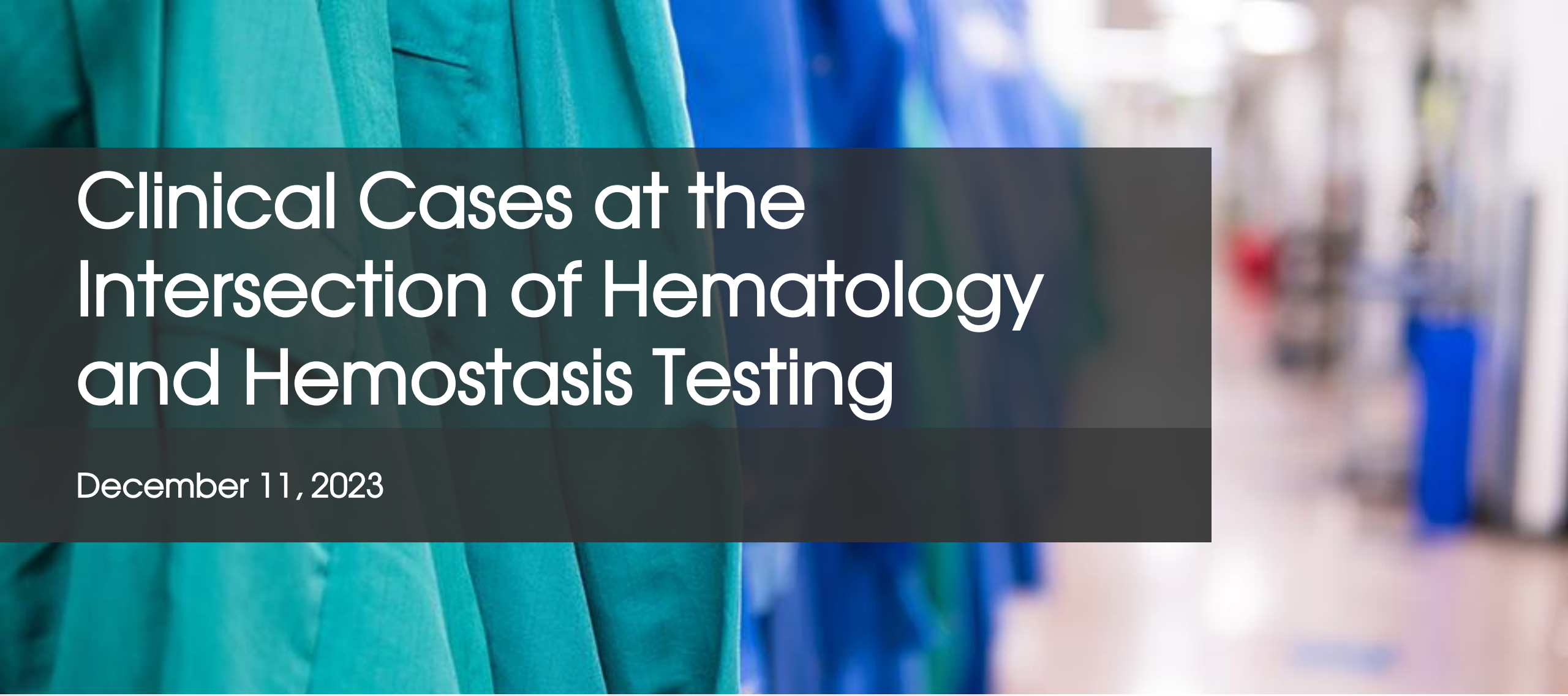

Clinical Cases at the Intersection of Hematology and Hemostasis Testing

December 11, 2023

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Objectives

- List diseases that may require both morphology assessment and hemostasis tests for diagnosis.
- Discuss how hematology and hemostasis tests can be interpreted together for comprehensive diagnosis of bleeding and clotting disorders.
- Identify key peripheral blood morphologic features that may suggest an underlying disorder of hemostasis.

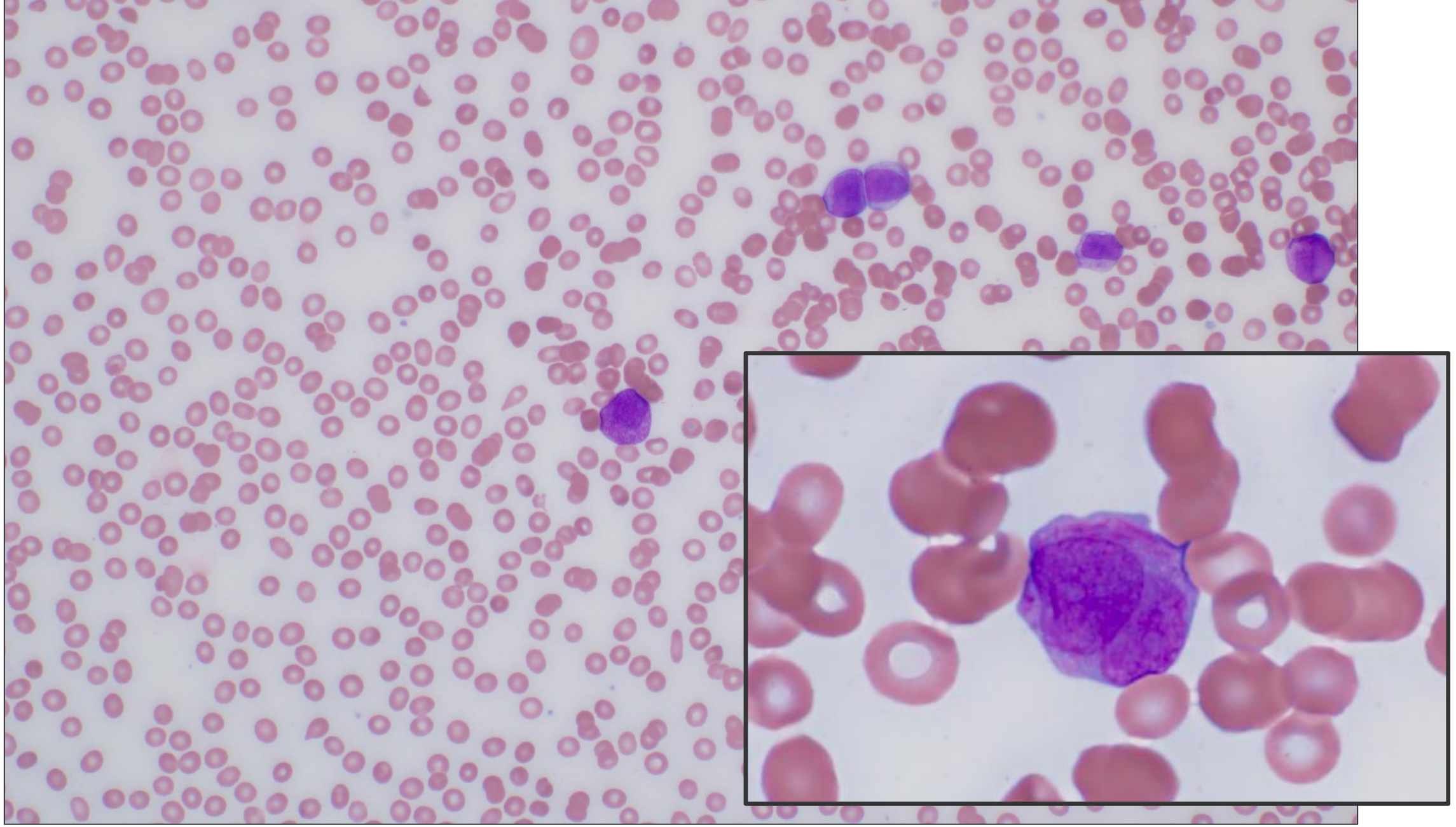
Case 1

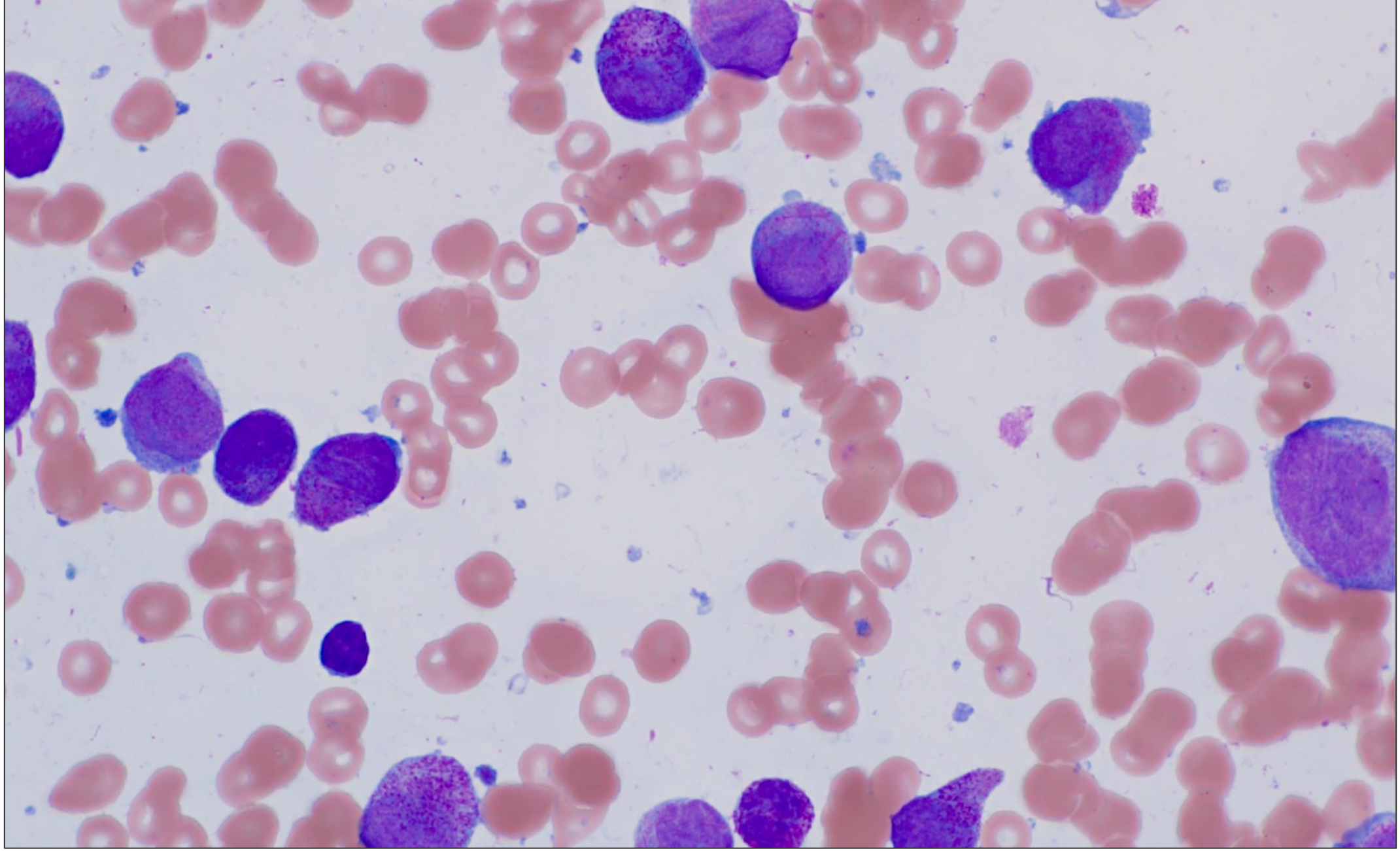
Clinical History

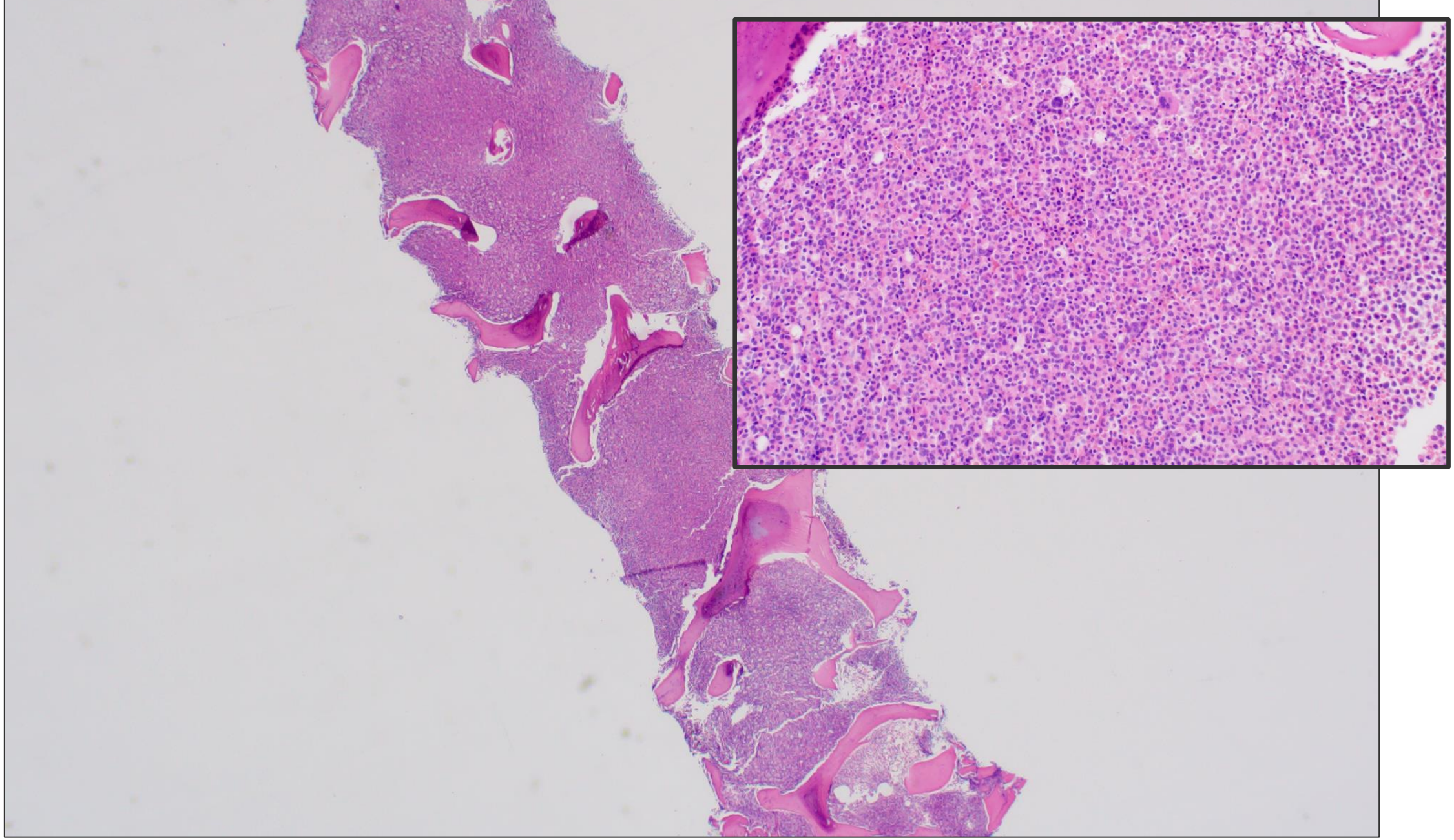
- 26-year-old male with history of
  - » Syncope
  - » Several days of gum bleeding following a dental procedure. He does not have a history of easy bruising or bleeding previously.

CBC data

Test	Result
WBC	16.86 K/µL
RBC	2.58 M/µL
Hgb	7.7 g/dL
Hct	22.3%
MCV	86.4 fL
MCHC	34.5 g/dL
RDW	14.4%
Platelets	59 K/µL







Flow Cytometry Data

- Atypical cell immunophenotype
  - » Positive for: partial CD11b, CD13, partial CD15, CD33, CD38, low CD45, partial CD65, CD117, CD123, myeloperoxidase
  - » Negative for: B- and T-cell markers, CD14, CD34, HLA-DR, TdT
- FISH detected t(15;17) PML::RARA
- Diagnosis: Acute promyelocytic leukemia (APL) with PML::RARA fusion

- But what about the bleeding presentation?
  - » Thrombocytopenia is part of the puzzle and could be due to decreased production from marrow replaced by blasts—is there anything else?

Additional Laboratory Data

Test	Result
D-dimer	68.7 ug/mL FEU
Fibrinogen activity	246 mg/dL
PT	20.0 s
aPTT	44 s

DIC in APL

- Prevalence varies in different reports, with some reports indicating features of DIC are present in up to 90% of cases
- Risk of bleeding complications is highest prior to start of treatment and in early days of treatment
- Risk factors for bleeding include
  - » Age >60 years
  - » WBC >10 10^9/L
  - » Blast count >30 10^9/L
  - » Fibrinogen <100 mg/dL
  - » Poor performance status (according to clinical scoring criteria)
  - » Impaired renal function

ten Cate H, Leader A. Haemostaseologie 2021; 41:120-126.
Kwaan HC, Weiss I, Tallman MS. Semin Thromb Hemost. 2019;45:612-621.

Activation of Coagulation in APL

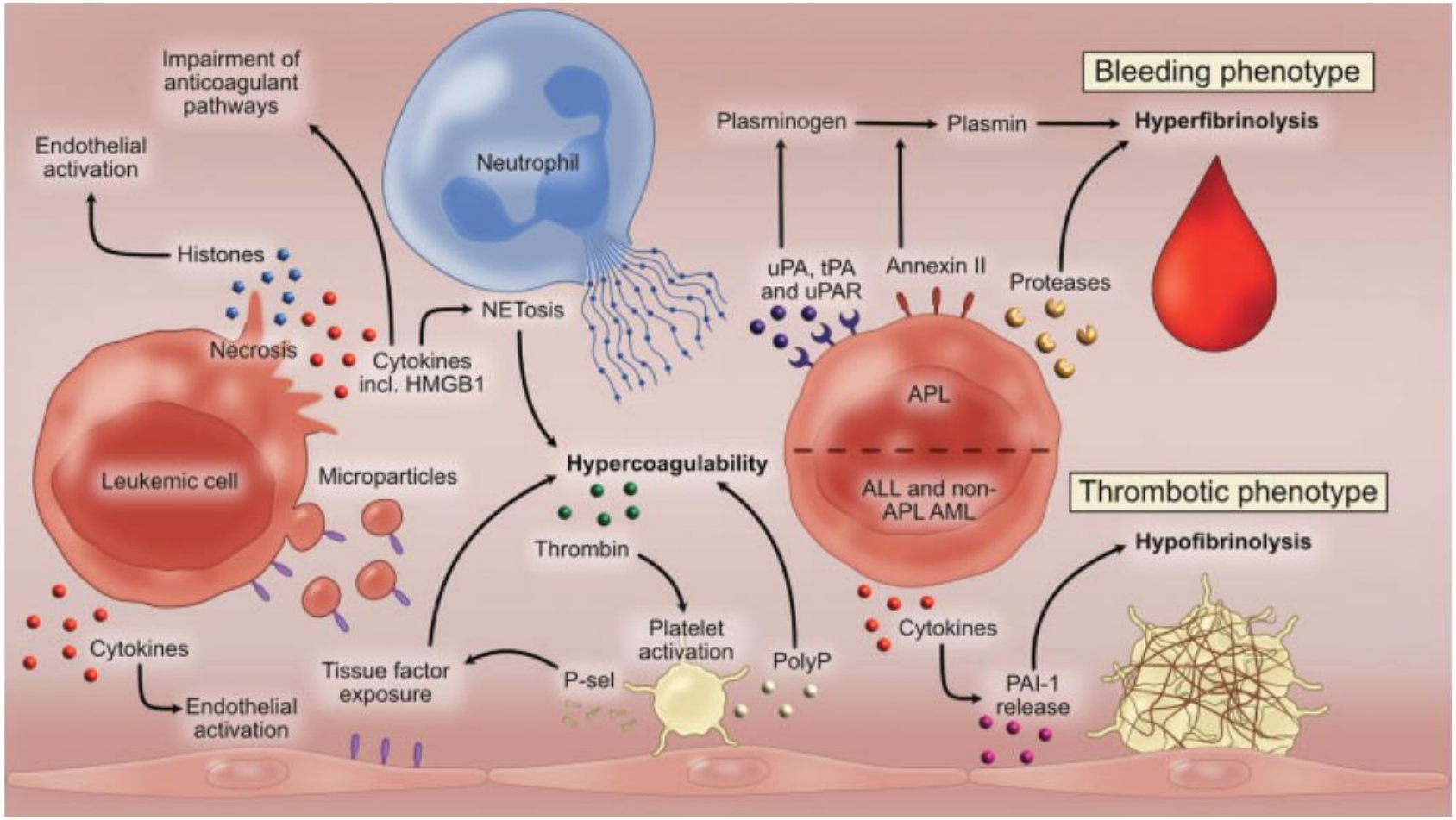


Figure: Figure 1 from ten Cate H, Leader A. Haemostaseologie 2021; 41:120-126.
Other references: Kwaan HC, Weiss I, Tallman MS. Semin Thromb Hemost. 2019;45:612-621.
Barbui T, Finazzi G, Falanga A. Blood. 1998; 91(9):3093-102.

Hematologic Malignancies and Coagulation Disorders

- Many different associations, including
  - » Acquired von Willebrand syndrome and myeloproliferative neoplasms (MPN)
  - » Thrombosis in MPN with high platelet counts
  - » Thrombosis in multiple myeloma (particularly with certain treatment combinations)
  - » Acquired factor X deficiency in multiple myeloma or amyloidosis

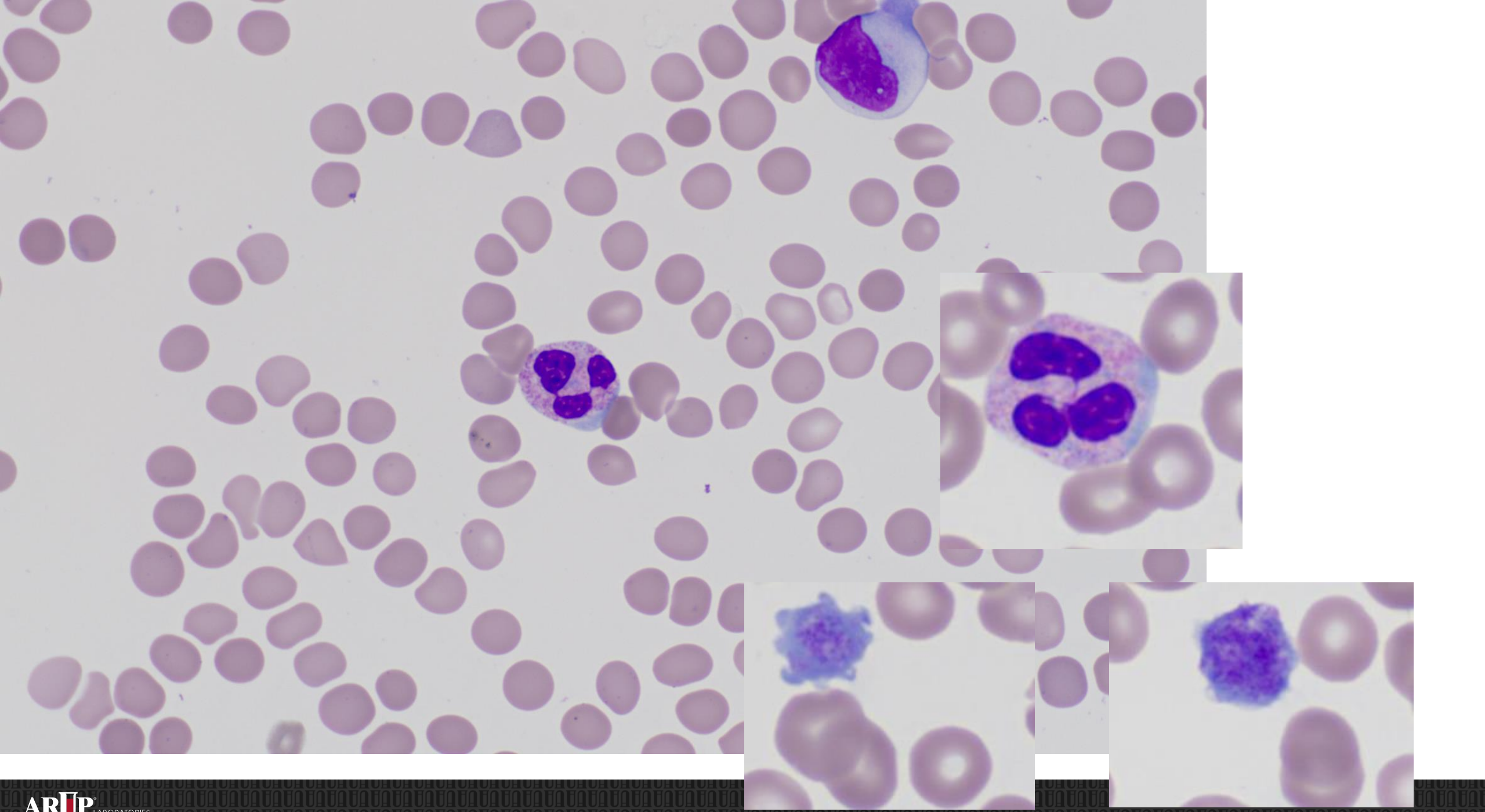
Case 2

Clinical History

- 20-year-old male with history of
  - » End stage renal disease (MPGN-like), s/p renal transplant 2012
  - » Sensorineural hearing loss, s/p cochlear implant
  - » Chronic anemia and thrombocytopenia (platelets often <10 K/µL)

CBC data

Test	Result
WBC	4.9 K/µL
RBC	3.56 M/µL
Hgb	9.6 g/dL
Hct	29.5%
MCV	82.9 fL
MCHC	32.5 g/dL
Platelets	1 K/µL
Absolute neutrophil count	3.4 K/µL
Absolute lymphocyte count	0.8 K/µL



What is the diagnosis?

- MYH9 related disorder
  - » Documented pathogenic mutation in MYH9

What are MYH9-related disorders?

- History
  - » 1909- May described family with large platelets but no-minor bleeding symptoms
  - » 1945- Hegglin described families with AD giant platelet disorder and Dohle body-like inclusions in leukocytes
    - May-Hegglin anomaly
  - » 1972- Epstein syndrome- giant platelets, deafness, nephritis
  - » 1985- Fechtner syndrome- large platelets, interstitial nephritis, cataracts, deafness, leukocyte inclusions (small)
  - » 1990- Sebastian platelet syndrome- large platelets and small leukocyte inclusions (cataracts and hearing loss identified in family members in follow up after 18 years)

What are MYH9-related disorders?

- 1999- inheritance of all these disorders linked to same region on chromosome 22q
- 2000- mutations in MYH9 gene identified as underlying cause

- Now unified category of MYH9-related disorders/ macrothrombocytopenias includes
  - » May-Hegglin anomaly
  - » Epstein syndrome
  - » Fechtner syndrome
  - » Sebastian platelet syndrome

What does MYH9 encode?

- Heavy chain of non-muscle myosin IIA (myosin-9)

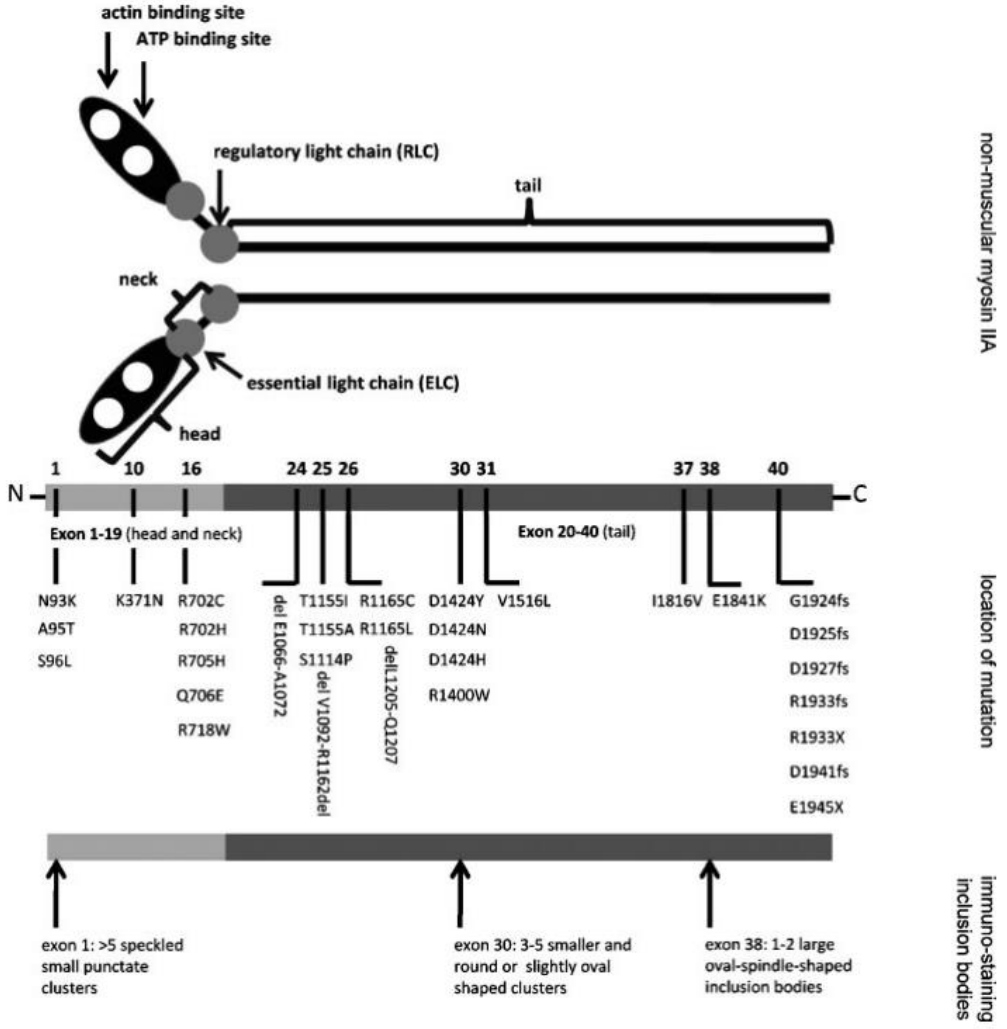
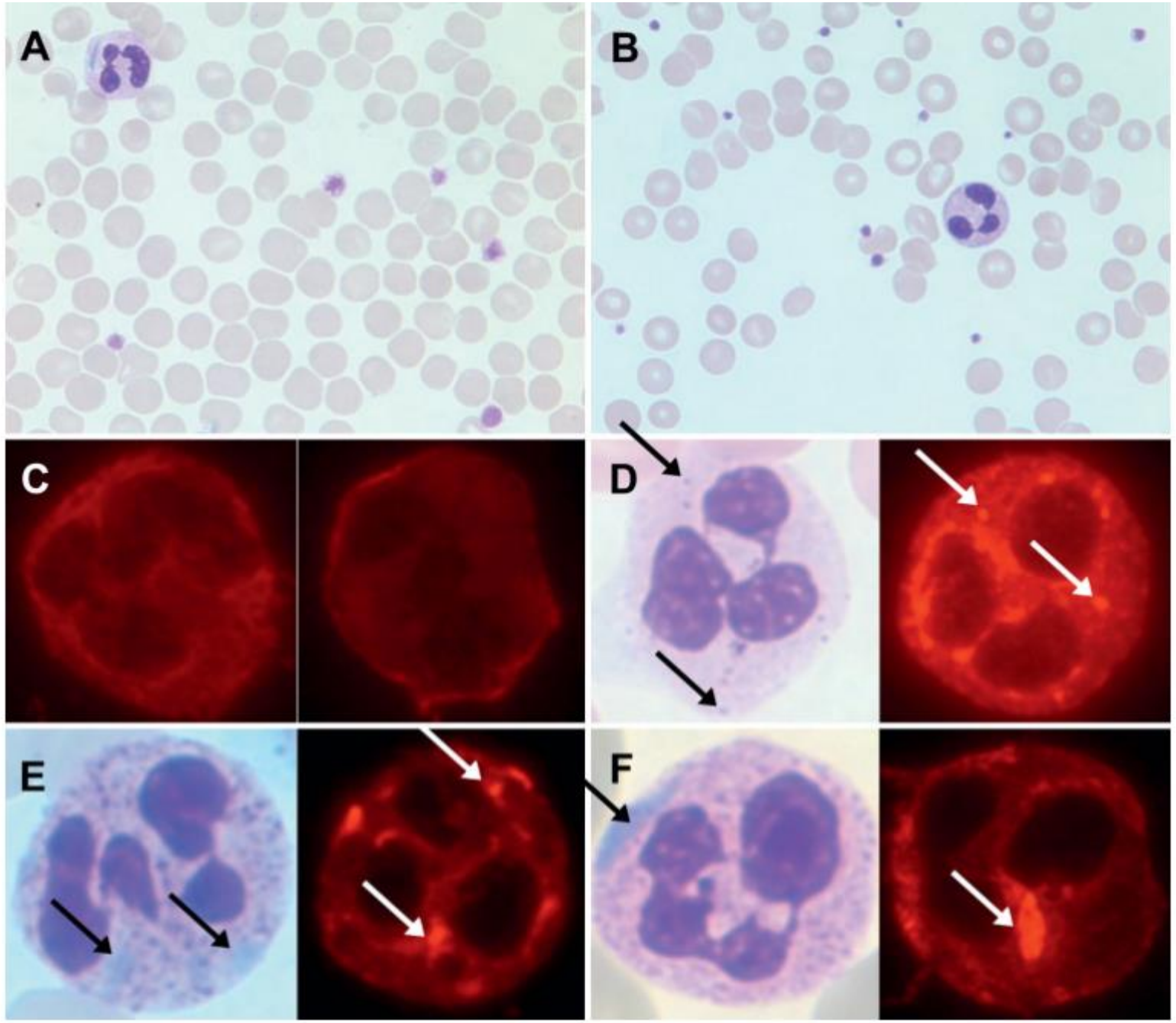


Figure from:
Althaus K, Greinacher A. Semin Thromb Hemost. 2009; 35:189-203.



Immunofluorescence with anti-NMM-IIA antibody shows abnormal deposits (D, E, F).

Figure from: Althaus K, Greinacher A. Semin Thromb Hemost. 2009; 35:189-203.

Why do patients with MYH9-related disorders bleed?

- Low platelet count
- Reduced clot stability- abnormal platelet cytoskeleton
- Giant platelets flow in the middle of vessels
- Anemia (if concurrent) makes things worse
  - » RBCs can't push platelets to vessel wall
  - » Less RBC contribution of arachidonic acid and ADP for platelet activation

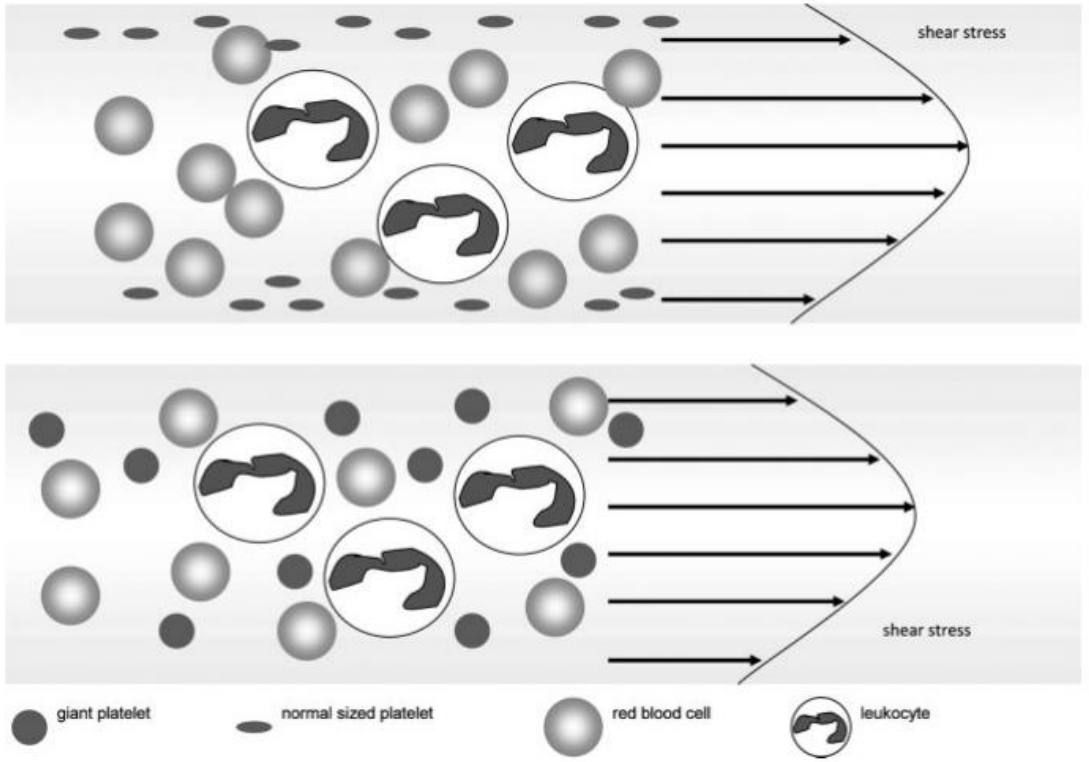


Figure from:
Althaus K, Greinacher A. Semin Thromb Hemost. 2009; 35:189-203.

What about platelet function?

- Platelet function studies usually normal in MYH9-related disorders
  - » Platelet aggregation
    - May not be possible to perform if platelet count is very low
  - » PFA-100/200

- Platelet aggregation testing can be helpful to distinguish MYH9-related disorders from Bernard-Soulier syndrome (BSS)
  - » BSS is an inherited platelet disorder with
    - Large platelets
    - Thrombocytopenia
    - No aggregation with ristocetin (but normal aggregation with other platelet agonists)
    - Caused by low or absent platelet glycoprotein Ib-IX-V receptor

What else can MYH9-related disorders be confused with?

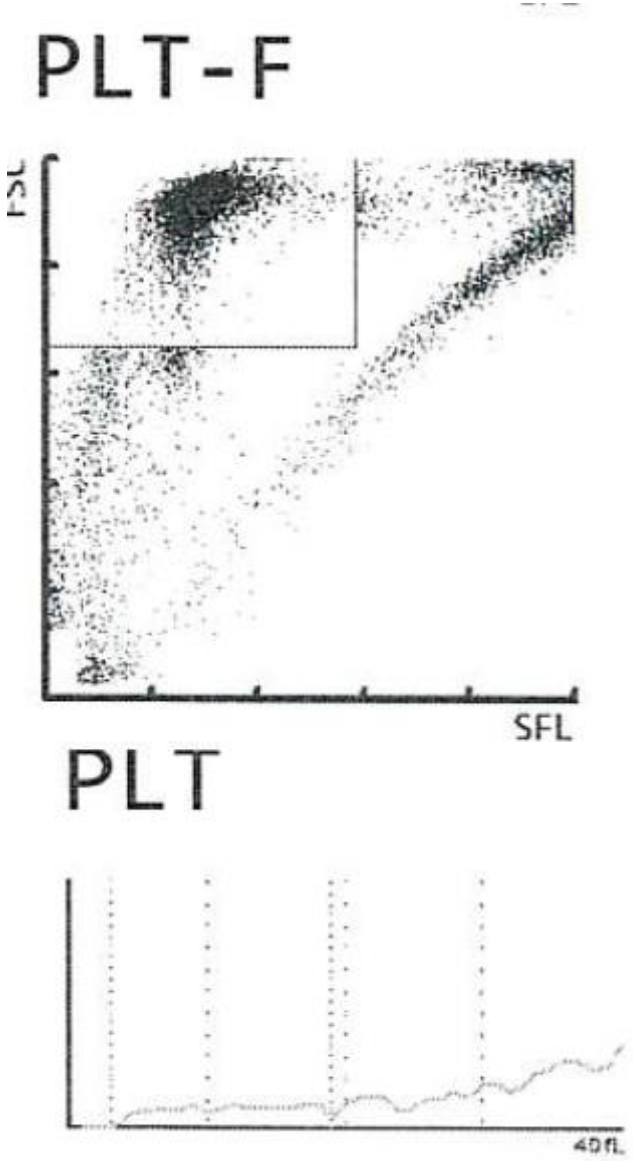
- Immune thrombocytopenia
  - » Particularly in adults
  - » Number of giant platelets < MYH9 disorders
  - » Family members tend to have normal platelet counts
  - » Platelet count improves with IVIG
- Other hereditary macrothrombocytopenias
  - » Bernard-Soulier syndrome
  - » Paris-Trousseau syndrome
  - » GATA1-related disease
  - » Gray platelet syndrome
  - » ITGA2B/ITGB3-related thrombocytopenia
  - » TUBB1-related thrombocytopenia

How do we treat patients with MYH9-related disorders?

- Avoid medications that impair platelet function
- Regular dental care
- Avoid drugs with potential renal toxicity
- Hearing aids and cochlear implants to improve hearing
- Eye examinations to monitor for cataracts
- Women tend not to have increased bleeding complications with childbirth
- Some medication support may help prevent bleeding with surgery
  - » DDAVP and tranexamic acid are examples of drugs that have been tried

Another Possible Pitfall

- From a different recent case in our laboratory—
  - » Patients with MYH9-related disorders may have underestimated platelet counts using automated methods
  - » Manual counts may be more accurate



Case 3

Clinical History

- 19-year-old female presents to ED with lower back pain, nausea, and bloody urine (hematuria) for one day.

- Past medical history- irritable bowel syndrome (IBS), renal stones, obesity

Clinical History- More Details

- Time Line
  - » 3 days prior- Polyethylene glycol treatment for constipation; successfully completed
  - » 2 days prior- acute onset of bilateral flank pain
    - Red-brown urine started at this time
  - » 1 day prior- seen at primary care office
    - UA- blood and protein in urine
      - › Given cephalexin for presumed UTI and toradol for flank pain
    - Additional lab values post-visit
      - › Platelets = 9,000/µL
      - › Creatinine = 1.65 mg/dL
    - Patient advised to go directly to ED on basis of laboratory values

Review of Systems

- No confusion or other neurologic symptoms
- Subjective fever and chills
- No skin rashes
- No bleeding other than hematuria
- Soft bowel movements but no diarrhea
- “Stuffy nose” for one day prior to presentation
- Nobody else in household is sick

Physical Examination

- Vital Signs (at ED presentation)
  - » BP 130/99
  - » Pulse 88
  - » Respirations 16
  - » Temperature 35.7°C (96.3°F)
  - » O_2 saturation 97%
- Physical Examination
  - » Tender to palpation over bilateral flanks
  - » Otherwise negative
  - » Patient alert and converses easily with physician

Initial Laboratory Studies

CMP (selected values)

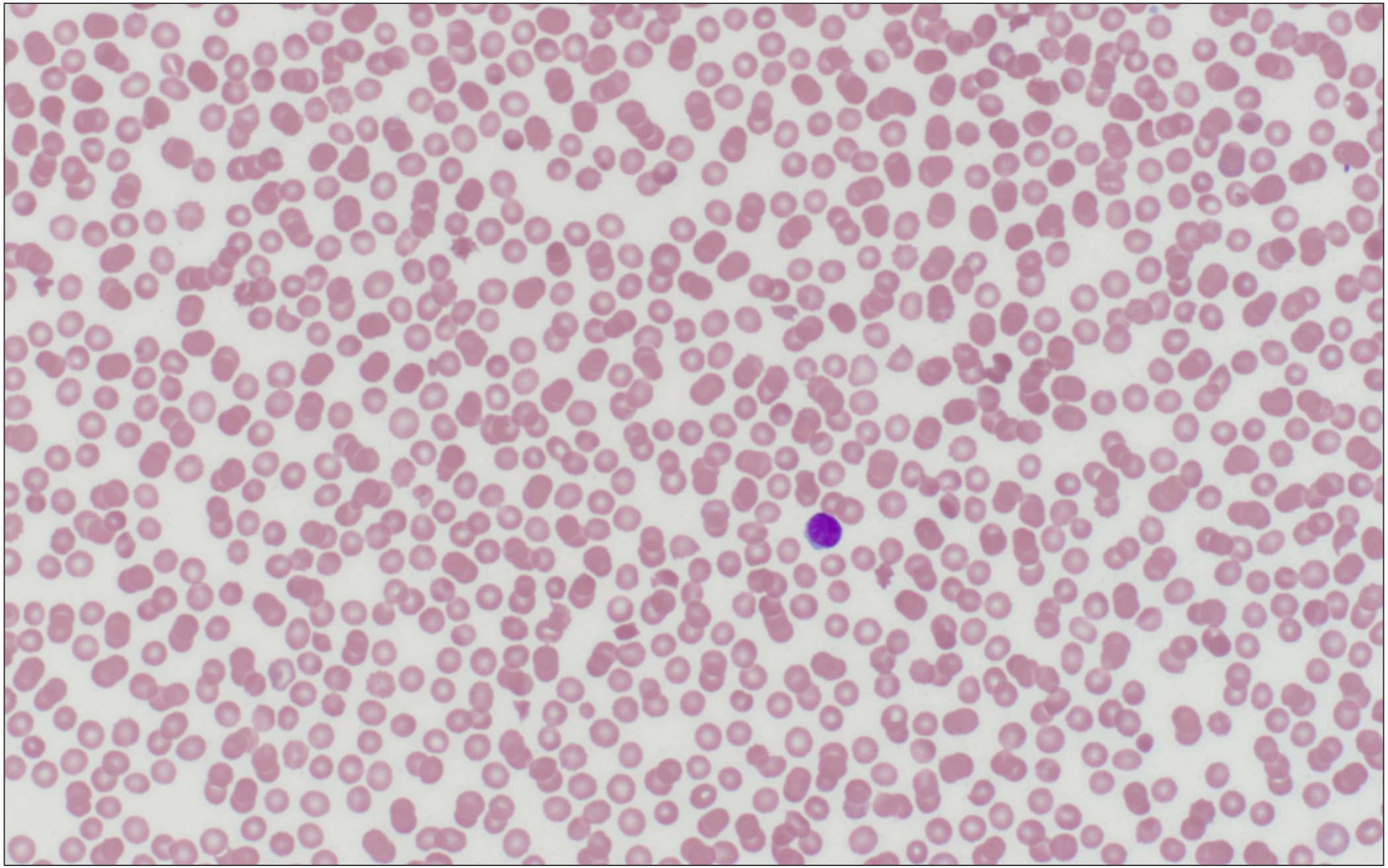
Test	Result
Na	136 mmol/L
K	3.8 mmol/L
Cl	107 mmol/L
CO_2	19 mmol/L
BUN	30 mg/dL
Creatinine	1.65 mg/dL
Total bilirubin	2.5 mg/dL
Alk phos	73 U/L
ALT	21 U/L
AST	40 U/L

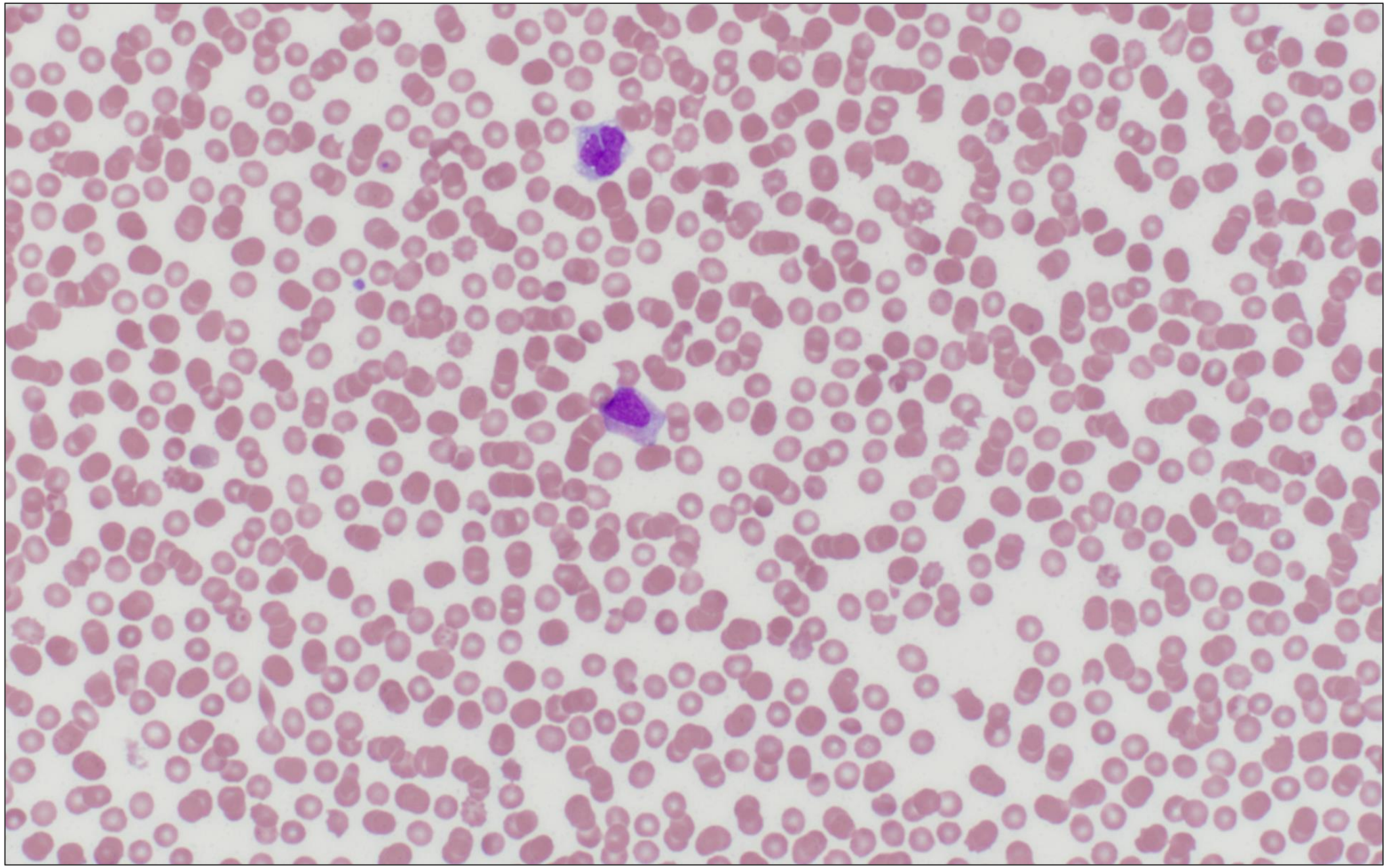
CBC

WBC	5.93 K/µL
RBC	4.39 M/µL
Hgb	12.8 g/dL
Hct	36.9%
MCV	84.1 fL
MCHC	34.7 g/dL
Platelets	<6 K/µL
Abs. neutrophil count	2.59 K/µL
Reticulocytes	1.4 % (60.3 K/µL)

Additional Laboratory Studies

- PT = 13.7 s, APTT = 26 s
- D-dimer = 6.6 µg/mL FEU, fibrinogen activity = 406 mg/dL
- LDH = 948 U/L
- Haptoglobin = <10 mg/dL
- DAT = negative
- Urine HCG = negative
- UA = positive for blood, protein, bilirubin; negative for leukocyte esterase
- Peripheral blood smear (see next slides)





Initial Imaging Studies

- CT abdomen/pelvis
  - » Bilateral perinephric fat stranding
  - » Small volume free fluid in perinephric space
  - » No renal calculi (stones)
  - » No hydronephrosis
  - » Differential diagnosis = bilateral pyelonephritis or recently passed calculi

What does our patient have?

- Microangiopathic hemolytic anemia
  - » Differential Diagnosis
    - Hemolytic uremic syndrome (HUS)
    - Thrombotic thrombocytopenic purpura (TTP)

    - Other thrombotic microangiopathy
      - › Disseminated intravascular coagulation (DIC)
      - › Cancer-related
      - › Pregnancy-related
        - Preeclampsia
        - HELLP syndrome
      - › Medication-related
      - › APS

What now???

- Tests to help distinguish HUS and TTP
  - » Shiga toxin, ADAMTS13 activity
- Plasma exchange
  - » (after we collect our sample for ADAMTS13 activity, of course)
- Steroids

What is a thrombotic microangiopathy?

- Group of disorders with
  - » Microvascular thrombosis leading to
    - Hemolytic anemia
      - › Mechanical RBC destruction = microangiopathic hemolytic anemia (MAHA)
    - Thrombocytopenia
    - Ischemic end-organ damage

TTP vs. HUS

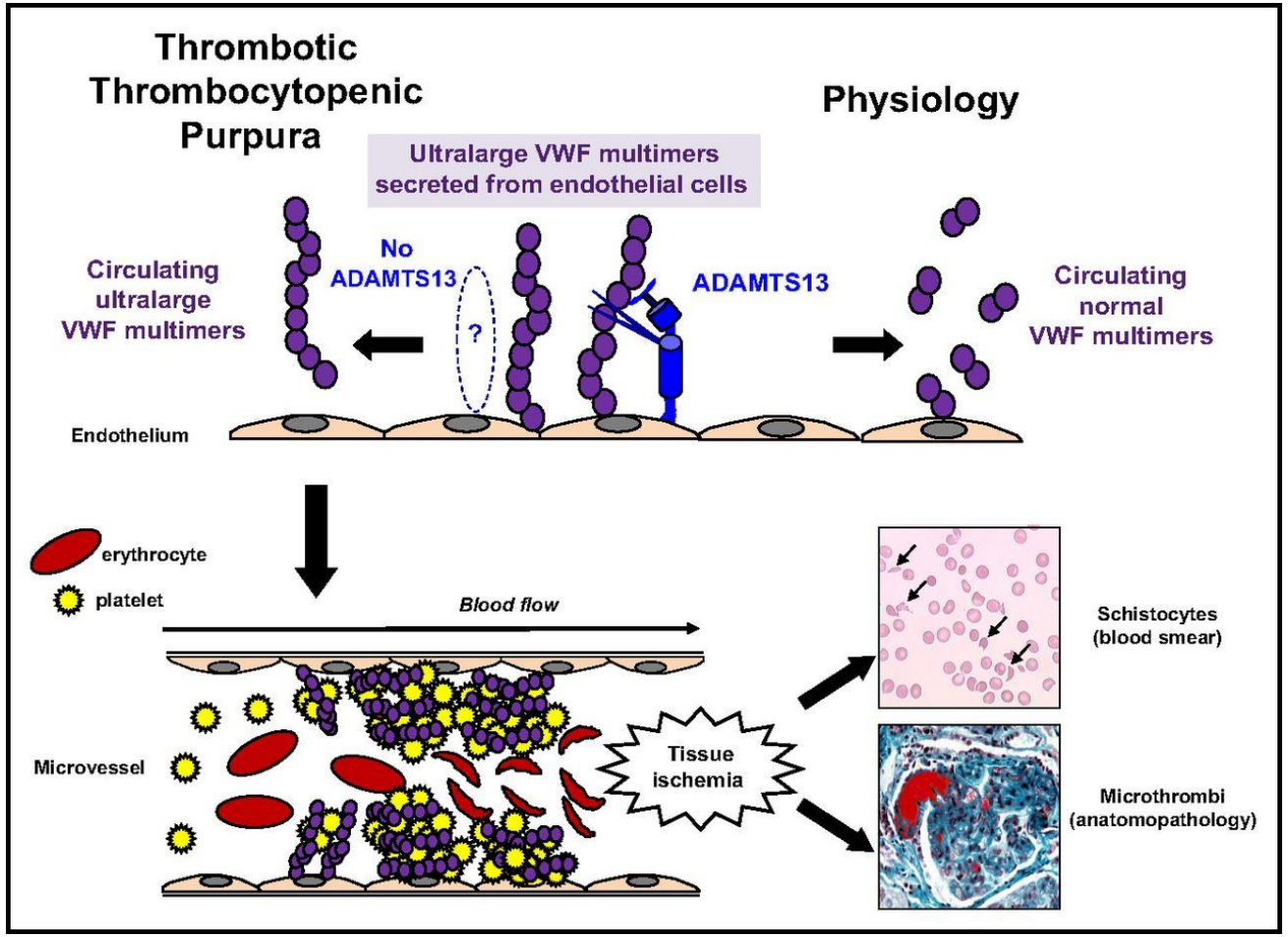
TTP

- "Pentad" of clinical findings
  - » Fever, thrombocytopenia, MAHA, neurologic symptoms, renal insufficiency
  - » Only seen in ~10% of TTP pts
- Caused by severe deficiency of functional ADAMTS13
- Excellent response to plasma exchange

HUS (typical)

- Preceding bloody diarrhea
  - » Caused by shiga toxin-producing *E. coli*
- Renal failure typically more prominent and severe than in TTP
- No severe ADAMTS13 deficiency
- Usually requires treatment other than plasma exchange

TTP- Pathophysiology



- TTP can be acquired or inherited. Acquired cases are most often related to autoantibodies (neutralizing or non-neutralizing) directed against ADAMTS13.
- Other precipitating factors may include infection, inflammation, and pregnancy—but the exact precipitating cause is not clear in every case.

Figure from Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. Blood 2017;129:2836-2846.

Final Diagnosis?

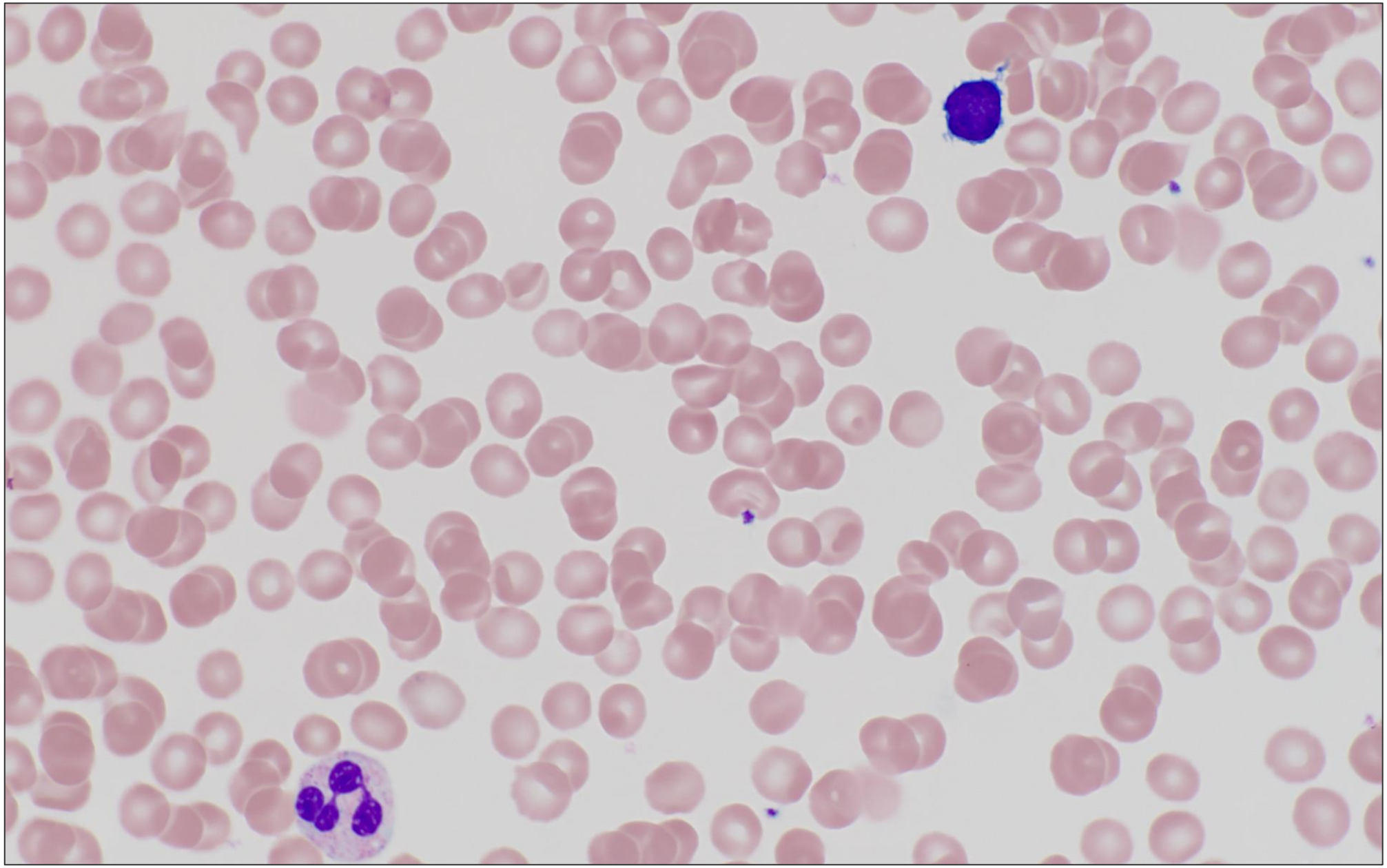
- ADAMTS 13 activity <5%
- Shiga toxin (stool) = negative

- TTP = final diagnosis
  - » Plasma exchange daily until platelets >150 K/µL
    - Total of 5 days of treatment
    - Final platelet count at discharge = 277 K/µL
  - » Rituximab weekly x 4 weeks
    - Proposed but patient declined this treatment
  - » At first hematology follow up, no signs of relapse
    - ADAMTS13 activity 89%

Case 4

Clinical History

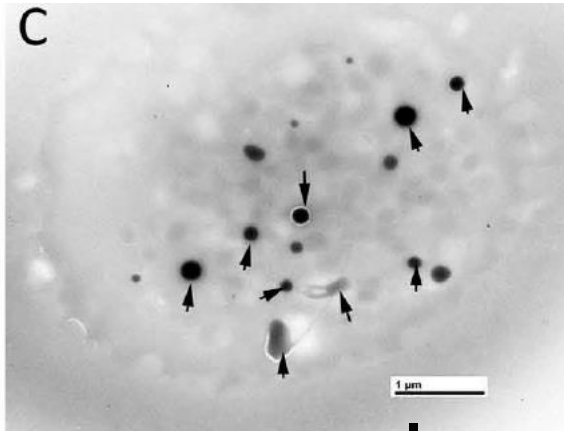
- 9-month-old male with a history of 1.5 months of respiratory symptoms and fatigue. He now presents with 5 days of fever, bloody diarrhea, and petechial rash.
- Current CBC indicates WBC 8.2 K/µL, RBC 4.16 M/uL, Hgb 11.3 g/dL, MCV 81.0 fL, MCHC 33.5 g/dL, platelets 23 k/µL, MPV 8.4 fL. Peripheral smear includes platelet features as shown in the image (next slide).



Wiskott Aldrich Syndrome

- X-linked recessive disorder due to *WAS* mutation
- Clinical features: immune deficiency, recurrent infections, eczema, microthrombocytopenia, platelet storage pool disorder (decreased dense granules)
    - Patients often present in infancy with petechiae and bloody diarrhea
    - Platelet aggregation shows decreased response to ADP, epinephrine, and collagen

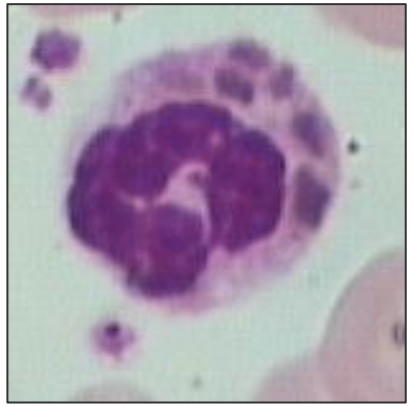
What do dense granules look like?



Platelet from normal donor.
Dense granules marked with arrows, characterized by sharp borders and perfectly round contours, uniform density, >50 nm diameter.

PTEM Images: Chen D, Uhl CB, Bryant SC, et al. *Platelets.* 2018; 29(6):574-582. Figure 4 C.

More Dense Granule Storage Pool Disorders



- Chediak-Higashi syndrome
- Due to mutations in *LYST*
- Clinical features: neutropenia, recurrent infections, albinism

- Hermansky-Pudlak syndrome
- Clinical features: albinism, pulmonary fibrosis
- Prevalent in Puerto Rico
- Defect in trafficking/sorting dense granule membrane proteins

Conclusion

- Peripheral blood smear findings may highlight the need for further consideration of disorders of hemostasis and thrombosis.
- Consideration of all available data (clinical, laboratory test results, morphologic appearance of blood smear) is necessary for diagnosis.

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Thank you!

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