	2:1 - 5:1	1:1 - 3:1
Nucleus	Round or slightly indented; coarse chromatin	Round to irregular; coarse chromatin
Cytoplasm	Blue; scant to moderate; no granules	Pale blue; moderate to abundant; few coarse azurophilic granules
Cell Types	CD4+ T cells, CD8+ T cell, B cell, NK cell	CD8+ T cell, NK cell

Causes of lymphocytosis

Neoplastic/Clonal

Acquired

- LGL
- CLL/MBL
- Other non-Hodgkin lymphomas
- ALL

Congenital B cell lymphocytosis

- *CARD11* mutation

Distribution of clonal *versus* reactive depends on patient's age

Secondary/Reactive

Viral infection

- EBV is most common

Pertussis

Stress

Drug hypersensitivity/DRESS

Persistent polyclonal lymphocytosis

- smoking

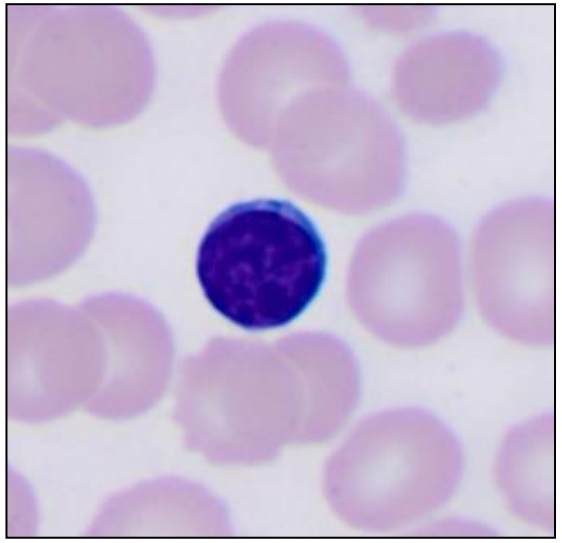
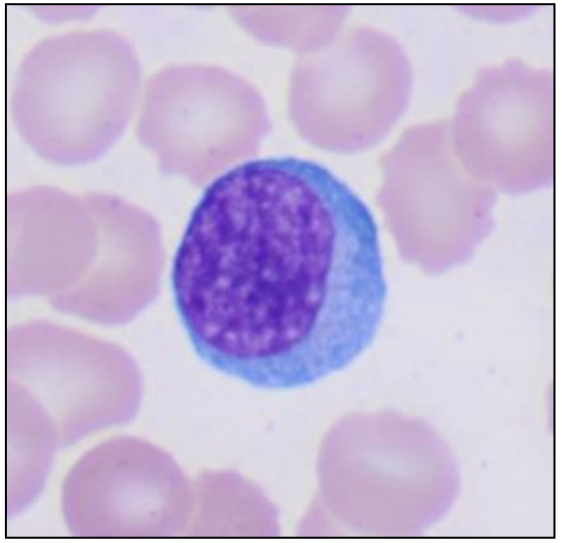
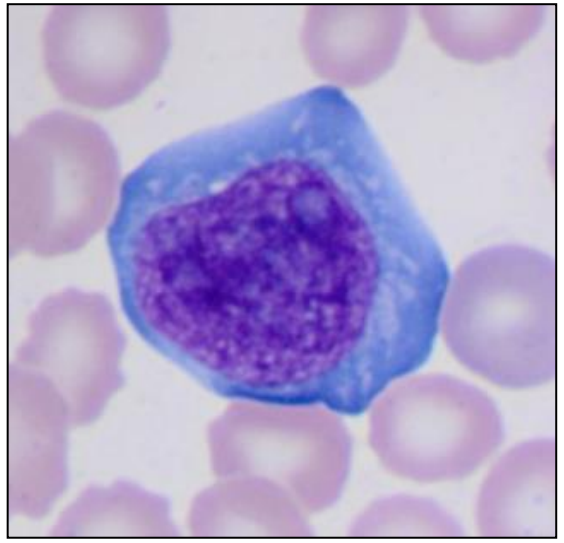
Paraneoplastic syndrome

- thymoma

Asplenia

Reactive Lymphocytosis

- Wide range of lymphocyte morphologies within a given blood smear!
- More abundant cytoplasm (lower N:C ratio), more irregular nuclei, more open chromatin, +/- nucleoli
- Some may be plasmacytoid

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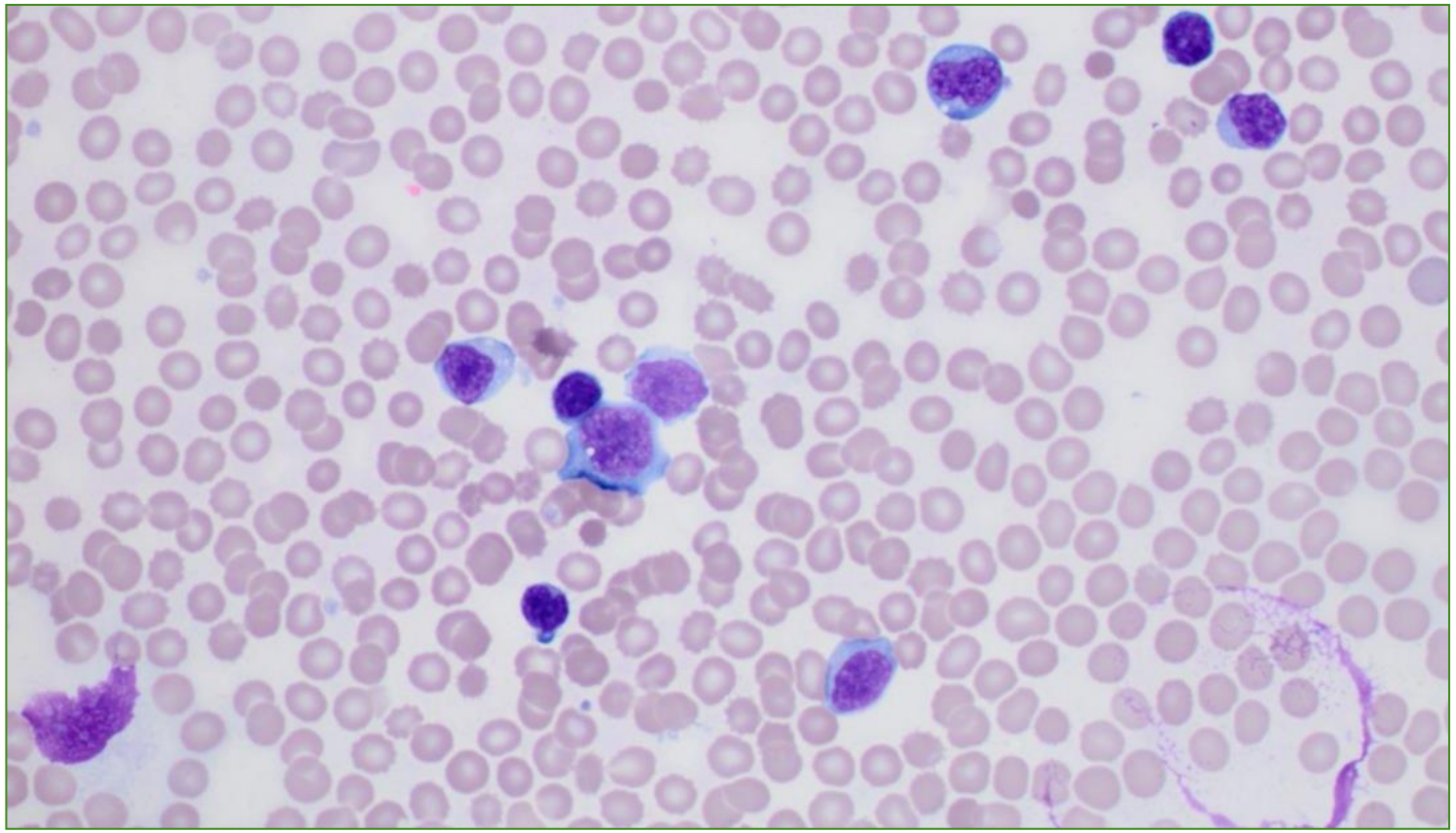
19-year-old college student presenting with lymphocytosis



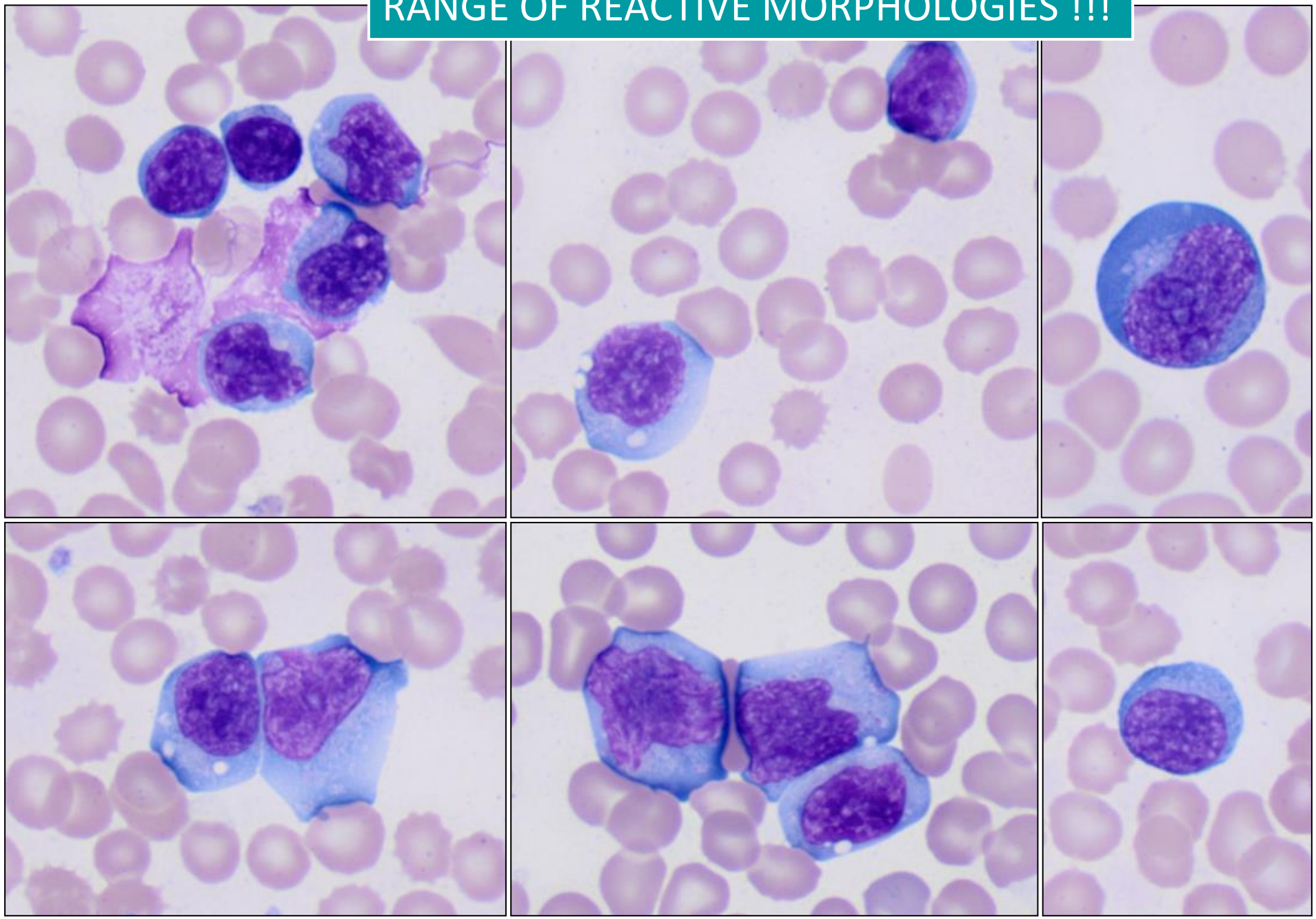
PMH
- Unremarkable

Family history
- Unremarkable

ROS
- Sore throat
- Cervical lymphadenopathy

CBC
- WBC = 26.0 x 10^9/L
- 84% lymphocytes
- PLT/RBC = normal

RANGE OF REACTIVE MORPHOLOGIES !!!



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Downey Classification of Reactive Lymphocytes

Type I

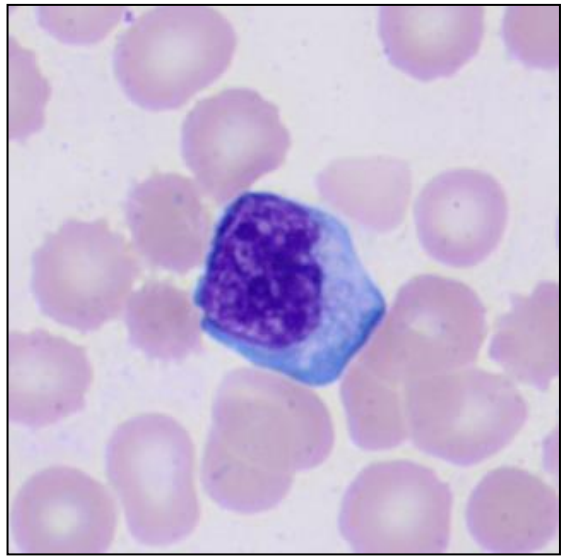
- Smaller size
- Indented to lobulated nucleus
- Cytoplasmic granules

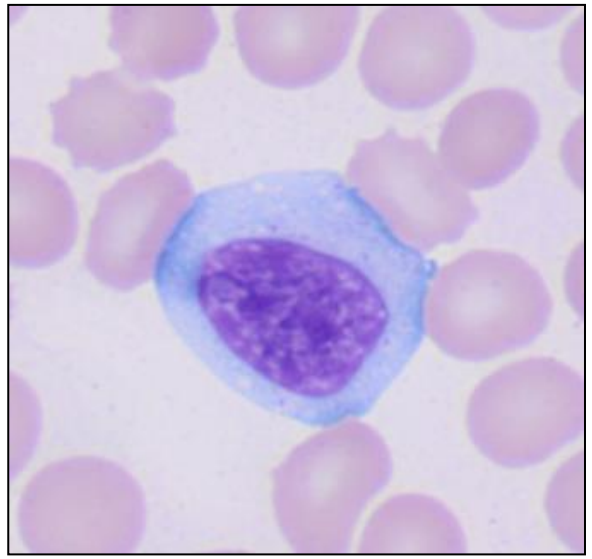
Type II

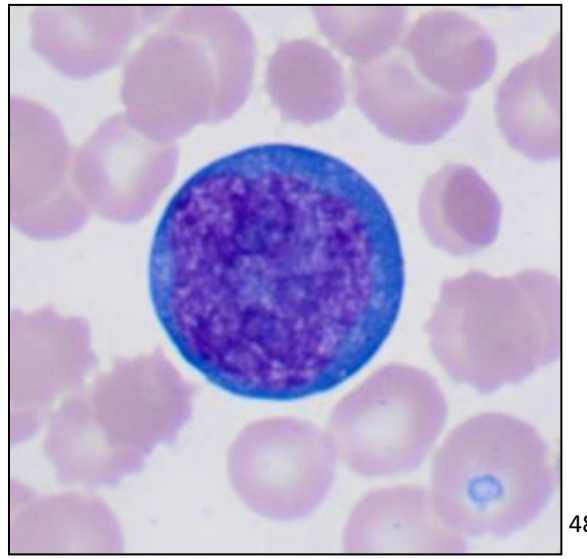
- Most common type
- Abundant agranular cytoplasm, darker at the periphery, molds around RBCs
- Radiating basophilia

Type III

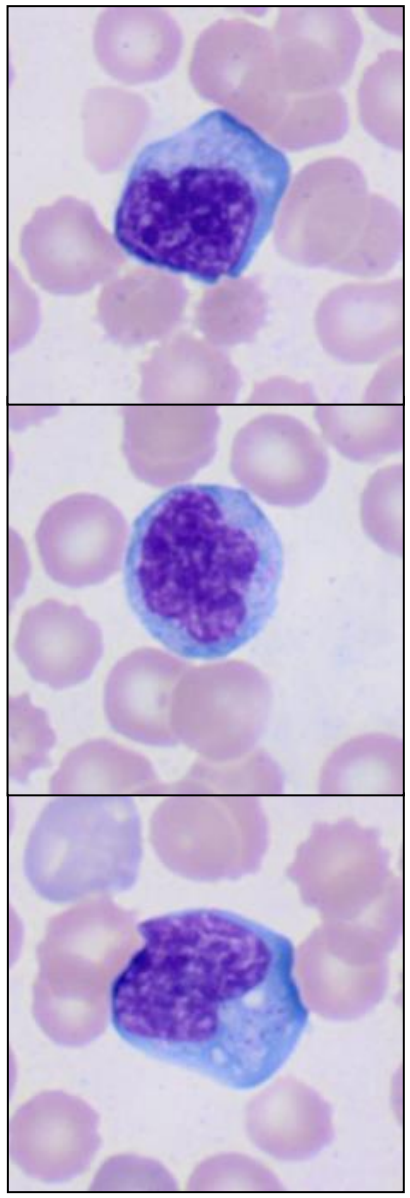
- "Immunoblasts"
- Fine to coarse chromatin
- Nucleoli
- Deeply basophilic cytoplasm



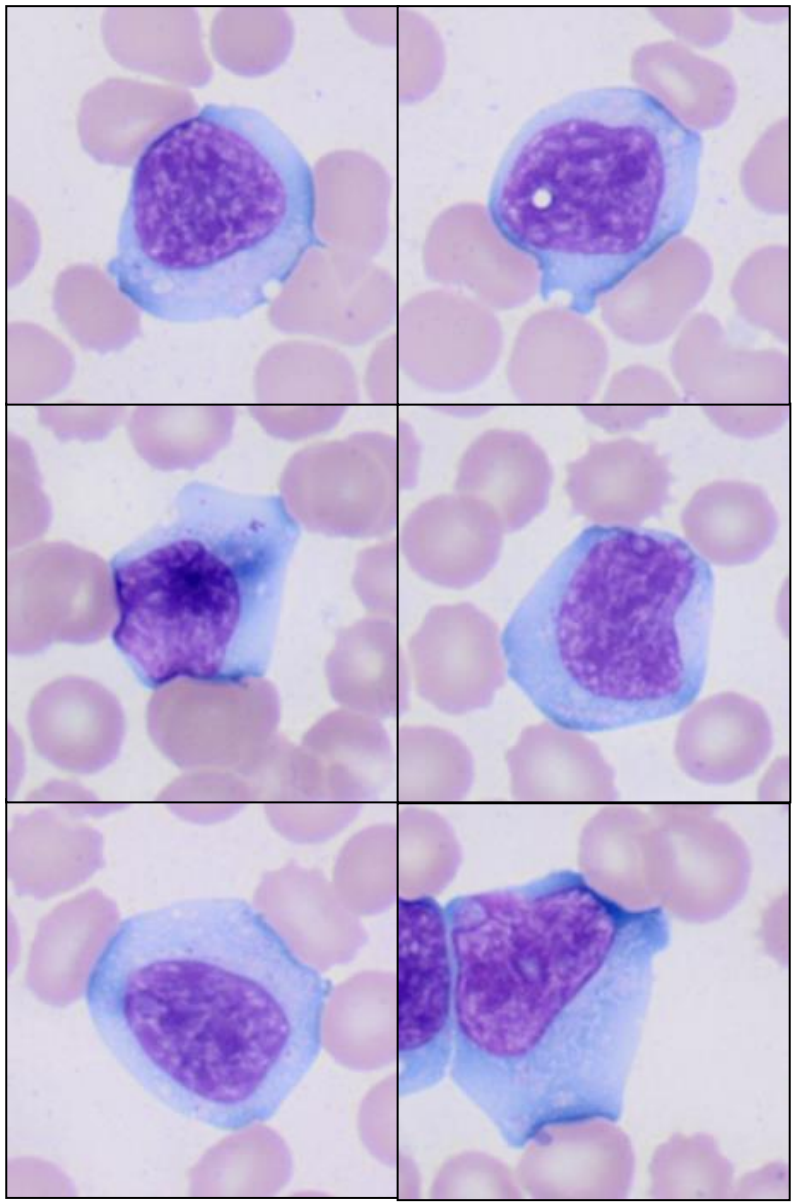




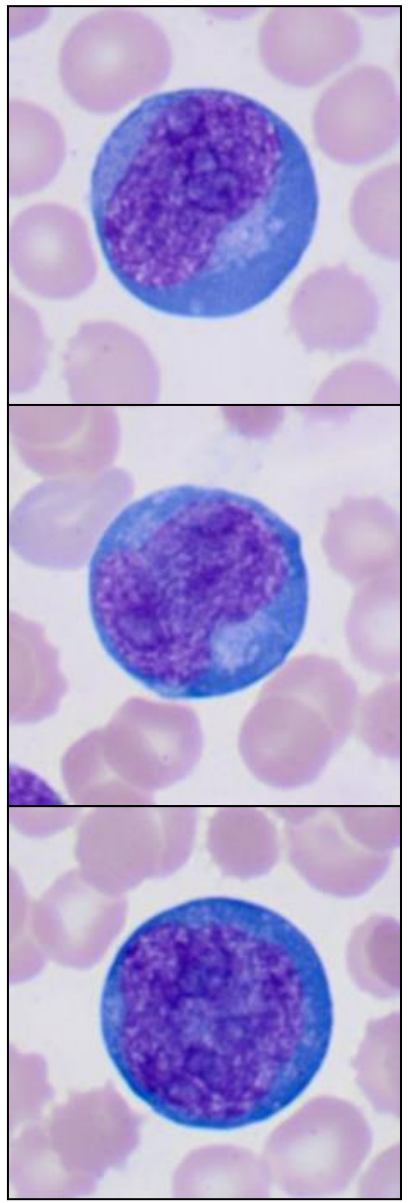
Type I



Type II



Type III



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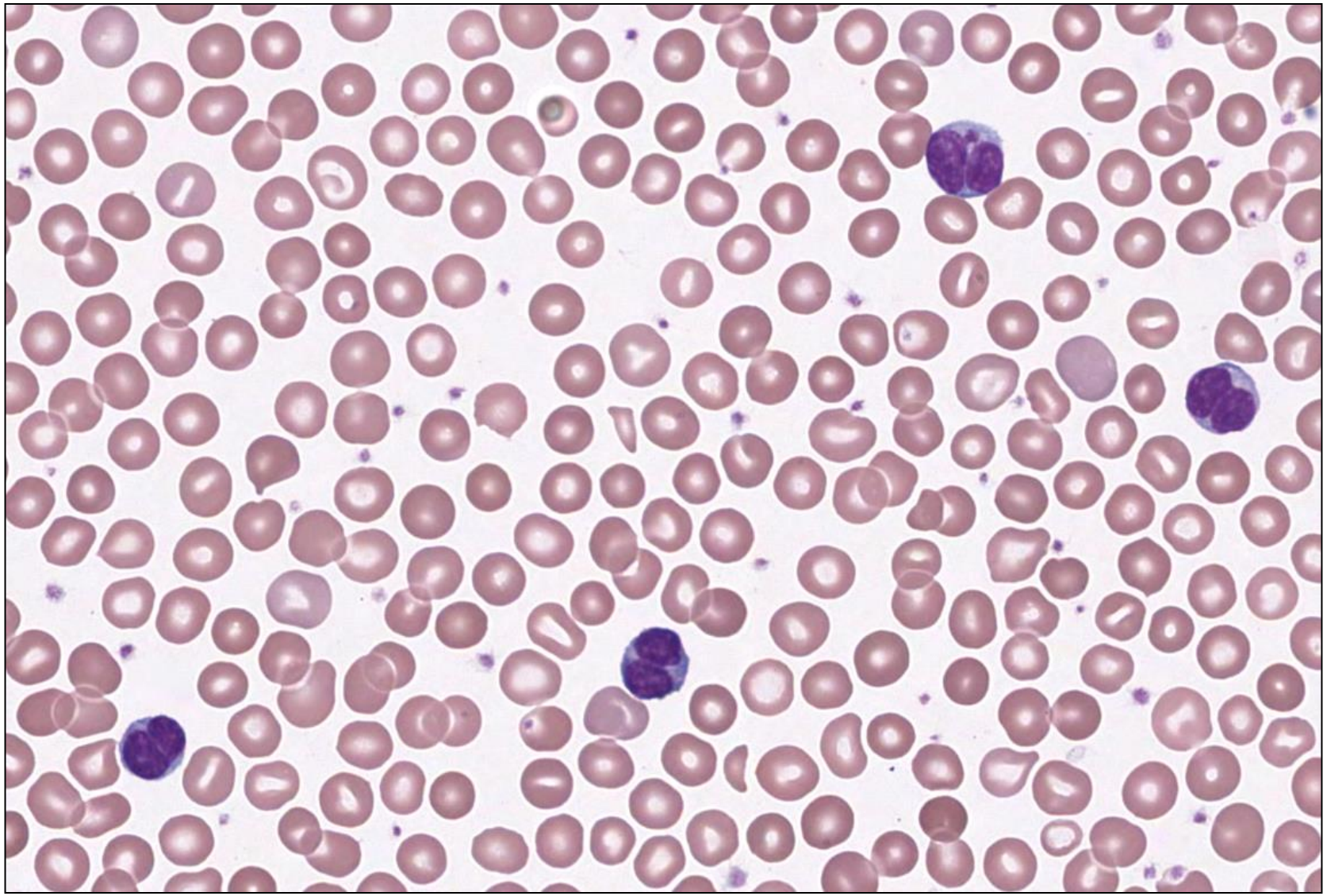
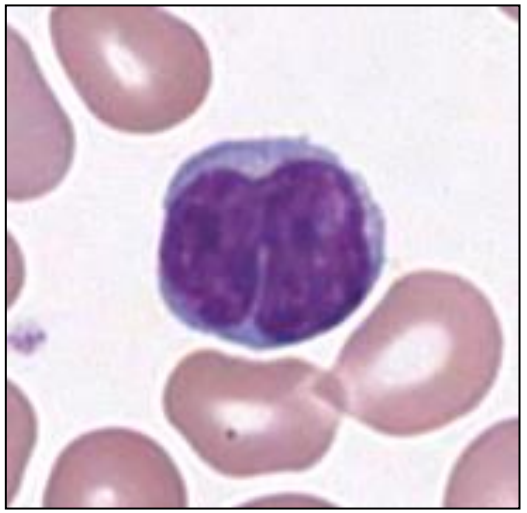
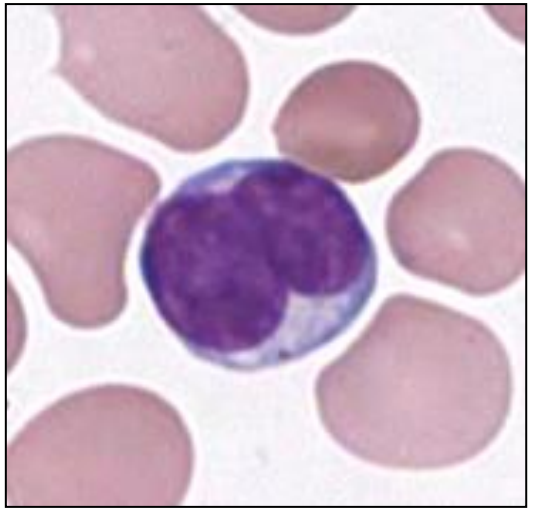
Bordetella Pertussis: Lymphocytosis with "non-reactive" morphology







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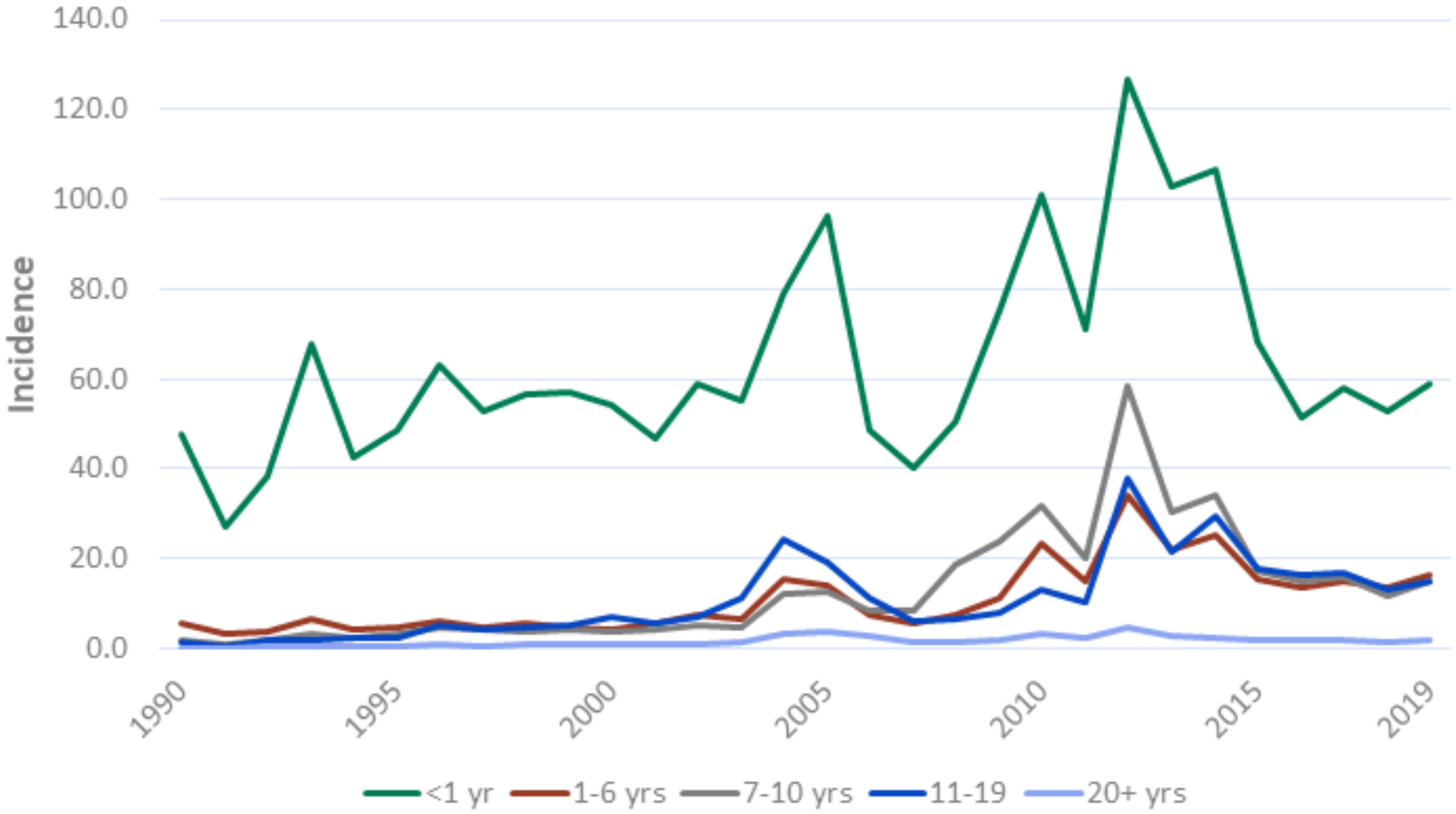
Reported pertussis incidence in the US (per 100,000 persons) by age



https://www.cdc.gov/pertussis/surv-reporting.html

Worrisome morphologic findings

Absence of morphologic range

- Monotonous lymphocytes

Cytoplasmic abnormalities

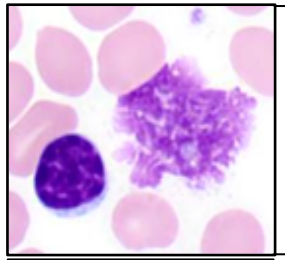
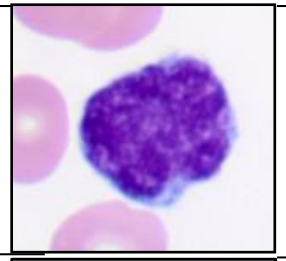
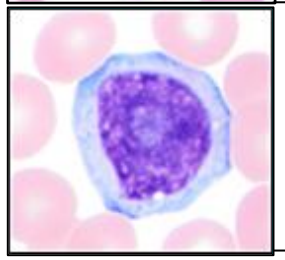
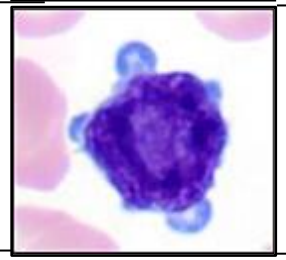
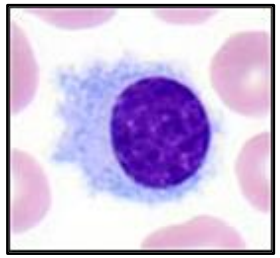
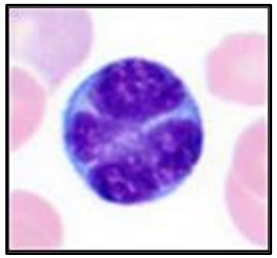
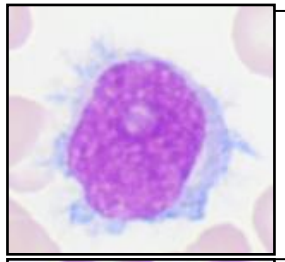
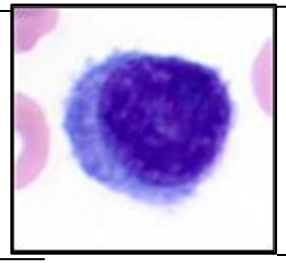
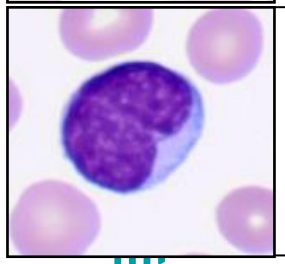
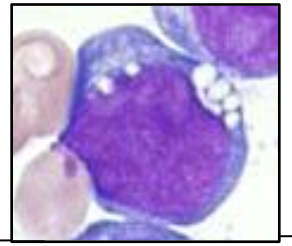
- Projections (villous, hair-like)
- Blebs
- Vacuoles

Abnormal nuclear shapes/structure

- Cleaved
- Cerebriform
- Prominent nucleoli
- Immature chromatin

Presence of unusual cell types or increased number

- Smudge cells
- Spherocytes
- Plasma cells
- Increased large granular lymphocytes

	Usually present in CLL but can be seen in any lymphoid neoplasm, especially presenting with high cell count		Nuclear irregularity, high N:C ratio (MCL, ALL)
	Large cells with prominent single, central nucleolus, may resemble blasts (PLL, MCL, PCL)		Cytoplasmic blebs (T-PLL)
	Often pancytopenia with monocytopenia; hair-like cytoplasmic projections are classic but variably prominent; oval to reniform nuclei (HCL)		“Flower-like” nuclei (ATLL)
	“Hairy prolymphocytes” (HCL-v)		Plasmacytoid, lacks paranuclear hof (PCL)
	Clefted nuclei, may be subtle (FL)		Cytoplasmic vacuoles (BL, ALL)

Flow cytometry plays an essential role in a lymphocytosis work-up

- Clonal versus reactive
- T or B or NK lineage
- Mature lymphoma versus acute leukemia
- Suggests a diagnosis based on immunophenotypic profile
- May suggest an underlying genetic alteration
- Helps with subsequent ancillary testing

PEARLS: Lymphocytosis

Distribution or reactive and neoplastic lymphocytosis depends on patient's age	More likely to be neoplastic in older age
Morphology is very important in lymphocytosis assessment	Accurate assessment reduces unnecessary testing
Wide range of morphologies within a smear is usually a sign of a reactive process	
Know morphologic abnormalities associated with a neoplastic process	Ancillary testing will be needed for diagnosis
Flow cytometry is essential	Suggests diagnosis Helps with triaging for further testing

Objectives

Correctly identify white blood cells morphologic abnormalities in peripheral blood smears.

What – Know what normal is (i.e. LGL)

When – Only a subset of PB need review

Where – Always check clinical history!

Why – Clues to patient's condition

Formulate an appropriate and comprehensive differential diagnosis for abnormal morphologic findings that includes neoplastic and reactive processes.

Reactive >>> Neoplastic

Worrisome findings – persistent, monotonous, abnormal morphology

Understand the role of ancillary testing, especially flow cytometry, in a work-up of patients presenting with abnormal peripheral blood morphologic findings

Neutrophilia and monocytosis – molecular > flow cytometry

Lymphocytosis – flow cytometry > molecular


Mass General Brigham