Mass General Brigham

# It Takes a Village: A Comprehensive Approach to Myeloproliferative Neoplasms

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# Disclosures

## Scopio:

- Consultant

## Sysmex:

- Speaker

# Objectives

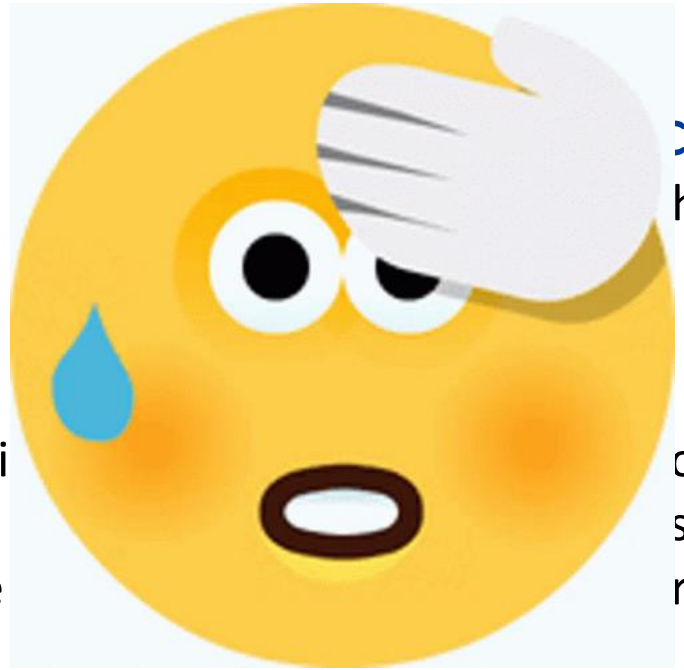
Recognize blood and/or bone marrow morphologic findings associated with myeloproliferative neoplasms.

Accurately apply bone marrow fibrosis grading and understand its significance.

Understand the importance of a comprehensive approach for diagnosis and prognosis of myeloproliferative neoplasms.

# Classification(s) Myeloproliferative Neoplasms: What's New?

| 5th ed WHO | ICC |
|---|---|
| Chronic myeloid leukemia | Chronic myeloid leukemia |
| Chronic phase | Chronic phase |
| Blast phase | Accelerated phase |
| Polycythemia vera | Blast phase |
| Essential thrombocythemi | olycythemia vera |
| Primary myelofibrosis | ssential thrombocythemia |
| Pre-fibrotic stage | rimary myelofibrosis |
| Fibrotic stage | Pre-fibrotic stage |
| Chronic neutro | Fibrotic stage |
| Chronic eosino | rophilic leukemia |
| Juvenile myelomonocytic leukemia | Chronic eosinophilic leukemia, NOS |
| Myeloproliferative neoplasm, NOS | Myeloproliferative neoplasm, unclassifiable |



Diagnostic criteria remain the same!

*Arber DA et al. Blood. 2022 Sep 15;140(11):1200-1228.PMID: 35767897; PMCID: PMC9479031.*
*Khoury JD et al. Leukemia. 2022 Jul;36(7):1703-1719. PMID: 35732831; PMCID: PMC9252913.*

# Ph- Myeloproliferative Neoplasms

Polycythemia vera (PV):

- Panmyelosis characterized by increased red cell production independent of the mechanisms that normally regulate erythropoiesis

Essential thrombocythemia (ET):

- Characterized by sustained non-reactive thrombocytosis

Primary myelofibrosis (PMF):

- Characterized by various degree of myeloid and megakaryocytic proliferation associated with deposition of fibrous connective tissue and extramedullary hematopoiesis
- Early/pre-fibrotic PMF and Overt PMF

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# PMF and ET and PV (35,000 ft view ….)

| | PV | PMF (early and overt) | ET |
|---|---|---|---|
| Survival | 6-18 mo (untreated)<br>>10 years | (very)low – 56-92% 10 years<br>Intermediate – 30% 10 years<br>(very)high – 0-13% 10 years | Overall - 18 years<br>(certain mutation affect prognosis) |
| Leukemic transformation | 3% at 10 years<br>5-8% at 15 years | ~20% at 10 years | <1% at 10 years |
| Fibrosis progression | 15-25% | 100% | ~15%<br>(MF presents at diagnosis) |
| Thrombosis, per 100 patients/year | 5.5 | 2 | 1-3<br>Mostly in with prior history, >60 years, *JAK2* mutation |
| Treatment | Phlebotomy (+/- HU, anagrelide, aspirin) | Allo-HST,<br>HU, JAK2 inhibitors, splenectomy | None<br>(+/- aspirin, HU for int or high risk) |

# MPN Diagnostic Challenges:

## Significant overlap within the MPN group

- Especially in disease early stages
  - ✓ PV, pre-polycythemic stage
  - ✓ Early/pre-fibrotic PMF
- Requires **integration** of clinical and molecular genetic data with morphology
- Important in predicting prognosis and dictating therapy

## Recognizing signs of progression

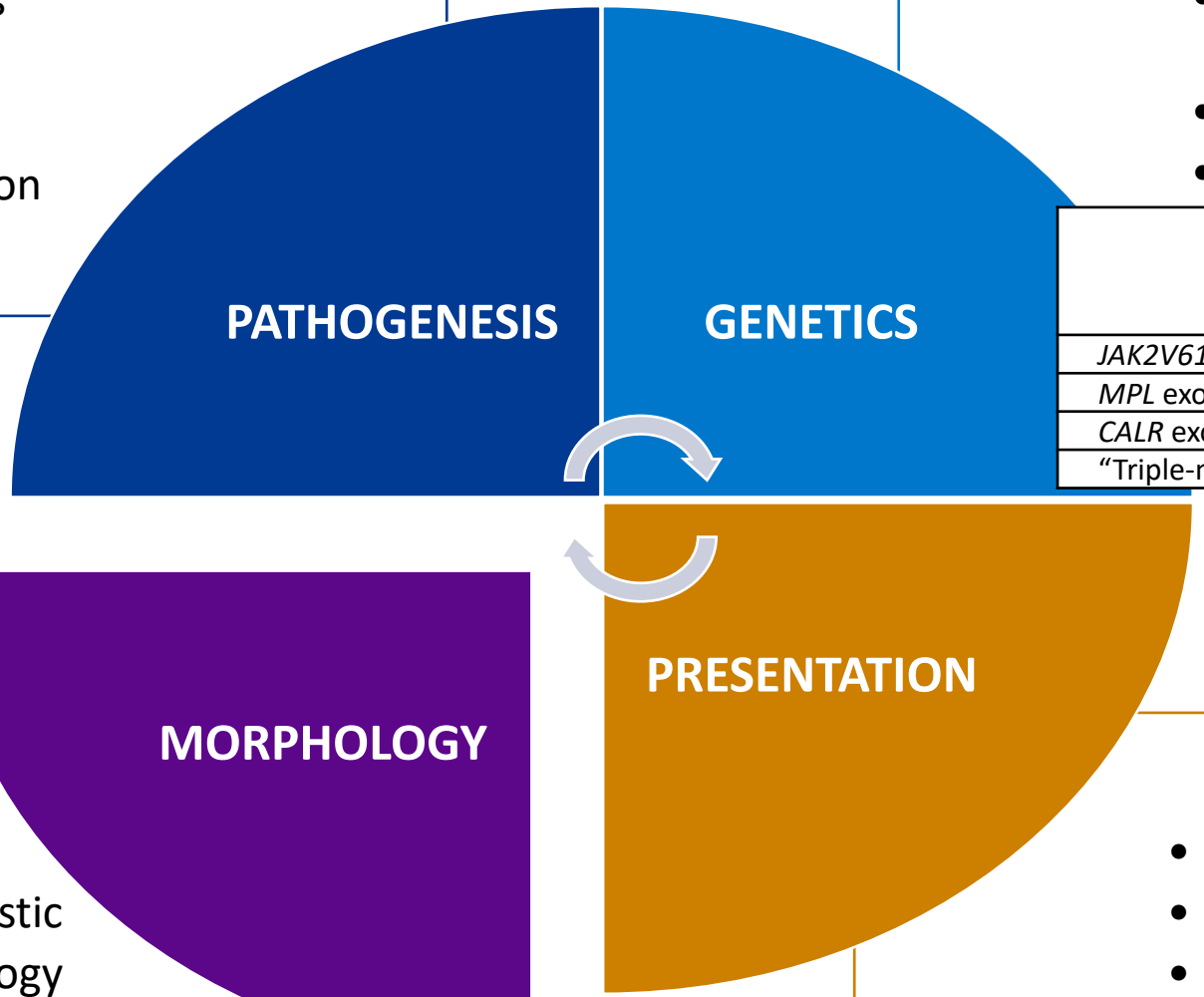
## Separating from other myeloid neoplasms (MDS and MDS/MPN)

## Distinguishing from reactive conditions that present with elevated counts

- Less of an issue with wide use of NGS

# Why Diagnostic Challenges?

- Abnormal hematopoiesis
- Myeloproliferation
  - ✓Fibrosis
  - ✓Leukemic transformation
- Cytokine overproduction




- Dysregulation of *JAK-STAT* signaling pathway
- Shared other mutations
- Karyotype

| | Driver mutations (mutually exclusive) | |
|---|---|---|
| | PMF | ET |
| *JAK2V617F* | 60-65% | 60-65% |
| *MPL* exon 10 (eg, *MPLW515*) | 5% | 5% |
| *CALR* exon 9 | 20-25% | 20-25% |
| "Triple-negative" | 12% | 5% |

- Cellularity
- What lineage is hyperplastic
- Megakaryocyte morphology
- Fibrosis

- Clinical symptoms (fatigue)
- CBC (WBC, HCT/Hgb, PLT, Diff)
- Other labs (LDH)

# Diagnostic Criteria for PMF and ET

Primary my
fibrotic) (Di
meeting all
criteria and
criterion)

Major criter

1. Typical m
changes,[a]
≥grade 2
fibrosis[b]

2. Presence
*MPL* muta
of other
absence
reactive b
fibrosis

smear

## Pre-PMF vs ET:

1. Megakaryocyte morphology
2. Fibrosis grading
3. Minor criteria
4. Platelet count

## Pre-PMF vs Overt PMF:

1. Fibrosis grading
2. Minor criteria

teria:

ot otherwise explained

osis ≥ 11 × $10^9$/L

splenomegaly

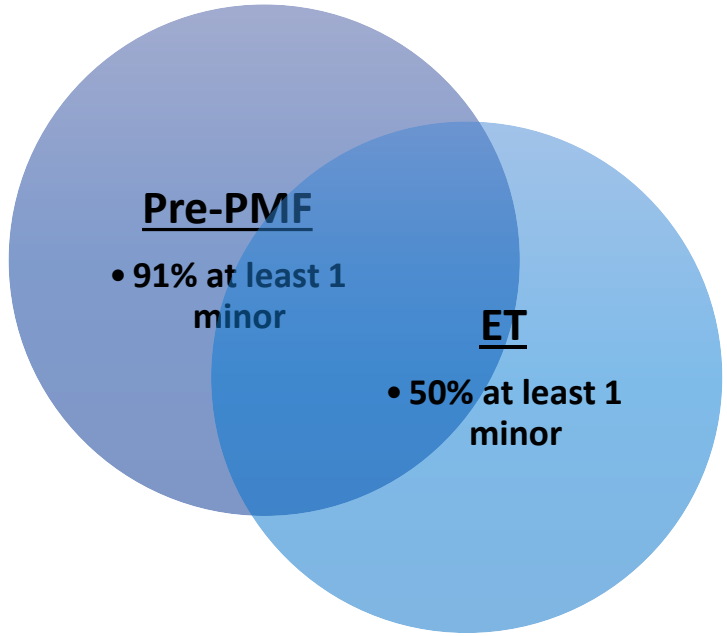
l serum lactate
ogenase

Minor criterion
Other clonal marker present or no evidence of reactive thrombocytosis

*Tefferi A. and Barbui T. Am J Hematol. 2020;95:1599–1613.*
*Tefferi A. Am J Hematol. 2021;96:145-162*

# Frequency of 2016 WHO non-morphologic criteria in pre-PMF and ET

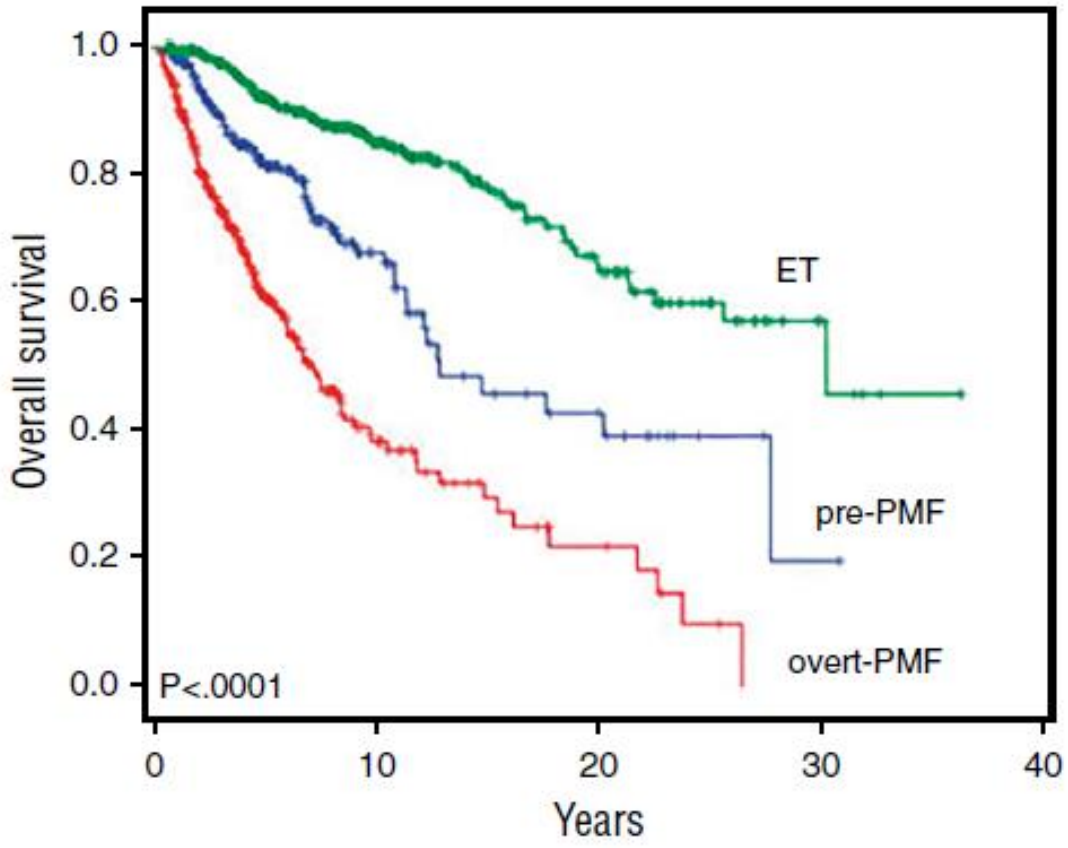
| Criteria | Pre-PMF n=170 | ET n=225 | P-value |
|---|---|---|---|
| Platelets ($10^9$/L) | 803 (541-1013) | 760 (622-921) | 0.995 |
| *JAK2* V617F | 62.6% | 68.3% | 0.473 |
| *CALR* | 29.7% | 20.3% | 0.157 |
| *MPL* | 3.3% | 2.4% | 0.707 |
| Triple negative | 4.4% | 9.0% | 0.309 |
| minor: Anemia | 24.7% | 6.2% | <0.001 |
| minor: Leukocytosis | 50.6% | 24.4% | <0.001 |
| minor: Increased LDH | 74.1% | 22.7% | <0.001 |
| minor: Palpable splenomegaly | 44.7% | 11.1% | <0.001 |

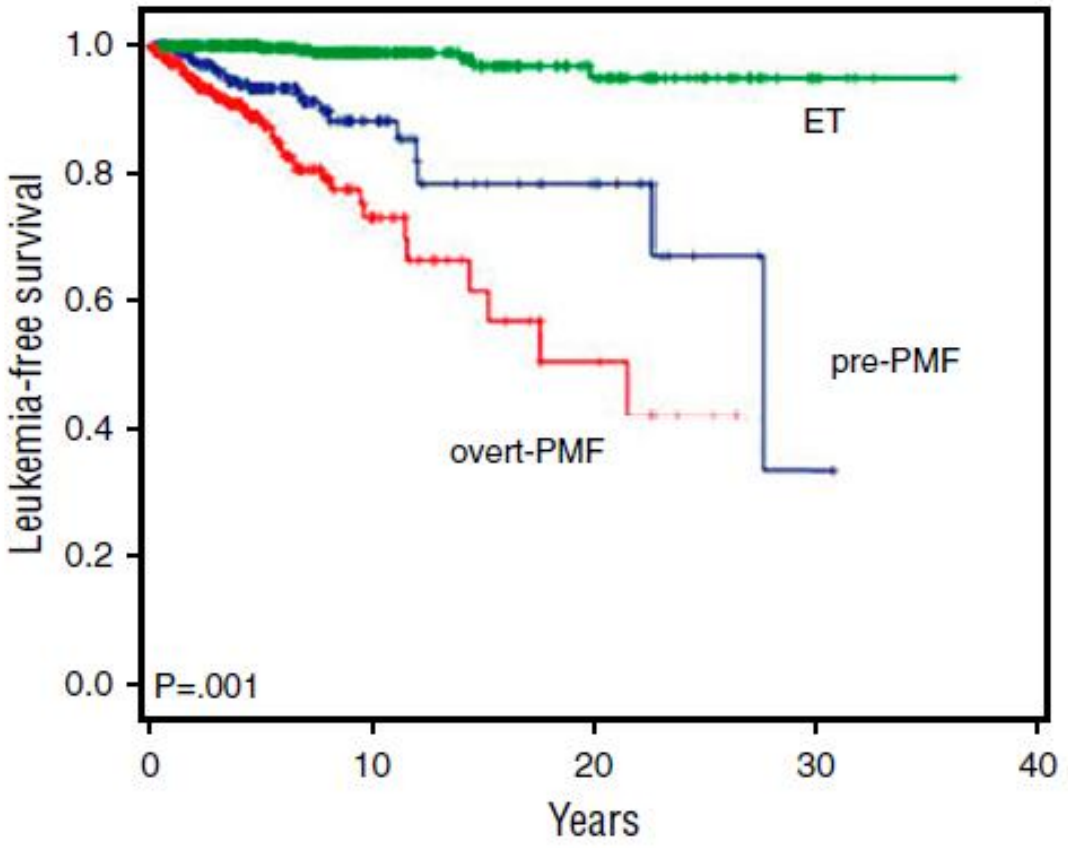





*G. Jeryczynski et al. Am J Hematol (2017) DOI 10.1002/ajh.24788*

# Overall and leukemia-free survival based on diagnosis (600 patients)

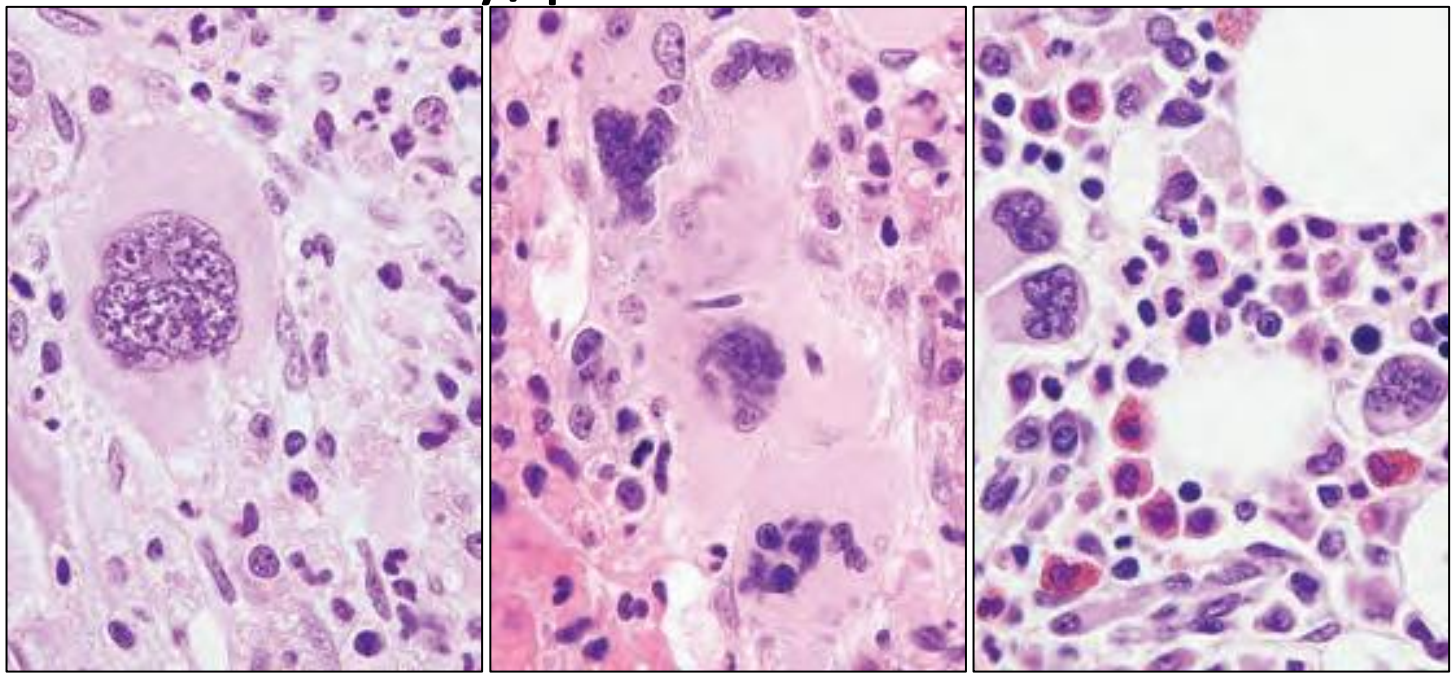


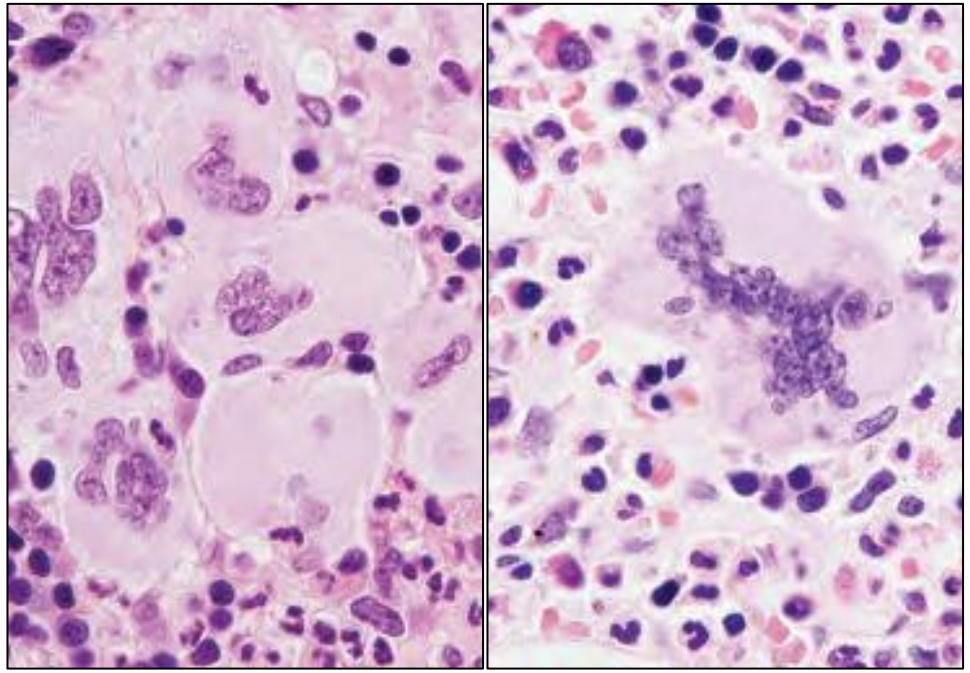

*P. Guglielmelli et al. Blood 2017;129(24):3227-3236*

# Megakaryocyte features

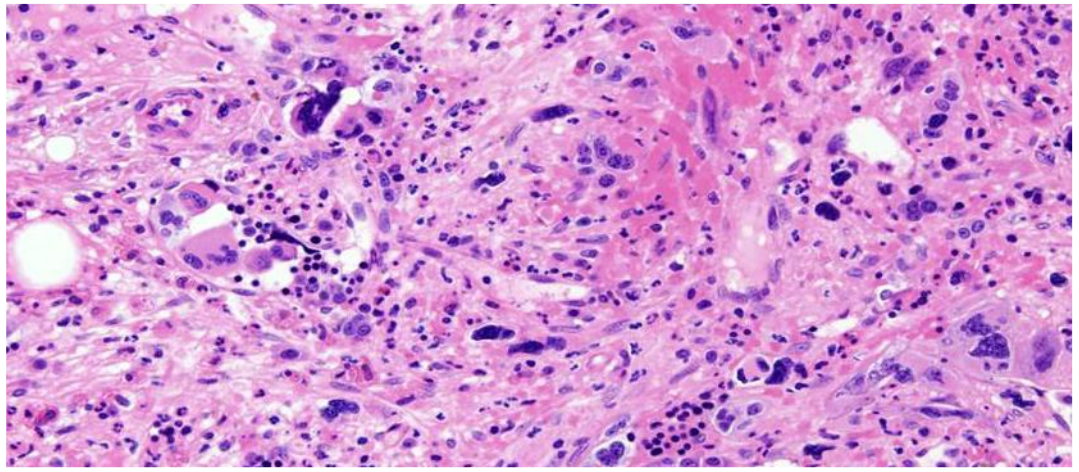
Early/pre-fibrotic PMF



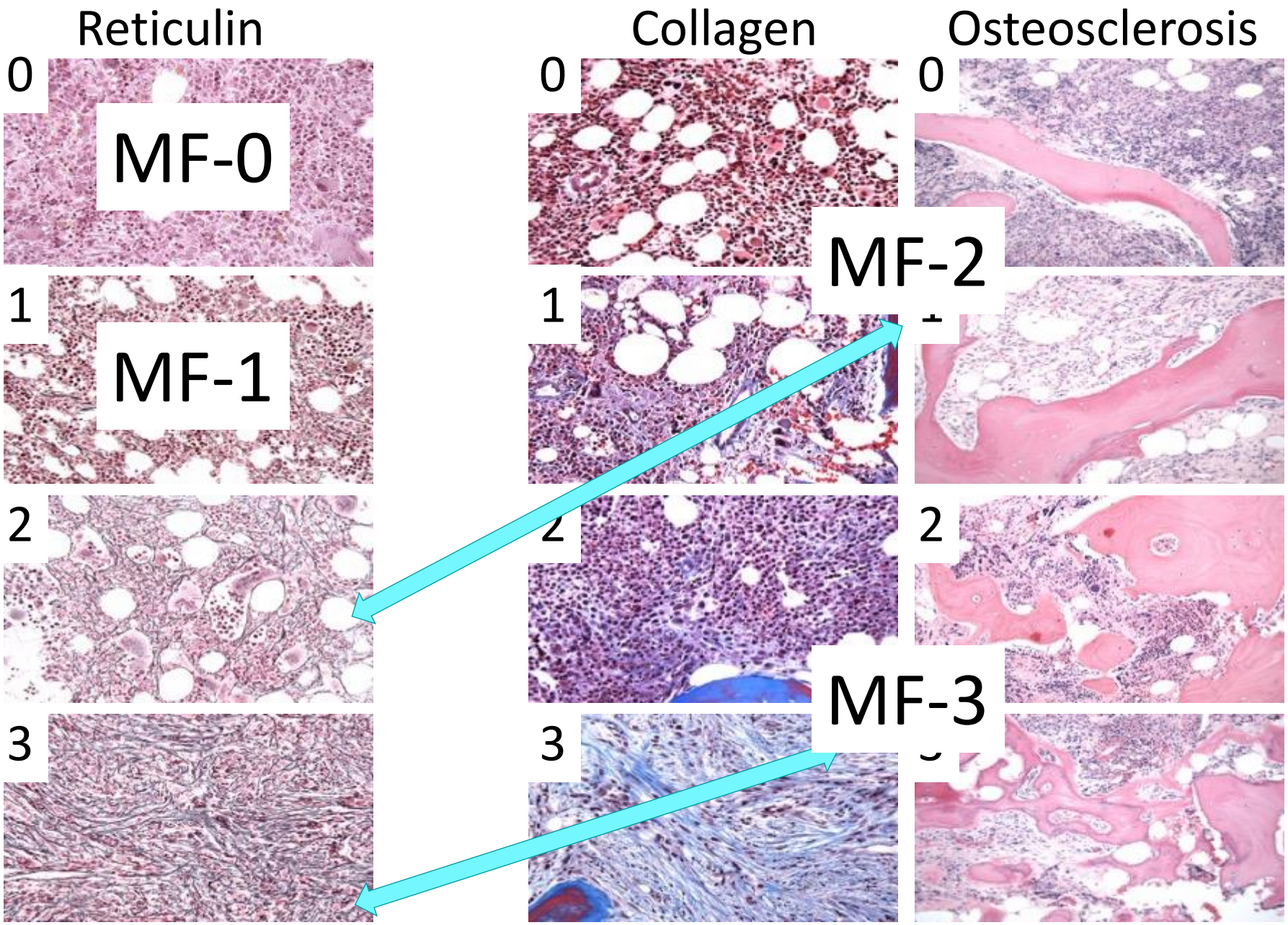
ET



Overt PMF



# WHO MF Grading at Presentation



# Importance of Fibrosis Grading

**Initial correct diagnosis:**

- PMF, especially early stages
- Increased reticulin grade at presentation is an adverse prognostic factor in ET, PV and PMF
- Increased fibrosis at CML diagnosis is associated with TKI resistance

**Disease progression:**

- post-PV-MF or post-ET-MF
- Potential inclusion in prognostic scoring system?

**Patient monitoring:**

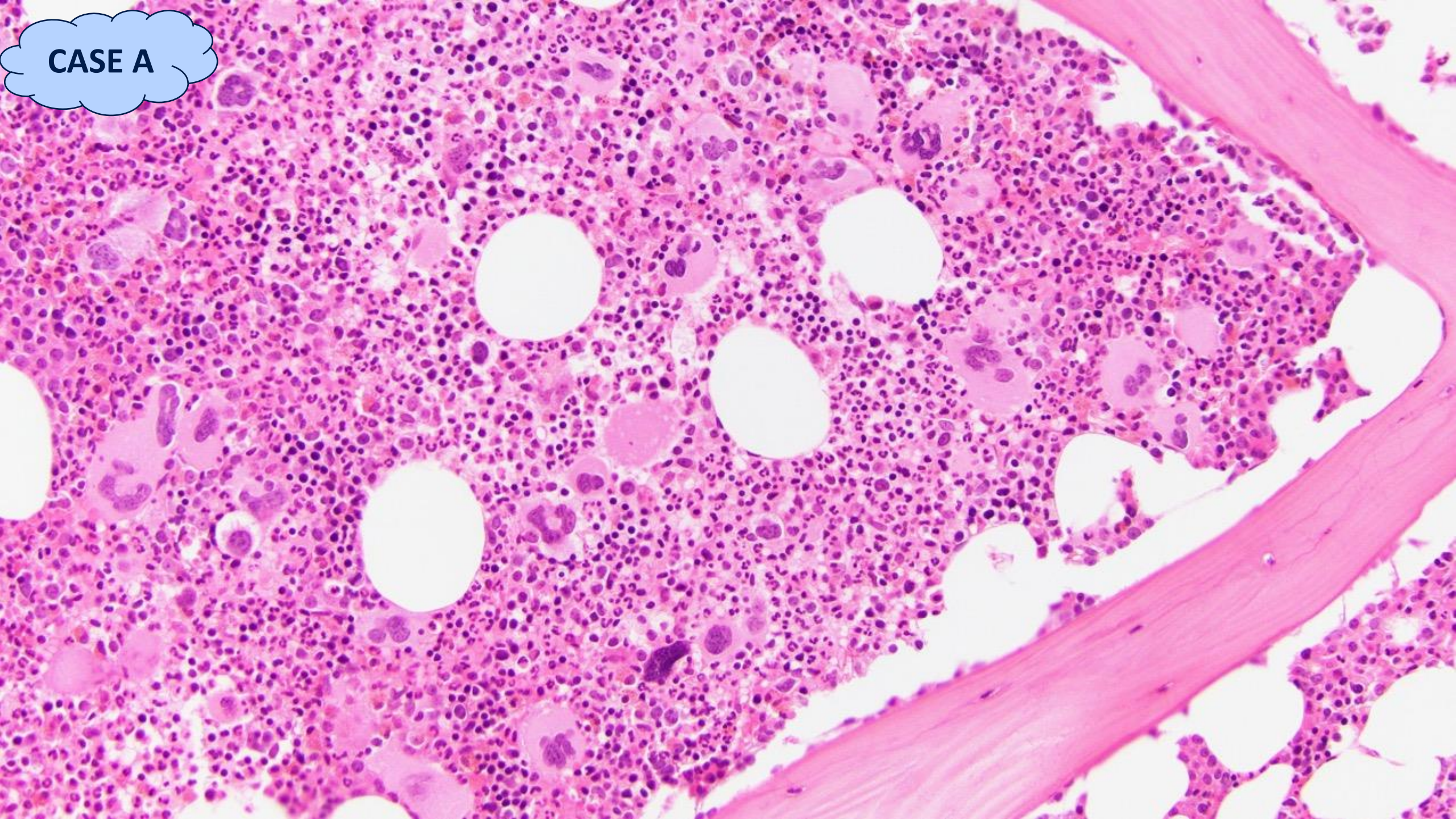
- Changes in fibrosis with disease-modifying agents

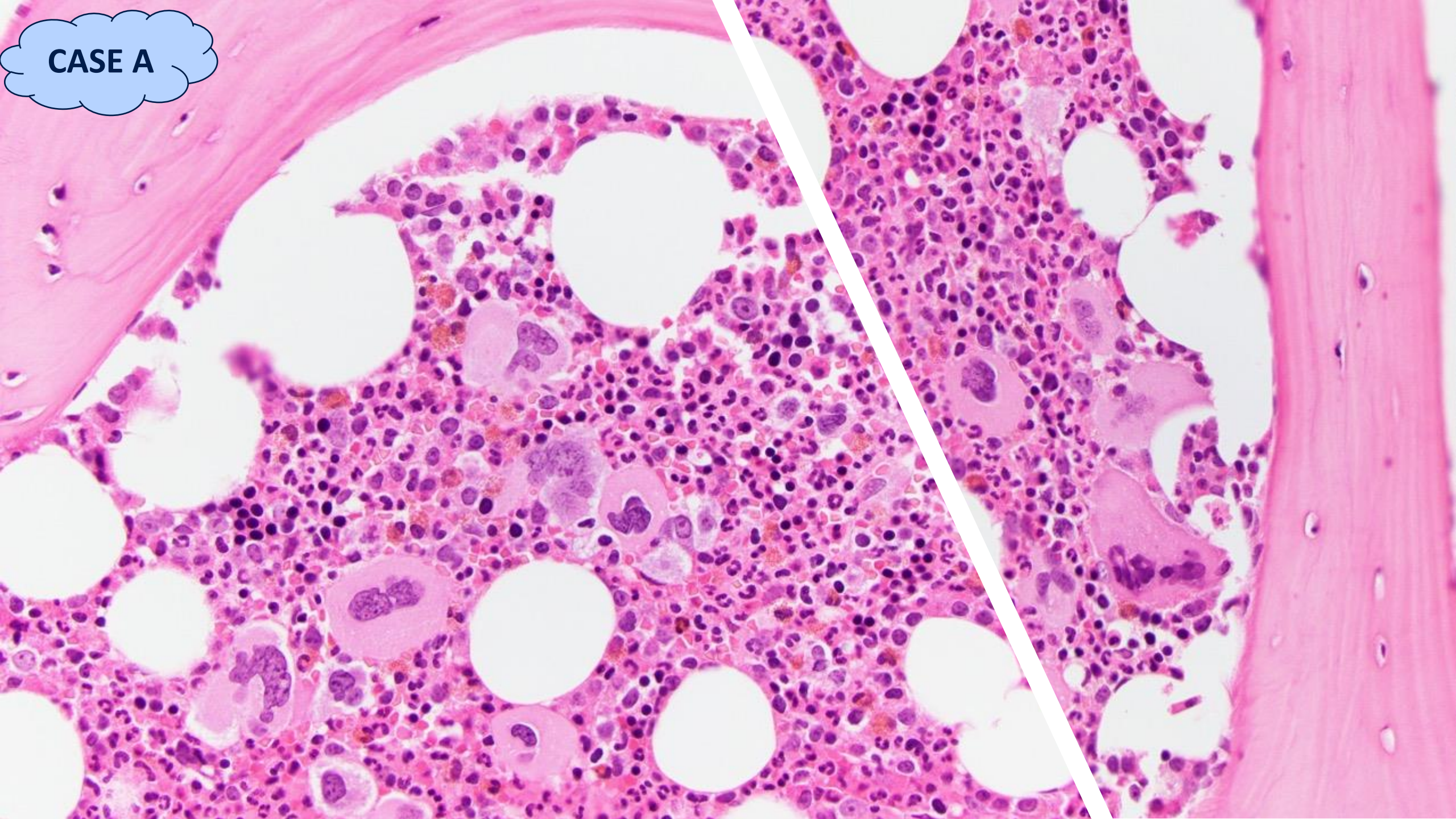
# A Tale of Two Cases

| CASE A | | CASE B |
|---|---|---|
| 61-year-old man<br>Asymptomatic<br>Upper limit normal spleen | **CLINICAL PRESENTATION** | 71-year-old woman<br>Fatigue, arthritis, hypertension<br>No splenomegaly |
| WBC 6.6 x $10^9$/L<br>RBC 4.43 x $10^{12}$/L<br>HGB 15.5 g/dL<br>HCT 46.8%<br>MCV 95.0 fL<br>PLT 1820 x $10^9$/L (H) | **CBC RESULTS** | WBC 11.3 x $10^9$/L (H)<br>RBC 3.05 x $10^{12}$/L (L)<br>HGB 11.0 g/dL (L)<br>HCT 33.2%<br>MCV 97.0 fL<br>PLT 1977 x $10^9$/L (H) |
| *CALR* c.1154_1155insTTGTC<br>p.K385fs* 30.3% VAF | **NGS RESULTS** | *CALR* c.1154_1155insTTGTC<br>p.K385fs* 26.6% VAF |

# Important Morphologic Features

| | Essential Thrombocythemia | Pre-fibrotic<br>Primary Myelofibrosis |
|---|---|---|
| Cellularity (age-adjusted) | Normal | Increased |
| Myeloid hyperplasia | Absent | Present |
| Erythroid hyperplasia | Absent | Absent |
| Megakaryocyte morphology | Staghorn | Bulbous, cloud-like, bizarre forms |
| Megakaryocyte size | Large | Small, medium and large |
| Megakaryocyte clusters | Loose>>>Tight | Tight>>>Loose |
| Megakaryocyte location | Evenly distributed | Paratrabecular |
| Fibrosis | MF-0 or MF-1 (rare) | MF-0 or MF-1 |


CASE A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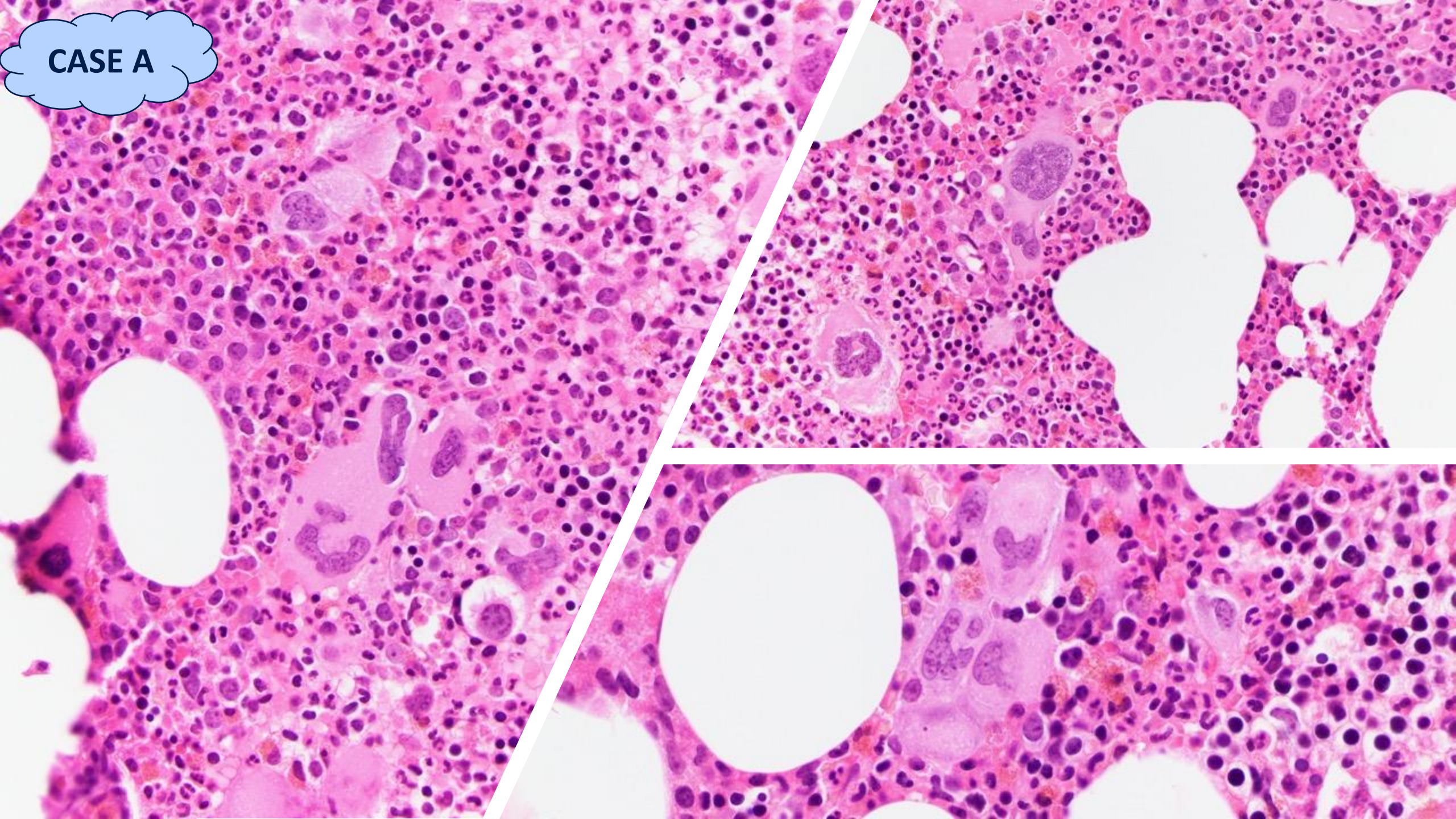
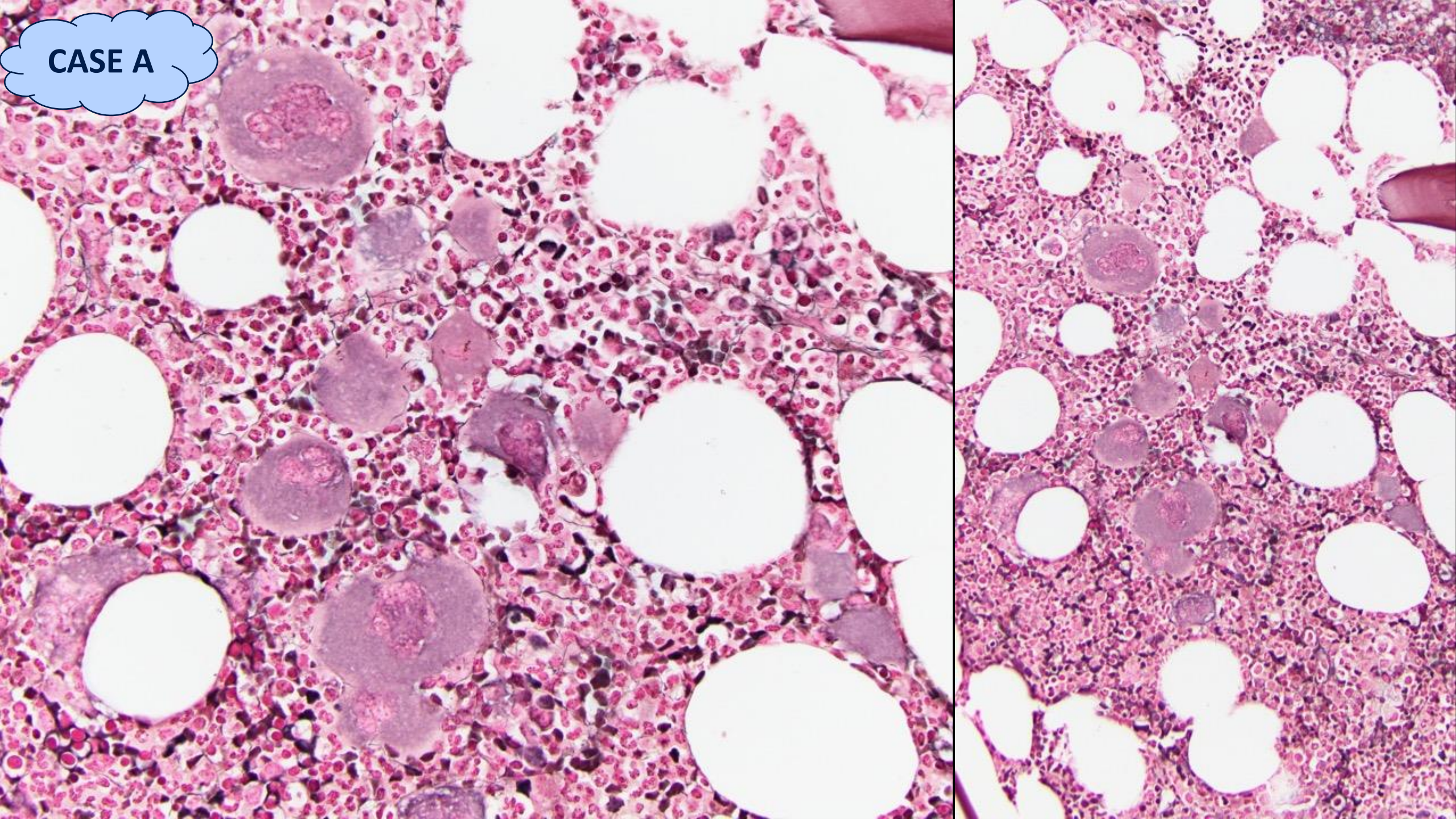
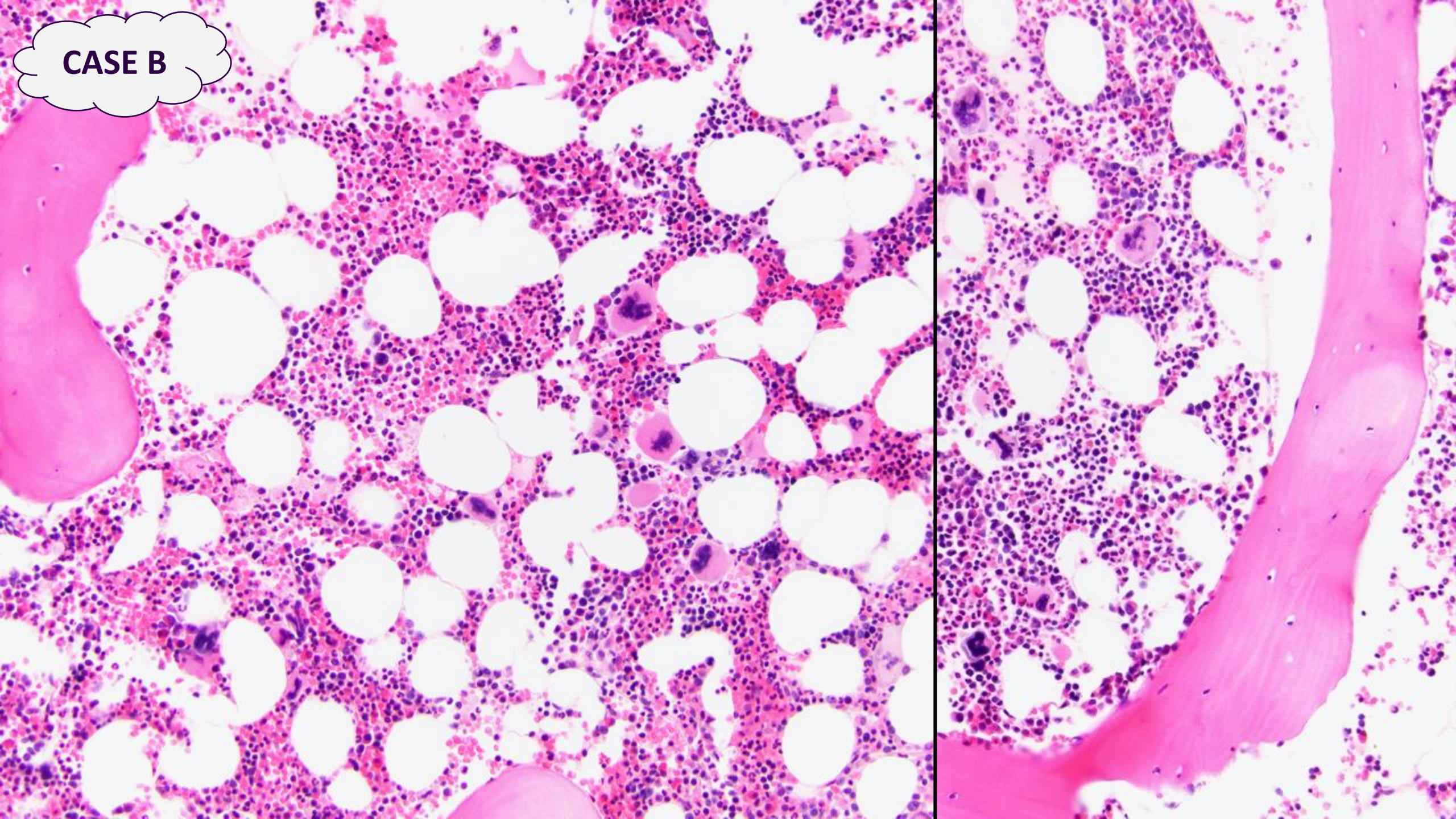
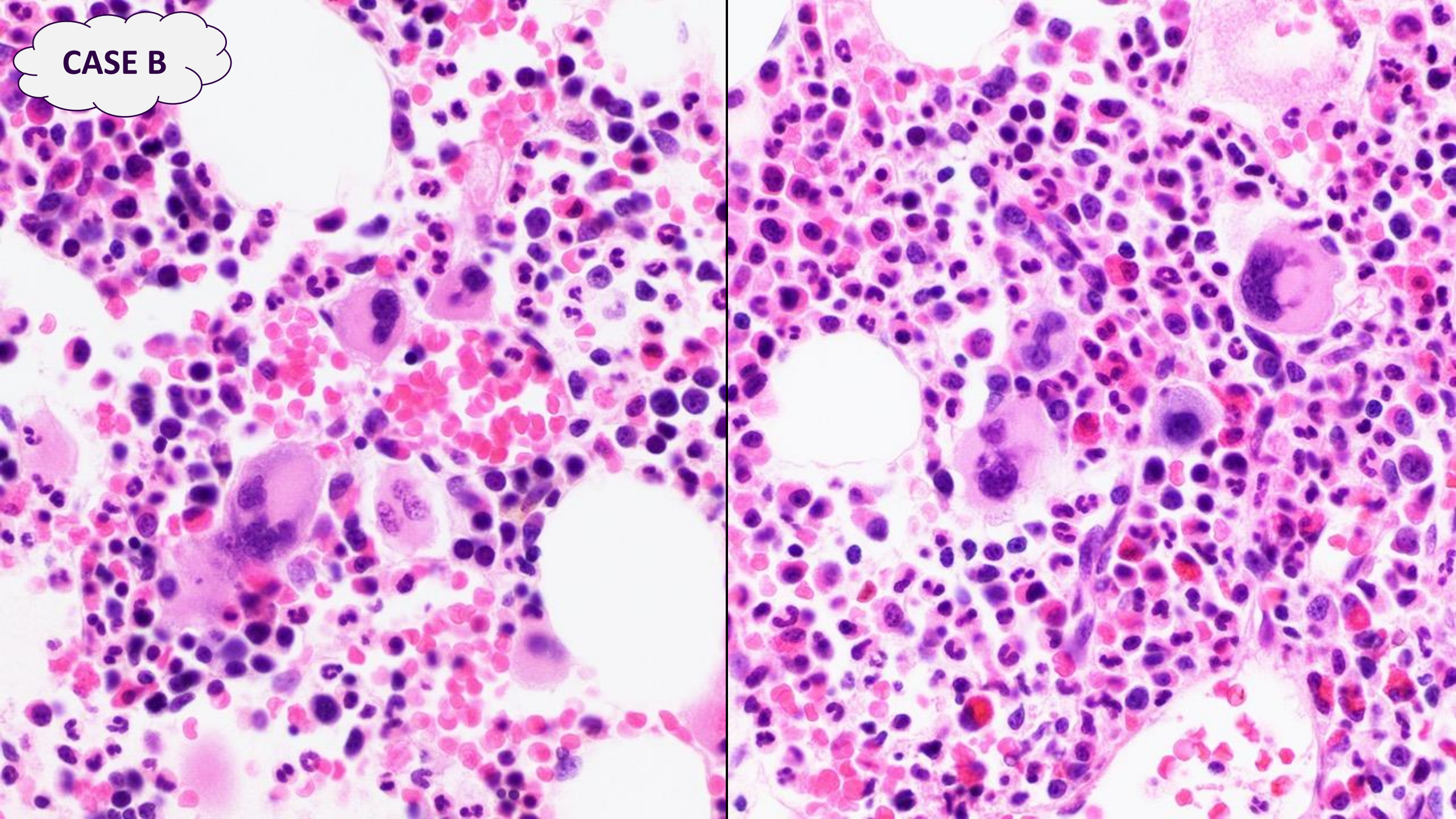
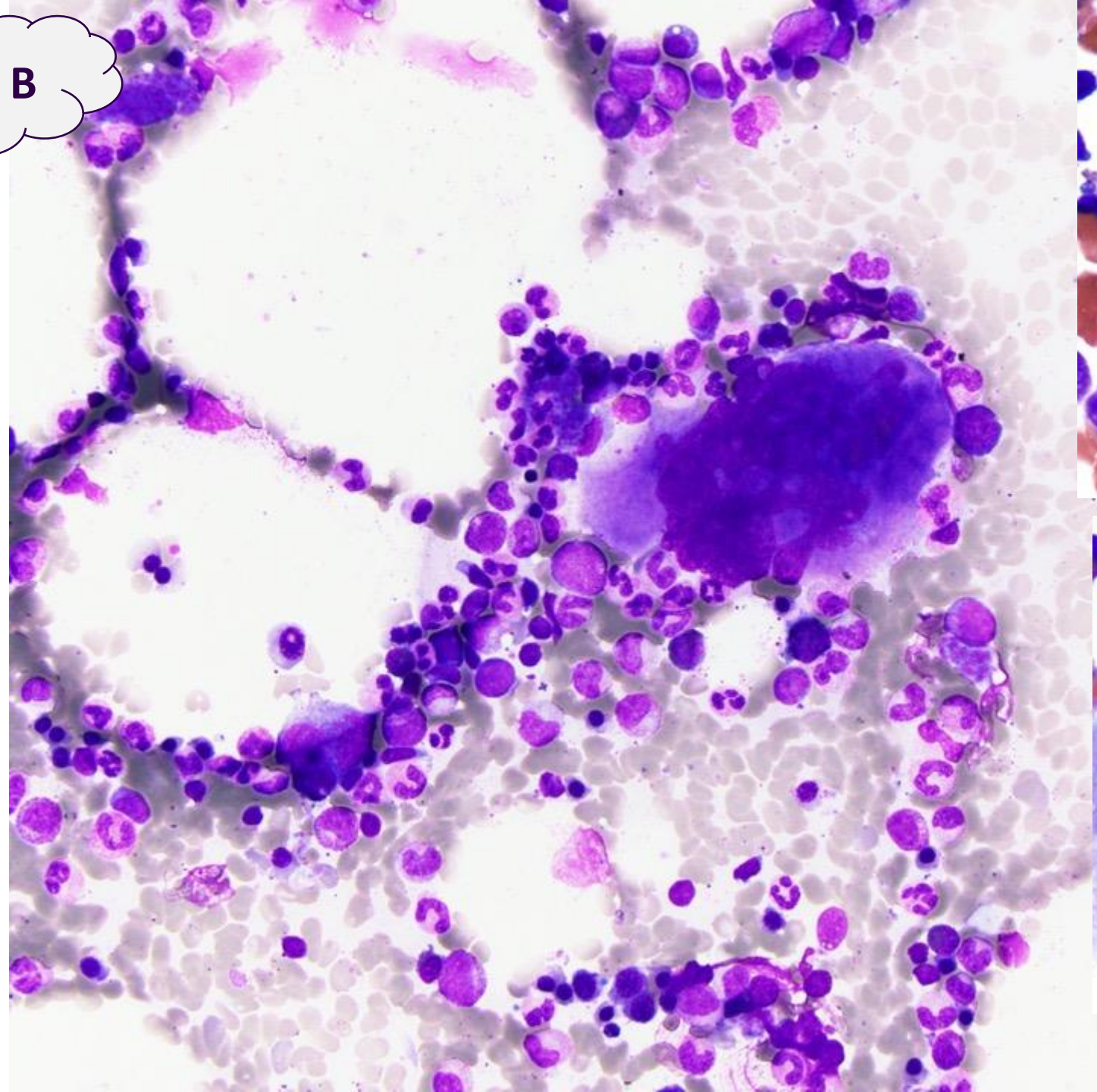

CASE A

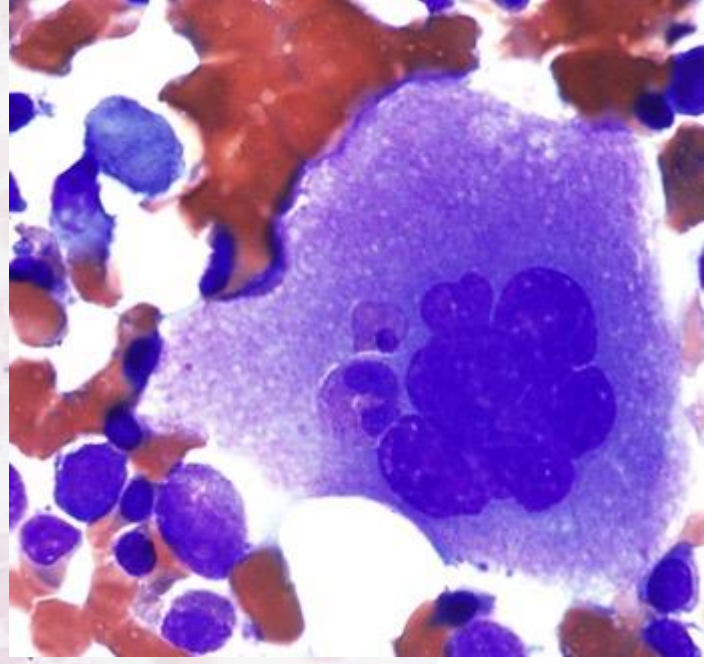

CASE A

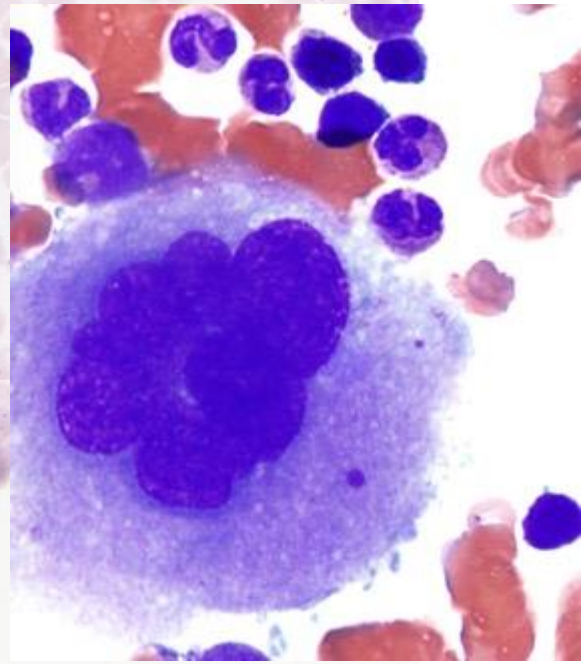

CASE B

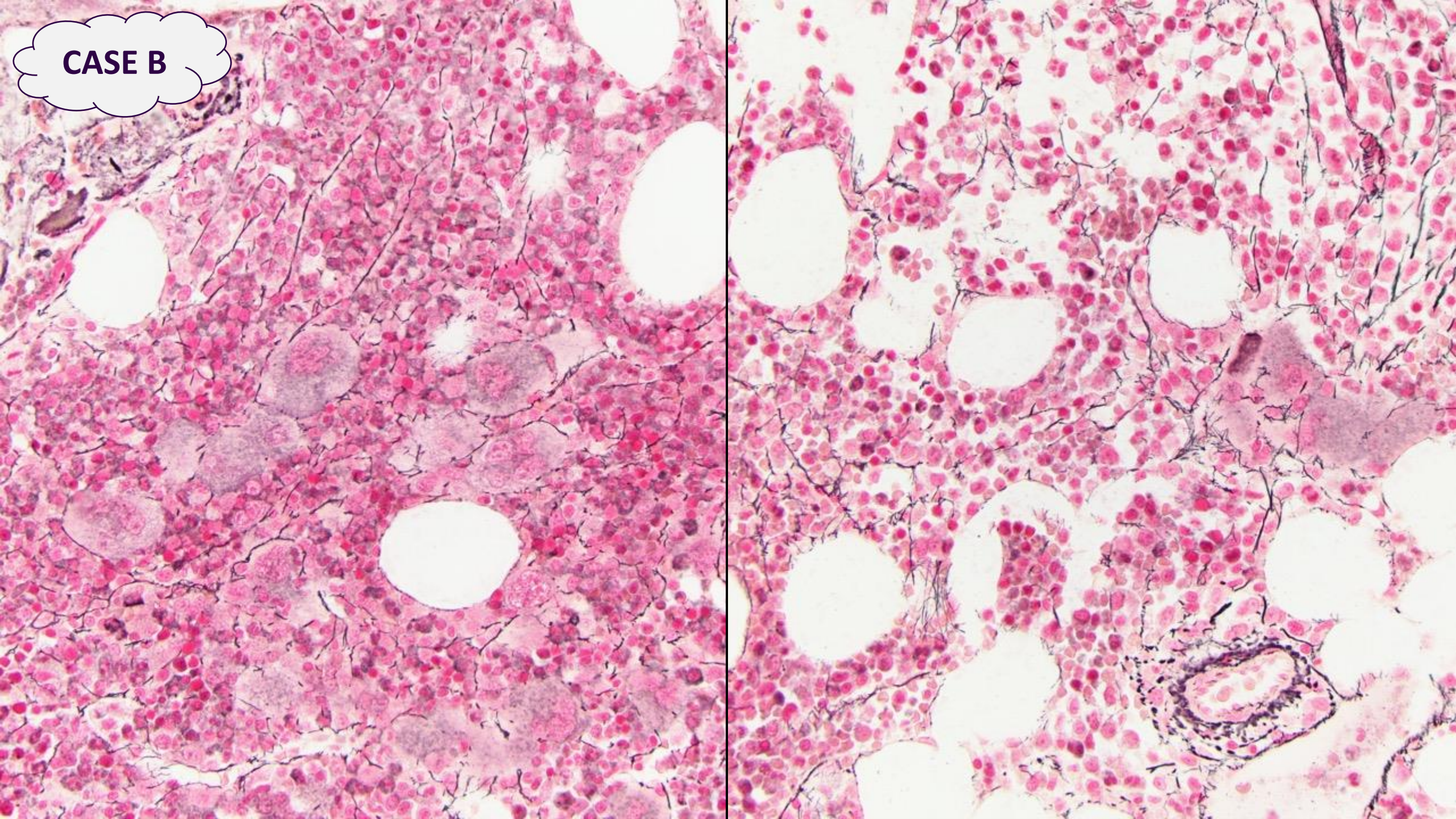

CASE B

# CASE B








CASE B

# WHICH IS WHICH?



| | **Essential Thrombocythemia** | **Pre-fibrotic Primary Myelofibrosis** |
|---|---|---|
| Cellularity (age-adjusted) | Normal | Increased |
| Myeloid hyperplasia | Absent | Present |
| Erythroid hyperplasia | Absent | Absent |
| Megakaryocyte morphology | Staghorn | Bulbous, cloud-like, bizarre forms |
| Megakaryocyte size | Large | Small, medium and large |
| Megakaryocyte clusters | Loose | Tight |
| Megakaryocyte location | Evenly distributed | Paratrabecular |
| Fibrosis | MF-1 (rare) | MF-0 |

# Clinical presentation of *CALR* mutated ET and PMF (compared to *JAK2*, *MPL* and TN)

| | ET | PMF |
|---|---|---|
| *CALR* type | 2>>>1 | 1 >>>2 |
| Age | younger | younger |
| Hemoglobin | lower | higher |
| Platelets | higher | higher |
| Leukocytosis | absent | lower |
| Thrombosis | decreased | decreased |
| MF | increased | similar |
| Prognosis | same | Improved* |

*OS: *CALR* – 17y; *JAK2* – 9y; TN – 3y

*CALR* mutation types:

Type 1: a 52-bp deletion (L367fs*46)

Type 2: a 5-bp insertion (K385fs*47)



*Tefferi A. and Barbui T. Am J Hematol. 2020;95:1599–1613.*
*How J, Hobbs GS, Mullally A. Blood, 2019;134 (25): 2242–2248.*

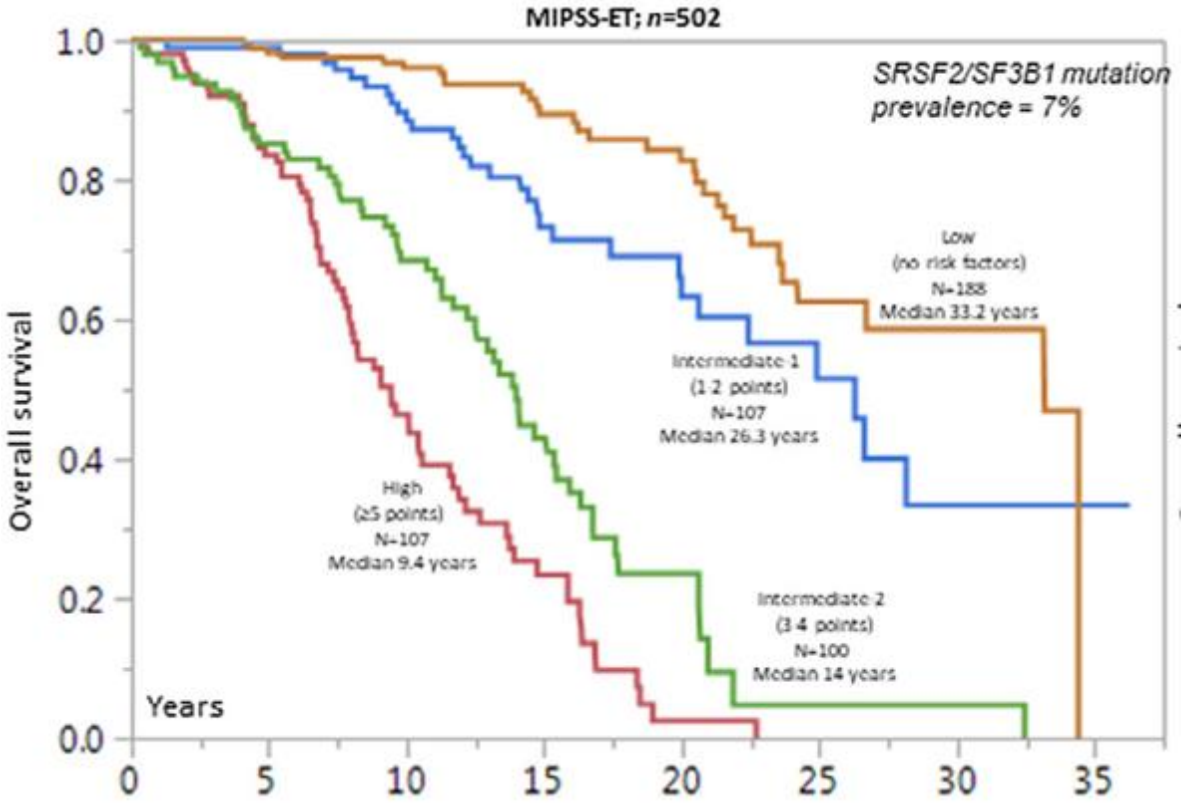
# Comparison of Three Prognostic Scoring Systems in PMF

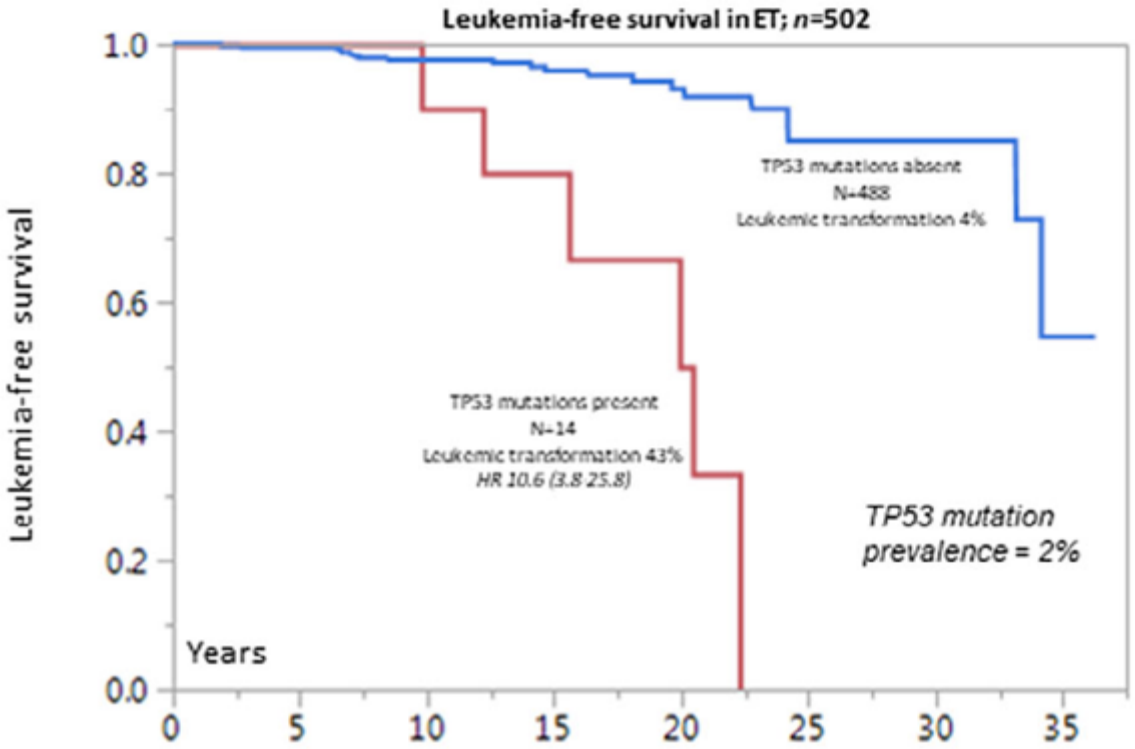
| | MIPSS70 (3-tiered) | | MIPSS70+ version 2.0 (5-tiered) | | GIPSS (4-tiered) |
|---|---|---|---|---|---|
| | Genetic variables | Clinical variables | Genetic variables | Clinical variables | Genetic variables |
| | One HMR mutation (1 point) | Hemoglobin < 10 g/dL (1 point) | VHR karyotype (4 points) | Severe anemia (2 points) | VHR karyotype (2 points) |
| | ≥ 2 HMR mutations (2 points) | Leukocytes > 25 × 10(9)/l (2 points) | Unfavorable karyotype (3 points) | Moderate anemia (1 point) | Unfavorable karyotype (1 point) |
| | Type 1/like CALR absent (1 point) | Platelets < 100 × 10(9)/L (2 points) | ≥ 2 HMR mutations (3 points) | Circulating blasts ≥ 2% (1 point) | Type 1/like CALR absent (1 point) |
| | | Circulating blasts ≥ 2% (1 point) | One HMR mutation (2 points) | Constitutional symptoms (2 points) | ASXL1mutation (1 point) |
| | | Constitutional symptoms (1 point) | Type 1/like CALR absent (2 points) | | SRSF2 mutation (1 point) |
| | | Bone marrow fibrosis grade ≥ 2 (1 point) | | | U2AF1Q157 mutation (1 point) |
| Very low risk (median survival) | | | Zero points (not reached) | | |
| Low risk (median survival) | 0-1 points (not reached) | | 1–2 points (16.4 y) | | Zero points (26.4 y) |
| Intermediate-1 risk (median survival) | | | | | One point (8 y) |
| Intermediate risk (median survival) | 2–4 points (6.3 y) | | 3-4 points (7.7 y) | | |
| Intermediate-2 risk (median survival) | | | | | Two points (4.2 y) |
| High risk (median survival) | ≥5 points (3.1 y) | | 5–8 points (4.1 y) | | ≥3 points (2 y) |
| Very high risk (median survival) | | | ≥9 points (1.8 y) | | |

Anemia (g/dL): Severe: Hgb<8(W) and <9(M); Moderate: Hgb8-9.9(W) and 9-10.9(M)
HMR - High molecular risk mutations: *ASXL1, SRSF2, EZH2, IDH1/2, U2AF1**



*Tefferi A. Am J Hematol. 2021;96:145-162*




# MIPSS-ET

ET survival risk factors: *SRSF2/SF3B1* mutations (2 points); age>60 years (4 points); male gender (1 point)





*Tefferi A. and Barbui T. Am J Hematol. 2020;95:1599–1613.*

CASE A
Pre-PMF

| Prognostic scoring system | Risk score | Median survival |
|---|---|---|
| MIPPS70 | 0 points – Low risk | Not reached |
| MIPSS70+ v2.0 | 0 points – Very low risk | Not reached |
| GIPSS | 0 points – Low risk | 26.4 years |

61-year-old man
Asymptomatic
Upper limit normal spleen

**CLINICAL PRESENTATION**

71-year-old woman
Fatigue, arthritis, hypertension
No splenomegaly

WBC 6.6 x $10^9$/L
PLT 1820 x $10^9$/L (H)

WBC 11.2 x $10^9$/L (H)
$10^{12}$/L (L)
/dL (L)
2%
0 fL
PLT 1977 x $10^9$/L (H)

*CALR* c.1154_1155insTTGTC
p.K385fs* 30.3% VAF

**NGS RESULTS**

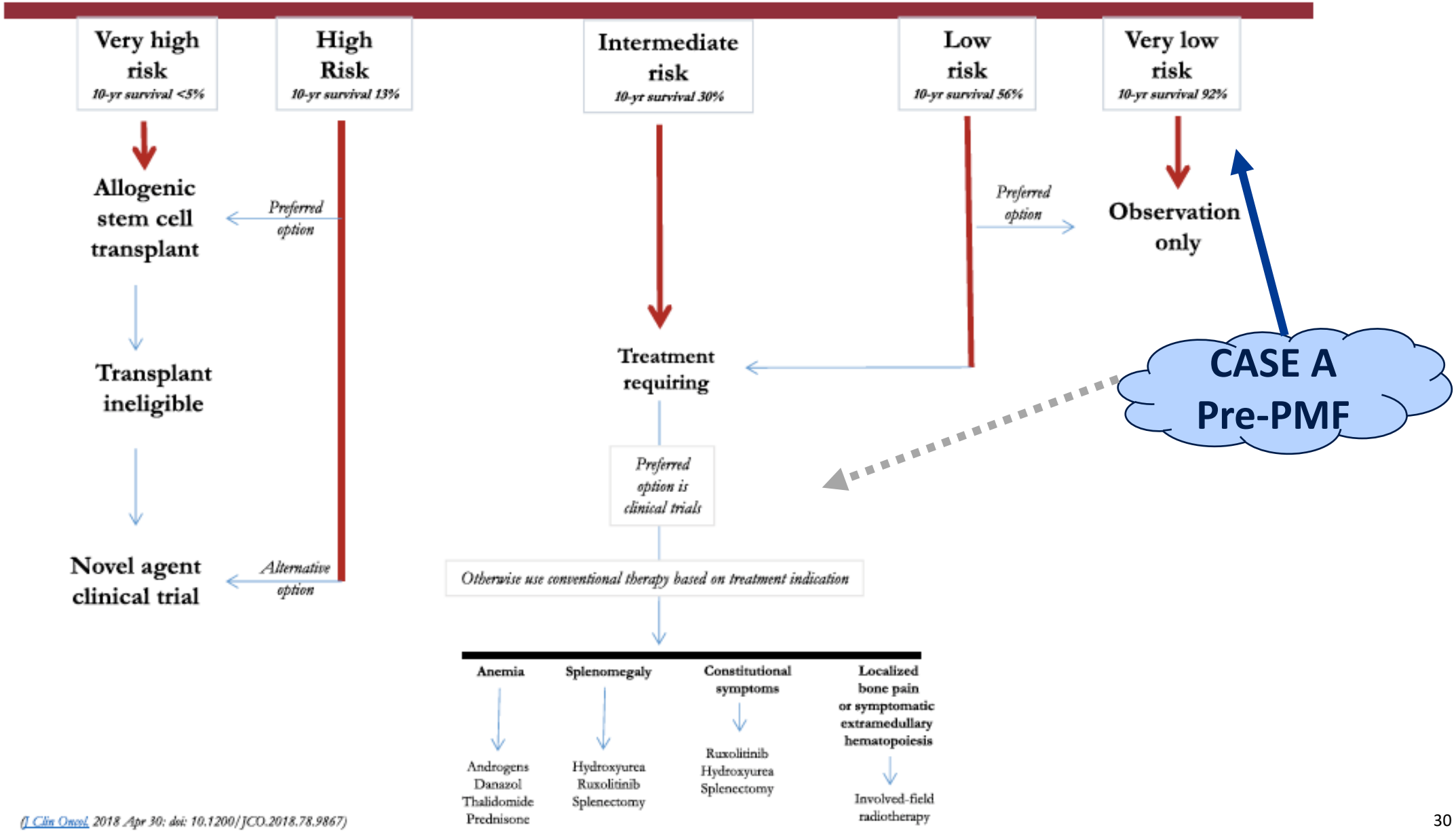
*CALR* c.1154_1155insTTGTC
p.K385fs* 26.6% VAF

What if we got it wrong?
CASE A (ET) – High risk (5 points) – 9.4 years
CASE B (pre-PMF) – Int risk (2-5 points) – 7.7 years

CASE B
ET

| Risk score | Median survival |
|---|---|
| 4 points – Int-2 | 14 years |

# Treatment Algorithm in Primary Myelofibrosis

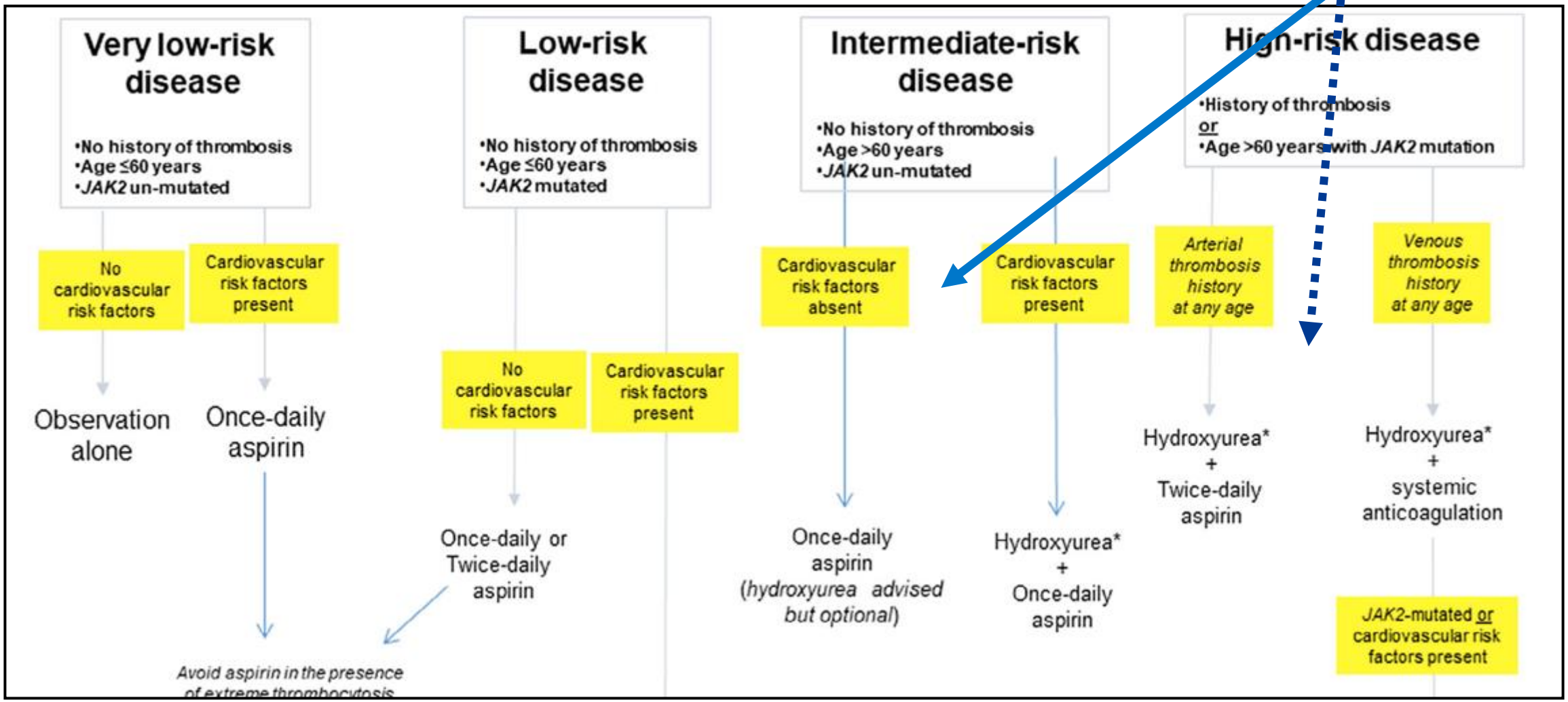



(J Clin Oncol 2018 Apr 30; doi: 10.1200/JCO.2018.78.9867)

http://www.mipss70score.it/

# ET Treatment Recommendations

CASE B
ET



# We are family

- ET and pre-fibrotic PMF belong to the MPN family
- Relationship is close but unclear (?cousins), but definitely **NOT** twins
- Correct diagnosis relies **HEAVILY** on morphology (but not prominent part of prognostic scoring system)
- Prognostic scoring system uses mutational status, CBC, gender, constitutional symptoms
- Treatment is guided by prognosis

# Case C.
# 71-year-old woman presents with severe anemia

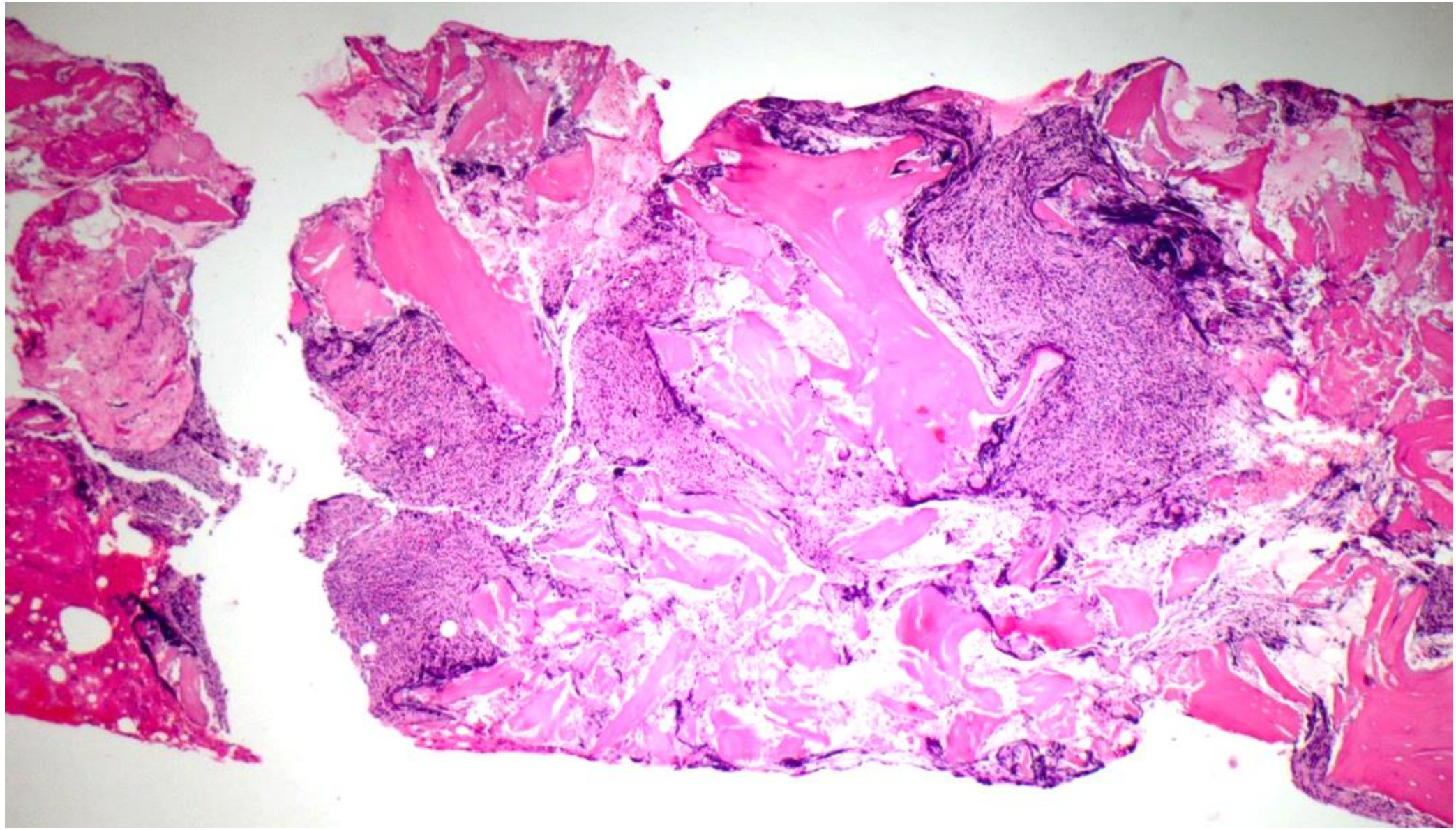
| Parameters | Result | Reference range |
|---|---|---|
| WBC | 12.9 (H) | 3.81 – 8.94 K/µL |
| - Neutrophils | 11.61 (H) | 2.23 - 6.11 K/µL |
| - Lymphocytes | 1.03 | 0.21 - 2.74 K/µL |
| - Monocytes | 0.13 | 0.20 - 0.87 K/µL |
| Hgb | **6.3 (L)** | 12.5 – 16.3 g/dL |
| HCT | **18.8 (L)** | 37.1 – 49.5% |
| MCV | **75 (L)** | 79.0 - 97.0 fL |
| PLT | 236 | 152 - 440 K/µL |
| RDW | 15.2 | 12.1-16.0% |

PMH
- Cough
- Fatigue and weight loss
- Hair loss, diarrhea, pedal edema
- Diverticulitis
- Hypertension
- Migraines

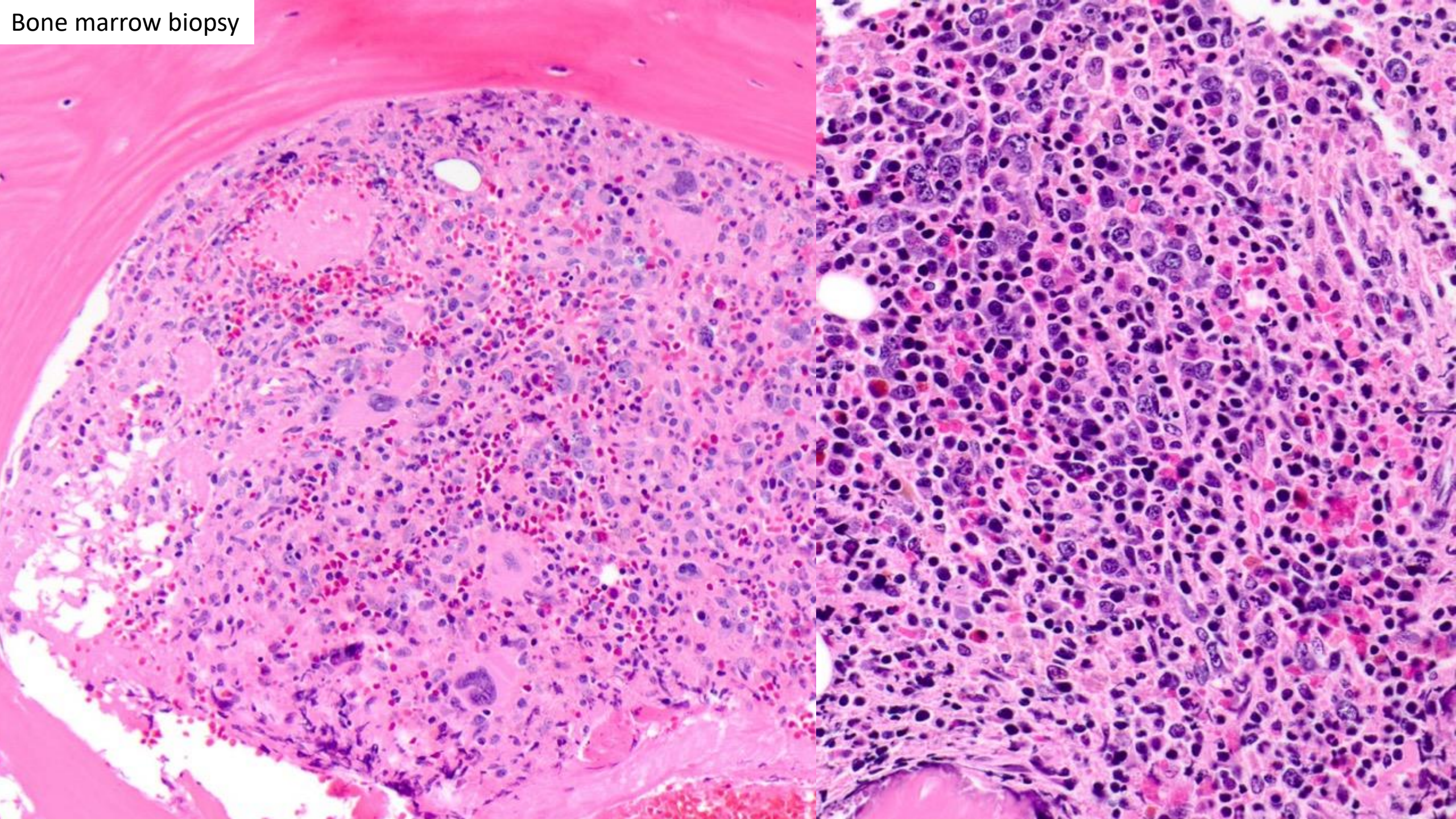
Family history
- Unremarkable

ROS
- No organomegaly or LAN

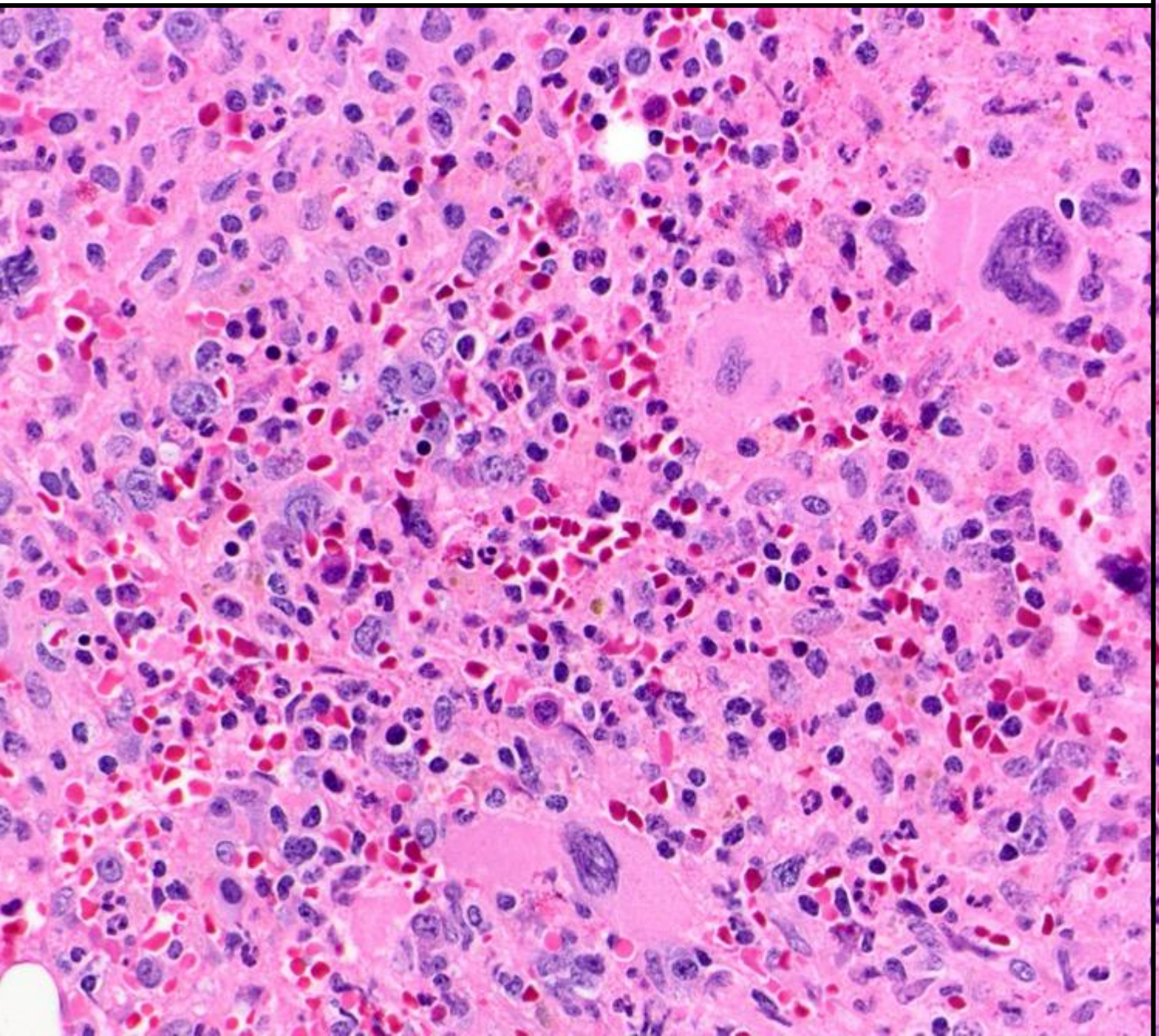
# Bone marrow biopsy

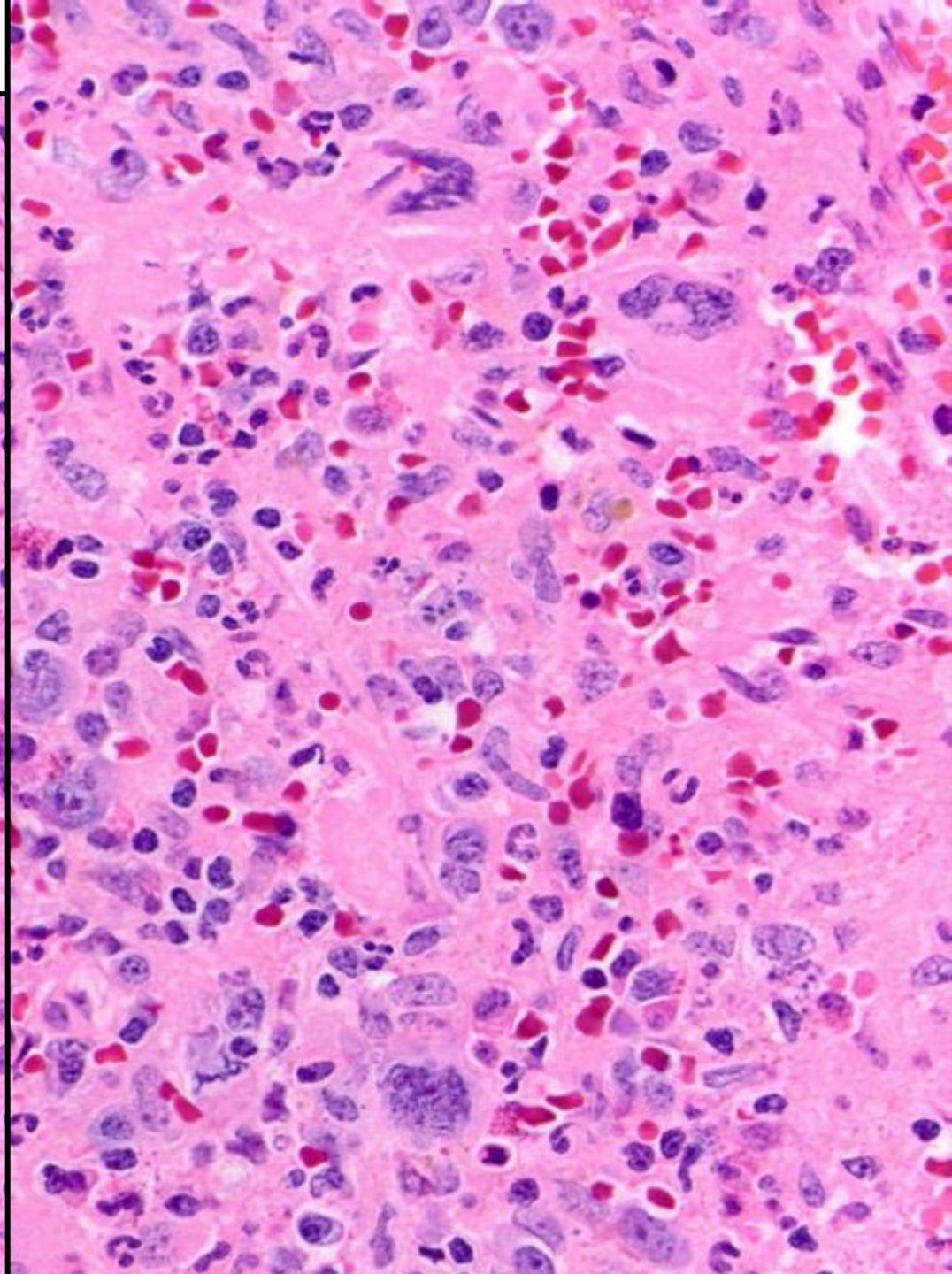


Bone marrow biopsy

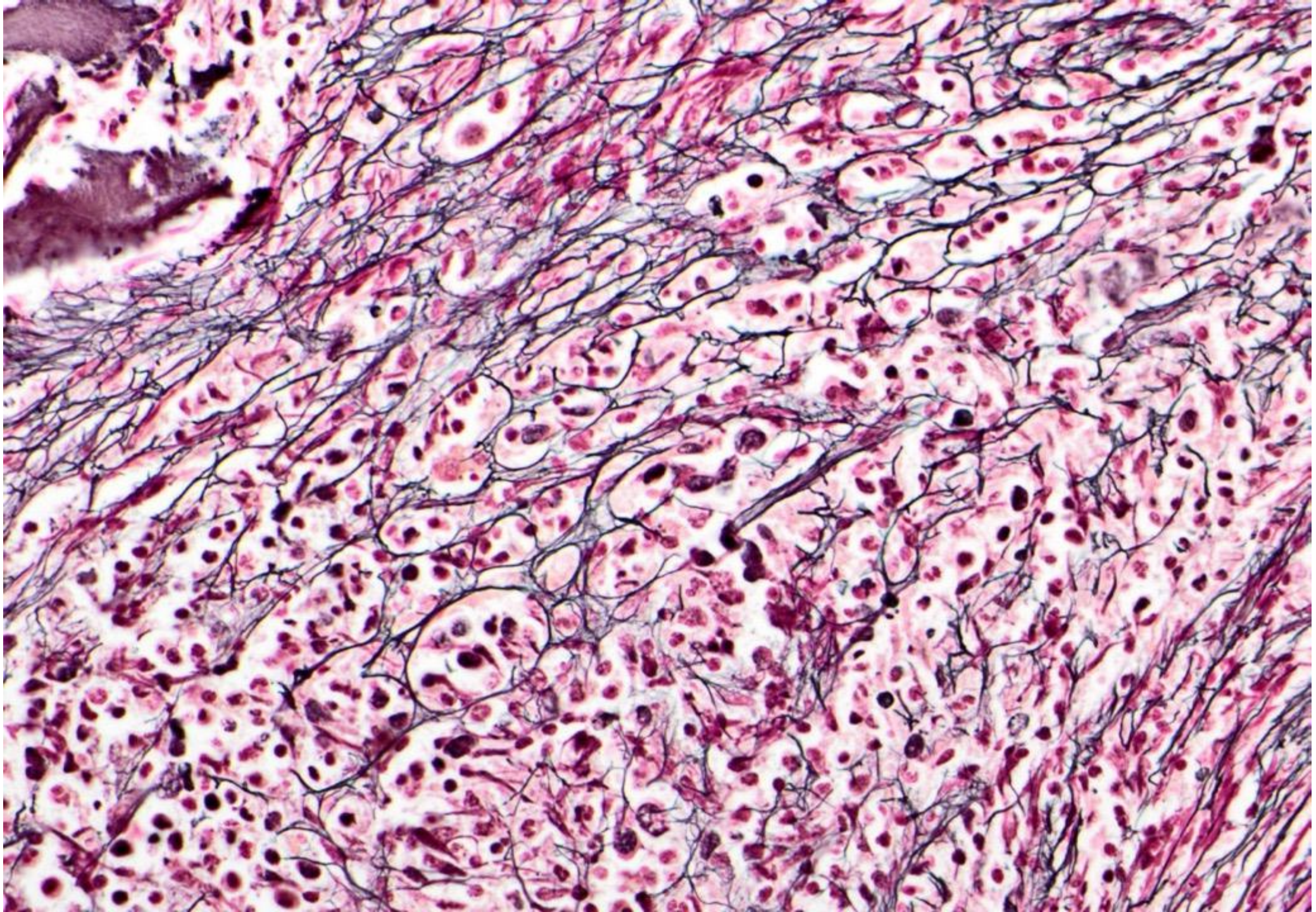


Bone marrow biopsy

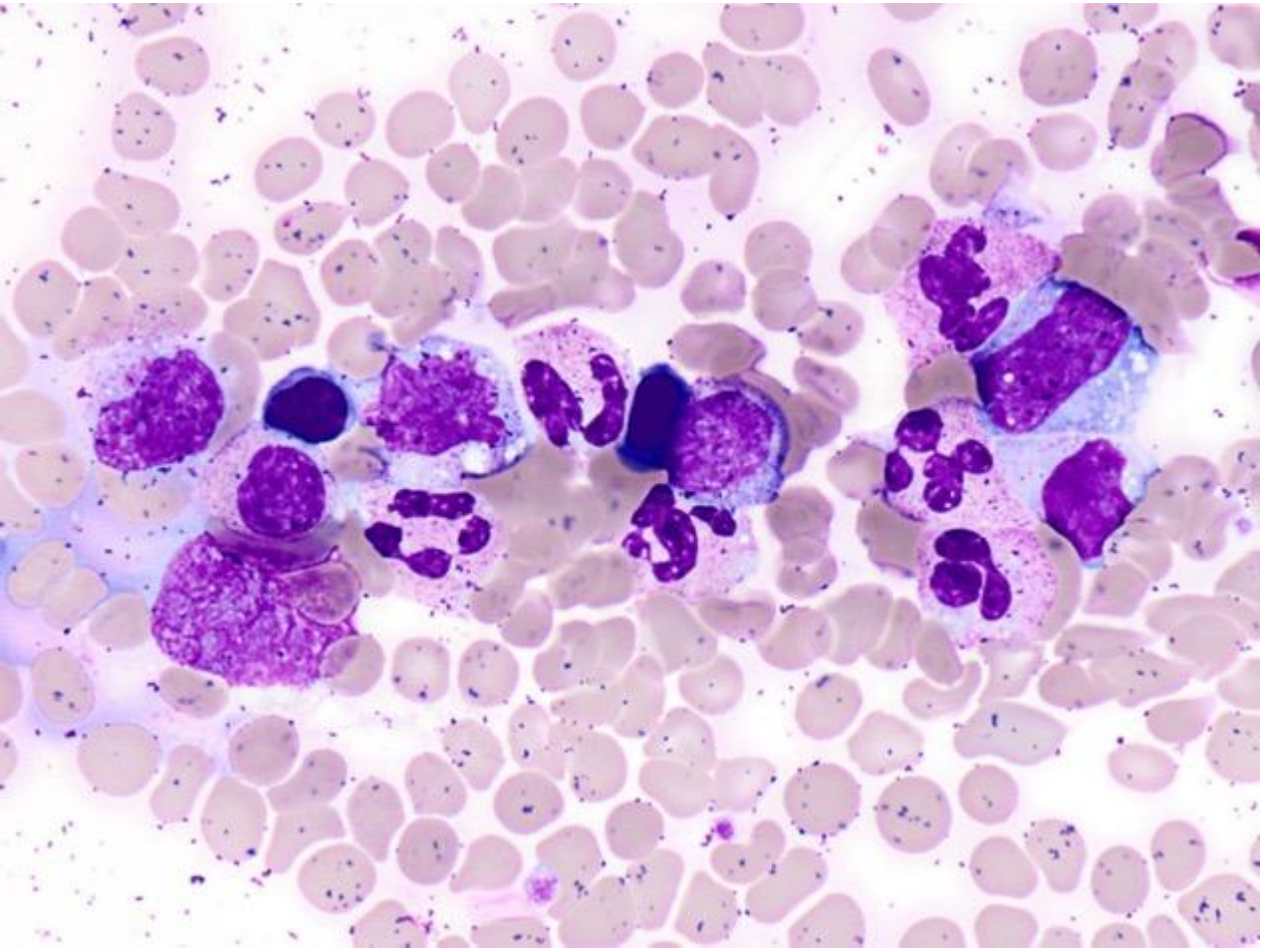


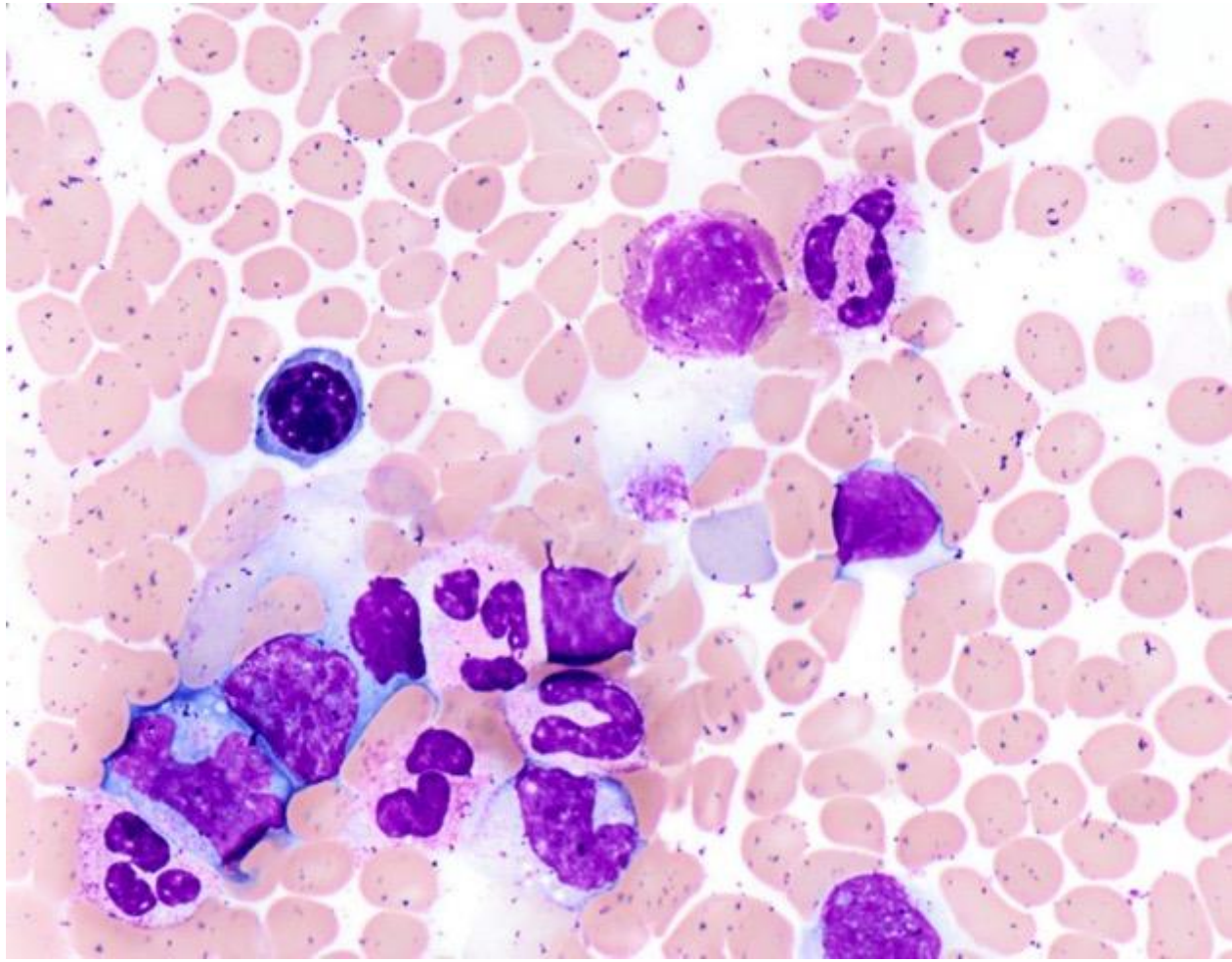


Reticulin



Bone marrow aspirate





Markedly hemodilute and paucicellular: 70% neutrophils and precursors, 8% erythroids, 14% lymphocytes, 5% monocytes, 2% eosinophils, 1% basophils, <1% blasts.

# Other information

Flow cytometry

- <1% myeloid blasts, normal immunophenotype
- 3% polyclonal B cells
- 9% T cells with CD4:CD8 ratio of 3 and no abnormalities

Cytogenetics

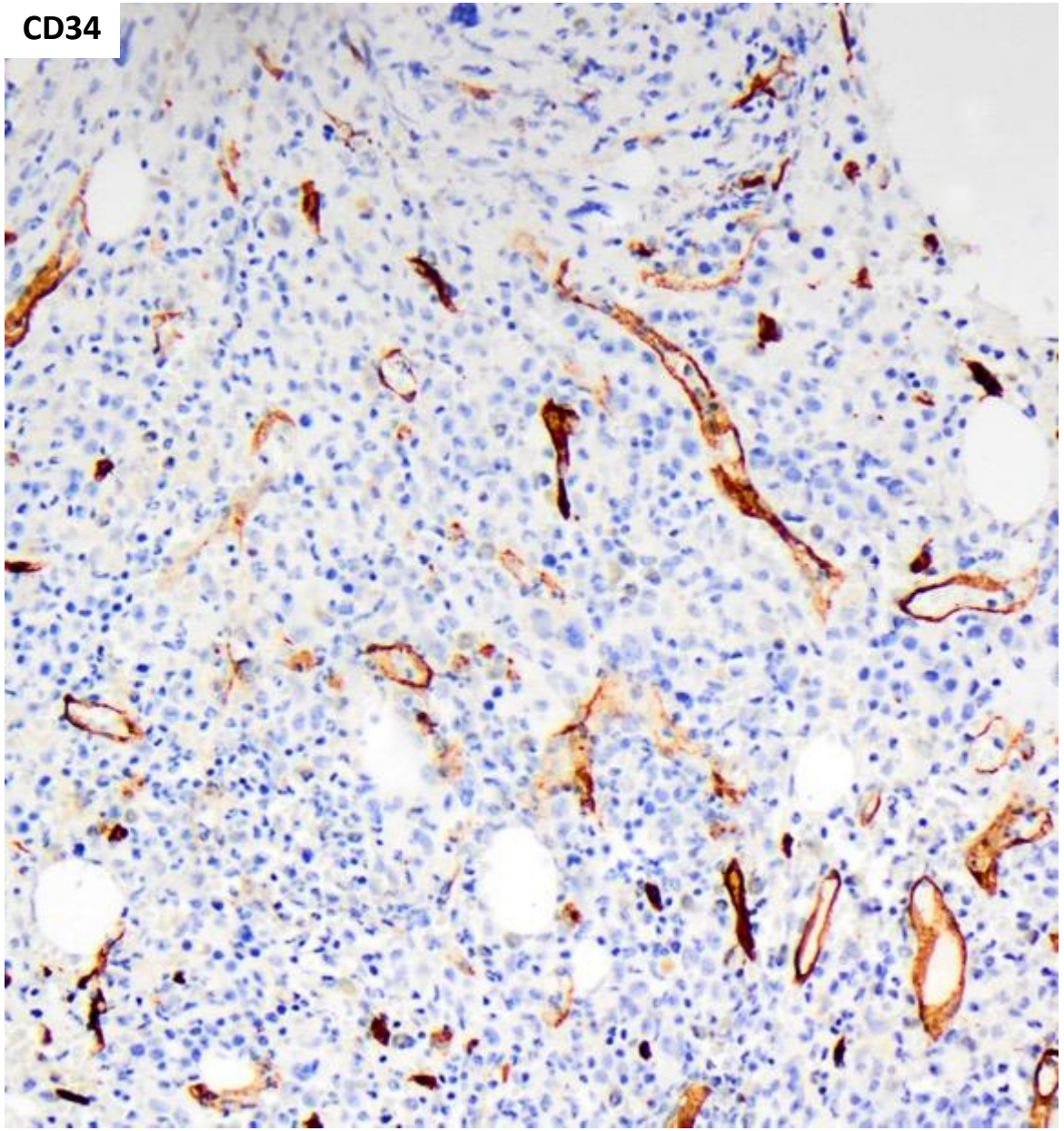
- 46,XX in 20/20 metaphases

NGS

- No pathogenic mutations

Reticulocytes 1.2%, haptoglobin normal

Ultrasound of abdomen: no splenomegaly



# What is your diagnosis?

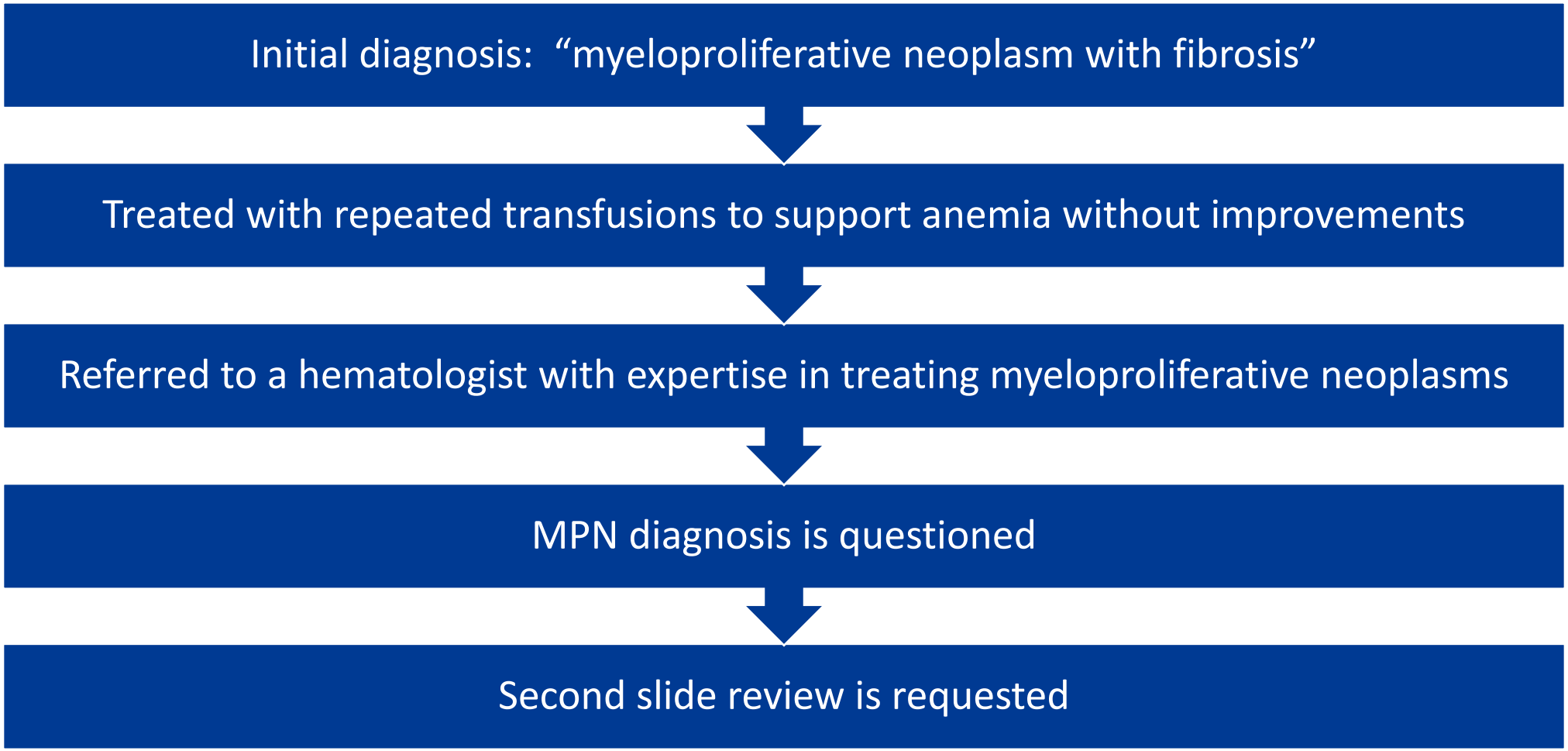
A. I don’t review bone marrows; ask a hematopathologist

B. Primary myelofibrosis, fibrotic stage

C. I need more data

D. Myeloproliferative neoplasm, NOS

# Diagnosis



# Causes of marrow fibrosis

**Neoplastic**

Lymphomas, especially classic Hodgkin lymphoma
Hairy cell leukemia
AML and ALL
Plasma cell myeloma
Mast cell disease
Metastatic solid tumors
MDS with fibrosis
Myeloproliferative neoplasms

- Ph-negative MPN
- CML

**Non-neoplastic**

Pulmonary arterial hypertension
Visceral leishmaniasis
Tuberculosis
Growth factors (thrombopoietin)
Paroxysmal nocturnal hemoglobinuria
Osteopetrosis
Hyperparathyroidism
Vitamin D deficiency
Autoimmune disorders



*Kuter D et al. Br J Haematol 2007;139:351*

# Neoplastic differential diagnoses in this patient

| Lymphoma/leukemia | Myeloproliferative neoplasm | Myelodysplastic syndrome with fibrosis (MDS-F) |
|---|---|---|
| • No neoplastic B-cell or T-cell population detected by flow cytometry<br>• BM eosinophilia, but lack of RS cells | • Leukocytosis but normal karyotype (not CML)<br>• *JAK2/MPL/CALR* negative; no other pathogenic mutations<br>• Lack of characteristic megakaryocyte morphology of Ph-negative myeloid neoplasms<br>• Lack of organomegaly<br>• BM eosinophilia but lack of aberrant mast cell population | • Lack of characteristic megakaryocyte morphology<br>• Severe anemia in absence of splenomegaly would be unusual |

*Tefferi A & Vardiman J Leukemia 2008;22:14, Maschek H et al. Eur J Haematol 1992;48:208, Della Porta MG et al. J Clin Oncol 2009;27:754*

# Remember the patient's presentation?

| |
|---|
| Cough |
| Fatigue and weight loss |
| Hair loss |
| Diverticulitis, diarrhea |
| Hypertension, pedal edema |
| Migraines |

# Additional lab results

ANA positive (high titer) and positive anti-Ro antibody
History of dry eye symptoms, but negative rheumatoid factor

Systemic lupus erythematosus
and
Autoimmune myelofibrosis (AIMF)

# Back to our patient. . .

Treated with prednisone (40 mg qd) —and anemia improved!
HGB 6.3 -> 9.6 g/dL
Patient no longer required transfusions

# Autoimmune myelofibrosis (AIMF)

Bone marrow fibrosis associated with cytopenias has been reported in patients with systemic lupus erythematosus

- Reversal of fibrosis and cytopenias can occur in response to immunosuppression

Also polyarteritis nodosa, scleroderma, psoriatic arthritis, Sjögren's, Hashimoto’s thyroiditis, ulcerative colitis, primary biliary cirrhosis

=“Secondary AIMF”



*Paquette et al. Medicine 1994;73:145, Rizzi et al. Leuk Lymph 2004;45:561*

# Autoimmune myelofibrosis in patients without a well-established autoimmune disease ("primary AIMF")

Grade MF2-MF3 reticulin fibrosis of bone marrow without osteosclerosis

Lack of clustered, atypical megakaryocytes or significant dysplasia to suggest MPN or MDS

Absence of pathogenic somatic myeloid-associated mutations

- Beware of clonal hematopoiesis, especially in older patients!

Lymphoid infiltration of bone marrow

- Often polytypic plasmacytosis, but no increased IgG4+ cells

Absent or mild splenomegaly

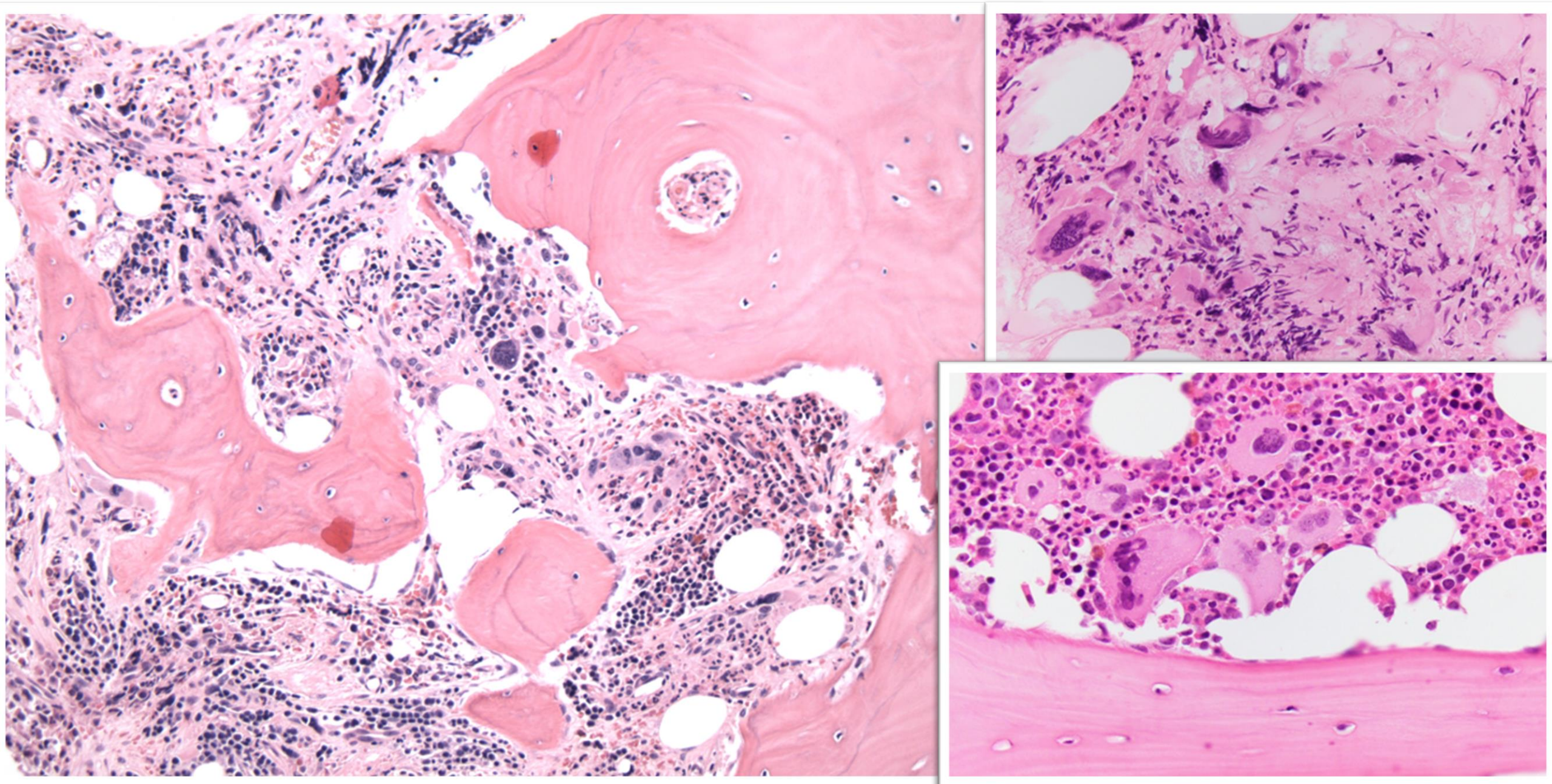
Usually, presence of autoantibodies

Response to immunosuppression



*Pullarkat et al Am J Hematol 2003;72:8, Bass et al. AJCP 2001;116:211, Rizzi et al. Leuk Lymph 2004;45:561, Vergara-Lluri ME et al. Hum Pathol 2014;45:2183-2191*

# Overt PMF



# Summary for case #C

| | |
|---|---|
| **YES,** | Most causes of increased BM reticulin (MF2-MF3) are neoplastic, but also consider non-neoplastic causes |
| **CONSIDER,** | Myelofibrosis may present as cytopenia in a patient with no known autoimmune disease, mimicking primary myelofibrosis or MPN |
| **PLEASE,** | Be VERY cautious making an MPN diagnosis without any pathogenetic variants |

# What we have covered:

| | |
|---|---|
|  Recognize bone marrow morphologic findings associated with myeloproliferative neoplasms: | Pay close attention to cellularity, individual lineages and megakaryocyte morphology and topography<br>Morphology could be a key to the correct diagnosis, especially in early stages |
|  Accurately apply bone marrow fibrosis grading and understand its significance: | Fibrosis grading is used for diagnosis, prognosis and treatment response |
|  Understand the importance of a comprehensive approach for diagnosis and prognosis of myeloproliferative neoplasms: | Never make an MPN diagnosis without careful review of clinical, laboratory, genetic and morphologic findings -> significant overlap<br>Question an MPN diagnosis in the absence of pathogenic variants and be mindful of CHIP |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