Disclosure belangen M.Wondergem

(potentiële) belangenverstrengeling	geen
Voor bijeenkomst mogelijk relevante relaties met bedrijven	Geen
• Sponsoring of onderzoeksgeld • Honorarium of andere (financiële) vergoeding • Aandeelhouder • Andere relatie, namelijk …	•nvt • • •

Disclosure belangen K. Hebeda

(potentiële) belangenverstrengeling	Geen
Voor bijeenkomst mogelijk relevante relaties met bedrijven	Geen
• Sponsoring of onderzoeksgeld • Honorarium of andere (financiële) vergoeding • Aandeelhouder • Andere relatie, namelijk …	• nvt • nvt • nvt •nvt

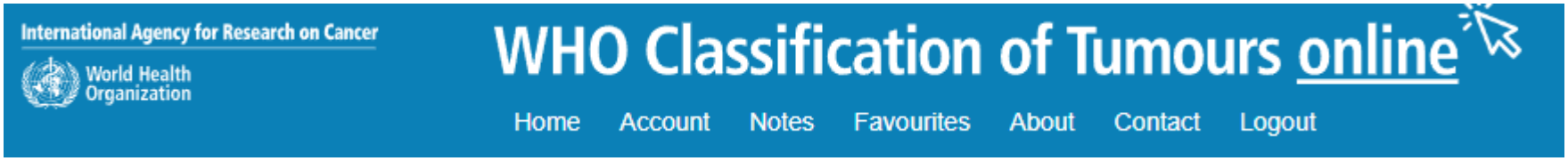
Myeloproliferatieve neoplasieën

Wenckebach basiscursus, September 2024

M. Wondergem, K. Hebeda

Met dank aan G Verhoef

WHO 2022



Myeloproliferative neoplasms

Myeloproliferative neoplasms

Introduction

Chronic myeloid leukaemia

Chronic neutrophilic leukaemia

Chronic eosinophilic leukaemia

Polycythaemia vera

Essential thrombocythaemia

Primary myelofibrosis

Juvenile myelomonocytic leukaemia

Myeloproliferative neoplasm, NOS

Usual features of myeloid disorders at diagnosis

Disease	BM cellularity	% marrow blasts	Maturation	morphology	hematopoiesis	Blood counts	Organo-megaly
Mypro	++	<10%	++	normal	effective	increased	common
MDS	++/-	<20%	+	dysplasia	ineffective	cytopenia	uncommon
Mypro/ MDS	++	<20%	+	dysplasia	Effective/ ineffective	variable	common
AML	++/-	≥20%	+/--	Normal/ dysplasia	ineffective	variable	uncommon

Myeloproliferatieve Neoplasieën: de rol van het beenmergbiopt

Wat willen hematologen weten

BMB bij diagnose:

- Past het beeld bij MPN of reactief?
- Welk type MPN
- Is er fibrose, gradering, extramedullaire hematopoiese
- Uitsluiten overig mn. mastocytose, lymfoproliferatief, MDS

Vervolgbiopt:

- Progressie of (bij therapie) afname fibrose?
- Dysplastische veranderingen, therapie effecten, aplasie
- Dry-tap: is er blastentoename: accelerated phase, blastic phase, fenotype
- Is er afname cellulariteit: spent phase

Wat wil de patholoog weten

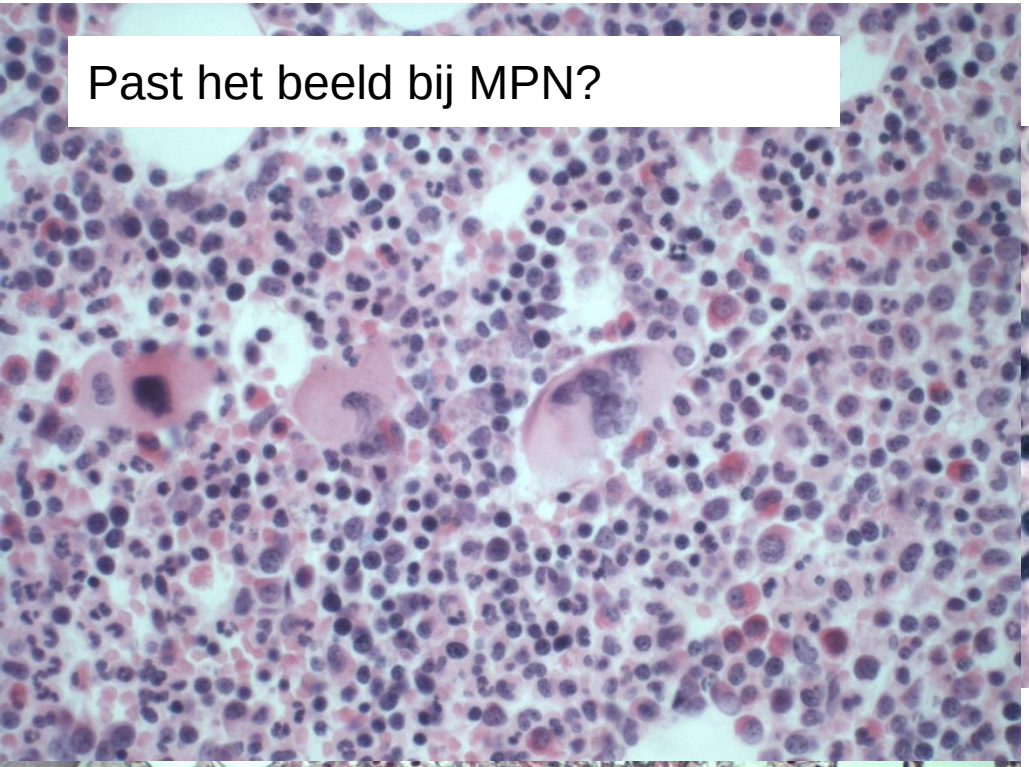
Bij diagnose:

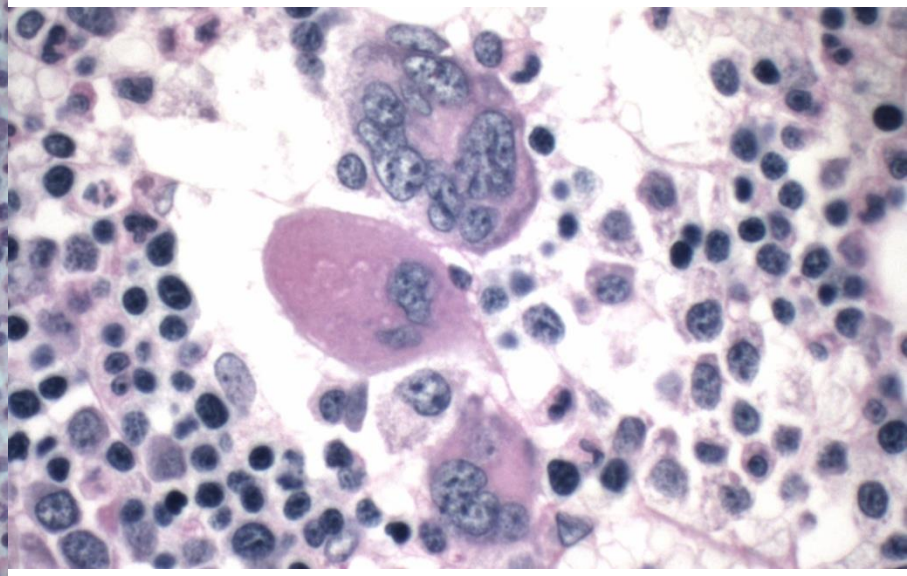
- Bloedwaarden
- *JAK2, CALR, MPL* mutatie aangetoond? *BCR-ABL* uitgesloten?
- Onbehandeld uitgangsbiopt of eerdere therapie?
- Relevante voorgeschiedenis

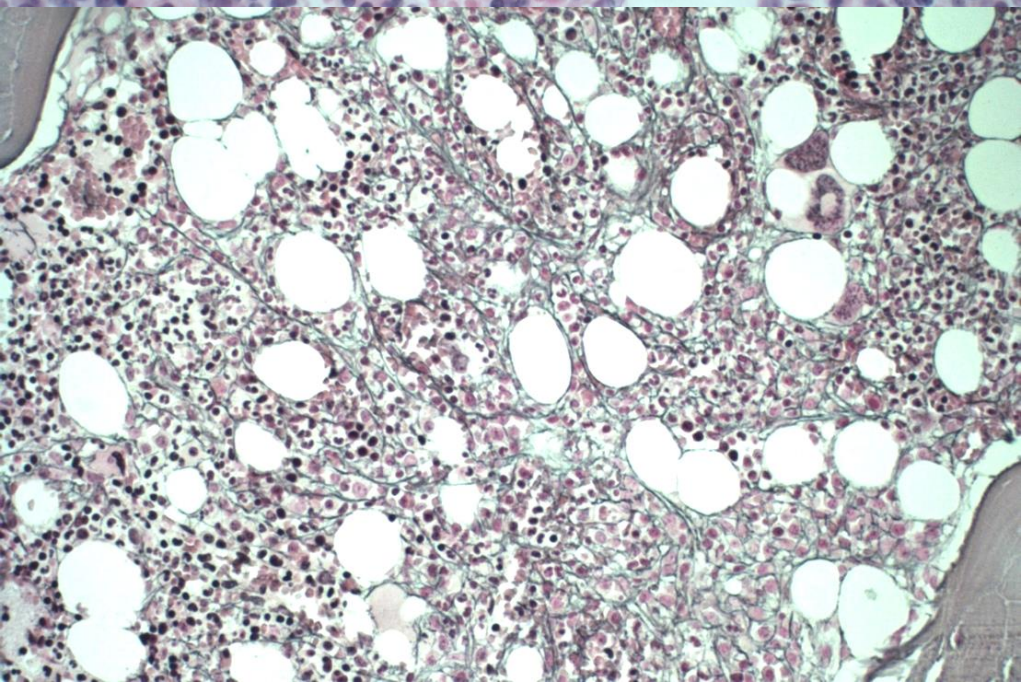
Vervolgbiopt:

- Eerdere diagnose
- Welke behandeling
- Nieuwe (cyto)genetische afwijkingen?
- Klinisch accelerated phase, blastic phase, fenotype blasten?

Past het beeld bij MPN?







Hypercellulair
Grote megakaryocyten
Clustering
Fibrose
Uitrijping

Past het beeld bij MPN?

Table 2.04 Relative incidence of discriminating features according to standardized WHO morphological criteria generating histological patterns in initially performed bone marrow biopsy specimens. Modified and adapted from Thiele J. and Kvasnicka H.M. {3969}

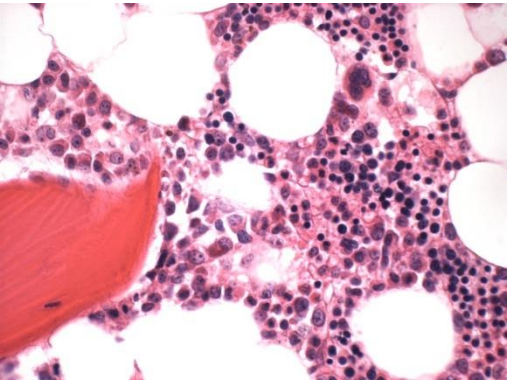
Bone marrow morphology features		Relative frequency of features			
		PV	ET	Pre-PMF	Overt PMF
Cellularity	Age-related increase	>80%	10–19%	>80%	10–19%
Granulopoiesis	Increased in quantity	>80%	<10%	50–80%	0%
	Left-shifted	<10%	<10%	20–49%	10–19%
Erythropoiesis	Increased in quantity	>80%	<10%	<10%	0%
	Left-shifted	>80%	<10%	10–19%	<10%
Megakaryopoiesis	Increased in quantity	50–80%	>80%	50–80%	20–49%
Size of cells	Small	20–49%	<5%	20–49%	20–49%
	Medium	20–49%	1		
	Large	20–49%	2		
	Giant	10–19%	2		
Histotopography	Endosteal translocation	10–19%	1		
Cluster formation	Small clusters (>3 cells)	10–19%	1		
	Large clusters (>7 cells)	<10%			
	Dense clusters	<10%			
	Loose clusters	20–49%	2		
Nuclear features	Hypolobulation (bulbous)	10–19%	<10%		
	Hyperlobulation (staghorn-like)	50–80%	50–80%	<10%	0%
	Maturation defects	0%	0%	50–80%	>80%
	Naked nuclei	20–49%	20–49%	50–80%	>80%
Fibrosis	Increased reticulin	10–19%	<5%	20–49%	>80%
	Increased collagen	0%	0%	0%	50–80%
	Osteosclerosis	0%	0%	0%	20–49%
Stroma	Iron deposits	0%	20–49%	10–19%	<10%
	Lymphoid nodules	10–19%	<5%	10–19%	<10%

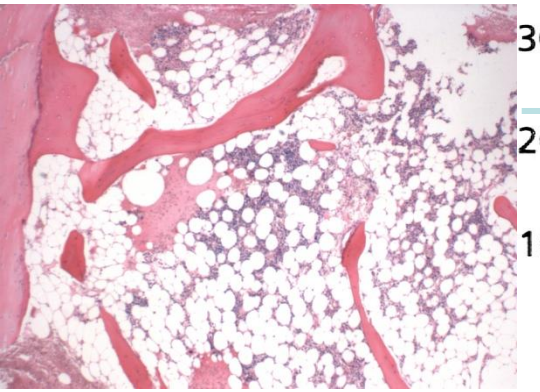
PV, polycythaemia vera; ET, essential thrombocythaemia; pre-PMF, prefibrotic/early primary myelofibrosis; Overt PMF, overt primary myelofibrosis

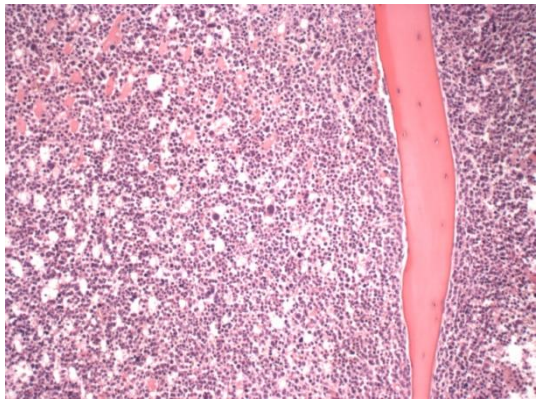
DD. histologie:
- reactieve hyperplasie (infectie, paraneoplastisch, therapie effect)
- MDS
- MDS/MPN
- PNH

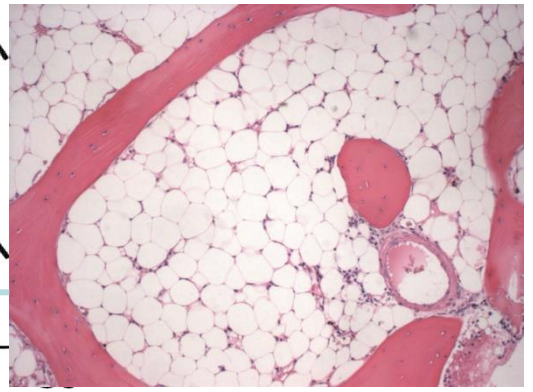
WHO 2016

Cellulariteit = relatief









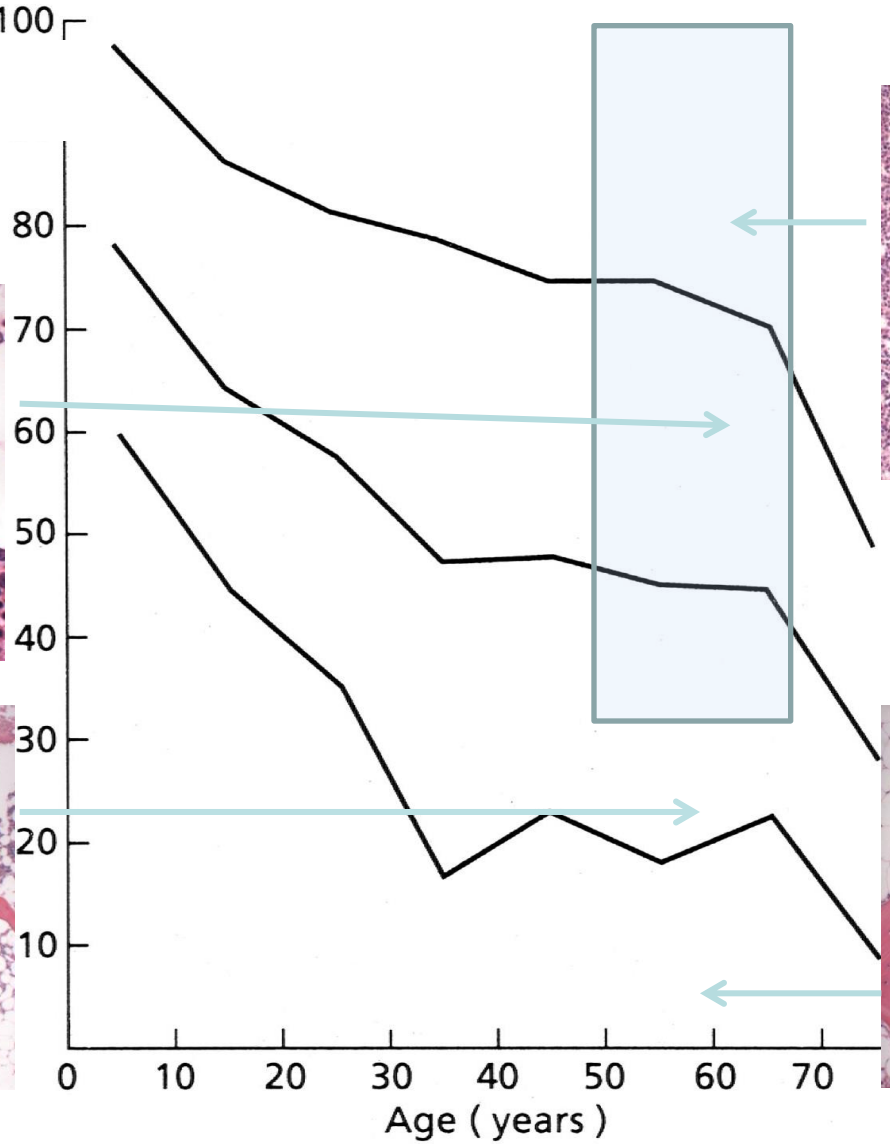
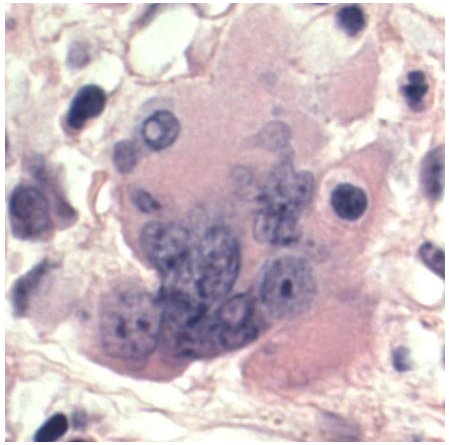
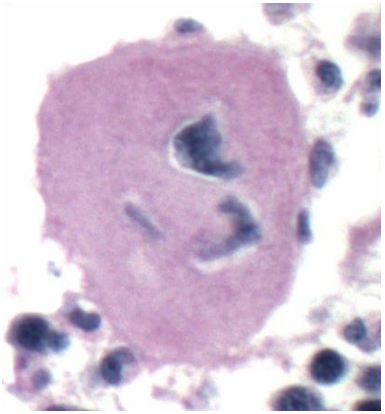
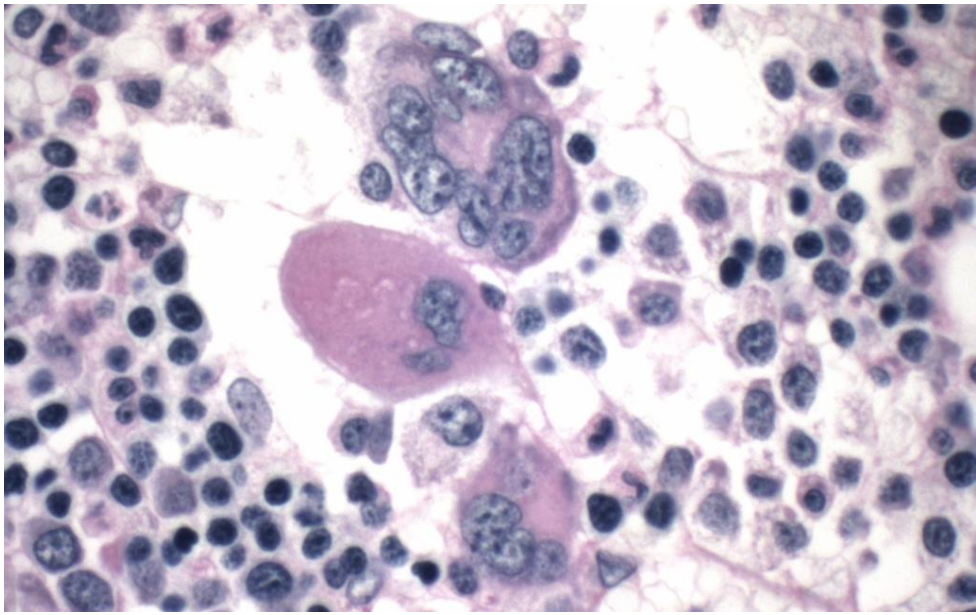


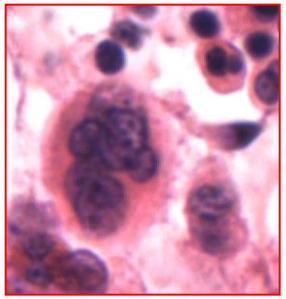
Fig. 1.3 Mean and 95 per cent range of cellularity at various ages of anterior iliac crest bone marrow which has been decalcified and paraffin-embedded. Cellularity is expressed as a percentage of the bone marrow cavity. Calculated from Hartsock.[8]

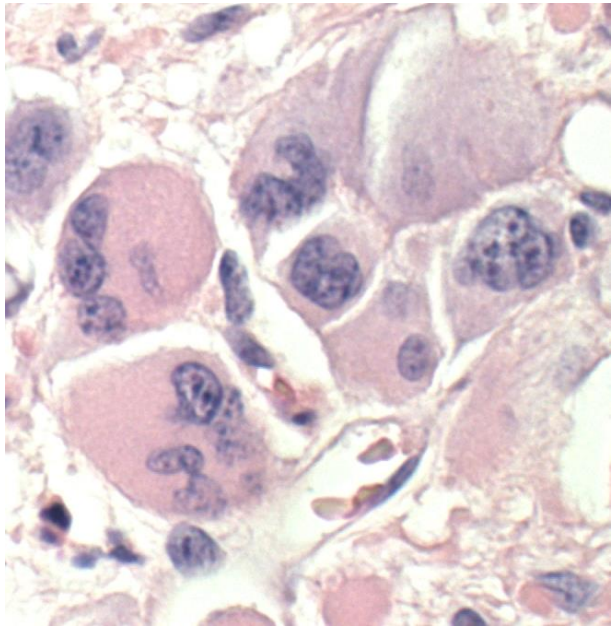
Morfologie van de megakaryocyten bij MPN

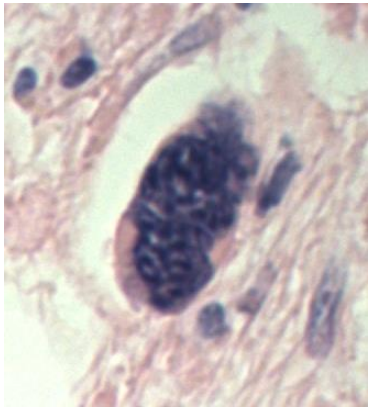


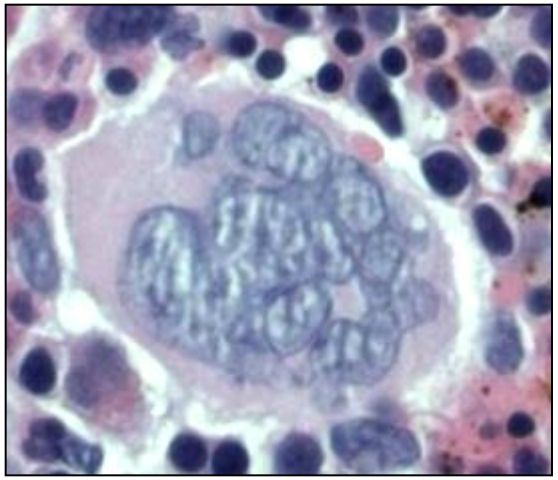




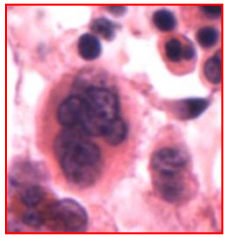


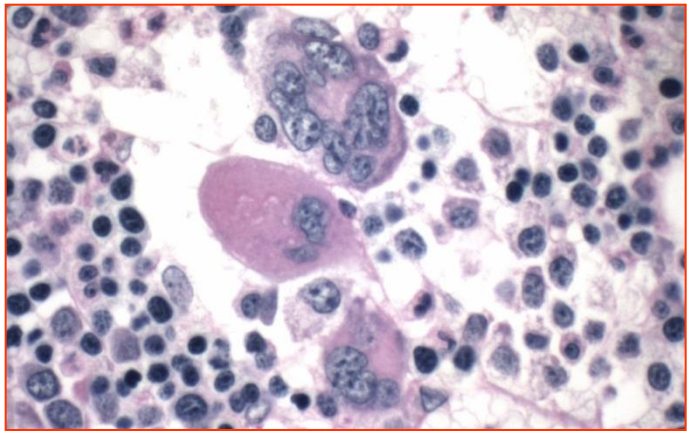


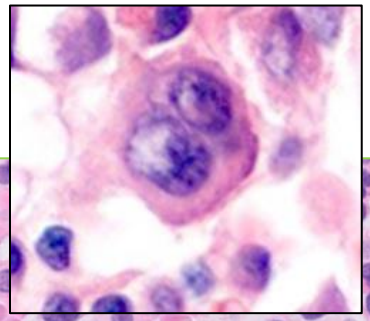


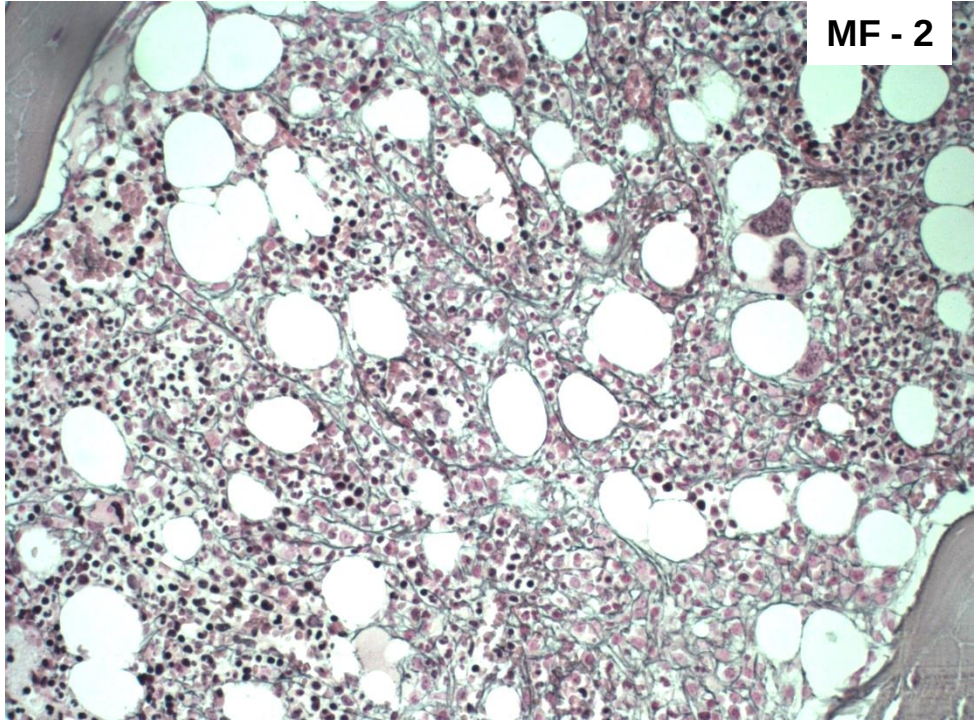


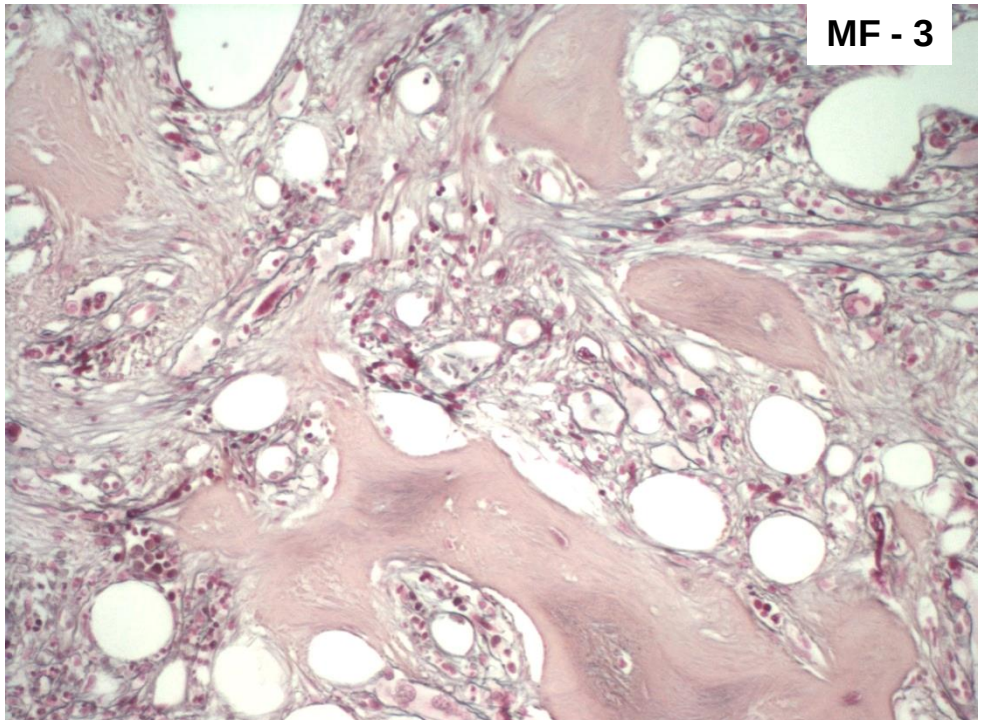
Morfologie van de megakaryocyten bij MDS











Gradering fibrose

MF - 0 Scattered linear reticulin with no intersections corresponding to normal bone marrow

MF - 1 Loose network of reticulin with many intersections, especially in perivascular areas

MF - 2 Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibres mostly consistent with collagen and/or focal osteosclerosis

MF - 3 Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibres consistent with collagen, usually associated with osteosclerosis

**Fiber density should be assessed in hematopoietic areas*
** If heterogeneous: highest grade in >30% of marrow area*
** Osteosclerosis can be graded, CAVE: cortical bone*

WHO2016

MyeloProliferatieve Neoplasie (MPN)

- **Polycythemia vera**
- **Essentiële trombocytemie**
- **Primaire myelofibrose**
- **Chronische myeloïde leukemie**
- **Differentiaal diagnosen**
- **Vervolgbiopten**

Klinische context, genetische afwijkingen

Casus

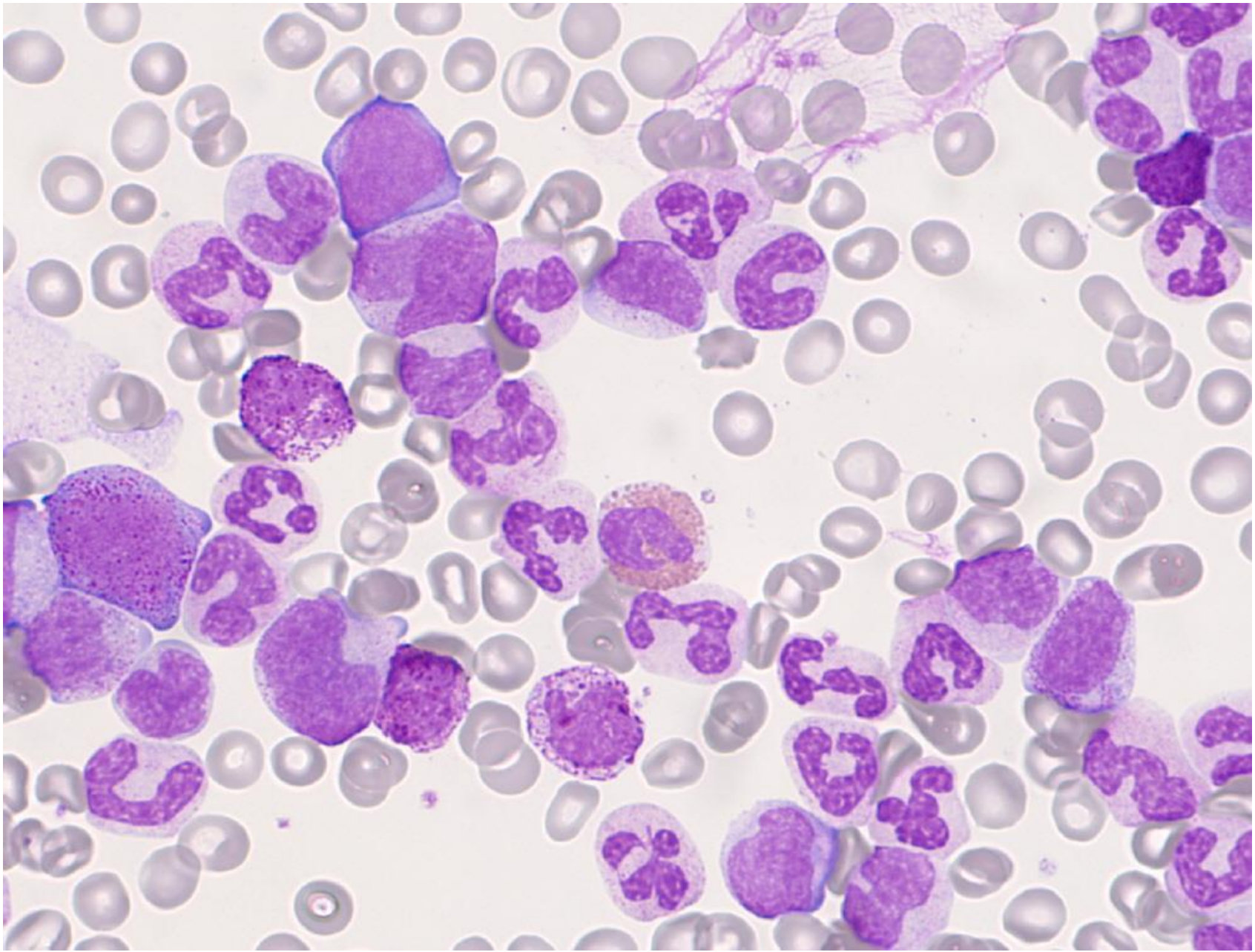
- Vrouw, 56 jaar, moe
- Hb 6,4 mmol/l, trombo 620 x 10^9/l, leuco 44 x 10^9/l
- In linker bovenbuik zwelling palpabel

- Wat is de differentiaal diagnose?
- Extra onderzoek nodig? Wat?

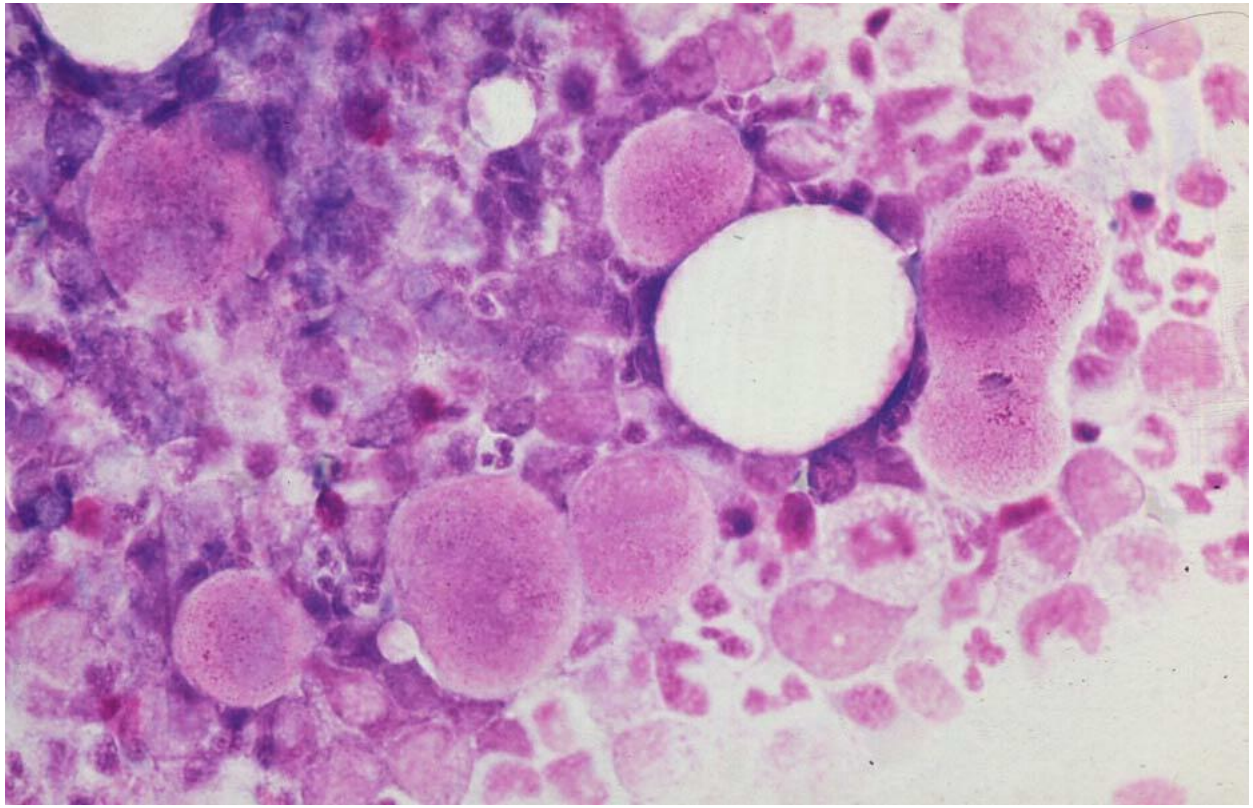
Stel: dit is de perifere diff

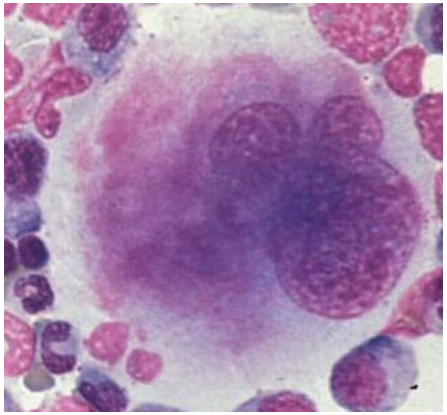
WBC	**44.7**	**4-10 x 10^9/L**
promyelocyten	**2**	**<0**
myelocyten	**13**	**<0**
metamyelocyten	**16**	**2-5 %**
staven	**7**	**2-5 %**
segmenten	**40**	**38-75 %**
eosinofielen	**1**	**<6 %**
basofielen	**5**	**<1 %**
lymfocyten	**13**	**20-50 %**
monocyten	**3**	**2-10**

CML: perifeer bloed

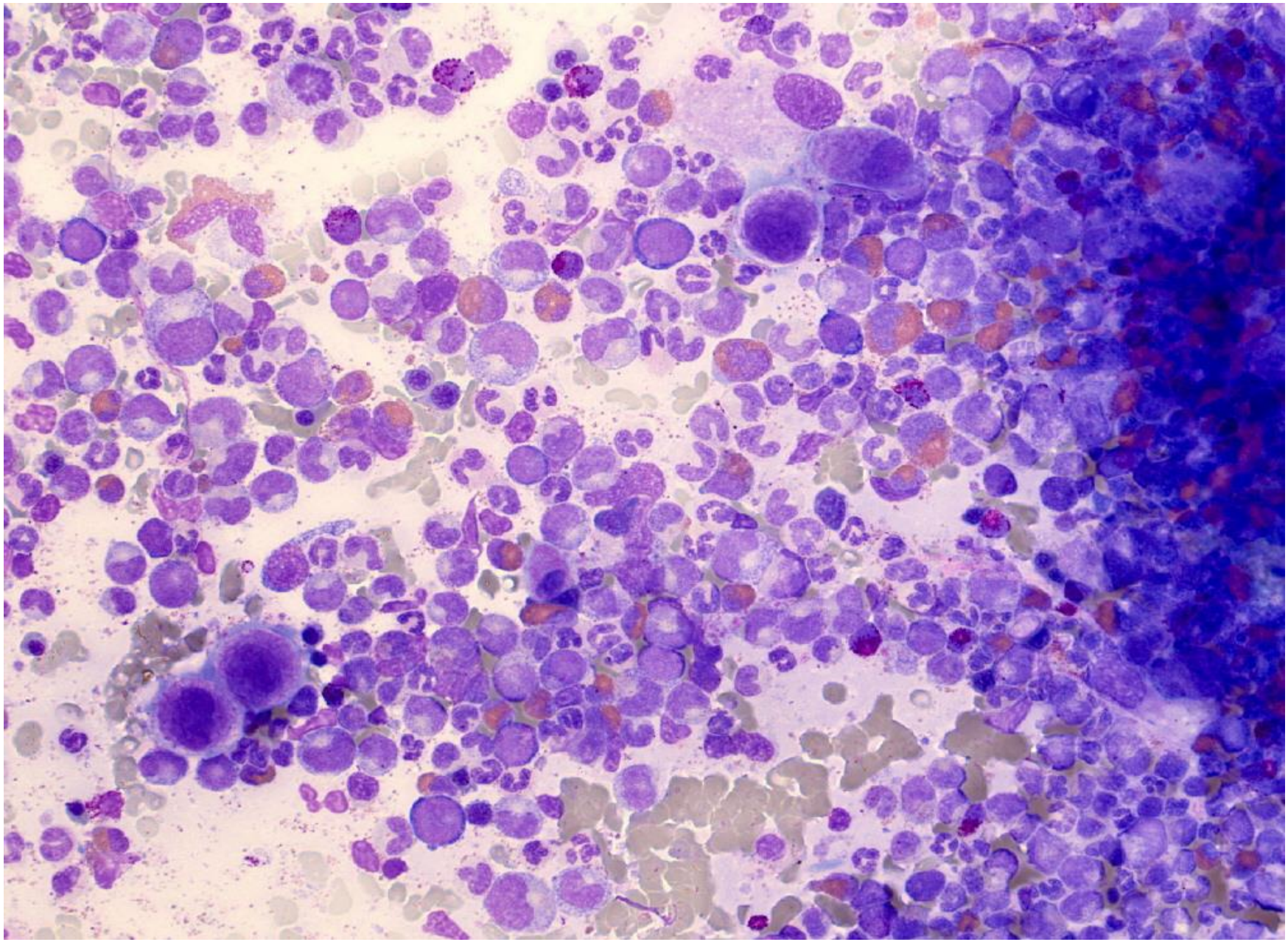


CML: kleine mega’s zonder dysplasie

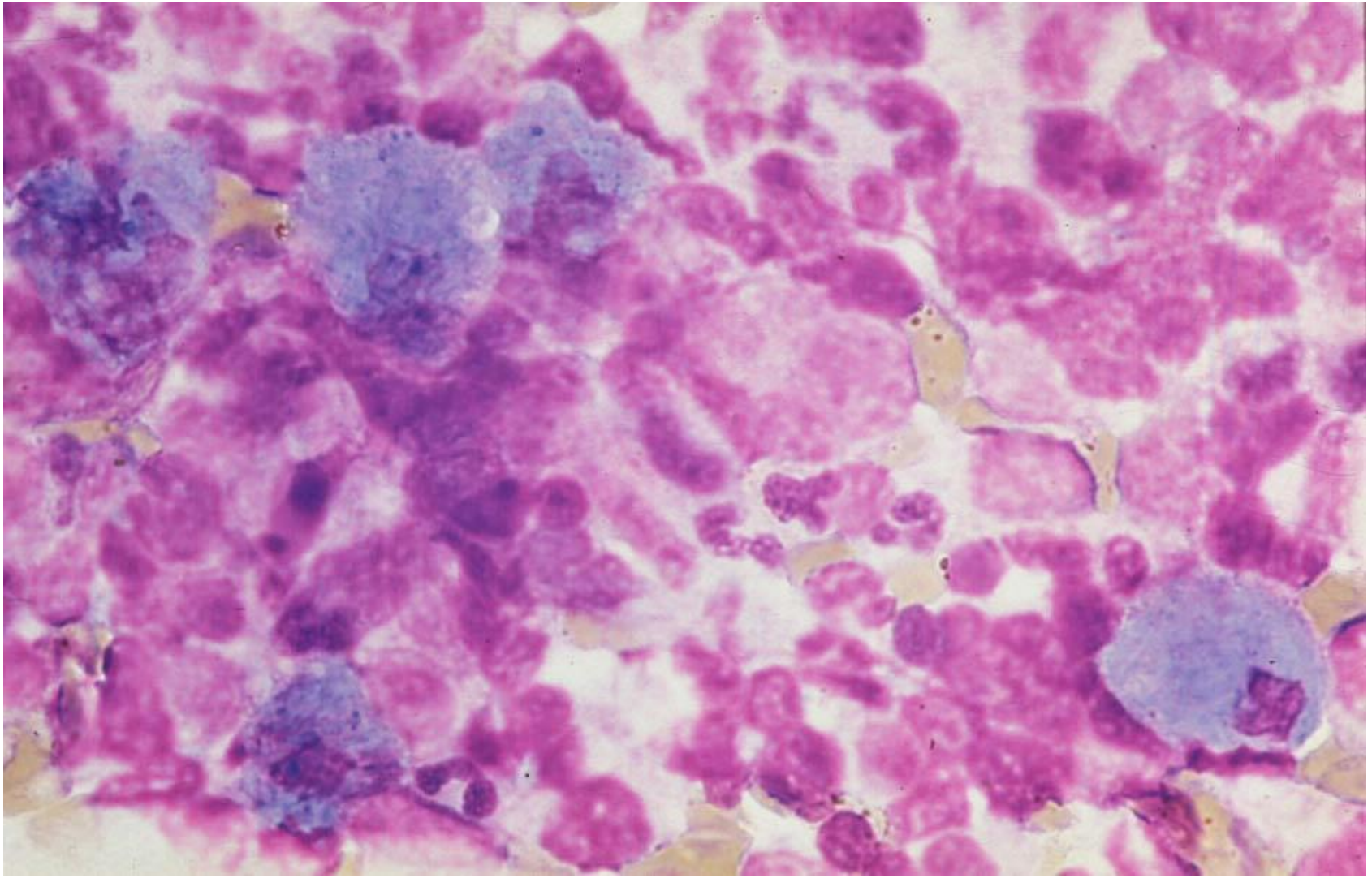




normaal

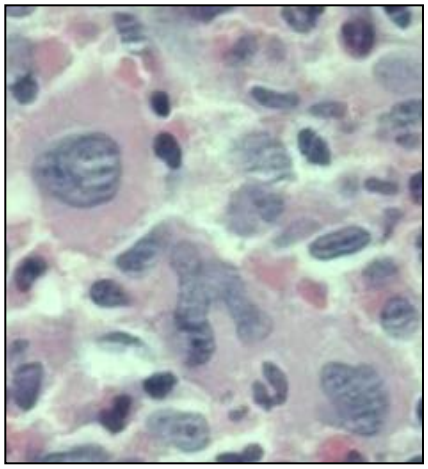


CML: sea blue histiocytes

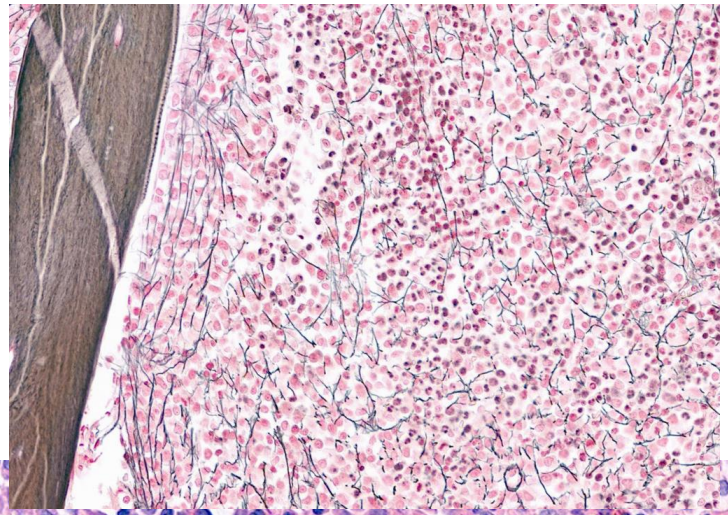


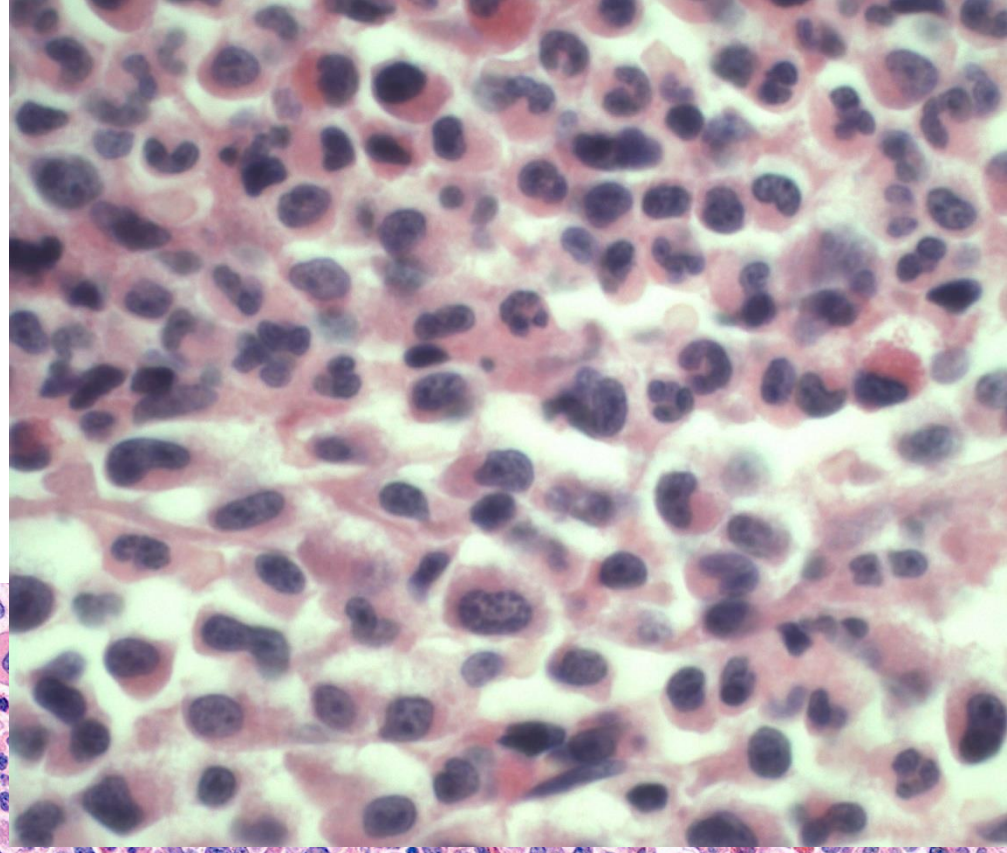
Botbiopt: Chronische myeloïde leukemie

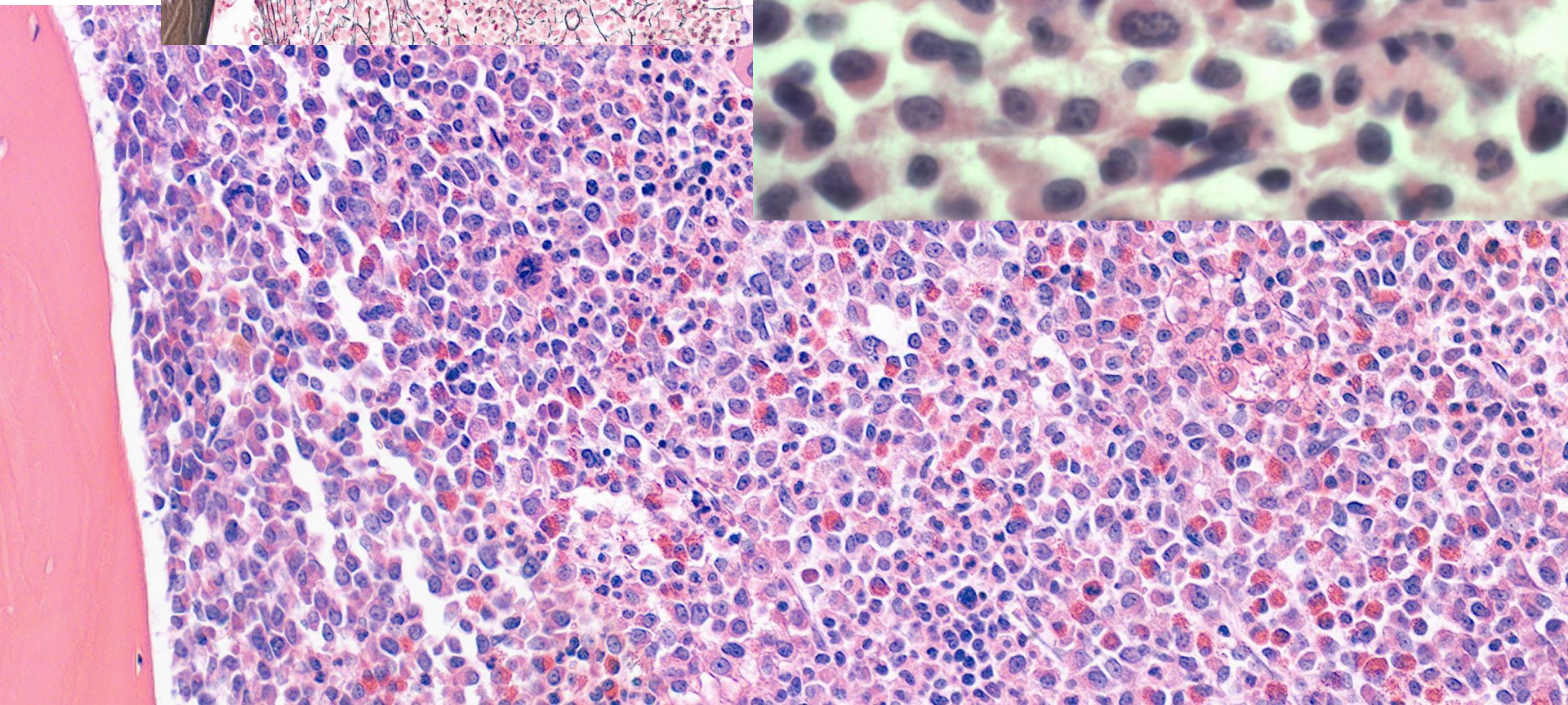
- 100% celrijk
- Hyperplasie myelopoiese
  - Brede onrijpe myeloïde zomen
  - Maturatie “gap”
  - Veel eosinofiele granulocyten (+baso’s)
  - Nauwelijks erytropoiese
- Megakaryocyten vrij klein en simpel
- Vaak enige reticuline fibrose (MF1)
- Pseudo-Gaucher cellen (hoge turn-over)



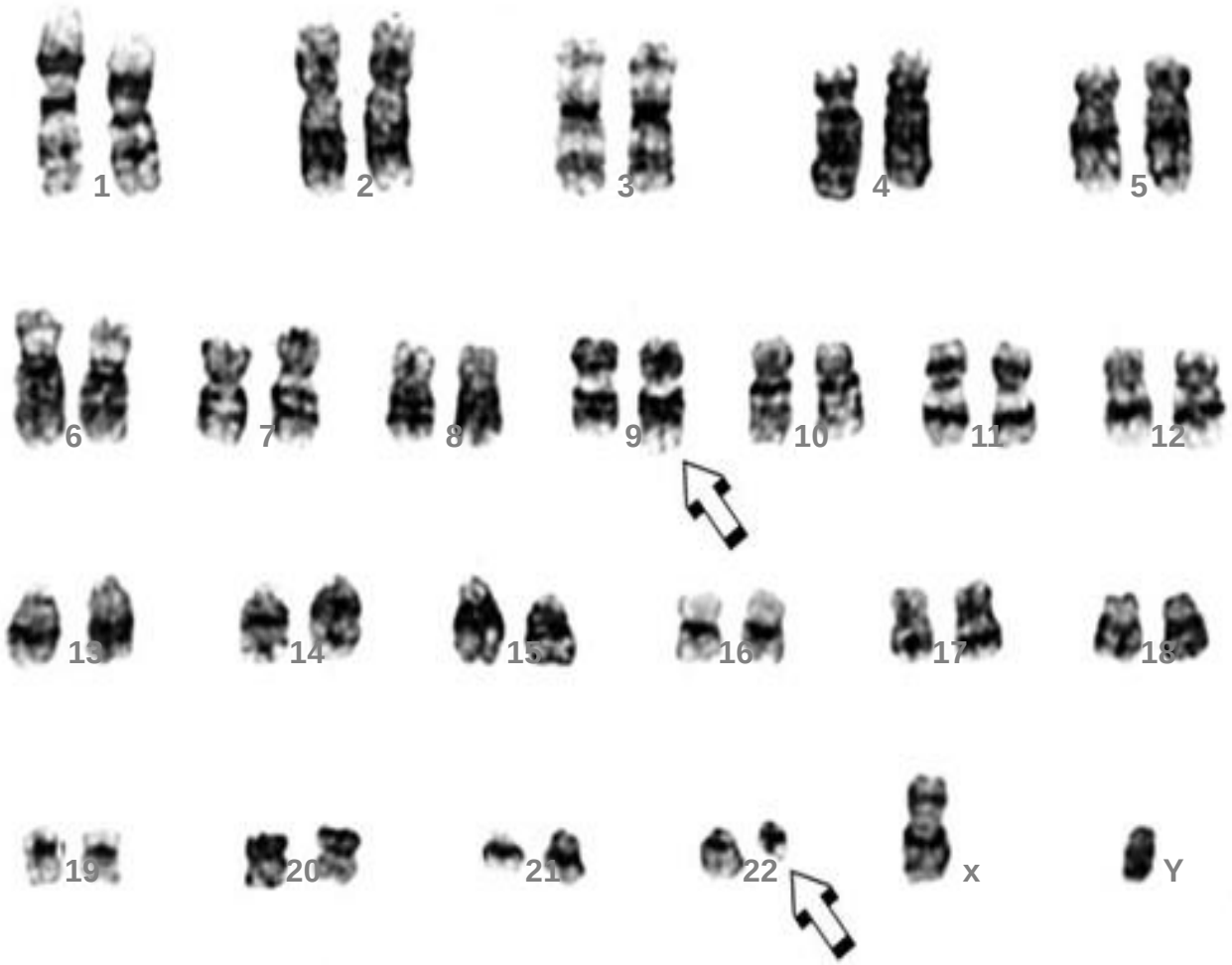
CML







The Philadelphia chromosome

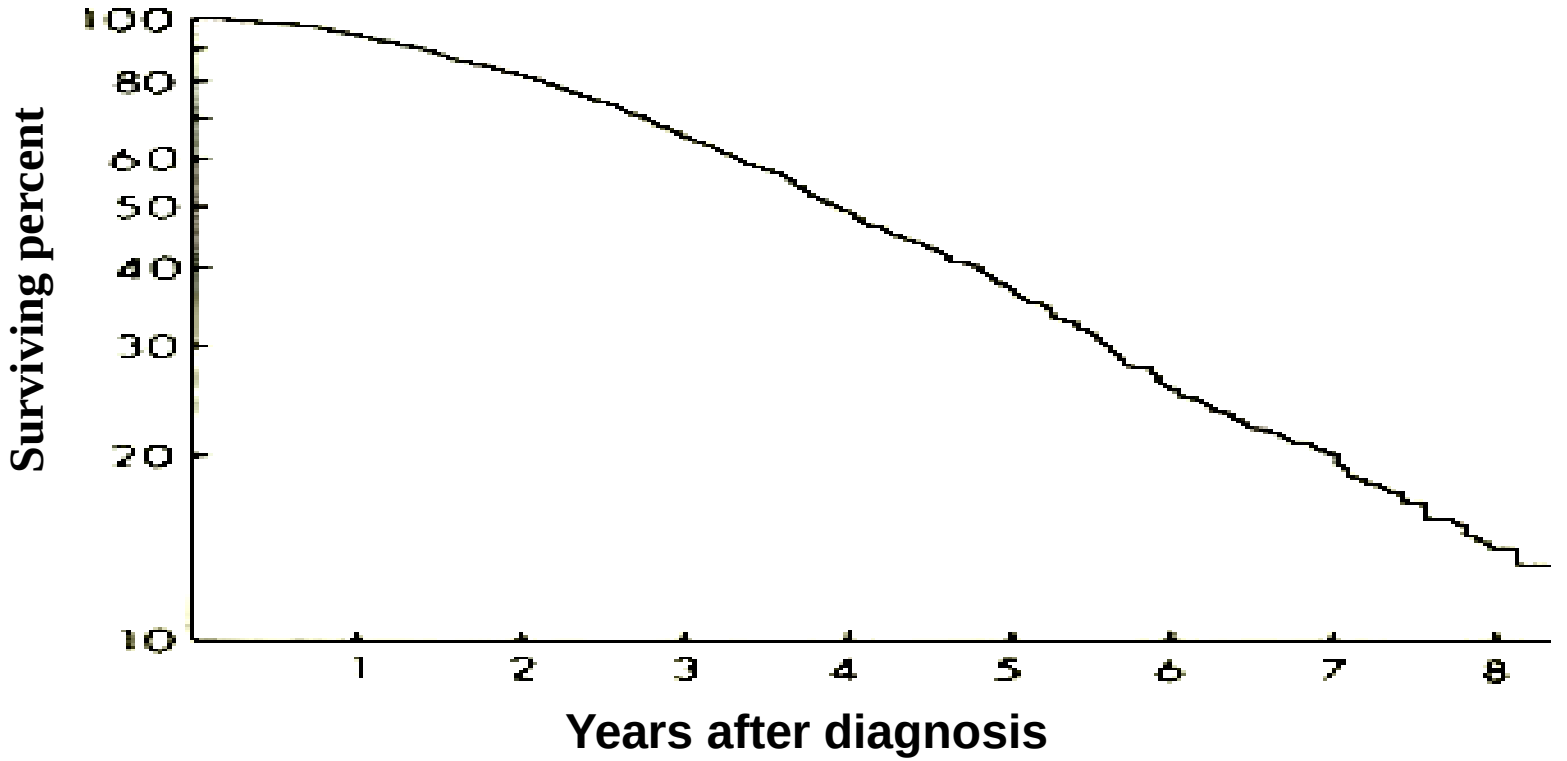


Three phases of the Disease

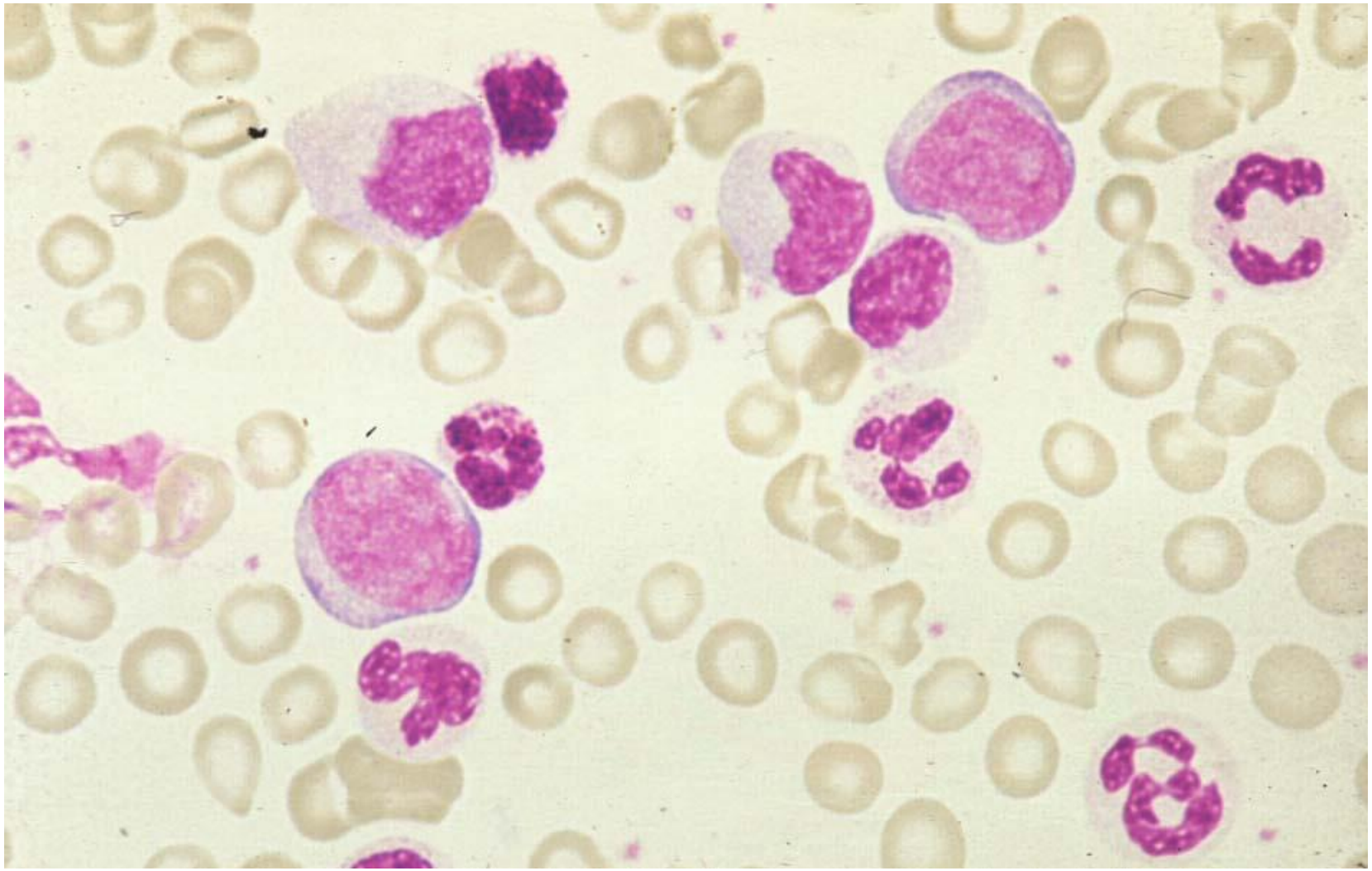
(before imatinib)

36-48 months **6 months** **3-6 months**

chronic phase **accelerated** **blast phase**



CML: blasten toegenomen



Criteria for CML, increased risk WHO 2022

At diagnosis

High ELTS score

10–19% blasts in the peripheral blood and/or bone marrow[ab]

≥20% basophils in the peripheral blood

Additional chromosomal abnormalities in Philadelphia (Ph) chromosome–positive (Ph+) cells, including 3q26.2 rearrangements, monosomy 7, isochromosome 17q and complex karyotype

Clusters of small megakaryocytes (including true micromegakaryocytes similar to those seen in myelodysplastic syndromes), associated with significant reticulin and/or collagen fibrosis, which is best assessed in biopsy sections.

a The finding of bona fide lymphoblasts in the peripheral blood or bone marrow (even if < 10%) is consistent with the diagnosis of blast phase
b ≥ 20% blasts in the peripheral blood or bone marrow, or an infiltrative proliferation of blasts in an extramedullary site, is diagnostic of blast phase

Criteria for CML, increased risk WHO 2022

Emerging on treatment

Failure to achieve a complete haematological response to the first TKI

Any haematological, cytogenetic, or molecular indications of resistance to two sequential TKIs (excluding explicable causes such as the presence of a kinase domain mutation resistant to the previous choice of TKI

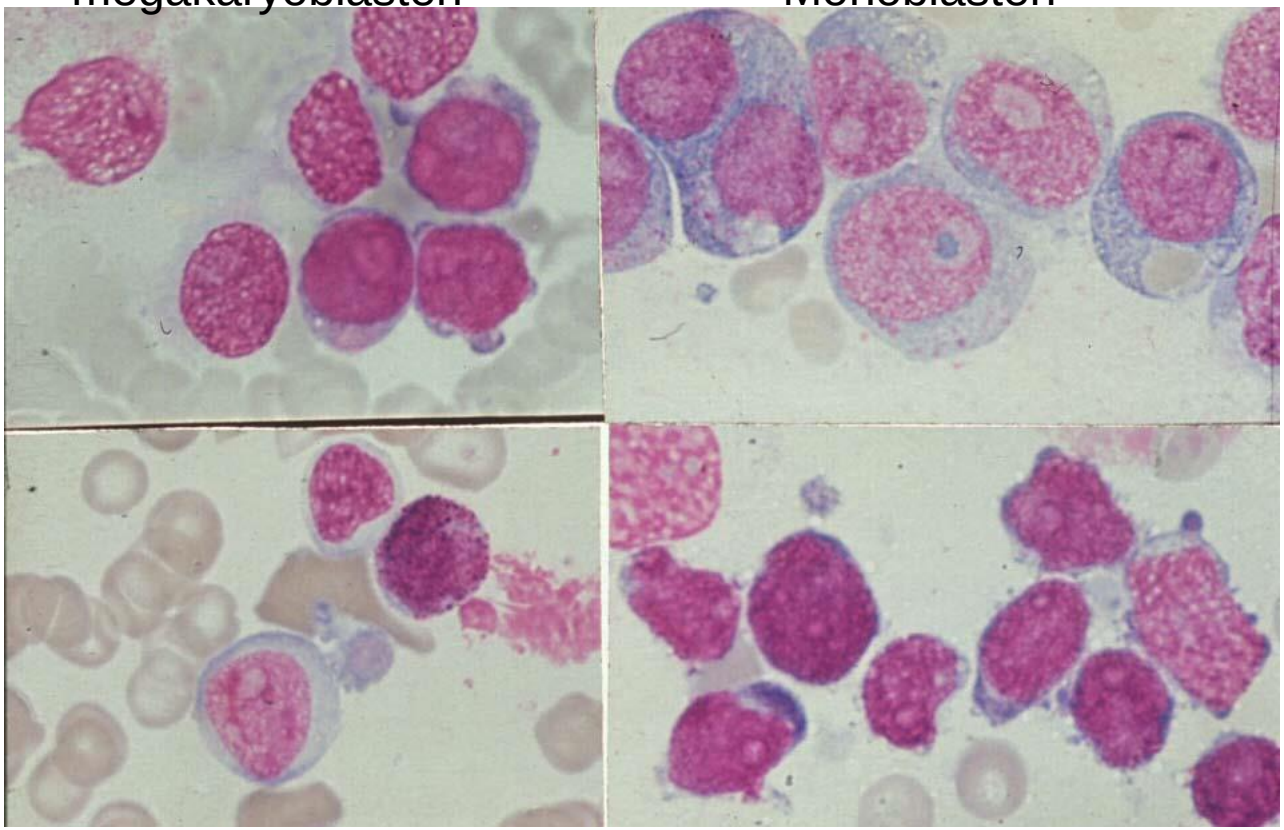
Development of new additional chromosomal abnormalities

Occurrence of compound mutations in the BCR-ABL1 fusion gene during TKI therapy

CML: blastaire fase

megakaryoblasten

Monoblasten



myeloblasten

lymfoblasten

> Immunoflowcytometrie!

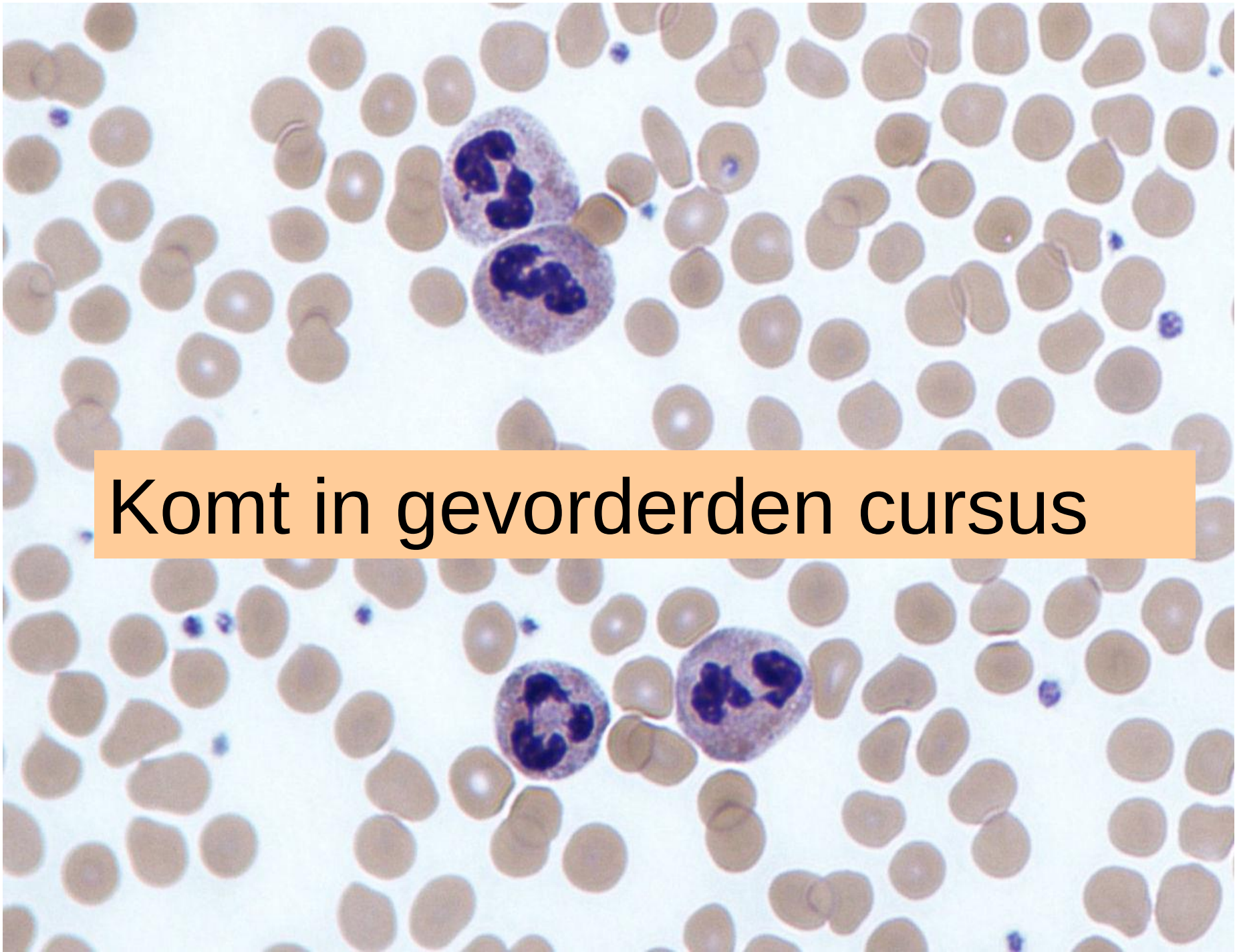
Differentiaal diagnose CML

- Leukemoïde reactive, reactieve hyperplasie
- Atypische CML, CMML, CNL, ET

Fusion gene		Translocation
BCR-ABL		t(9;22)(q34;q11)
ETV6-ABL		t(9;12)(q34;p13)
ZNF198-FGFR1	**Myeloid/lymphoid neoplasms with *FGFR1* (8p11 mypro)**	t(8;13)(p11;q12)
FOP-FGFRI		t(6;8)(q27;p11)
CEP110-FGFR1		t(8;9)(p11;q33)
BCR-FDGFR1		t(8;22)(p11;q22)
ETV6-PDGFRB	**Myeloid/lymphoid neoplasms with *PDGFRB* (5q33 mypro)**	t(5;12)(q33;p13)
HIP1-PDGFRB		t(5;7)(q33;q11)
H4-PDGFRB		t(5;10)(q33;q21)
RAB5-PDGFRB		t(5;17)(q33:p13)
ETV6-JAK2		t(9;12)(p24;p13)
BCR-JAK2		t(9;22)(p24;q11)
ETV6-SYK2		t(9;12)(q22;p12)

CML, aCML, CMML

	CML	aCML	CMML
Baso's	≥2%	<2%	<2%
Monocyten	<3%	≥3-10%	≥10%
Granulocytaire dysplasie	---	++++	+
Immature voorlopers	>20%	10-20%	≤10%
Blasten	≤2%	>2%<20%	<2%
BM erytropoïese	---	--	++


Komt in gevorderden cursus

Wat als perifere diff dit zou zijn?

- Eo 4%
- Baso 3%
- Blast 2%
- Promyelo 1%
- Myelo 2%
- Metamyelo 6%
- Staafkernig granulocyt 10%
- Segmentkernige granulocyt 50%
- Lymfocyt 12%
- Monocyt 10%
- Erytroblast: 2/100 cellen
- traandruppelcellen

Myelofibrose beloop

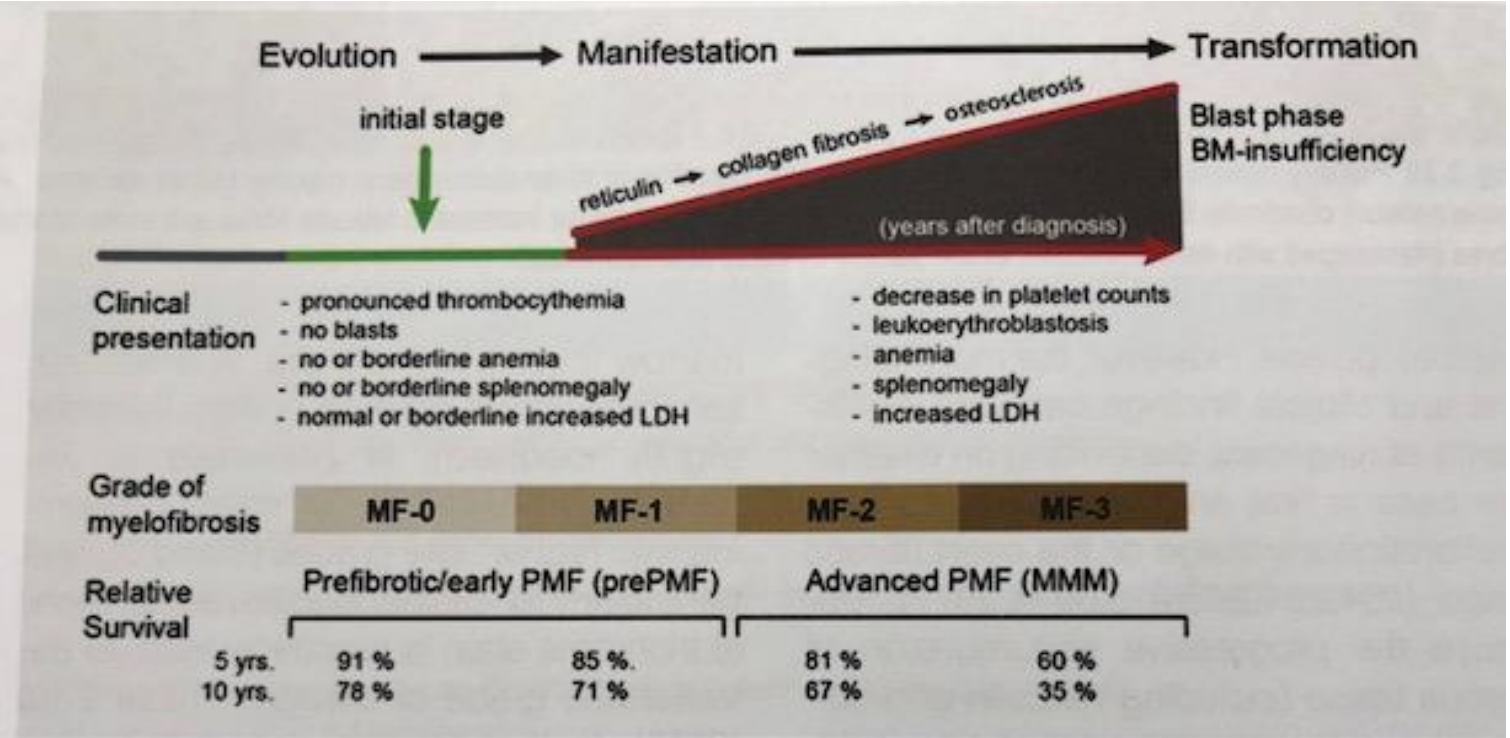
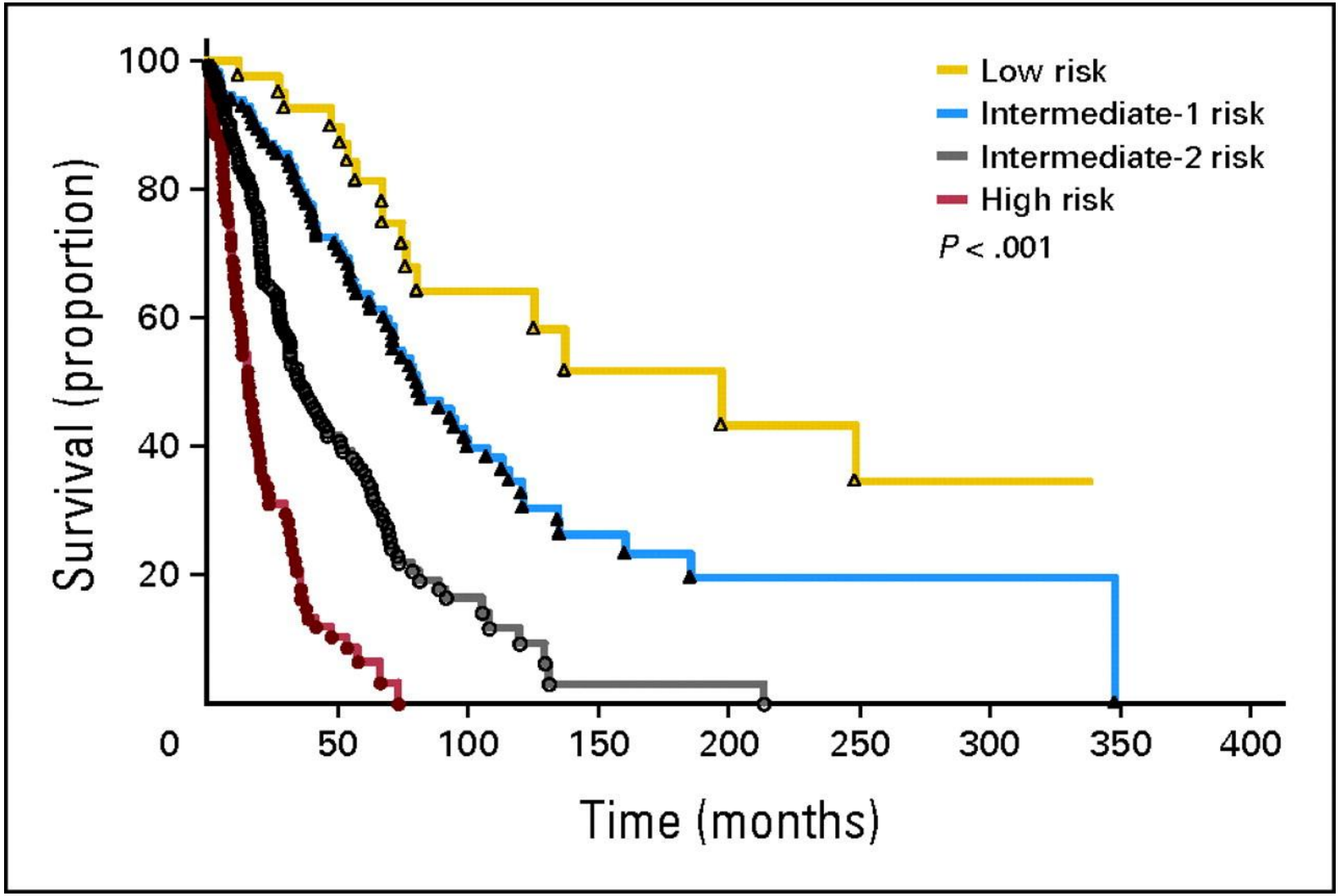


Fig. 2.18 Primary myelofibrosis (PMF). Dynamics of the disease process, with haematological presentation a associated bone marrow fibrosis (MF) grading and relative survival rates.
BM, bone marrow; LDH, lactate dehydrogenase; MMM, myelofibrosis with myeloid metaplasia.

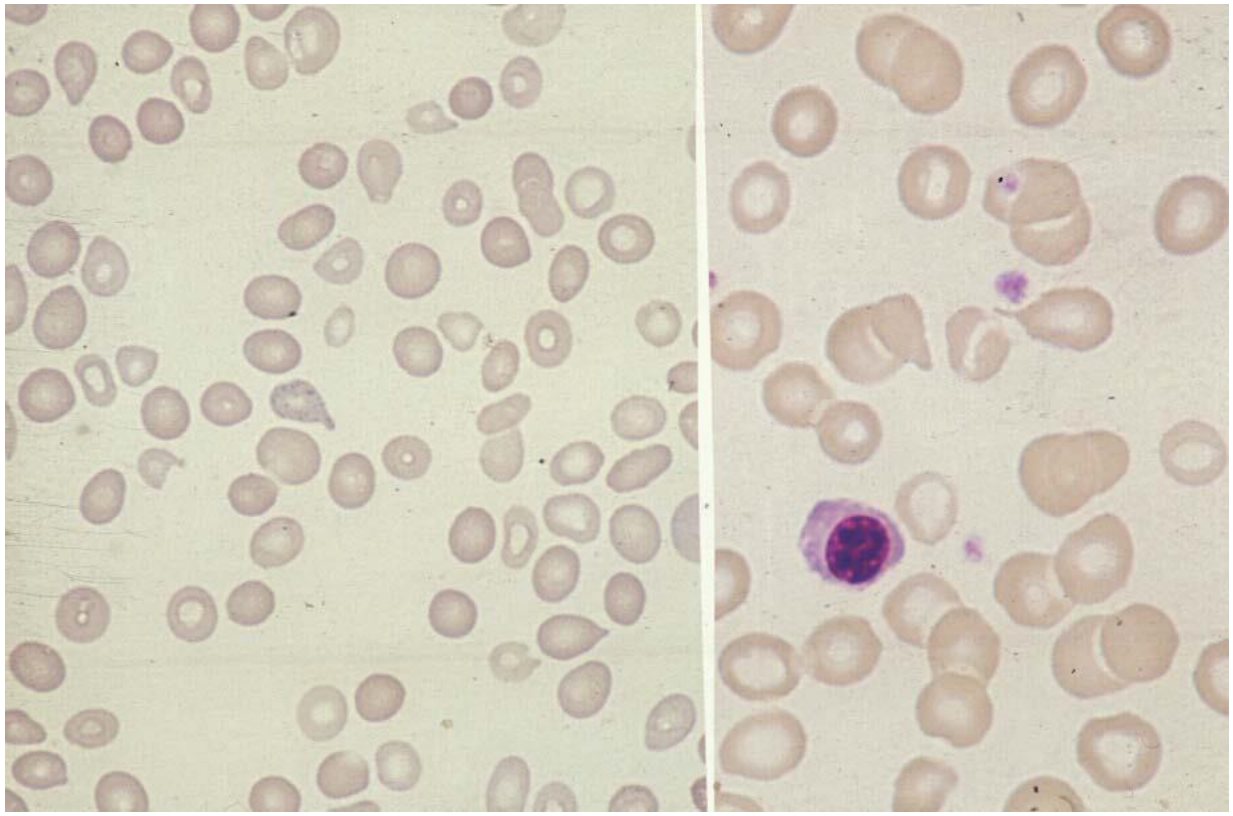
WHO 2016/2022 criteria for PMF

PMF criteria
Majeure criteria
1. Toename van afwijkende megakaryocyten met reticuline en/of collageen fibrose (MF2-3)
2. Voldoet niet aan PV, *BCR-ABL1*-positieve CML, MDS of andere myeloïde neoplasie
3. Aantonen van *JAK2, CALR of MPL* mutatie of andere clonale marker, bij ontbreken hiervan geen aanwijzingen voor reactieve beenmergfibrose
Mineure criteria
Aanwezigheid van minstens één van de volgende en tweemaal aangetoond:
1. Anemie niet anders verklaard
2. Leukocytose ≥11x10^9/L
3. Splenomegalie palpabel of bij beeldvorming
4. Toename LDH
5. leukoerythroblastose
Diagnose PMF vereist 3 majeure en minstens 1 mineur criterium

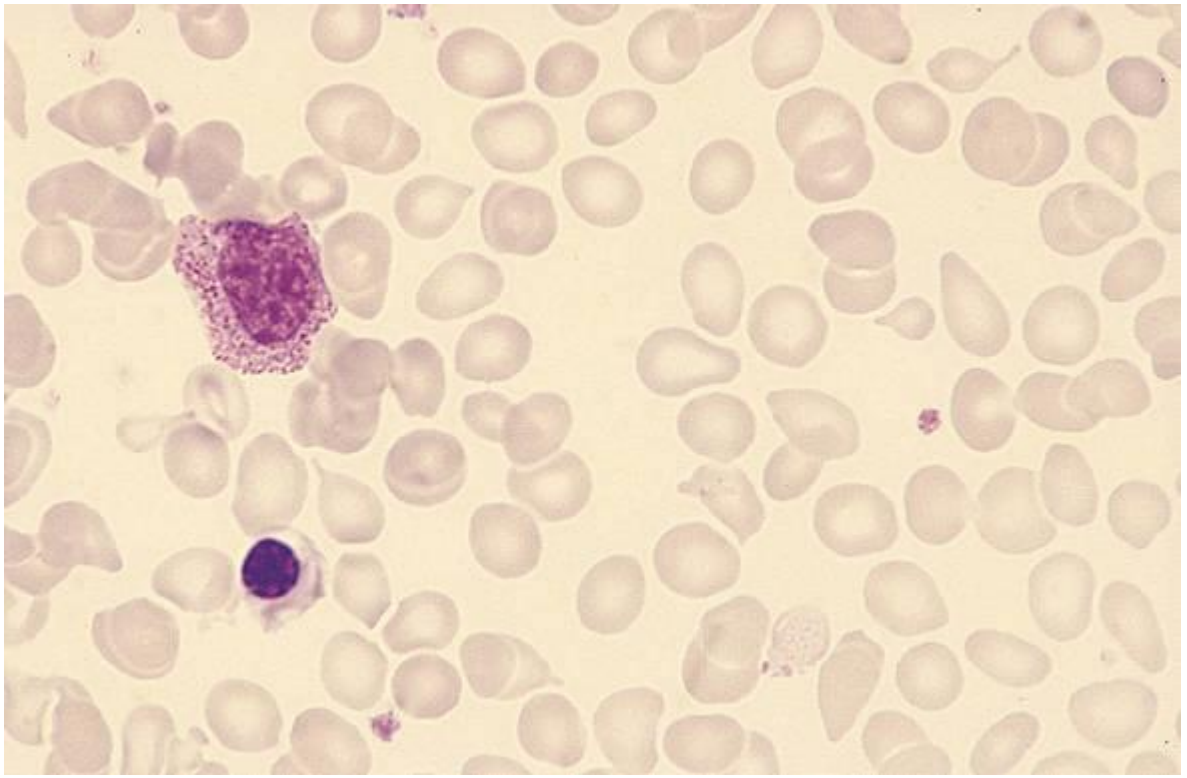


Risk factors: Age>65 yrs, Hb<10g/dL, WBC≥25x10^9/L, circ. blasts≥1%, B-symtoms

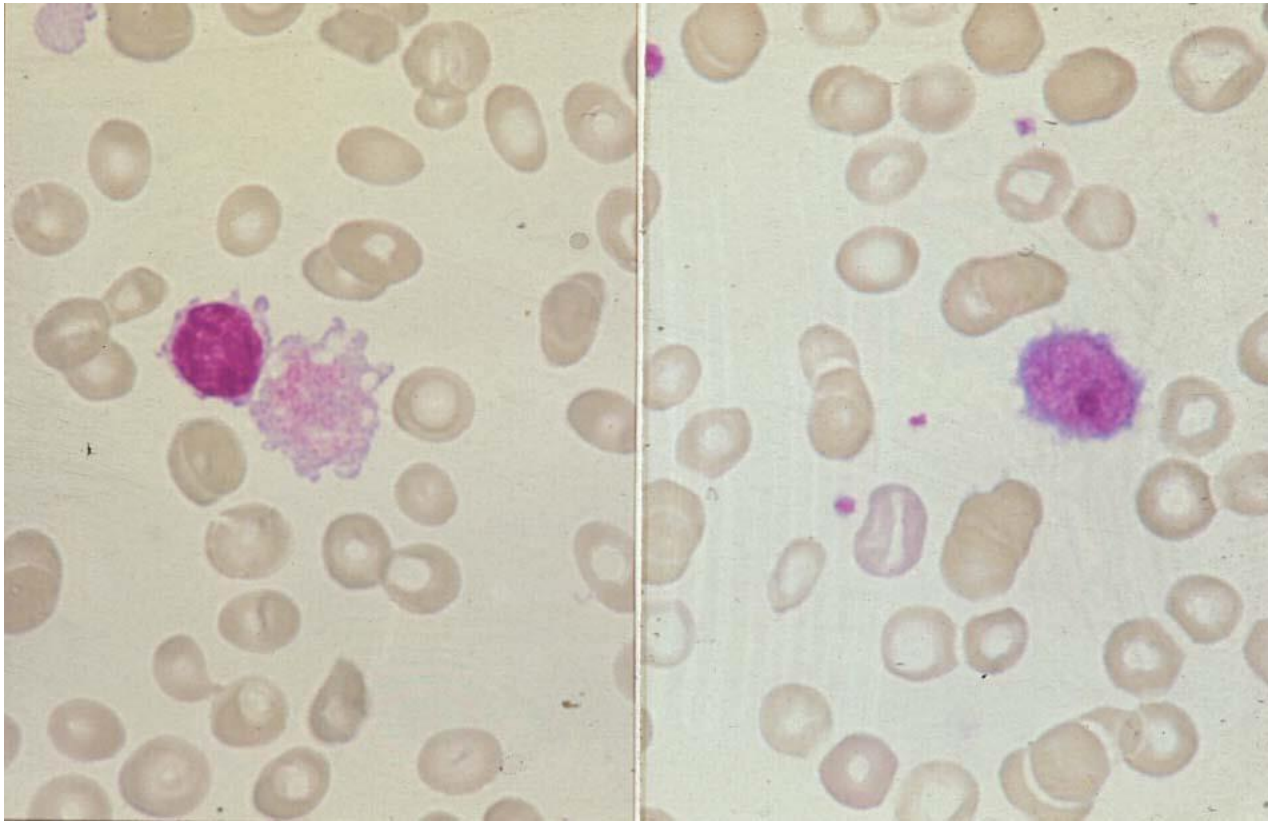
PMF bloed: teardrop cells, erytroblast



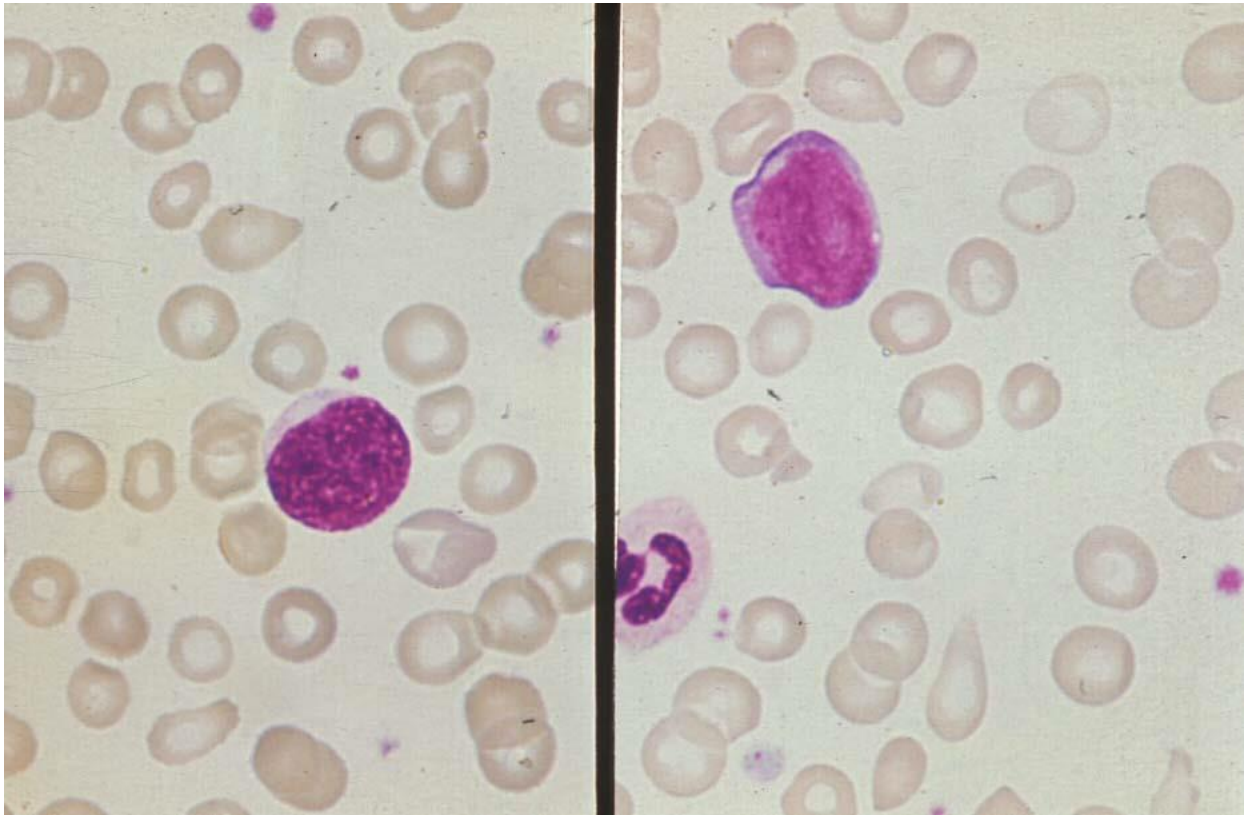
PMF bloed: Leuko-erytroblastair bloedbeeld



PMF: reuze trombocyten



Primary myelofibrosis



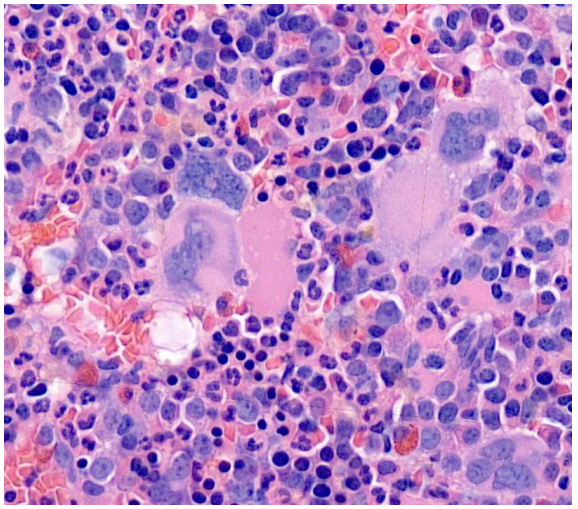
Mega?

blast

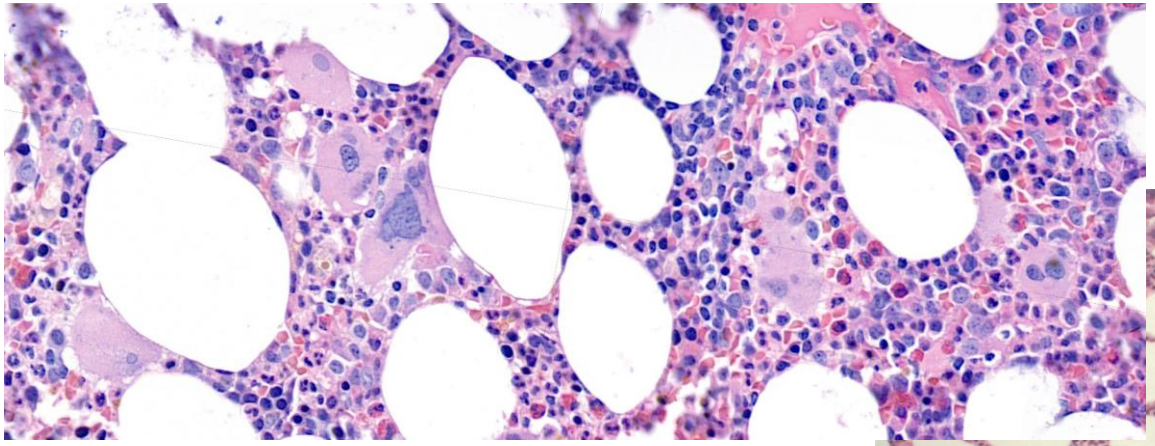
Aspiraat:

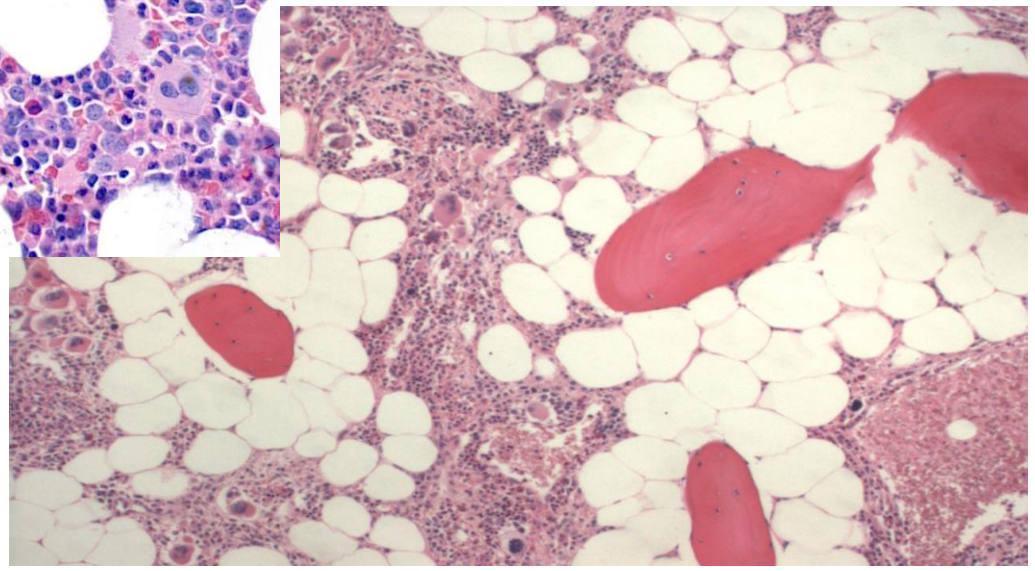
- Lukt vaak niet, dry tap

Botbiopt: Primaire myelofibrose (PMF)

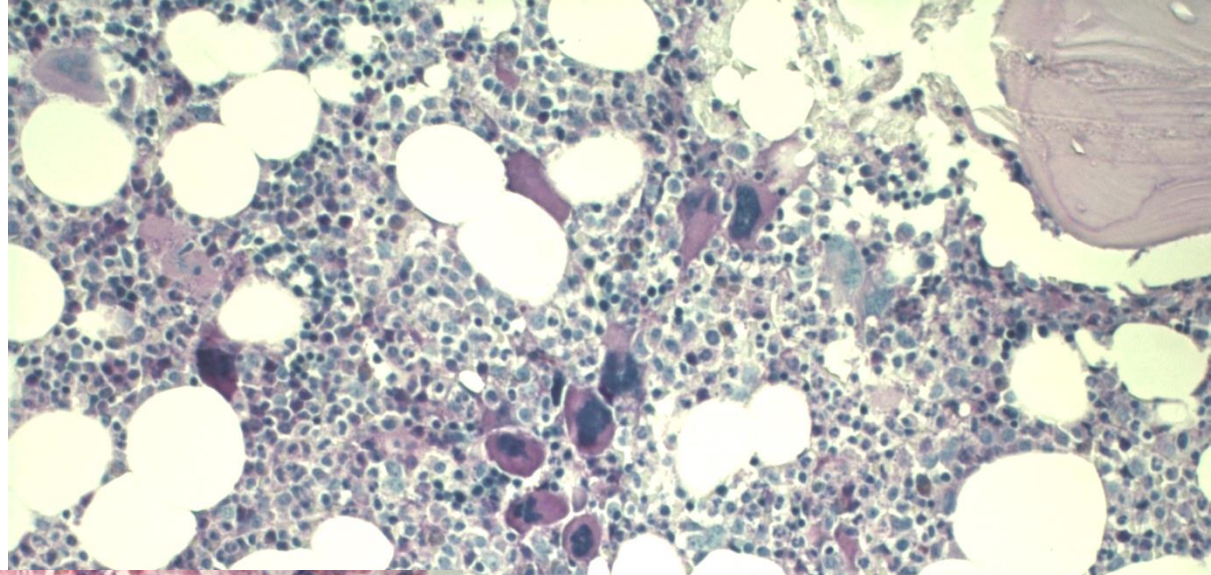


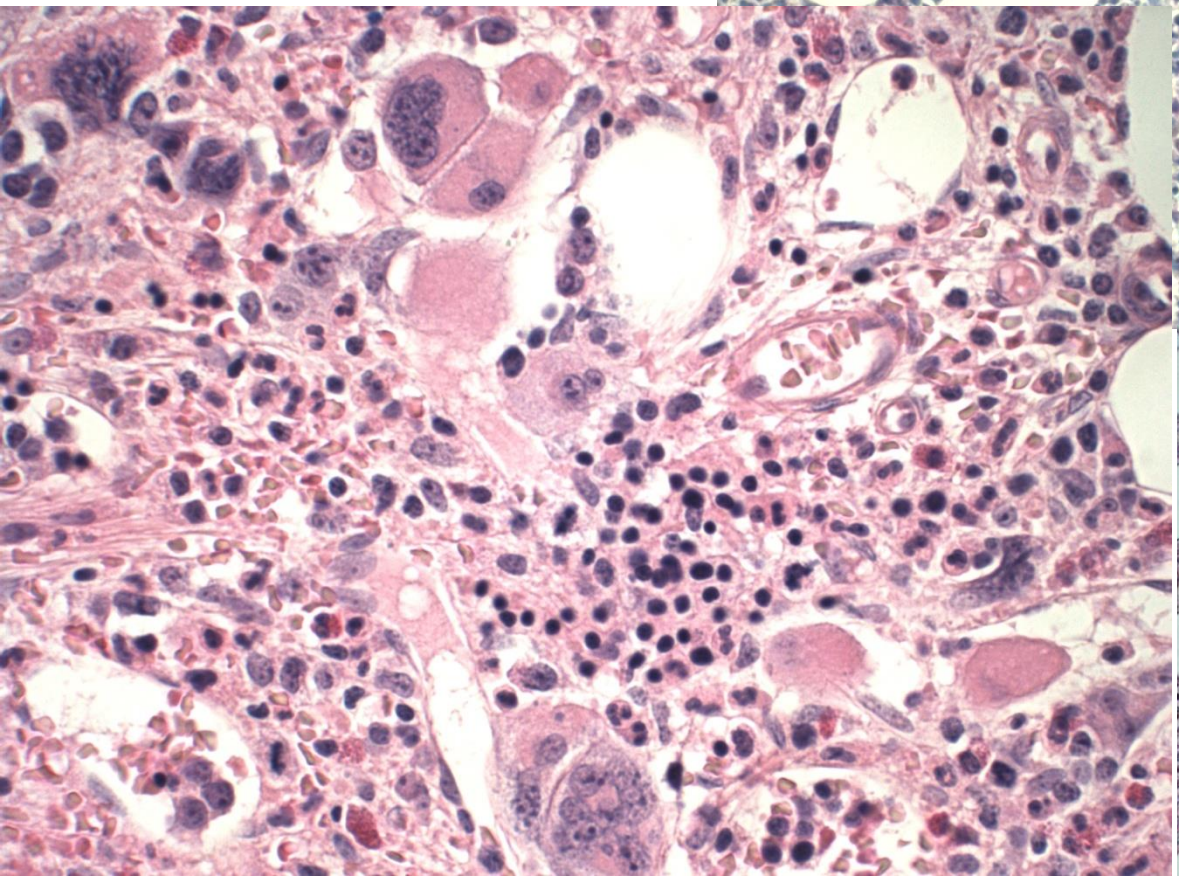
- Trilineaire/myeloide hyperplasie
- Wisselende cellulariteit, patchy BM
- Hyperplasie afwijkende megakaryocyten
  - Variabele grootte, vorm, kernlobulering, atypie
  - Ligging: compacte clusters , paratrabeculair
- Sinusoïden wijd, extramedullaire hematopoiese
- Reticuline, collageen en osteosclerose MF2-3, “streaming”

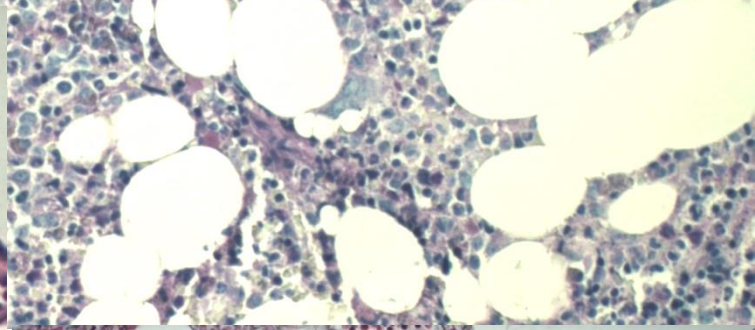


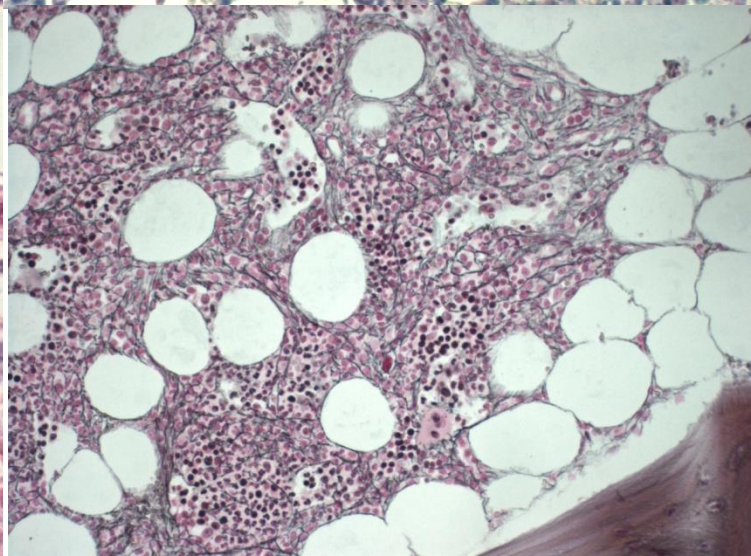


PMF-MF2

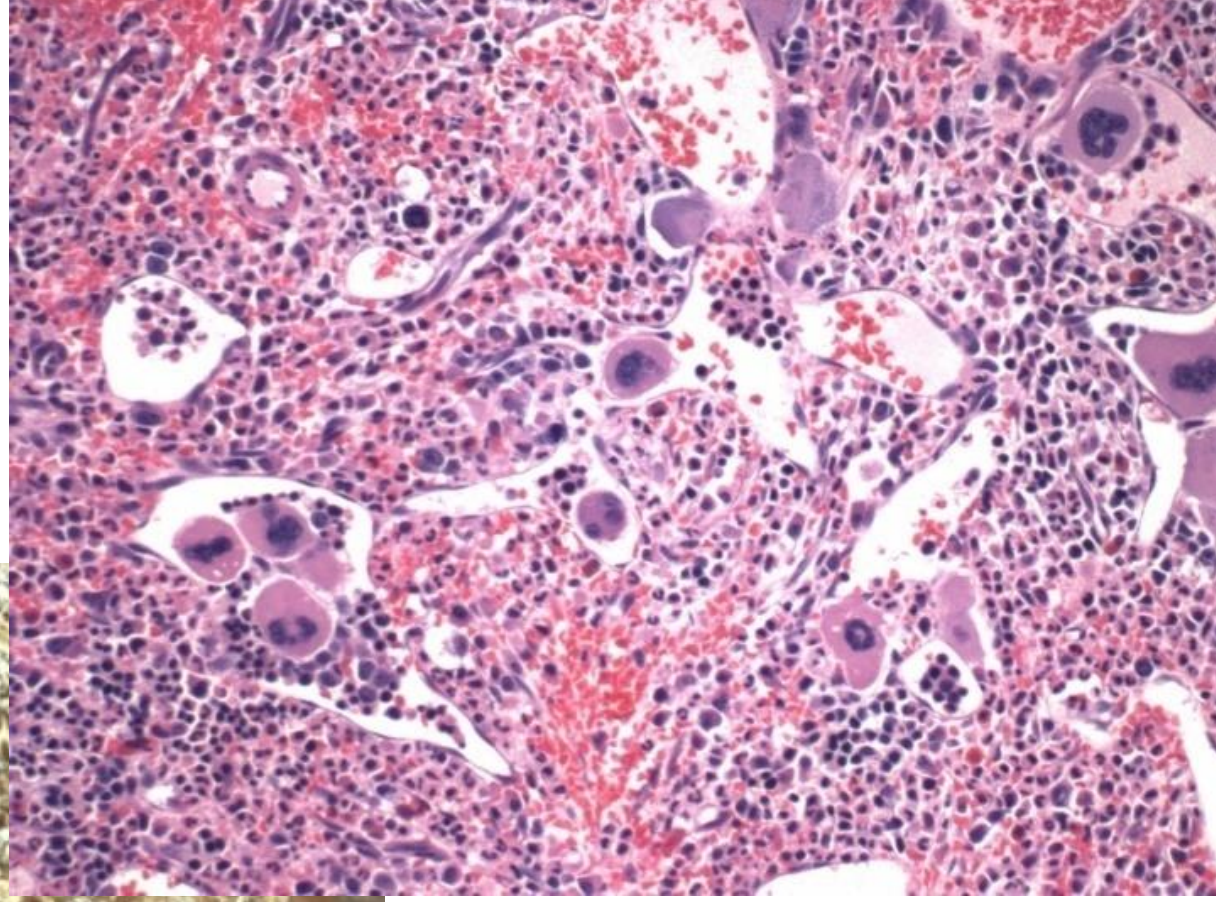


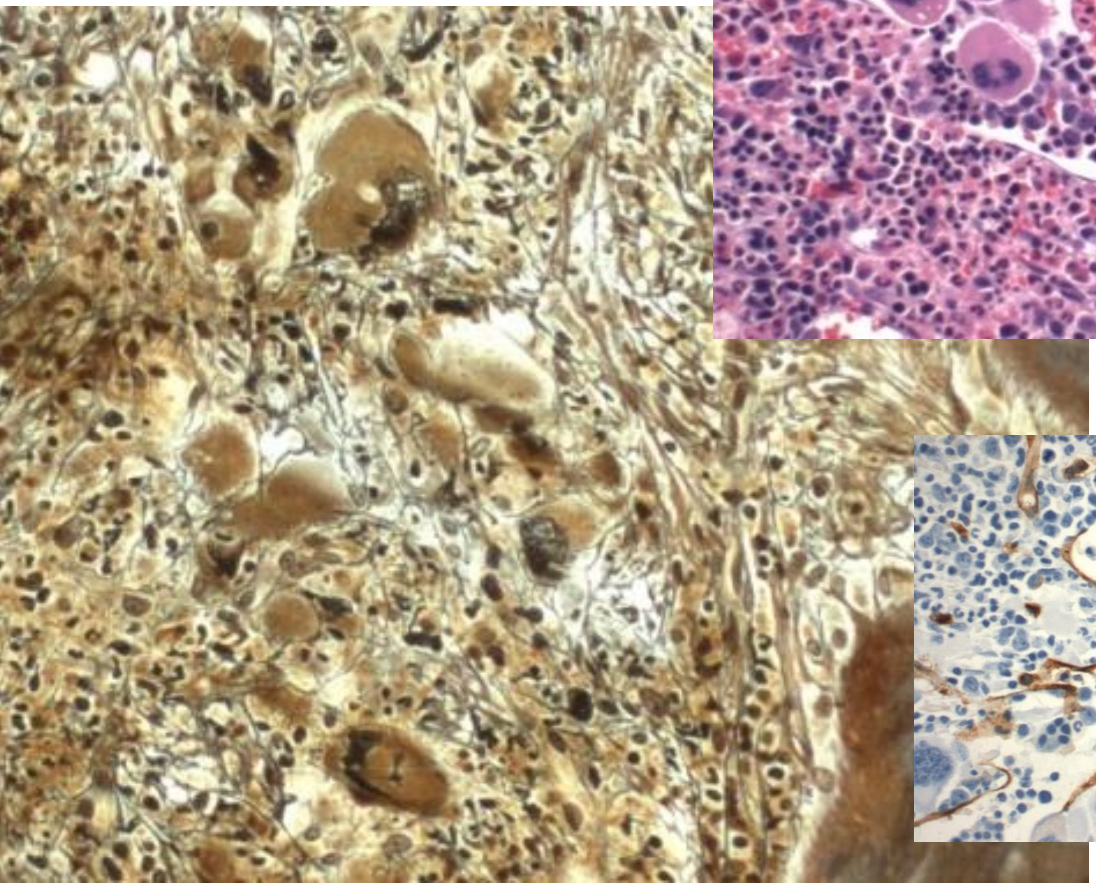


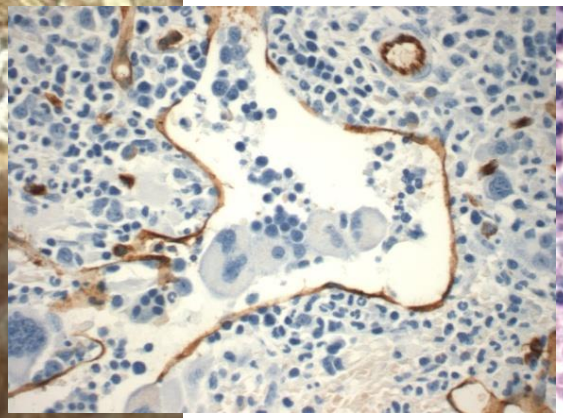


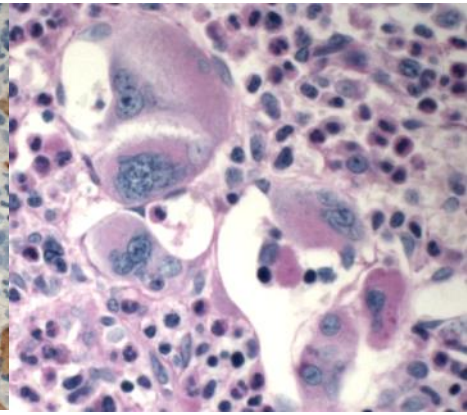


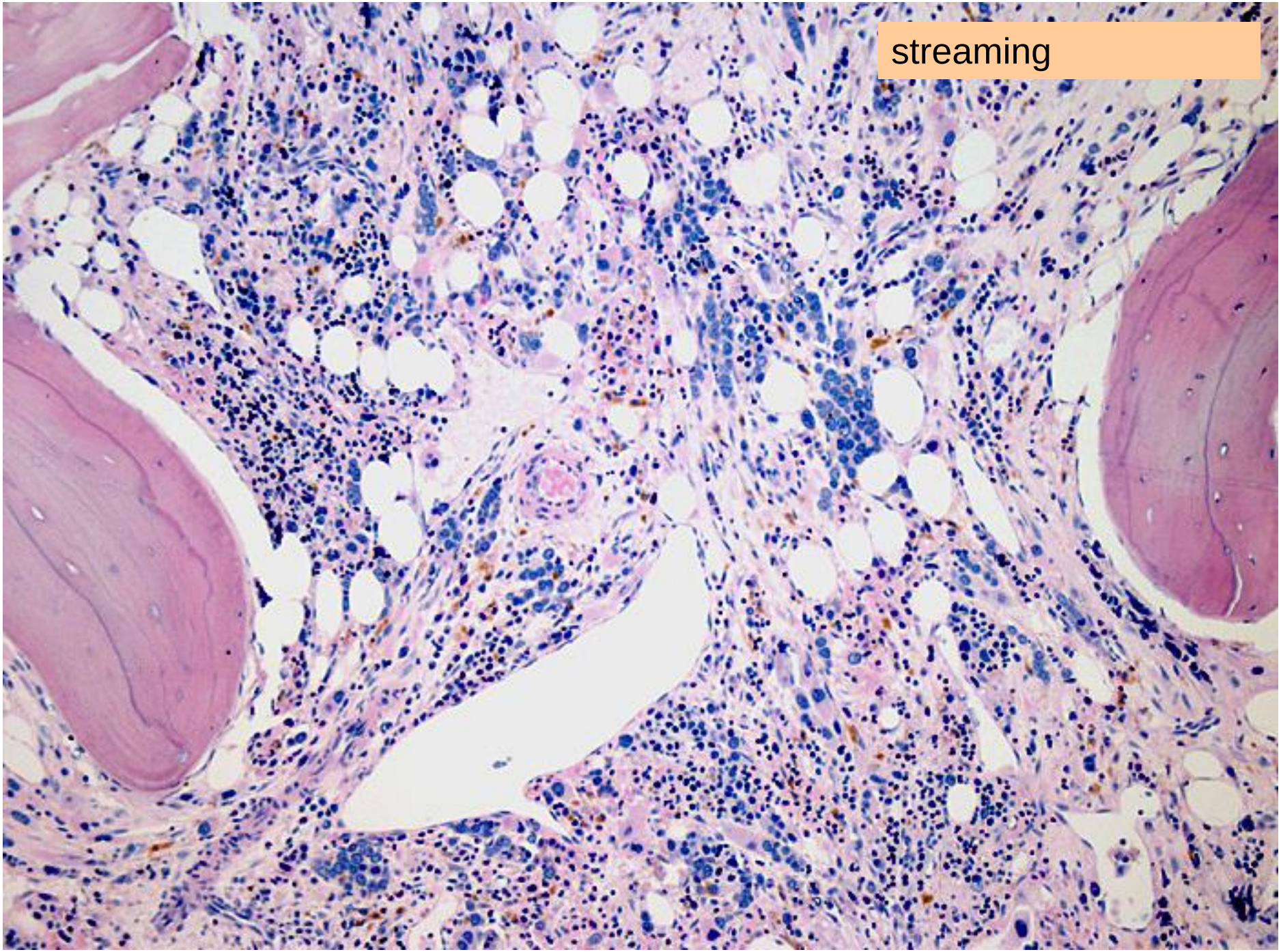
PMF MF-3
Celrijk fibrotisch stadium







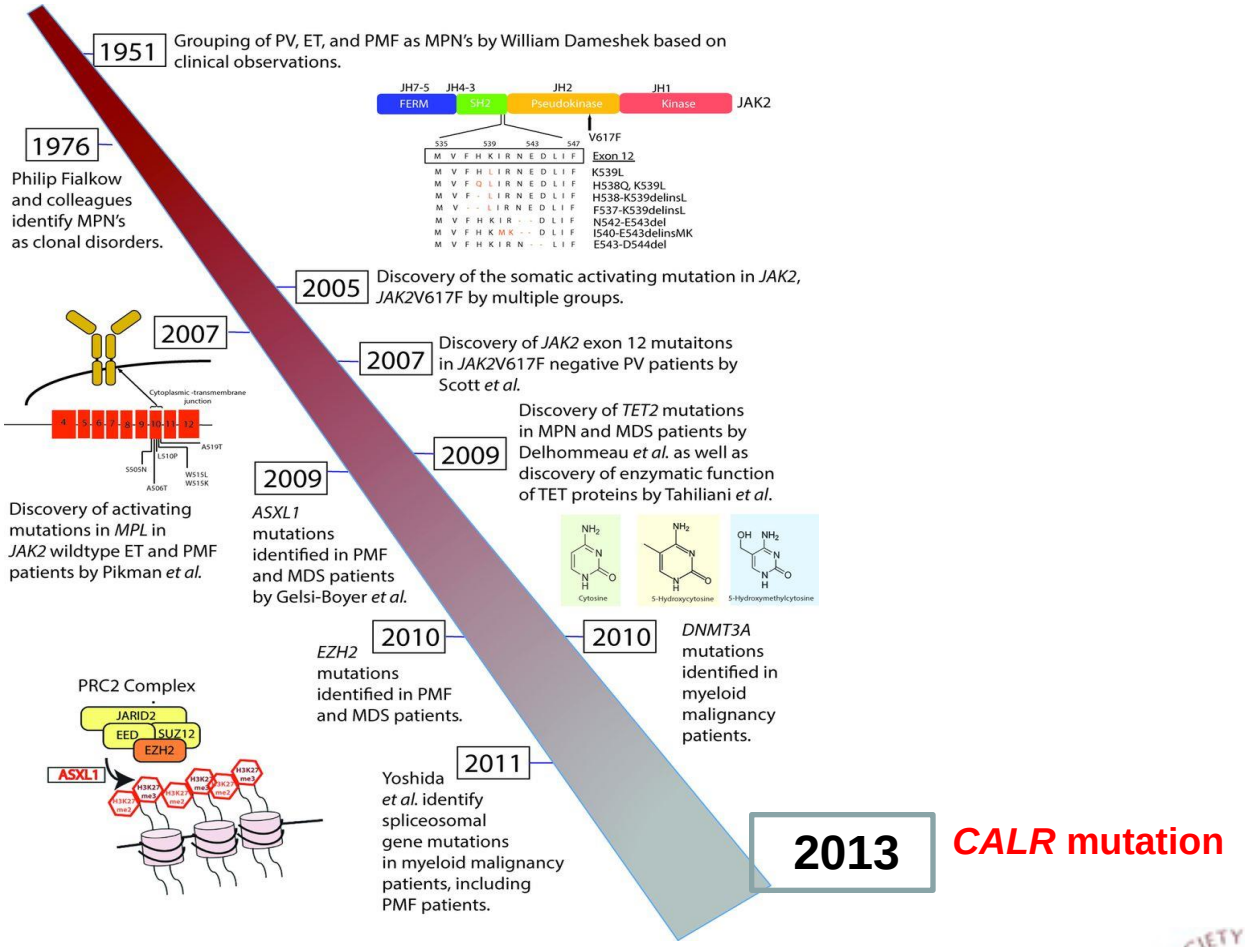



streaming

Recurring genetic abnormalities in MPN

Disease	Specific abnormality	%	Recurring, nonspecific abnormalities	%
CML, CP	**t(9;22), BCR/ABL**	**100**		
CML, AP/BP	**t(9;22), BCR/ABL**	**100**	**+8, +Ph, +19, I(17q), t(3;21), EVI1/AML1**	**80**
CNL	**none**		**+8, +9, del(20q), del(11q14)**	**10**
CEL	**none**		**+8, t(5;12), TEL/PDGFβR, dic(1;7), 8p11, FIP1L1-PDGFRα**	**?**
PV	**none**		**+8, +9, del(20q), del(13q), del(1p11)**	**15**
PMF	**none**		**+8, del(20q), -7/del(7q), del(11q), del(13q), Der(6)t(1;6)(q21-23;p21.3)**	**35**
ET	**none**		**+8, del(13q)**	**5**

Timeline of gene discovery efforts in patients with MPNs. The MPNs were initially grouped together based on prescient clinical insights by William Dameshek in 1951.54 The earliest insights into the genetic causes for the MPNs were then made in 1976 to 1981,...

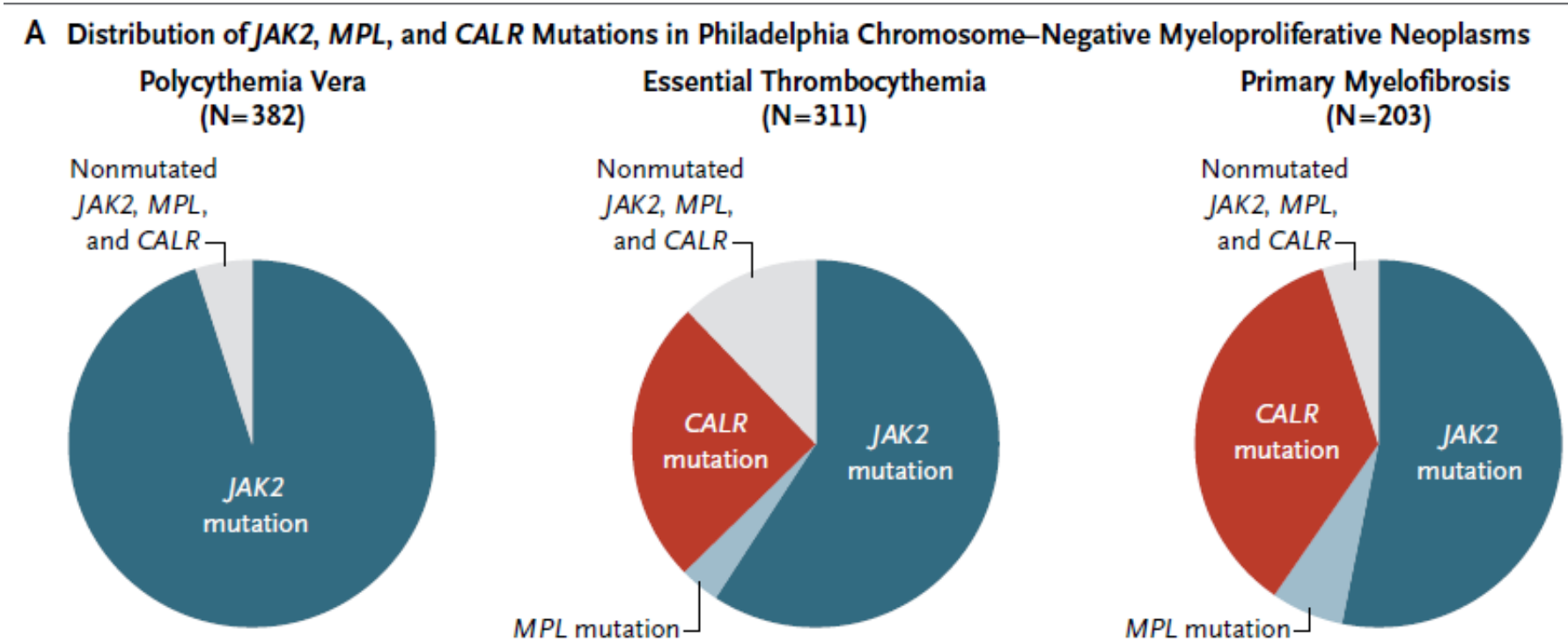


Kim E , and Abdel-Wahab O Hematology 2013;2013:538-544

JAK2, CALR, MPL

VU medisch centrum

Met name bij primaire myelofibrose en essentiële thrombocytose



Klampfl T. et al, *N Engl J Med*. 2013 Dec 19;369(25):2379-90

Casus 2

- Man, 42 jaar
- Mesenteriaal trombose
- MDL deed JAK2 mutatie: was positief

- Hb 7,8 mmol/l, (Ht 0,40)
- Trombocyten 460 x 10^9/l
- Leucocyten 14 x 10^9/l

Kans op ET vs. PV bij (diepe) trombose

- Venous thromboembolism in 10-40% PV, 10–30% ET, and <10% PMF at presentation
- >80% *JAK2* mutation
- *DNMT3A, TET2*, and *ASXL1* mutations may be an independent risk factor for thrombosis in patients with PV

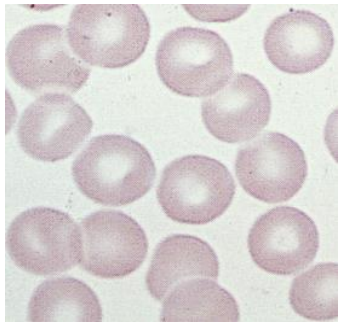
- Splanchnic vein thromboses (SVT) are rare, estimated incidence rate 0.7 per 100,000 patients per year for portal vein thrombosis and 0.8 per million patients per year for Budd-Chiari syndrome,
- 30-40% SVT *JAK2* mutation
- SVT 5–10% PV, 9–13% ET, 0.6–1% PMF, deel onvoldoende criteria voor MPN diagnose

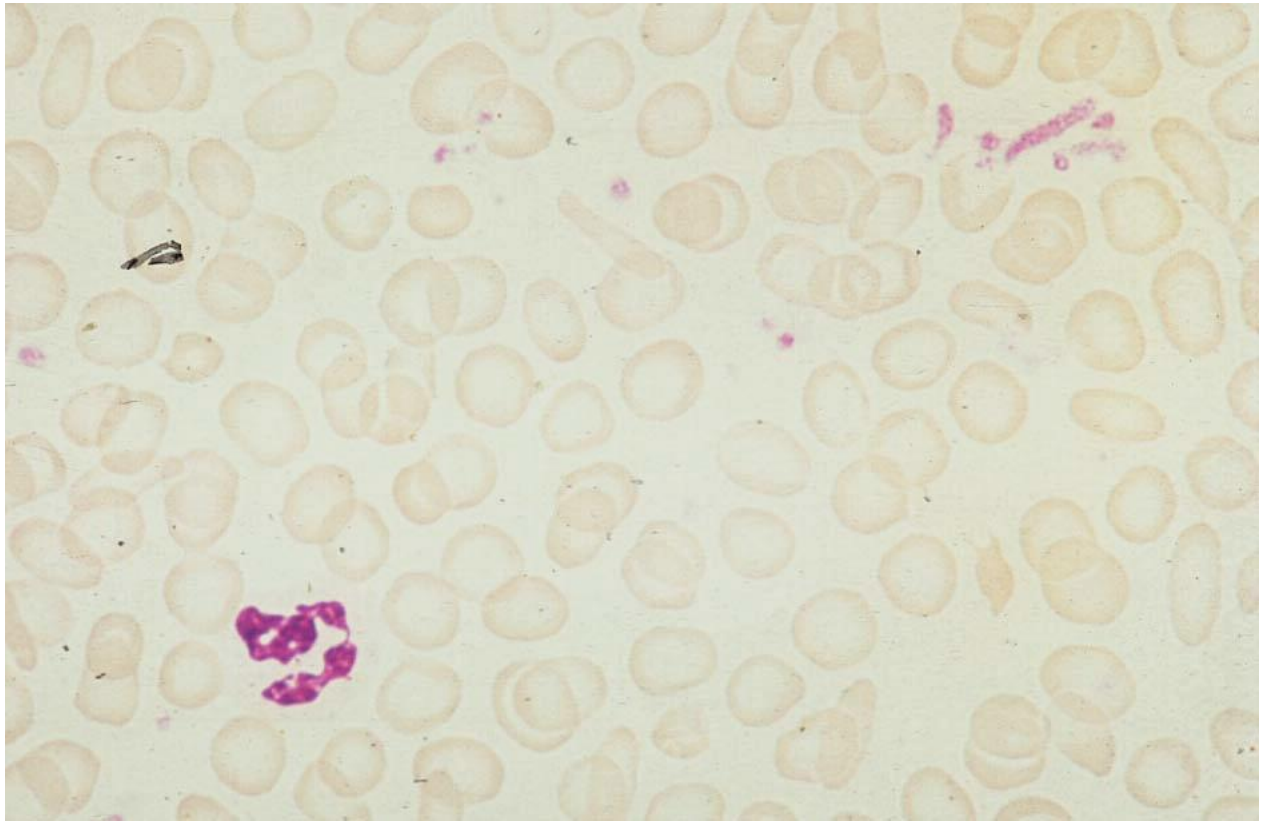
How et al. 2017 https://dx.doi.org/10.1177%2F2040620716680333 , Segura-Díaz et al2020

WHO criteria Polycythemia Vera 2022

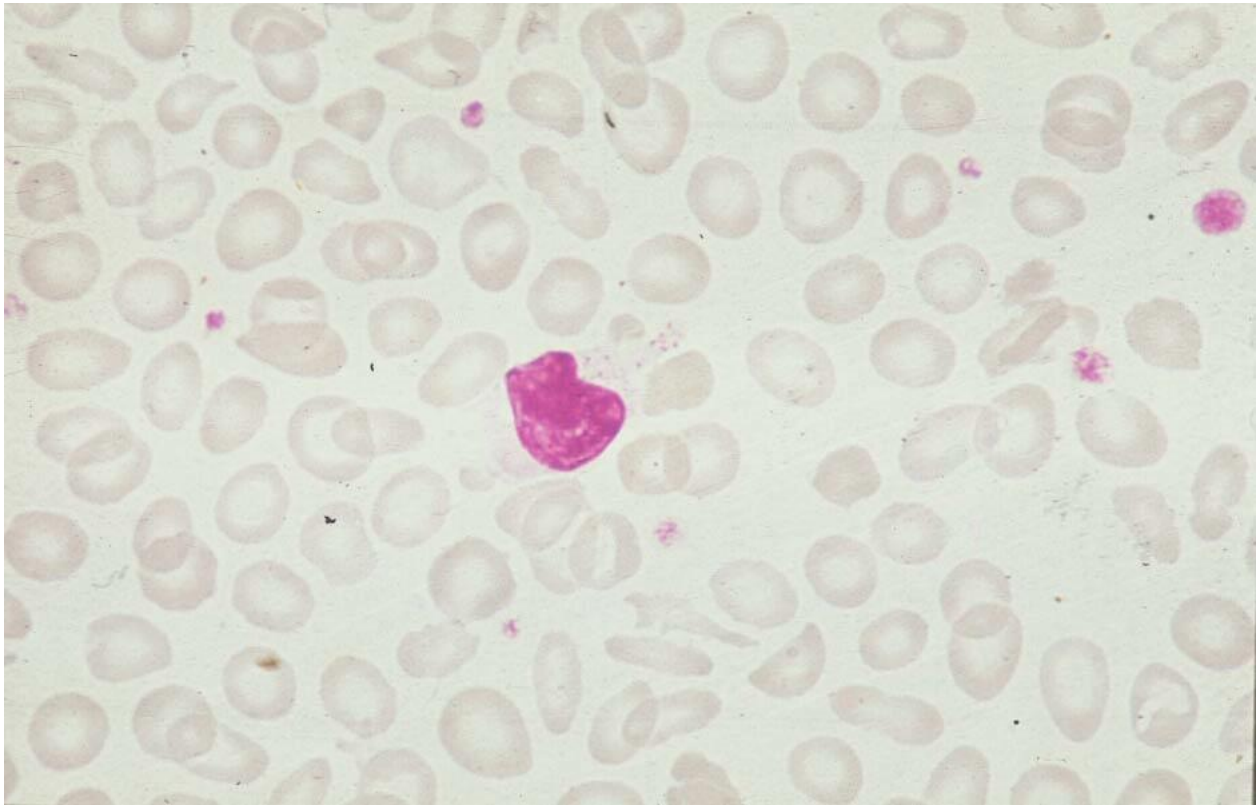
PV criteria
Majeure criteria
1. Toegenomen rode bloedcel massa >25% boven gemiddelde, of Hb >16.5 g/dL (11.5 mmol/l) (mannen) of >16.0 g/dl (10.2 mmol/l)(vrouwen) of hematocriet>49% (man), 48% (vrouw)
2. Beenmergbiopt met prominente toename erytro-, myeloide en megakaryocytaire reeks met grote megakaryocyten
3. Aanwezigheid van *JAK2V617F* or *JAK2* exon 12
Mineur criterium
1. laag serum EPO
Diagnose PV vereist 3 majeure of 2 majeure en 1 mineur criterium

PV

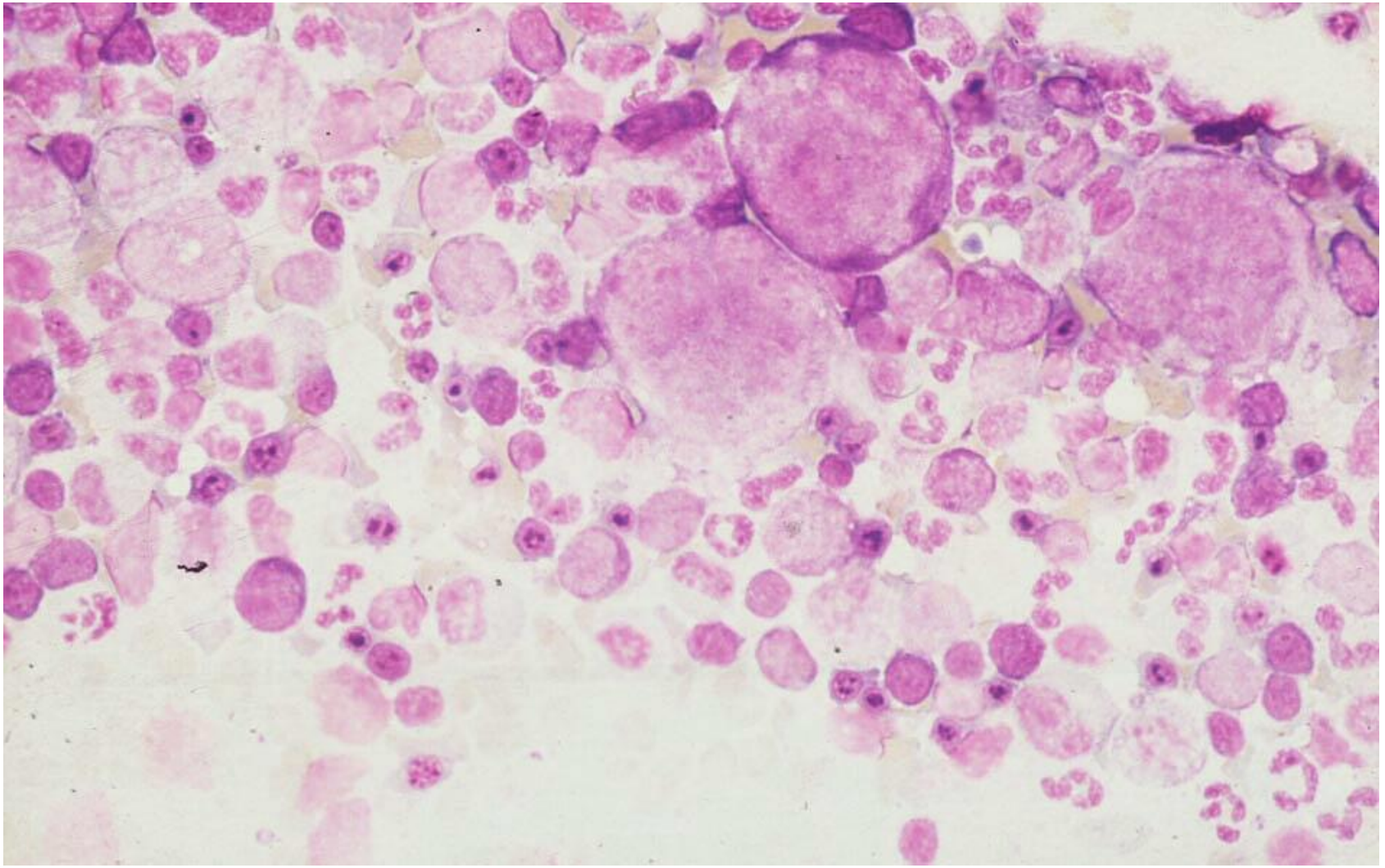




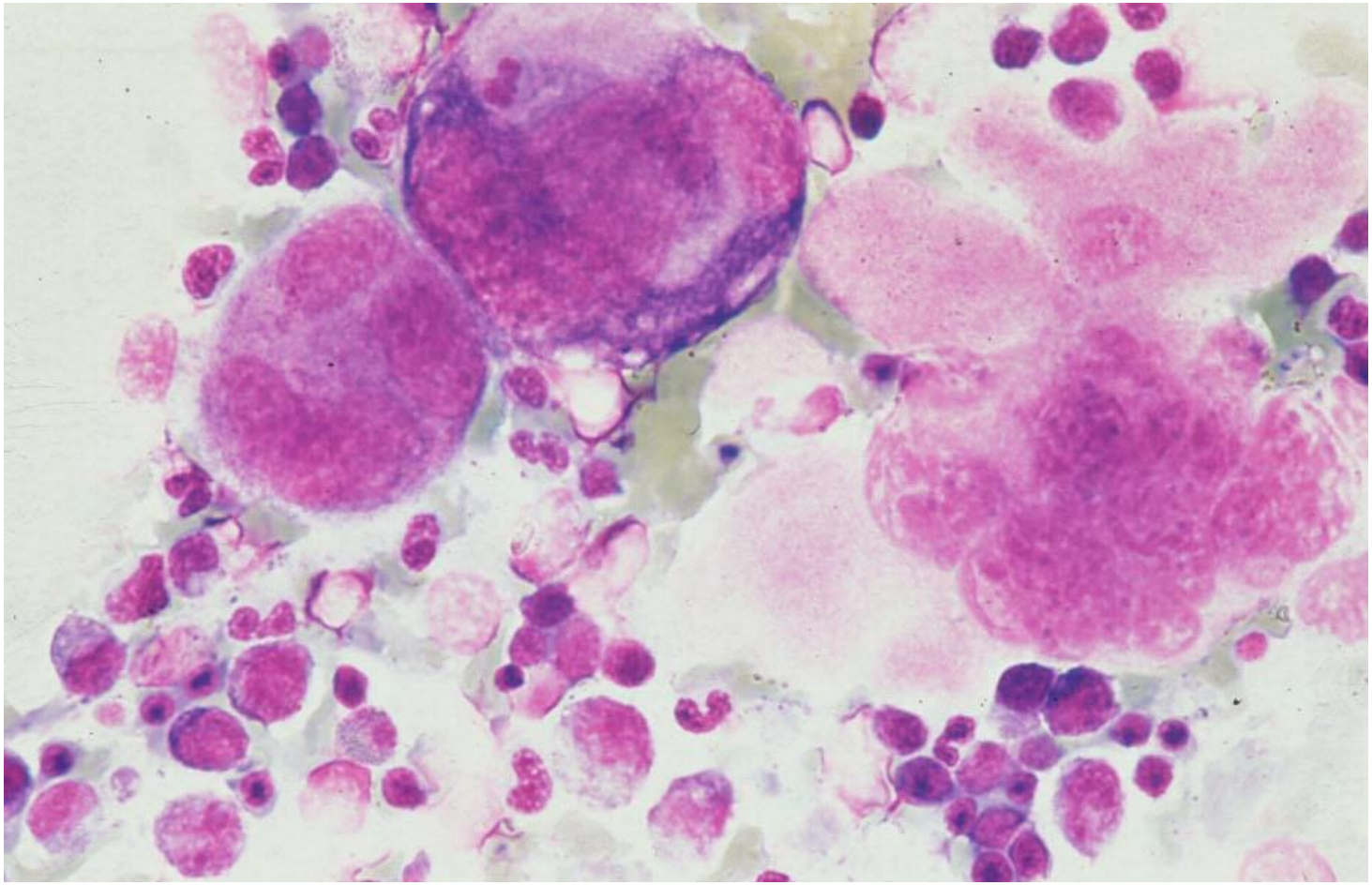
PV, bloed: mega??

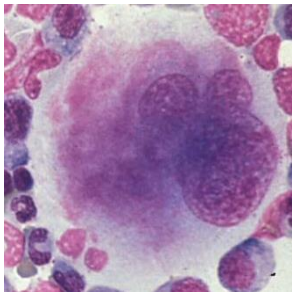


PV aspiraat: grote megakaryocyten, trilineage proliferatie



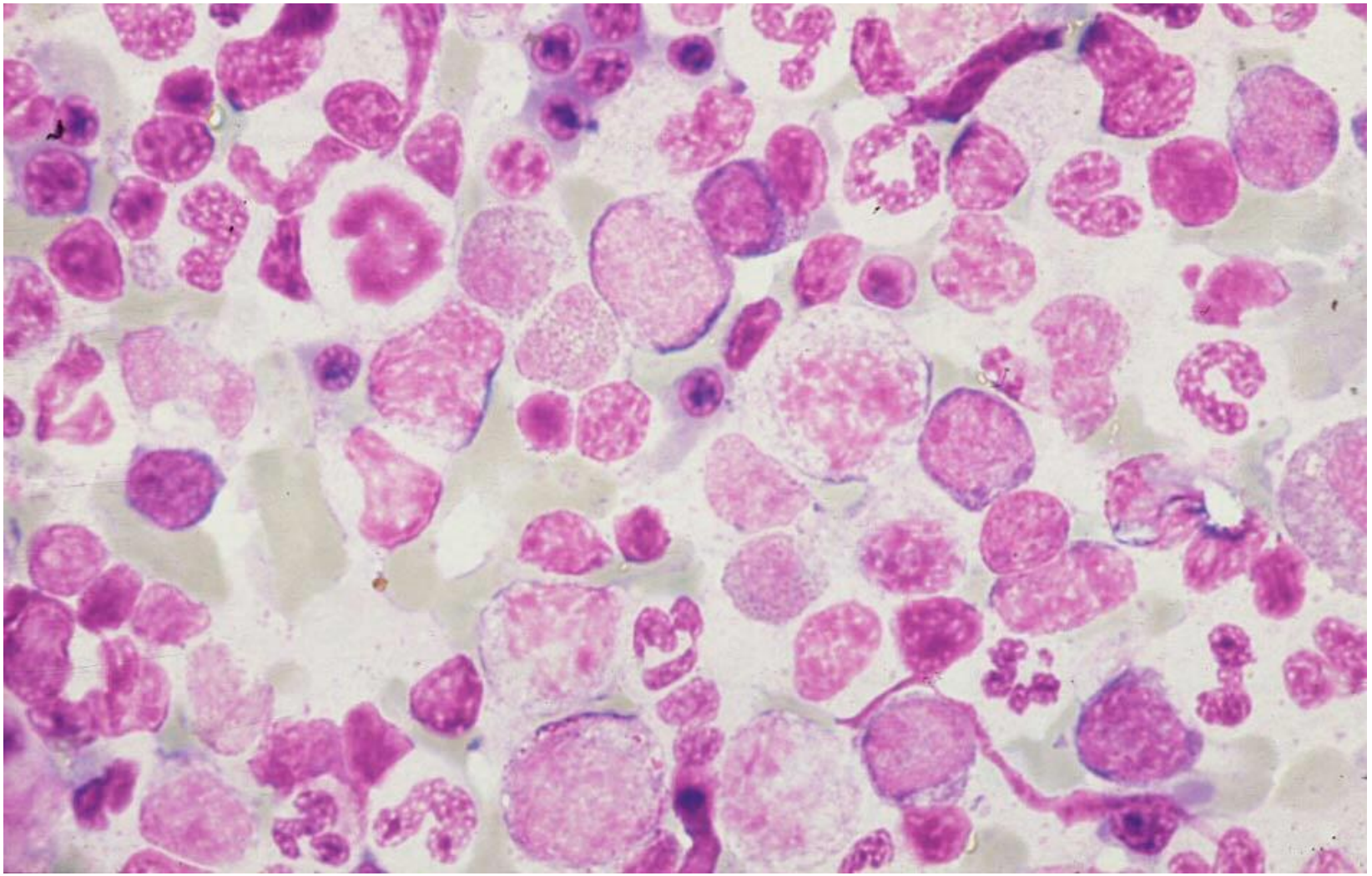
PV: megakaryocyten (groot!!)



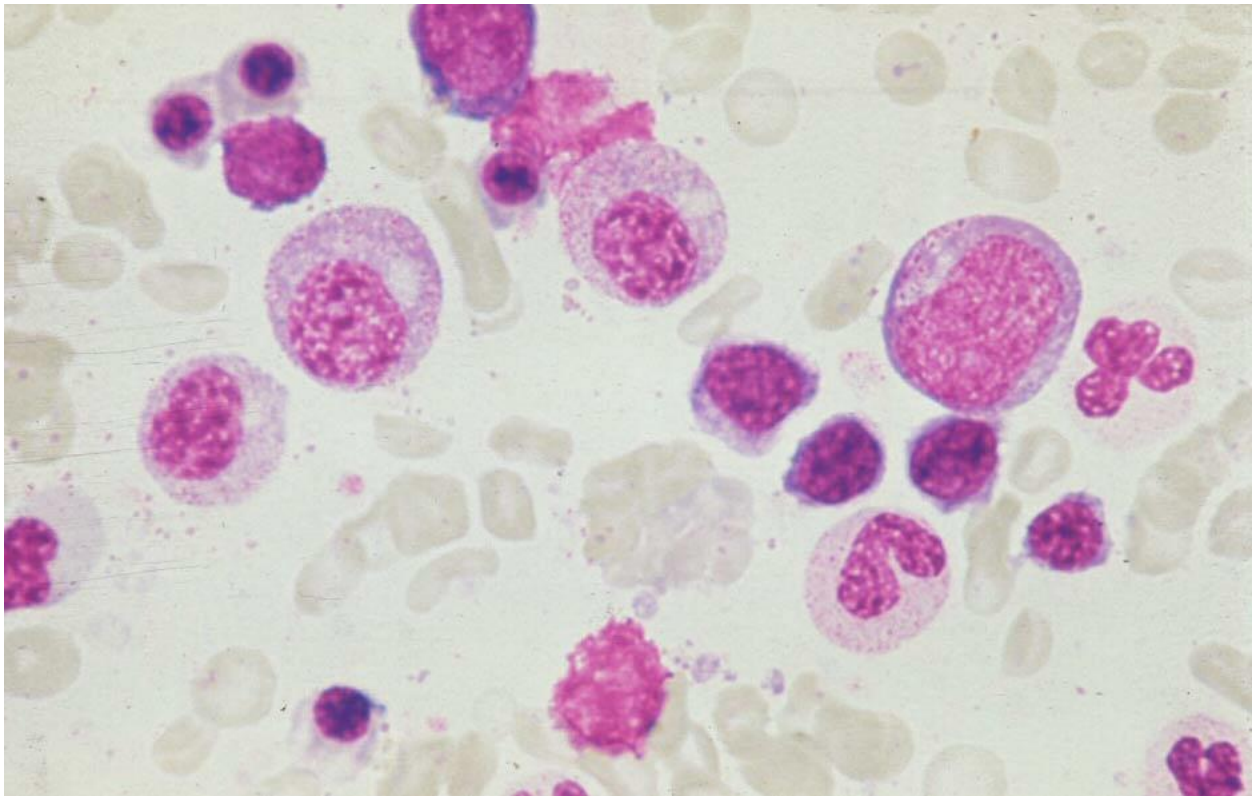


normaal

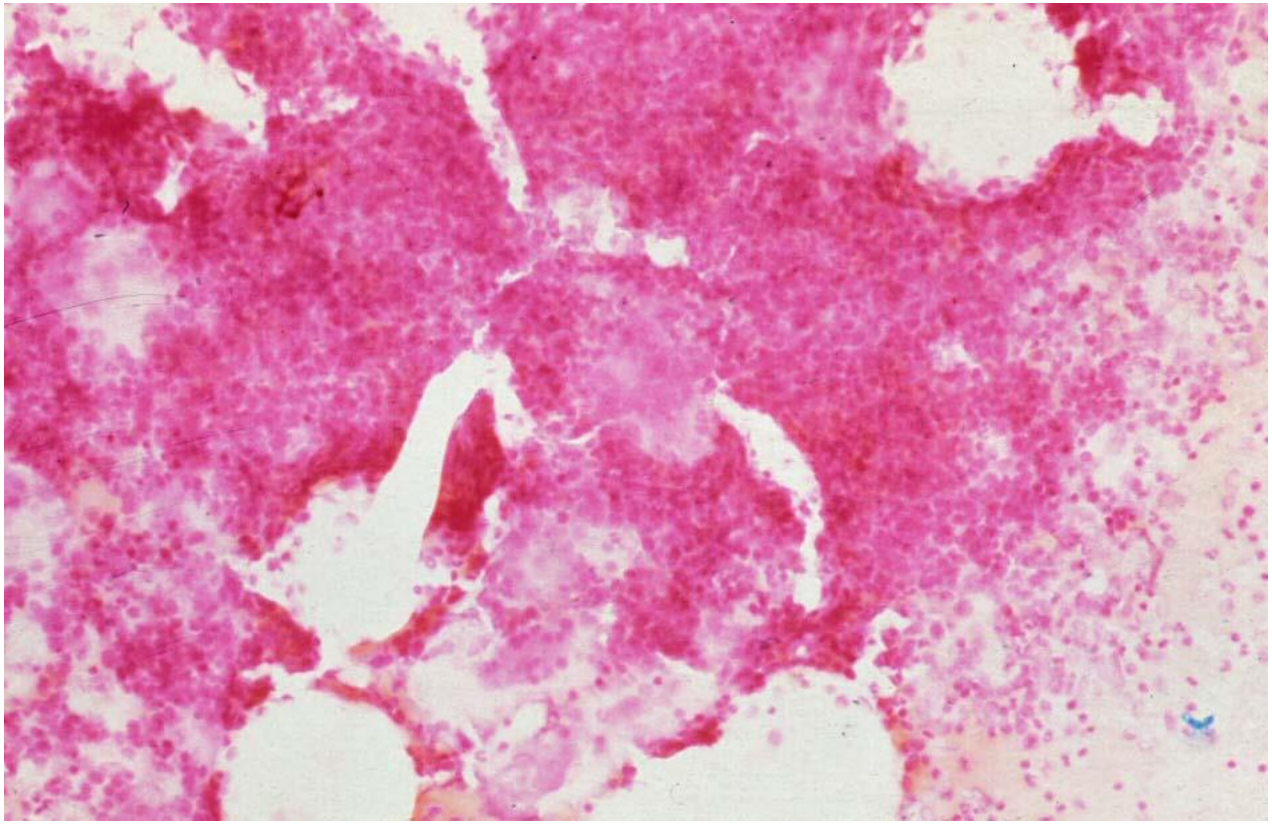
PV: proliferatie erythropoiese en granulopoiese



PV: erythropoiese “rafelig”

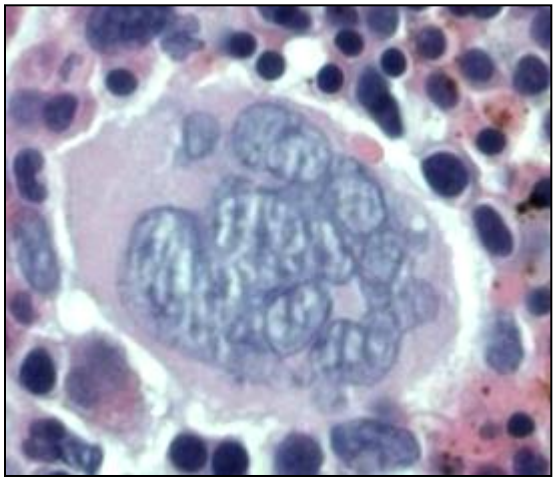


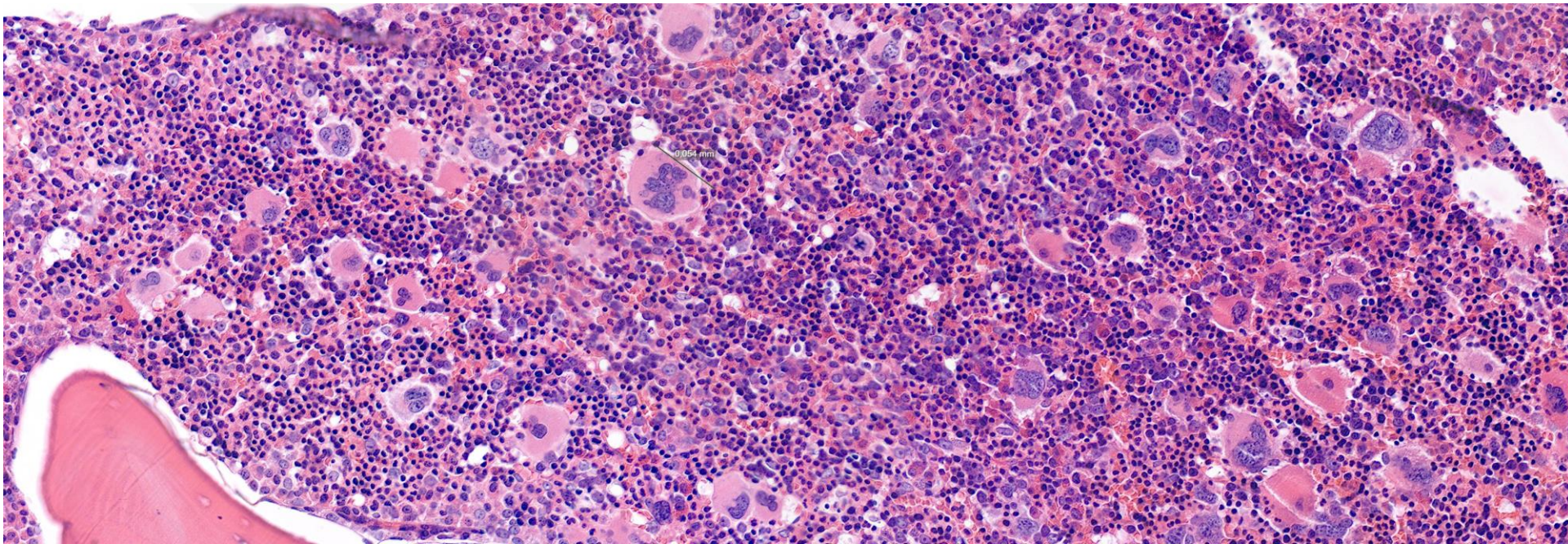
PV: ijzerkleuring



Botbiopt: Polycythemia Vera

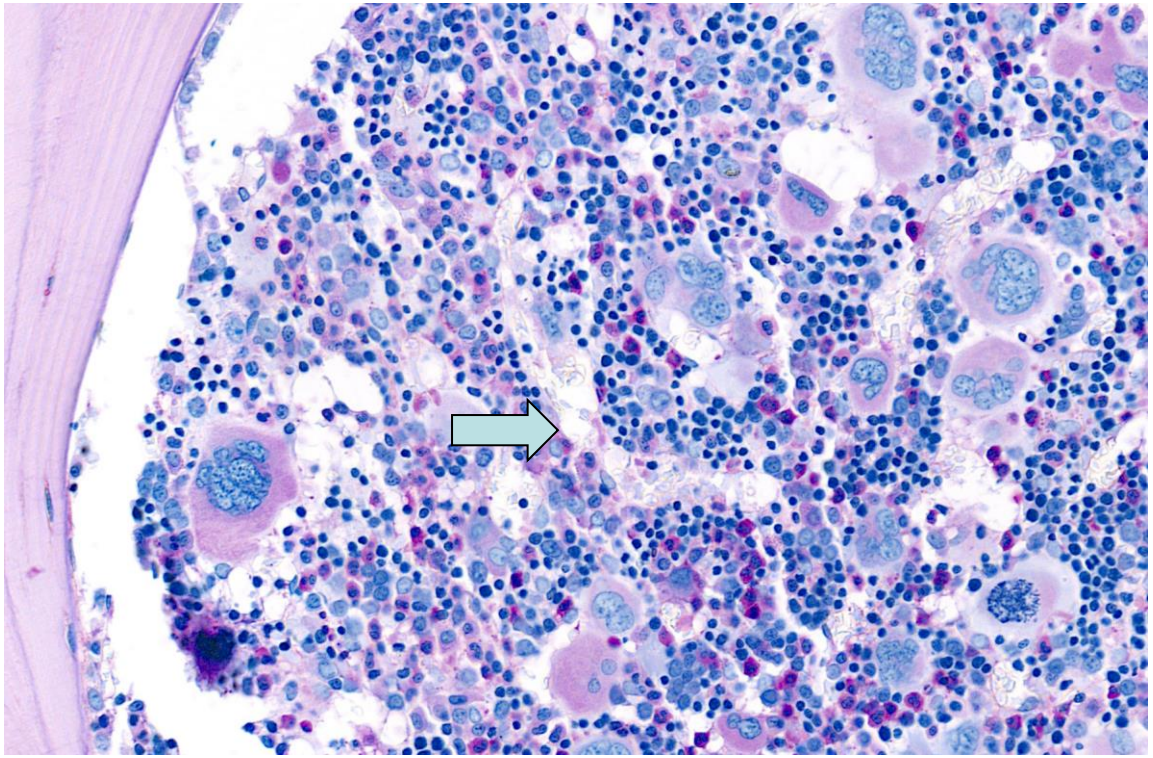
- 70-100% celrijk
- Trilineaire hyperplasie
- Megakaryocyten:
  - zeer groot
  - losjes geclusterd
  - hypersegmentatie, “staghorn”
- Wijde gestuwde sinussen
- Soms fibrose gr.1-2
- Geen ijzer

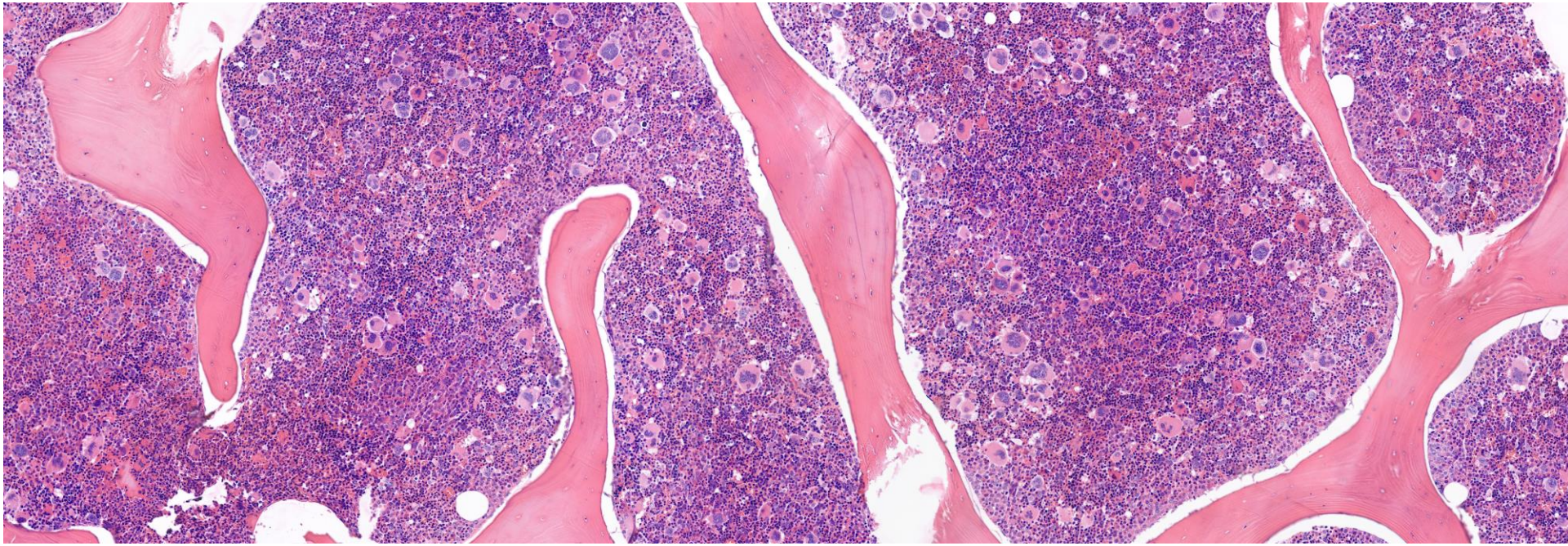




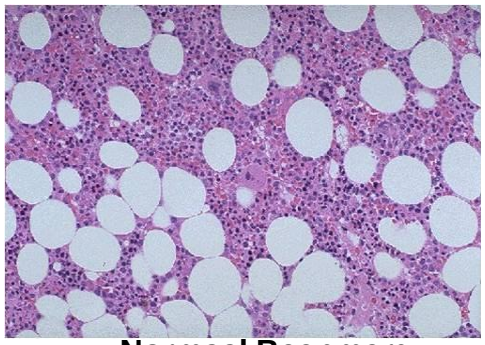
Polycythemia Vera

PAS kleuring:
ME ratio <1
Gestuwde sinussen

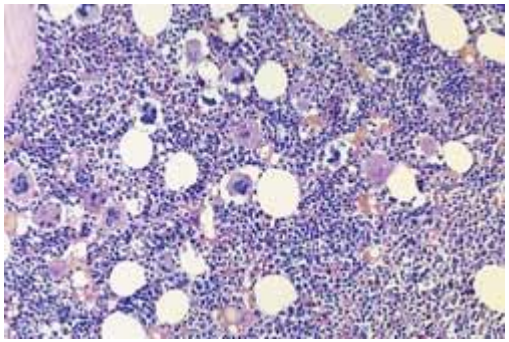




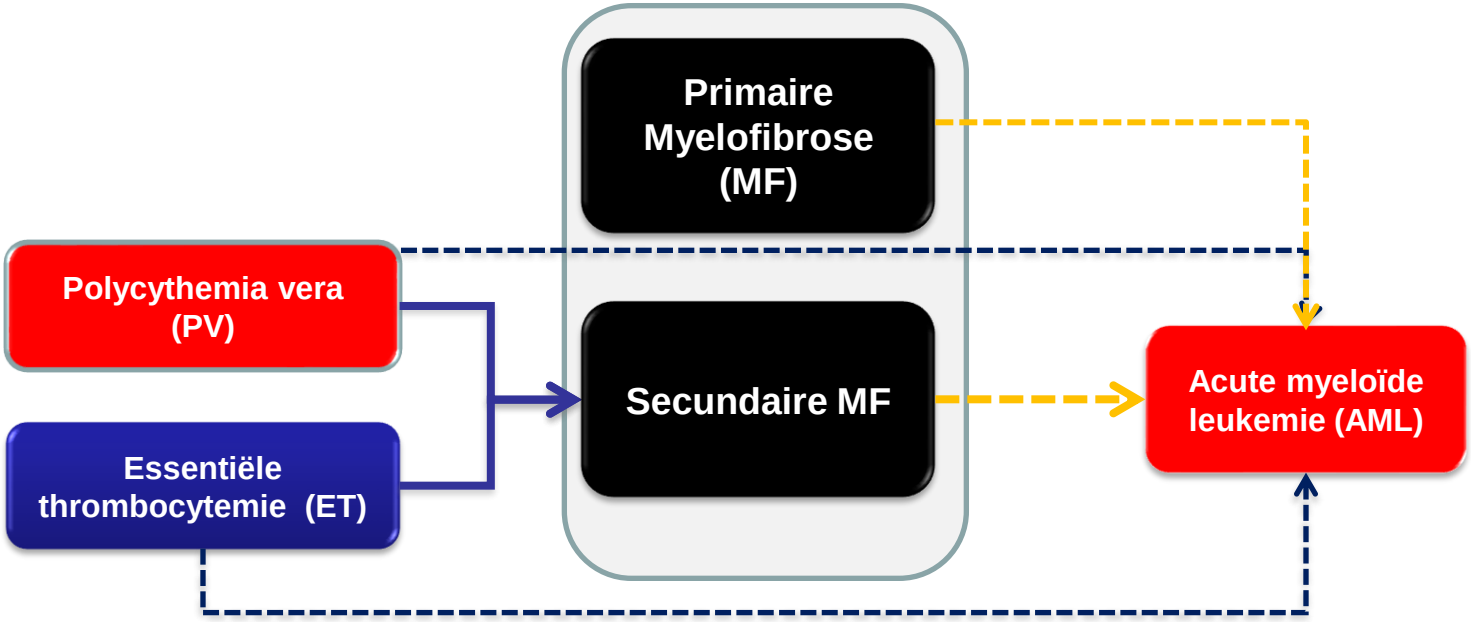
PV naar post-PV MF



Normaal Beenmerg



Polycythemia vera



Abdel-Wahab OI, Levine RL. Ann Rev Med. 2009;60:233-245.

Post-PV MF

Required criteria
1. Documentation of previous diagnosis of WHO-defined PV
2. Bone marrow fibrosis grade 2-3
Additional criteria (2 required)
1. Anemia or sustained loss of either phlebotomy or cytoreductive treatment
2. Leukoerythroblastic peripheral blood picture
3. Increasing splenomegaly
4. Development of > 10% weight loss in 6 months, night sweats of unexplained fever >37.5 ^{0}C (>1 required)

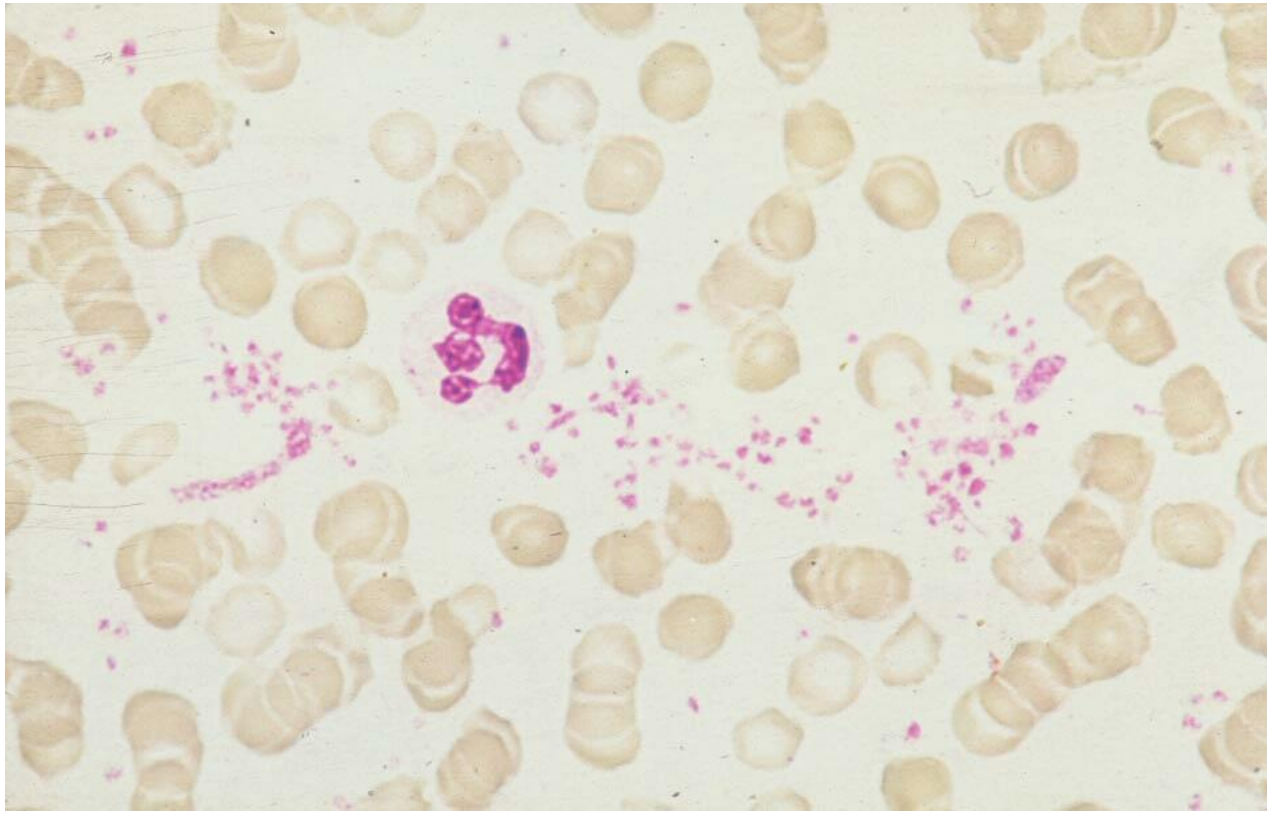
Of is het een essentiele trombocytose?

WHO criteria Essentiële Trombocytemie 2022

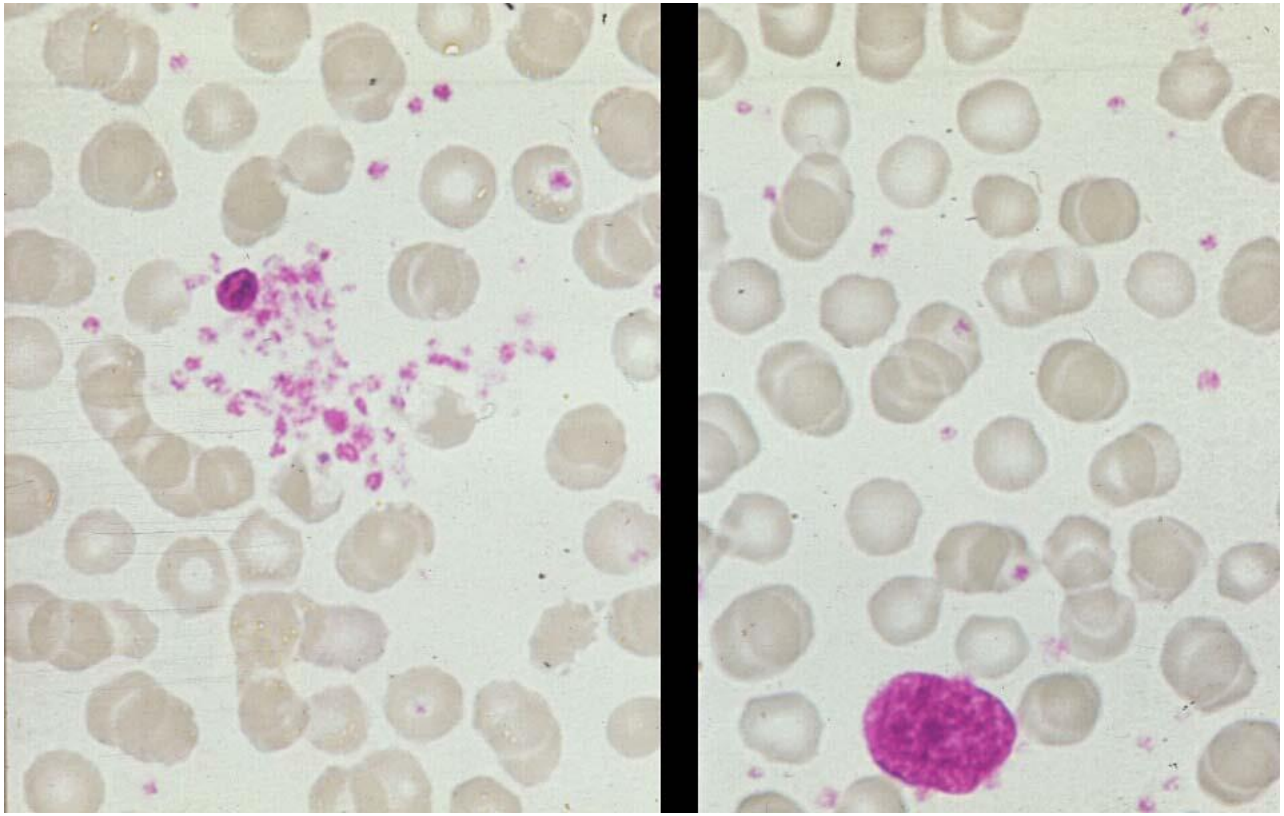
Majeure criteria
1. Trombocyten ≥450 x 10^9/L
2. Beenmergbiopt met voornamelijk megakaryocytaire toename met atypische grote megakaryocyten. Geen belangrijke afwijkingen aan rood en wit, geen vezeltoename (≤ graad 1 toename in reticuline vezels)
3. Voldoet niet aan WHO criteria PV, IMF, CML, MDS of andere entiteit
4. Aanwezigheid van *JAK2V167F, CALR of MPL* mutatie
Mineur criterium
1. Aanwezigheid van andere clonale merker of geen aanwijzingen voor reactieve trombocytose

Voor diagnose ET zijn alle vier majeure criteria noodzakelijk, of 3 majeure en 1 mineur

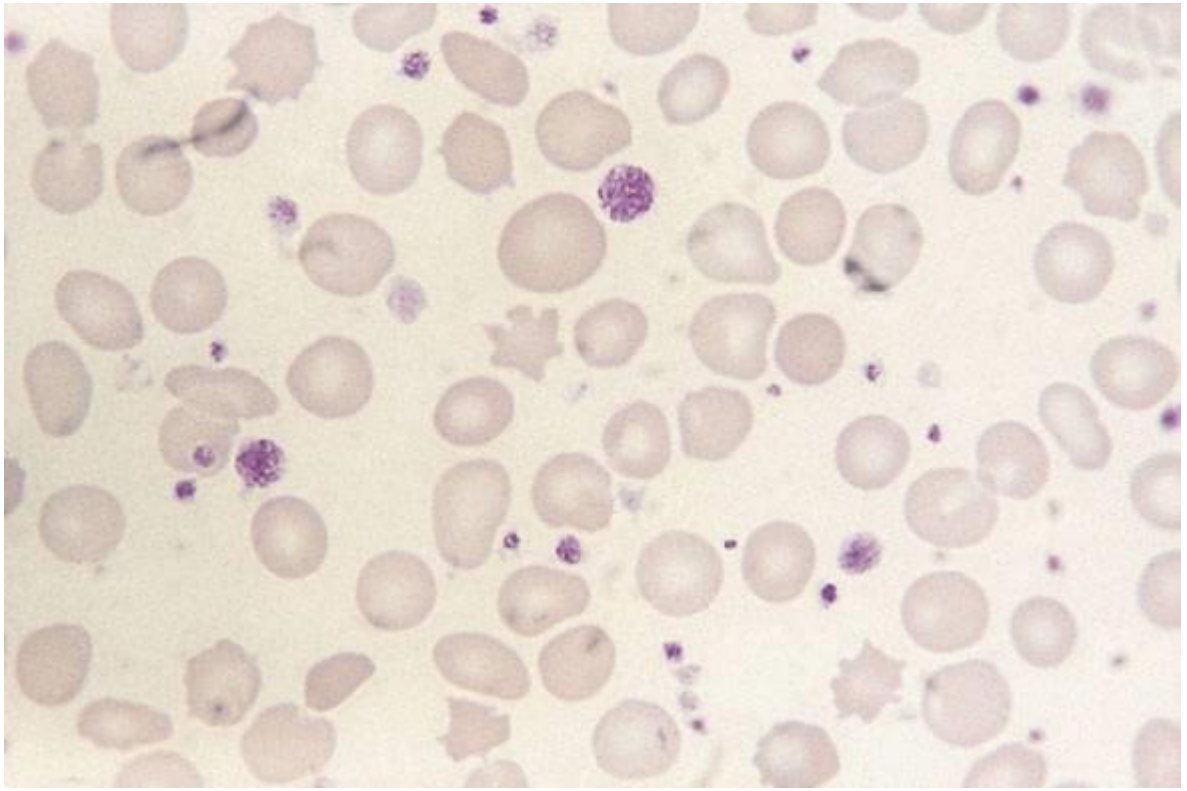
Essentiële trombocytose: bloed



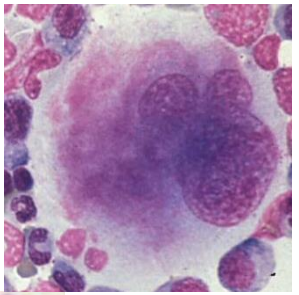
ET, bloed: kern van megakaryocyt

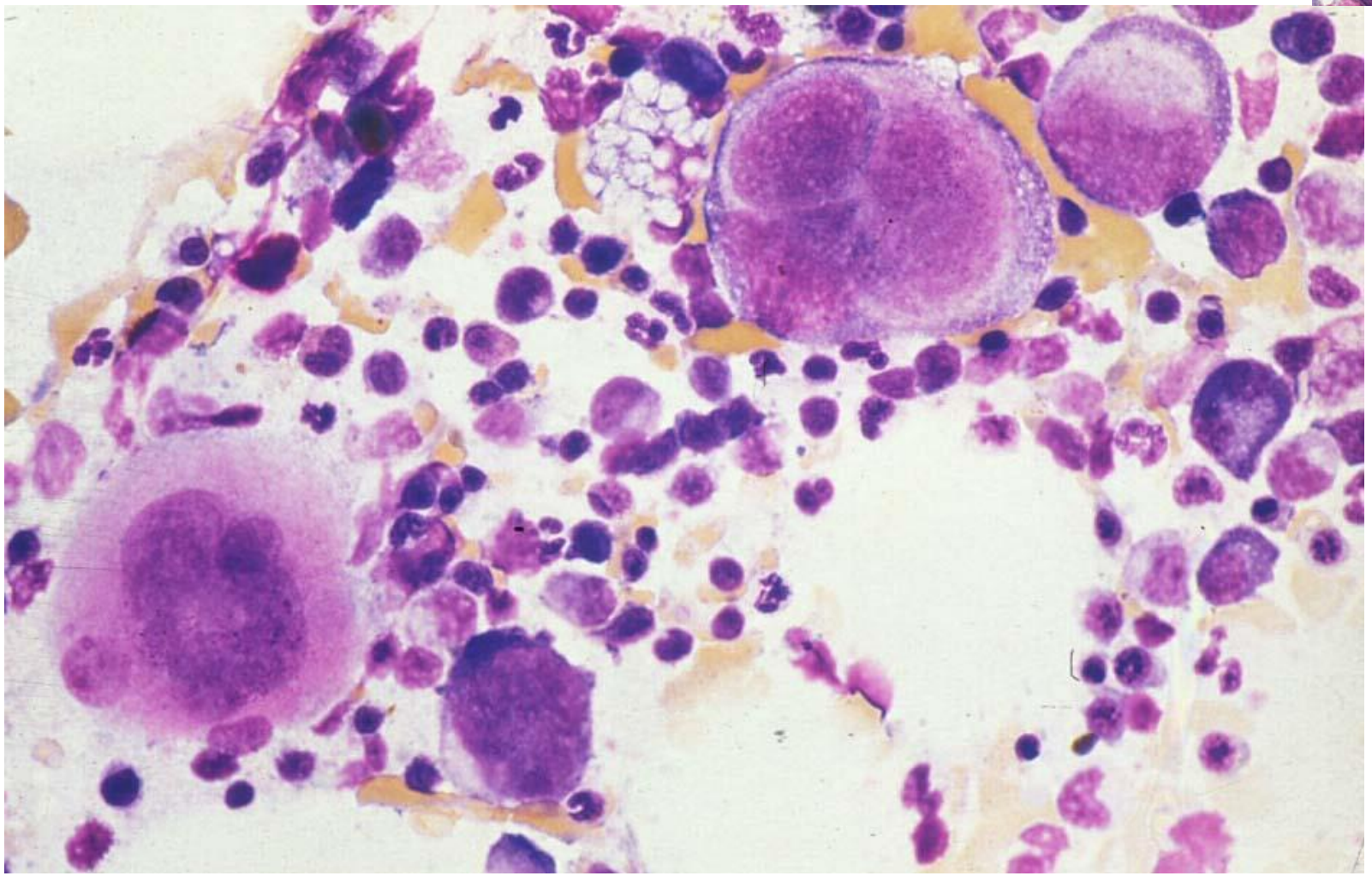


Essentiële trombocytose: bloed

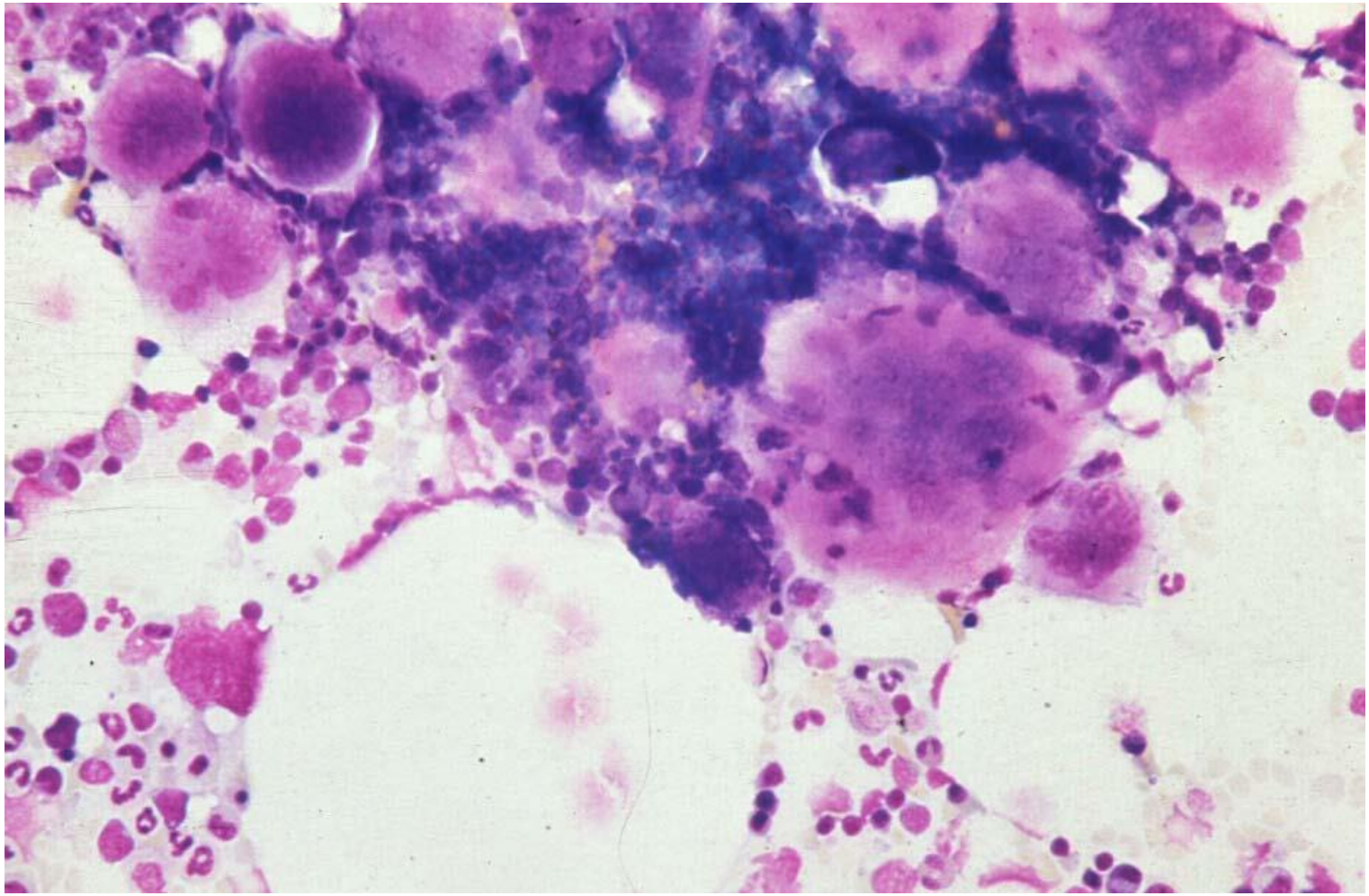


ET: beenmergaspiraat

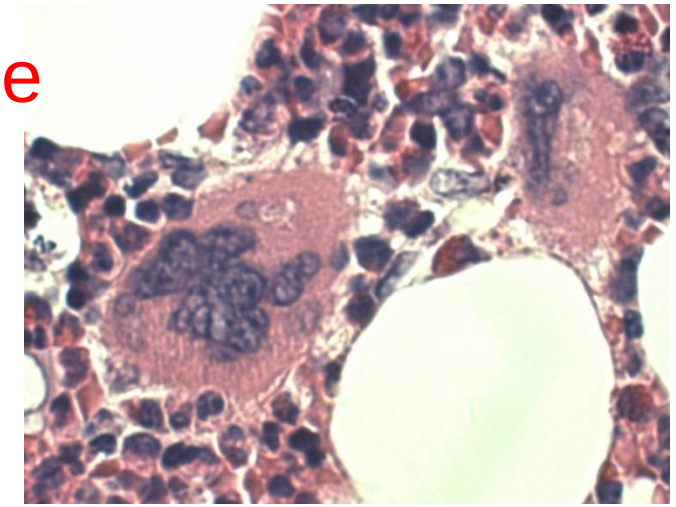




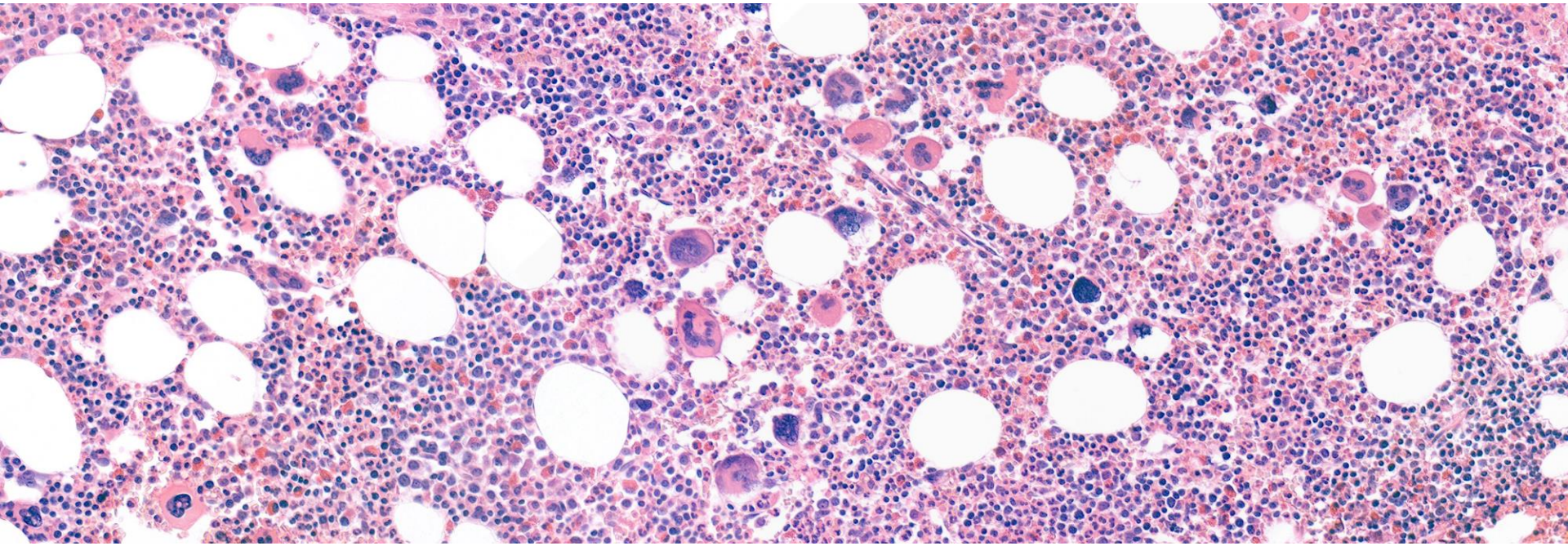
ET: beenmerg



Botbiopt: Essentiële trombocytemie



- Matig toegenomen celrijk
- Alleen megakaryocytaire hyperplasie
- Megakaryocyten:
  - normaal tot zeer groot
  - Solitair/losse groepen gelegen
  - Hypersegmentatie



WHO 2022 criteria for prePMF

prePMF criteria
Majeure criteria
1. Toename van afwijkende megakaryocyten zonder reticuline fibrose > graad 1, en toegenomen celrijkdom met granulocytaire hyperplasie en vaak onderdrukte erytropoiese
2. Voldoet niet aan PV, *BCR-ABL1*-positieve CML, MDS of andere myeloïde neoplasie
3. Aantonen van *JAK2, CALR of MPL* mutatie of andere clonale marker, bij ontbreken hiervan geen aanwijzingen voor reactieve beenmergfibrose
Mineure criteria
Aanwezigheid van minstens één van de volgende en tweemaal aangetoond:
1. Anemie niet anders verklaard
2. Leukocytose ≥11x10^9/L
3. Splenomegalie palpabel of bij beeldvorming
4. Toename LDH
5. Leukoerythroblastose

Diagnose prePMF vereist 3 majeure en minstens 1 mineur criterium

Post-ET MF

Required criteria
1. Documentation of previous diagnosis of WHO-defined ET
2. Bone marrow fibrosis grade 2-3
Additional criteria (2 required)
1. Anemia or ≥ 2 g/dL decrease from baseline Hb level
2. Leukoerythroblastic peripheral blood picture
3. Increasing splenomegaly
4. Increased LDH
5. Development of a/weight loss, b/ night sweats of c/ unexplained fever (>1 required)

Frequencies of the JAK2^{V617F} mutation in patients with myeloproliferative disorders

Authors	PV (%)	PMF (%)	ET (%)
James et al.	89	43	43
Baxter et al.	73	44	12
Levine et al.	74	35	32
Kralovics et al.	65	57	23
Zhao et al.	83	ND	ND
Jones et al.	81	43	41
Steensma et al.	ND	ND	ND
Goerttler et al.	100	57	33
Jelinek et al.	86	95	30
Levine et al.	ND	ND	ND
Total	**422/557 76%)**	**80/160 (50%)**	**114/391 (29%)**
Data obtained by allele-specific PCR*	71/93 (97%)	8/16 (50%)	29/51 (57%)

* Baxter et al.

The END