MYELODYSPLASTIC SYNDROME AND ITS DIFFERENTIAL DIAGNOSIS

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Outline of lecture

- Review the overall pathway to diagnosing myelodysplastic syndromes (MDS), including appropriate application of current diagnostic criteria
- Present pitfalls in applying current criteria to cytopenic patients undergoing bone marrow evaluation
  - Including differential diagnosis of neoplastic and non-neoplastic mimickers of MDS

Myelodysplastic syndromes

- Hypercellular, clonal marrow proliferations
- Ineffective hematopoiesis
  - Intramedullary cell death of maturing hematopoietic elements
  - Peripheral cytopenias
- Disordered maturation
  - Dysplastic morphology
- Characteristic portfolio of somatically acquired genetic abnormalities in the malignant clone

Involvement of the pathologist in diagnosing MDS

Hematologist evaluates the patient
--Identifiable secondary cause of cytopenia?
--Would the patient be treated if MDS is diagnosed?

Bone marrow biopsy and aspirate are performed

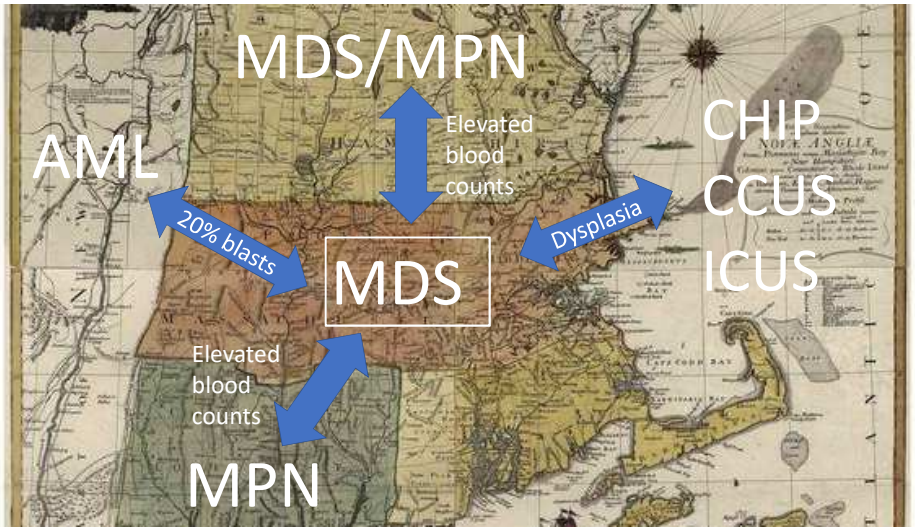
Pathologist is tasked to integrate these diverse data and make a determination of MDS versus other

- Clinical information
- Peripheral blood and marrow morphology
- Flow cytometry
- Cytogenetics
- Molecular genetics (NGS)

Criteria for diagnosing MDS

- Cytopenia(s)(at least 1)
  - Hemoglobin <12 g/dL (women) or <13 g/dL (men)
  - ANC <1.8 x 10^9/L
  - Platelets <150 x 10^9/L
- Dysplasia
  - At least 10% of cells appear dysplastic in at least 1 hematopoietic lineage
  - More dysplasia allows a more confident diagnosis
- Clonality:
  - Cytogenetic abnormality in ~50%
  - Somatic mutation found by NGS in ~90%

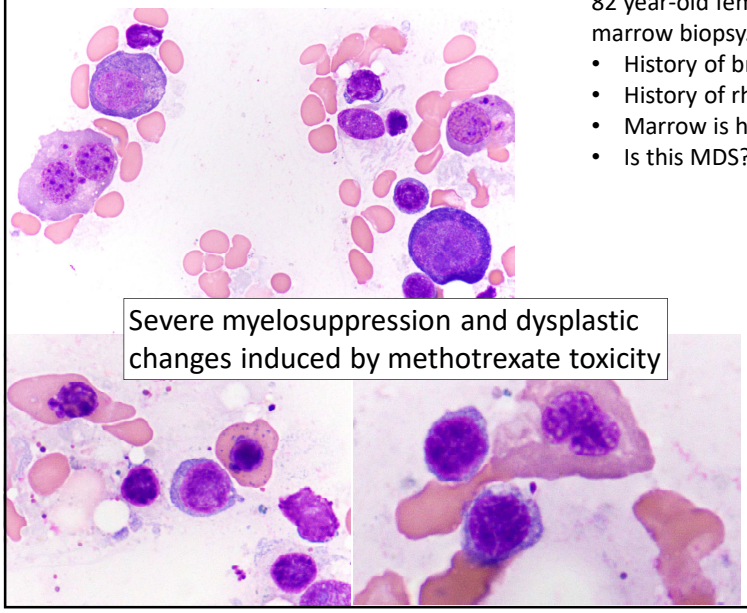
The boundaries of MDS present many opportunities to 'get lost'!



Nuanced assessment of cytopenia is an important first step in the diagnosis of MDS

- Know the complete CBC results (including WBC differential) at the time the bone marrow sample was taken
- Know the features that can support MDS over and above a simple 'cytopenia present or absent' assessment
  - Duration of cytopenia: MDS cytopenias are chronic and stable or inexorably worsening
  - Comorbid conditions that can cause cytopenia: can steer away from MDS diagnosis
  - Type and depth of cytopenia
    - Mild (hemoglobin 10-12 g/dL) isolated anemia not uncommon in early MDS, but isolated mild thrombocytopenia or neutropenia would be very unusual

Longitudinal review of blood counts can help avoid a misdiagnosis of MDS: Case 1



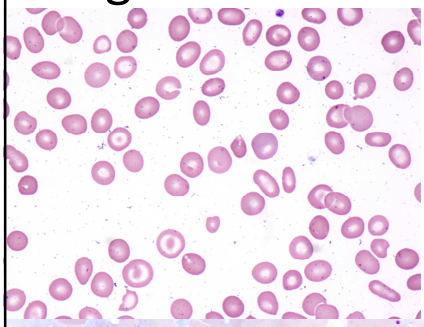
Severe myelosuppression and dysplastic changes induced by methotrexate toxicity

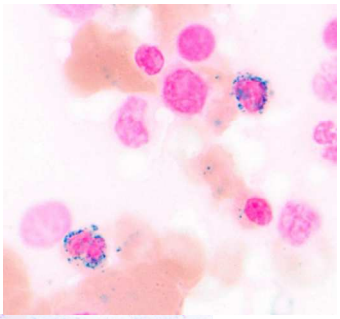
82 year-old female presents with pancytopenia, underwent a bone marrow biopsy.

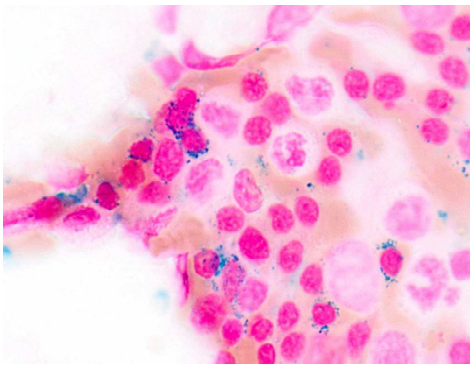
- History of breast cancer treated with chemotherapy
- History of rheumatoid arthritis treated with methotrexate
- Marrow is hypocellular marrow with severe dyserythropoiesis
- Is this MDS?

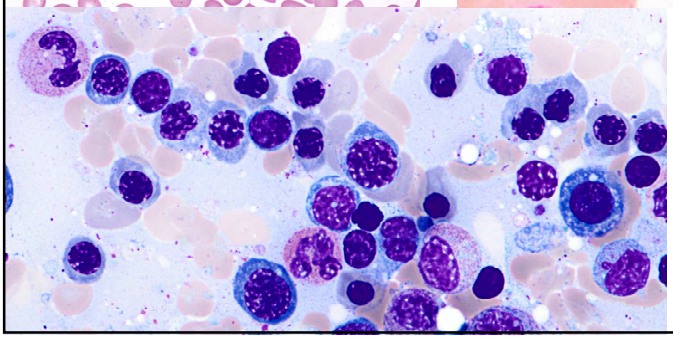
	WBC	Hgb	Hct	Plts
12/26/20	0.6	6.7	19.8	13
12/28/20	0.9	8.5	25.2	30
12/29/20	1.9	9.6	28.6	16
12/30/20	4.1	8.6	25.1	18
12/31/20	9.6	7.6	22.0	48
1/1/21	13.1	7.3	21.7	54
1/2/21	15.3	7.3	22.2	70
1/3/21	12.9	6.9	20.3	91
1/4/21	11.9	8.4	24.7	125
1/7/21	10.7	9.9	31.8	261

Longitudinal review of blood counts can help support a diagnosis of MDS: Case 2



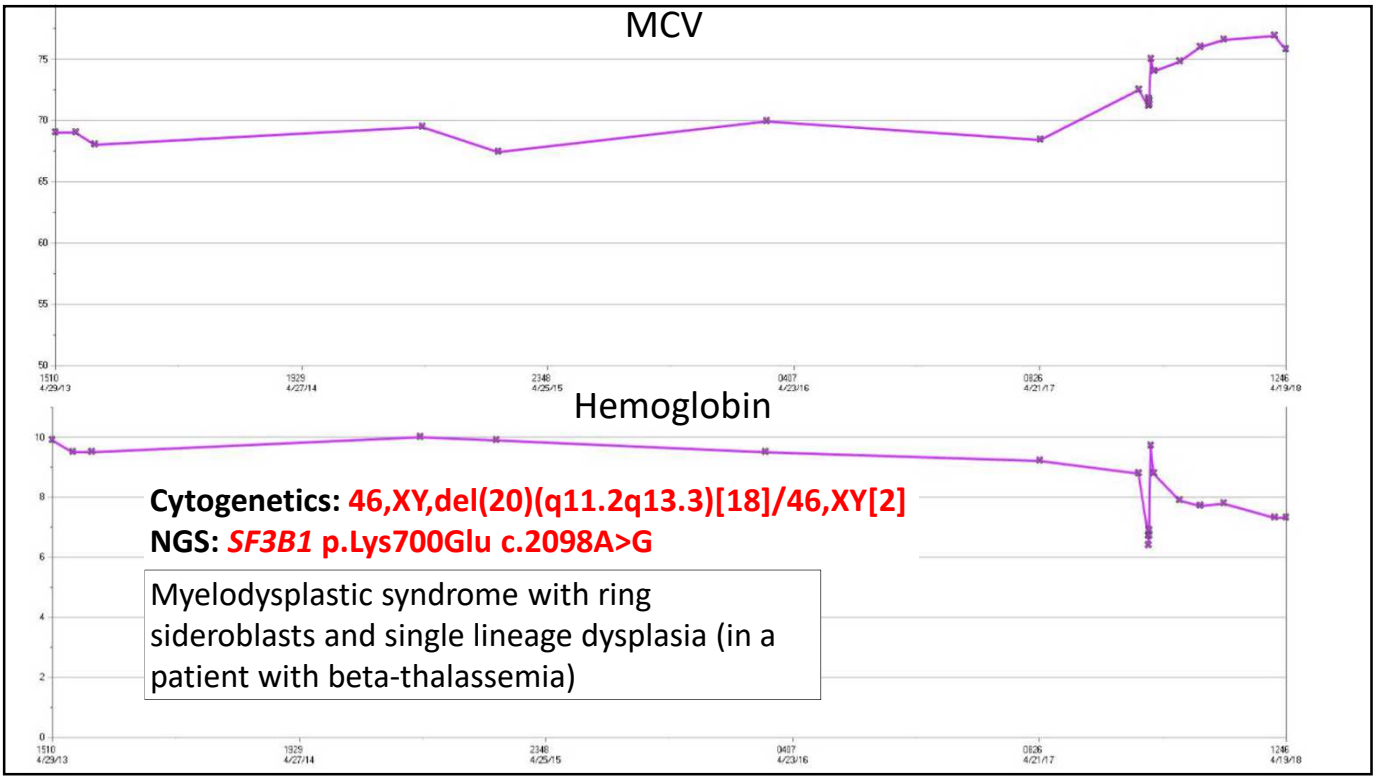






84 year-old man presents with anemia and fatigue

- WBC 3.8, HGB 7.3 g/dL (MCV 77), PLT 162
- Review of the clinical history discloses that the man has a history of beta thalassemia with chronic (asymptomatic) anemia and low MCV



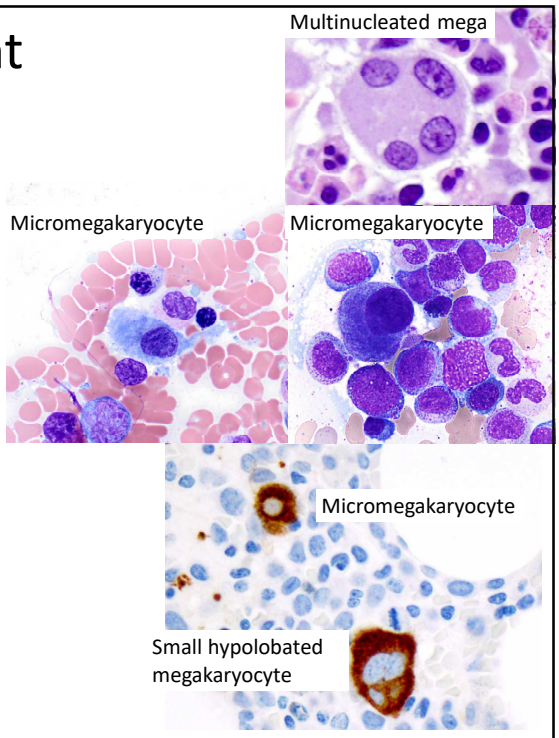
Cytogenetics: **46,XY,del(20)(q11.2q13.3)[18]/46,XY[2]**

NGS: ***SF3B1*** **p.Lys700Glu c.2098A>G**

Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia (in a patient with beta-thalassemia)

Morphologic dysplasia assessment

- Current threshold of 10% dysplastic cells is arbitrary and is too low for megakaryocytes
  - Several studies suggest that >10% <u>micromegakaryocytes</u> are highly specific for MDS, but 30-40% is a more appropriate threshold when considering all types of megakaryocyte dysplasia
  - 10% dysplasia cutoff appears to be appropriate for erythroid and granulocytic lineage
- Keep in mind that even dysplasia above these levels is not specific for MDS
  - Significant dysplasia in bone marrow of normal volunteers and even more frequently in patients with non-neoplastic cytopenias



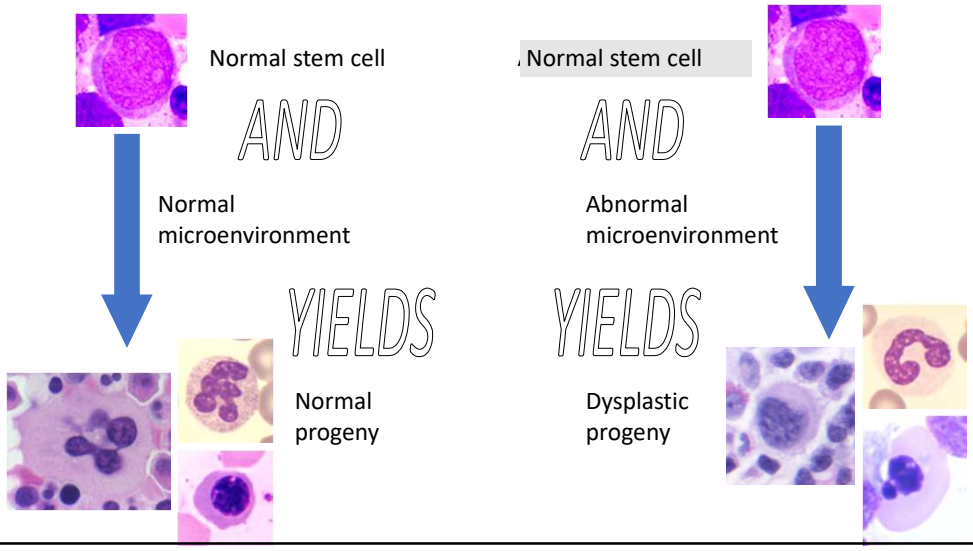
Font P Ann Hematol 2013;92:19, Parmentier S Haematologica 2012;97:723, Matsuda A Leukemia 2007;21;678; Della Porta MG Leukemia 2015;29:66

Specificity of dysplastic findings

Morphological abnormalities[a]	*Cutoff values*[b]	*AUC*	*Cohen's K-coefficient (inter-observer agreement)*[c]
Erythroid lineage *9% false positive*			
Megaloblastoid changes	>5%	0.814, $P<0.001$	0.83
Bi- or multinuclearity	>3%	0.679, $P<0.001$	0.87
	>5%	0.698, $P<0.001$	
Nuclear lobulation or irregular contours	>3%	0.674, $P<0.001$	0.84
Pyknosis	>5%	0.677, $P<0.001$	0.81
Cytoplasmic fraying	⩾7%	0.602, $P<0.001$	0.82
Ring sideroblasts	>5%	0.650, $P<0.001$	0.95
	⩾15%	0.719, $P<0.001$	
Ferritin sideroblasts	⩾30%	0.670, $P<0.001$	0.92
Granulocytic lineage *5% false positive*			
Myeloblasts	>3%	0.777, $P<0.001$	0.92
	>5%	0.723, $P<0.001$	
Auer rods	⩾1%	0.524, $P=0.001$	0.90
Pseudo Pelger–Hüet anomaly	>3%	0.714, $P<0.001$	0.87
	>5%	0.814, $P<0.001$	
Abnormal nuclear shape	⩾7%	0.700, $P<0.001$	0.86
Neutrophil hypogranulation	>3%	0.791, $P<0.001$	0.81
11% false positive	>5%	0.821, $P<0.001$	
Megakaryocytic lineage			
Micromegakaryocytes	>5%	0.916, $P<0.001$	0.88
Small binucleated megakaryocytes	>5%	0.845, $P=0.001$	0.81
Megakaryocytes with multiple separated nuclei	>5%	0.750, $P<0.001$	0.84
Hypolobated or monolobar megakaryocytes	>5%	0.646, $P<0.001$	0.86

Della Porta MG et al. Leukemia 2015;29:66

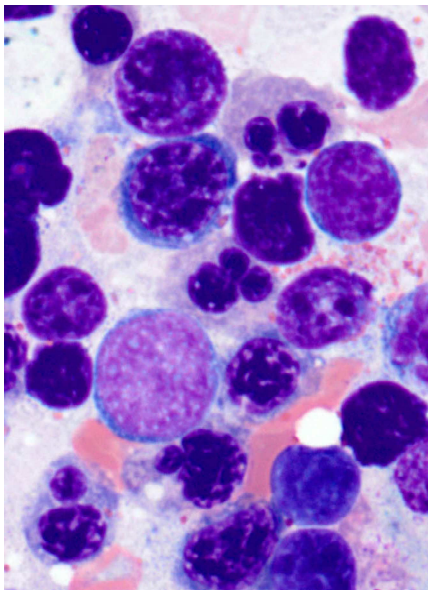
Neoplastic versus ‘reactive’ dysplasia



Navigating the minefield of ‘false dysplasia’: stress dyserythropoiesis

- Left shift and erythroid dysplasia due to exuberant non-neoplastic erythroid hyperplasia
  - Hemolytic anemia (immune or inherited)
  - Megaloblastic anemia
  - Recovery post-chemotherapy
- Lack of dysplasia in other lineages and lack of other cytopenias are helpful clues to avoid misdiagnosing as MDS

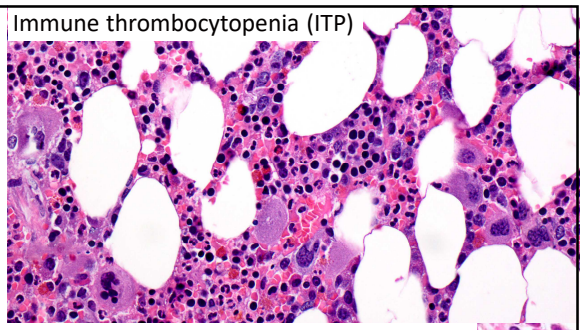
Stress erythropoiesis in β-thalassemia



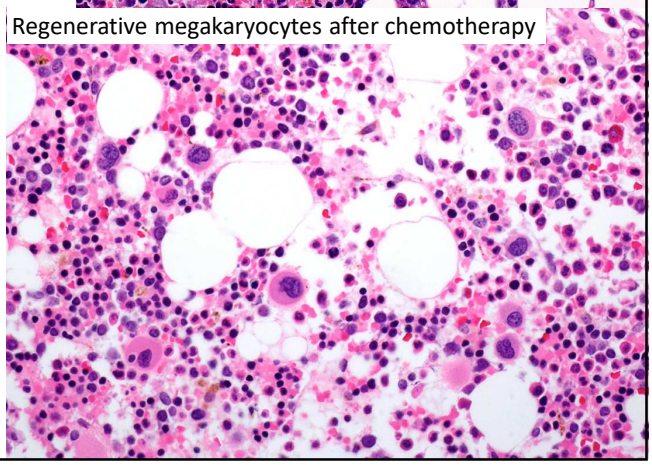
Regenerative 'dysmegakaryopoiesis'

- Small, hypolobated megakaryocytes are frequently seen in reactive settings
  - Peripheral platelet destruction
  - Post chemotherapy regeneration
  - Other acute/subacute marrow injury
- In reactive megakaryocyte proliferations such as ITP, a spectrum of morphologies is usually present

Immune thrombocytopenia (ITP)



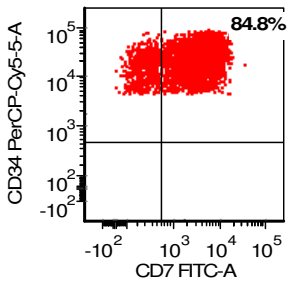
Regenerative megakaryocytes after chemotherapy



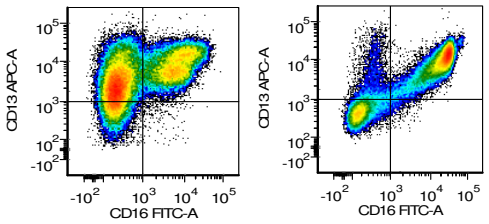
Using supportive evidence: flow cytometry

- Evaluate for a lymphoma or myeloma that could mimic MDS in clinical presentation
  - B-cell clonality assessment
  - T-cell assessment
  - Plasma cell clonality assessment
- Do not read too much into blast %
  - Usually correlates with the aspirate blast count, but should never be used in lieu of the morphologic blast enumeration
  - Blasts can be expressed as % of all events or % of non-erythroid events
- Antigenic aberration in blasts or myeloid/monocytic compartments can help support a diagnosis of MDS

Aberrancy in blasts



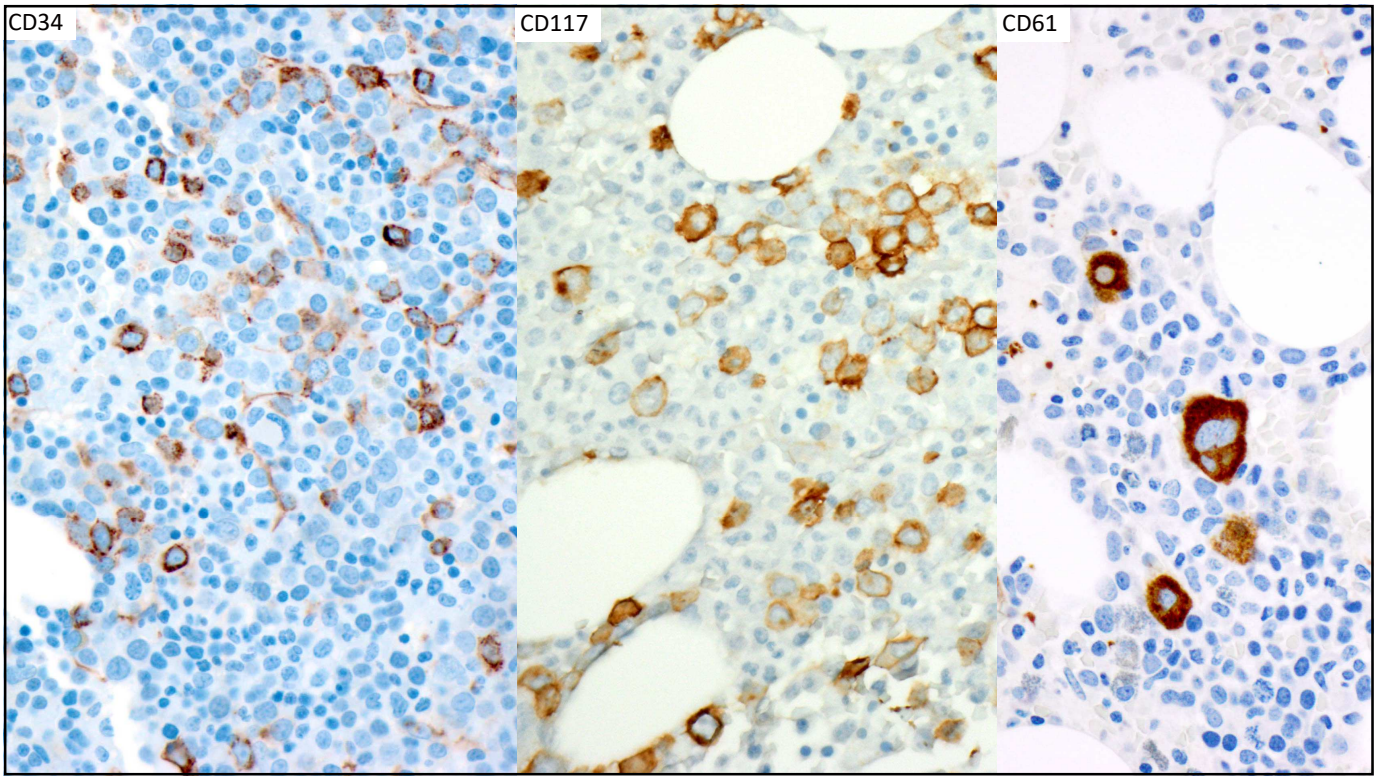
Aberrancy in maturing granulocytes



Courtesy of Sa Wang, MD Anderson Cancer Center

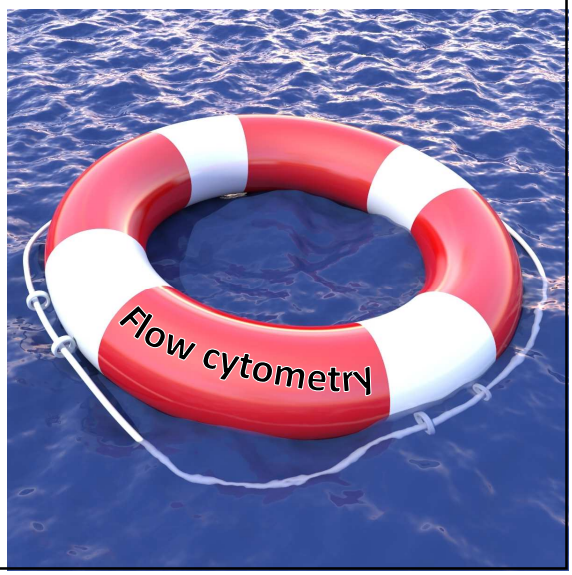
Immunohistochemistry in MDS diagnosis

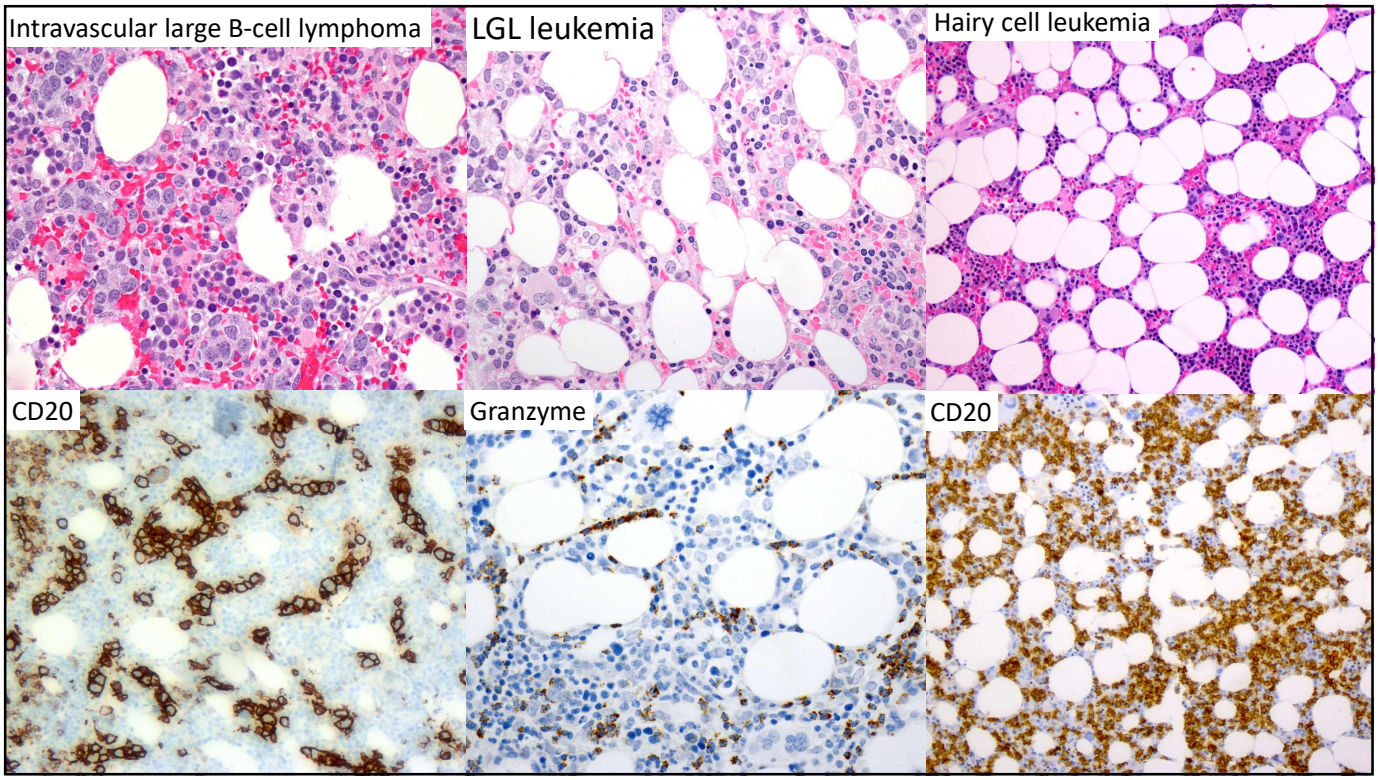
- May not be necessary if adequate flow cytometry and high-quality aspirate smears are obtained
- Essential if biopsy is crushed and/or aspirate is markedly hemodilute
- Useful to identify abnormal cells that may be subtle on routine histology or poorly detected by flow cytometry
  - Facilitate the identification of micromegakaryocytes (CD61)
  - Enumerate blasts to corroborate aspirate blast count
  - Disclose subtle neoplastic infiltrates that may mimic MDS clinically
    - Large cell lymphomas (CD20, PAX5, CD3)
    - Plasma cells (CD138, kappa, lambda)
    - Mast cells (CD117, tryptase)



Lymphomas presenting with cytopenias and no or minimal lymphocytosis/lymphadenopathy

- Hairy cell leukemia
- Large granular lymphocyte leukemia
  - Cytopenias are often more severe than the degree of infiltration would suggest
- Diffuse large B-cell lymphoma
  - More common in elderly and immunosuppressed
  - Patient may lack lymphadenopathy
- Classic Hodgkin lymphoma
  - More common in elderly and immunosuppressed



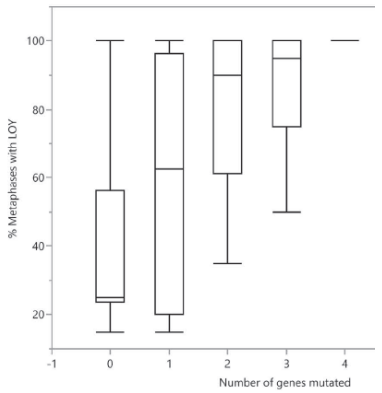


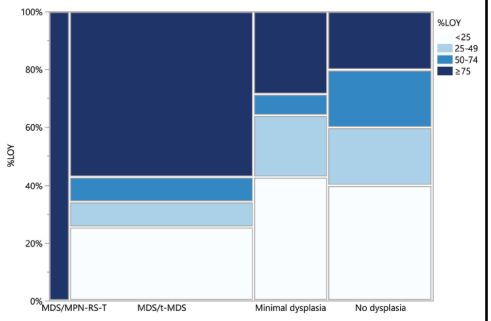
Using supportive evidence: Karyotype

MDS-defining irrespective of morphologic dysplasia

Unbalanced
-7 or del(7q)
del(5q) or t(5q)
i(17q) or t(17p)
-13 or del(13q)
del(11q)
del(12p) or t(12p)
del(9q)
idic(X)(q13)
Balanced
t(11;16)(q23;p13.3)
t(3;21)(q26.2;q22.1)
t(1;3)(p36.3;q21.2) 1%
t(2;11)(p21;q23)
inv(3)(q21q26.2)
t(6;9)(p23;q34)

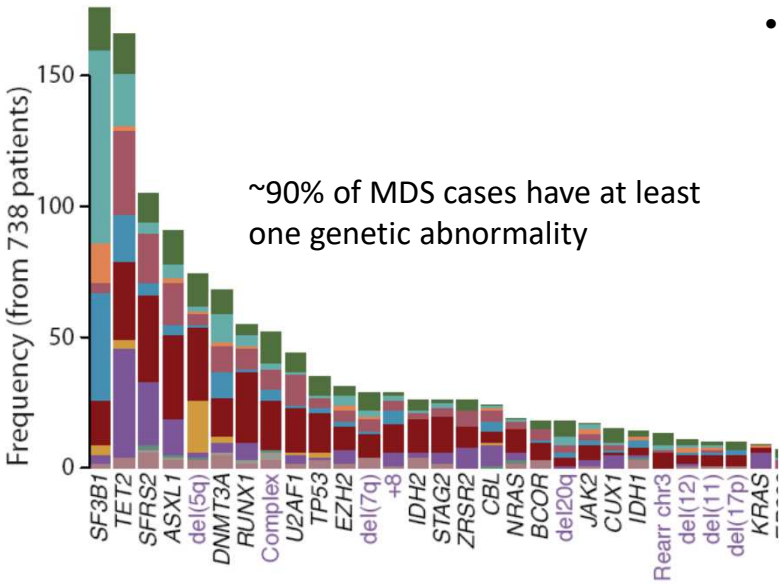
- ~50% of MDS cases have a normal karyotype
- +8, -Y, and del(20q) are common in MDS, but can occur in non-neoplastic conditions and are not MDS-defining
  - Greater –Y metaphases more suggestive of MDS
- Beware findings only seen by FISH
  - False positives may occur, especially low-level near cutoff threshold (typically ~5%)





Ouseph M Haematologica 2021;106:555

Using supportive evidence: Next-generation sequencing

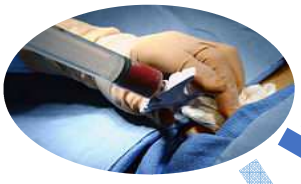


~90% of MDS cases have at least one genetic abnormality

- However, many apparently healthy individuals harbor somatic MDS-type mutations in hematopoietic cells ("CHIP")
  - Incidence increases with age
  - Associated with increased risk of mortality, but many patients never develop MDS even after many years

Papaemmanuil et al. Blood. 2013;122:3616, Jaiswal S et al. NEJM 2014;371:2488, Genovese G et al. NEJM 2014;371:2477, Xie M et al. Nature Med 2014;20:1472; Steensma D et al. Blood 2015;126:9

Integration of genetic testing into bone marrow diagnosis process at MGH



Biopsy, aspirate smear, peripheral blood smear morphology

Flow cytometry from aspirate smear

Stored fresh marrow aspirate (4°)

NGS on aspirate

3 weeks: addendum with final classification

2-3 days: Issue pathology report

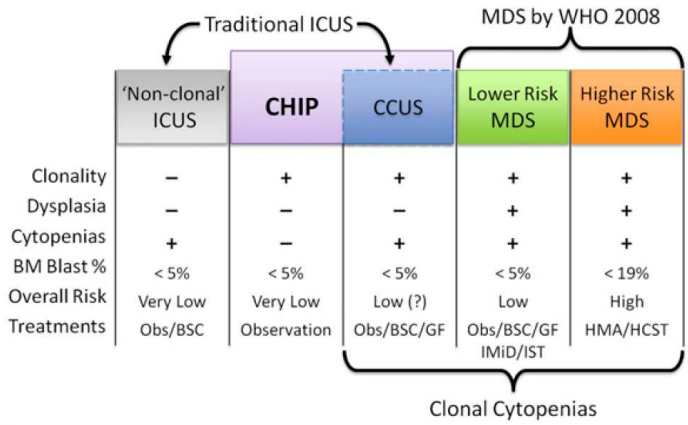
Cytogenetics on aspirate

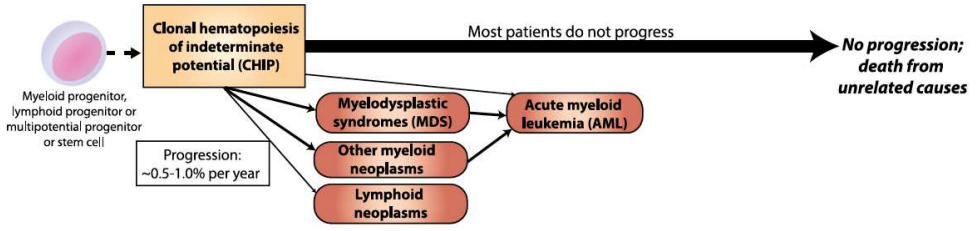
2 weeks: Genetic reports

Hematopathology fellows and faculty, molecular pathologist, clinicians

MDS and other clonal/cytopenic conditions

- Clonal hematopoiesis is a precursor state to MDS, but in and of itself does not define MDS
- Morphologic dysplasia is the main feature that separates MDS from CCUS (clonal cytopenia of undetermined significance)





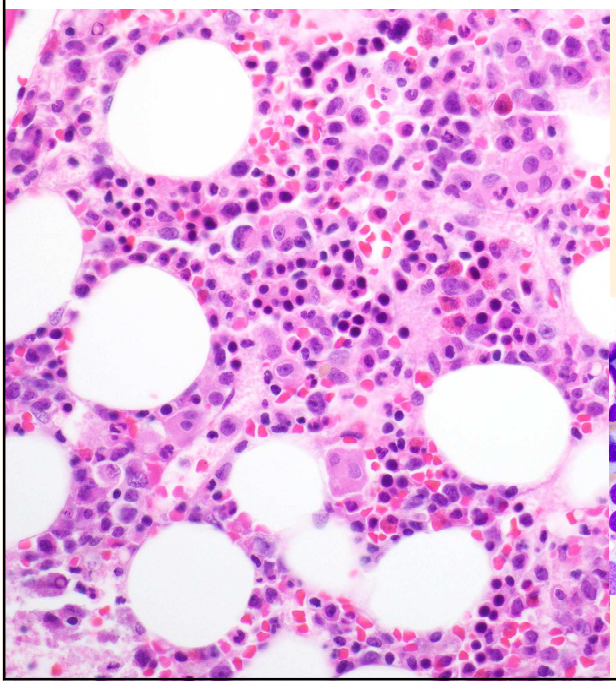
Steensma et al. Blood 2015; 126:9

Role of NGS in evaluating cytopenias

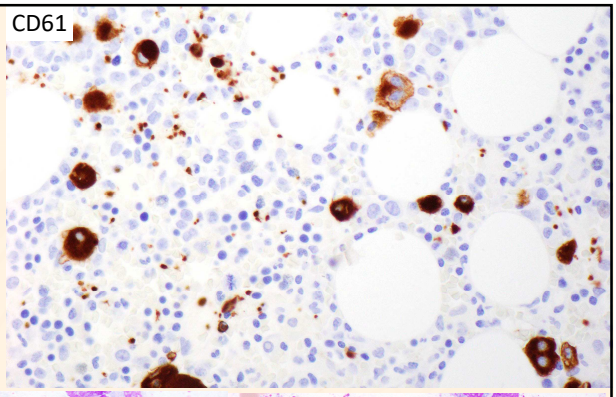
- Currently, there are no mutations or mutation patterns that are considered diagnostic of MDS in isolation
  - Many apparently healthy aging individuals harbor somatic MDS-type mutations in hematopoietic cells
  - Termed “Clonal hematopoiesis of indeterminate potential” AKA “CHIP”
    - *DNMT3A, TET2, ASXL1, TP53, JAK2, SF3B1* most common
- Mutation data can be used to support an MDS diagnosis suspected on morphology (especially if multiple and at high VAF)
  - Some dysplasia (>10%) still required to establish an MDS diagnosis
- Lack of mutations can be reassuring and stimulate a deeper search for non-MDS causes of the cytopenia
  - However, keep in mind that 5-10% of bona fide MDS cases may have normal cytogenetics and lack mutations on current NGS panels

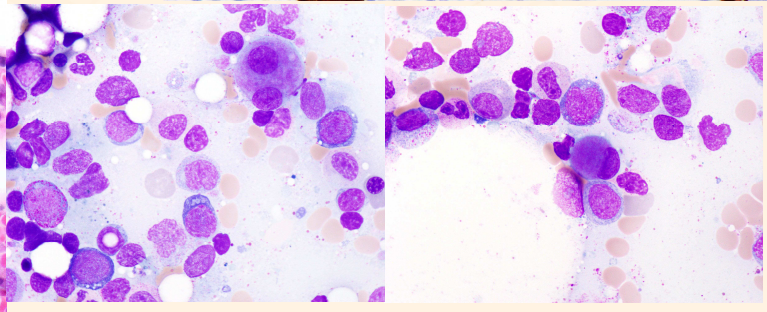
Jaiswal S NEJM 2014;371:2488, Genovese G NEJM 2014;371:2477, Xie M Nature Med 2014;20:1472, Malcovati L et al. Blood 2017;129:3371, Wang SA AJH 2021;96:E420

65 year-old presenting with WBC 2.3, HGB 11.8 (MCV 105), PLT 93
46, XY, del(20)(q11.2q13.1)[4]46,XY [16]
No mutations on NGS



CD61



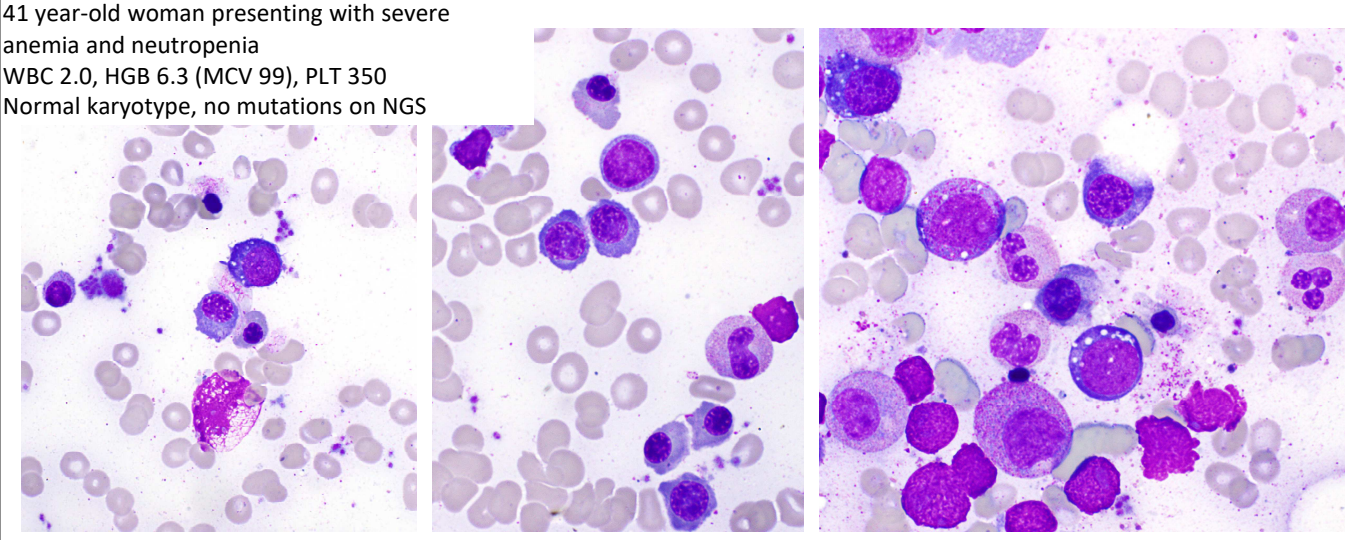


MDS with single lineage dysplasia

Non-neoplastic causes of cytopenia and dysplasia

- Drugs/toxins
  - Recent (<6 months) chemotherapy
  - Heavy alcohol intake
- Metabolic deficiencies: B12, folate, copper
- 'Stress erythropoiesis' due to hemoglobinopathies or acquired/congenital hemolytic anemias
- Infections, especially HIV and Hepatitis C
- Autoimmune diseases
- Marrow failure
  - Acquired aplastic anemia
  - Congenital bone marrow failure syndromes
- Germline mutation associated with altered hematopoiesis
  - *RUNX1, ANKRD26, ETV6*

41 year-old woman presenting with severe anemia and neutropenia
WBC 2.0, HGB 6.3 (MCV 99), PLT 350
Normal karyotype, no mutations on NGS



Patient had been taking zinc supplements and was found to be severely copper deficient (<0.10 mcg/mL), blood counts normalized after copper supplementation

Diagnosis: Copper deficiency

Case courtesy of Elizabeth Courville, University of Virginia

Diagnosis of MDS is a balancing act. . .

- Morphologic dysplasia
  - ↑ Lineages involved
  - ↑ Number of dysplastic forms
  - ↑ Severity of dysplasia
- Severity and persistence of cytopenia(s)
- Unexplained ↑MCV
- Flow cytometry abnormalities
- MDS-type mutations

- Younger patients
- Co-morbid conditions
- Paucity of clinical history

What if it's not clearly MDS, but there's no specific diagnosis?

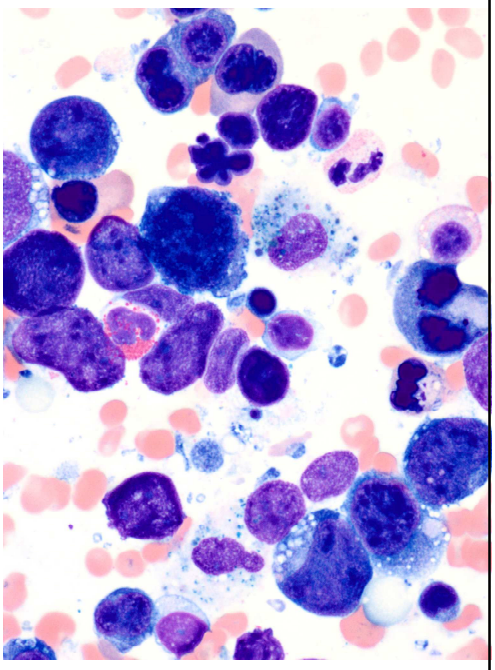
- A common occurrence in the workup of the cytopenic patient!
- Anemia of chronic inflammation
  - Often increased iron in marrow histiocytes
- Reactive causes which may or may not become evident later
  - Test of time: transient causes will eventually resolve
- Early MDS cases which are not well-developed enough for definitive diagnosis
  - Test of time: cytopenia is refractory or worsens
  - OK to hedge on the initial marrow in these situations: marrow can be repeated at a later date if cytopenias persist or worsen
- Clonal cytopenia of undetermined significance (CCUS)
  - Clinicians are now more aware of this entity as a pre-MDS and should follow the patient closely

Problems posed by specific scenarios

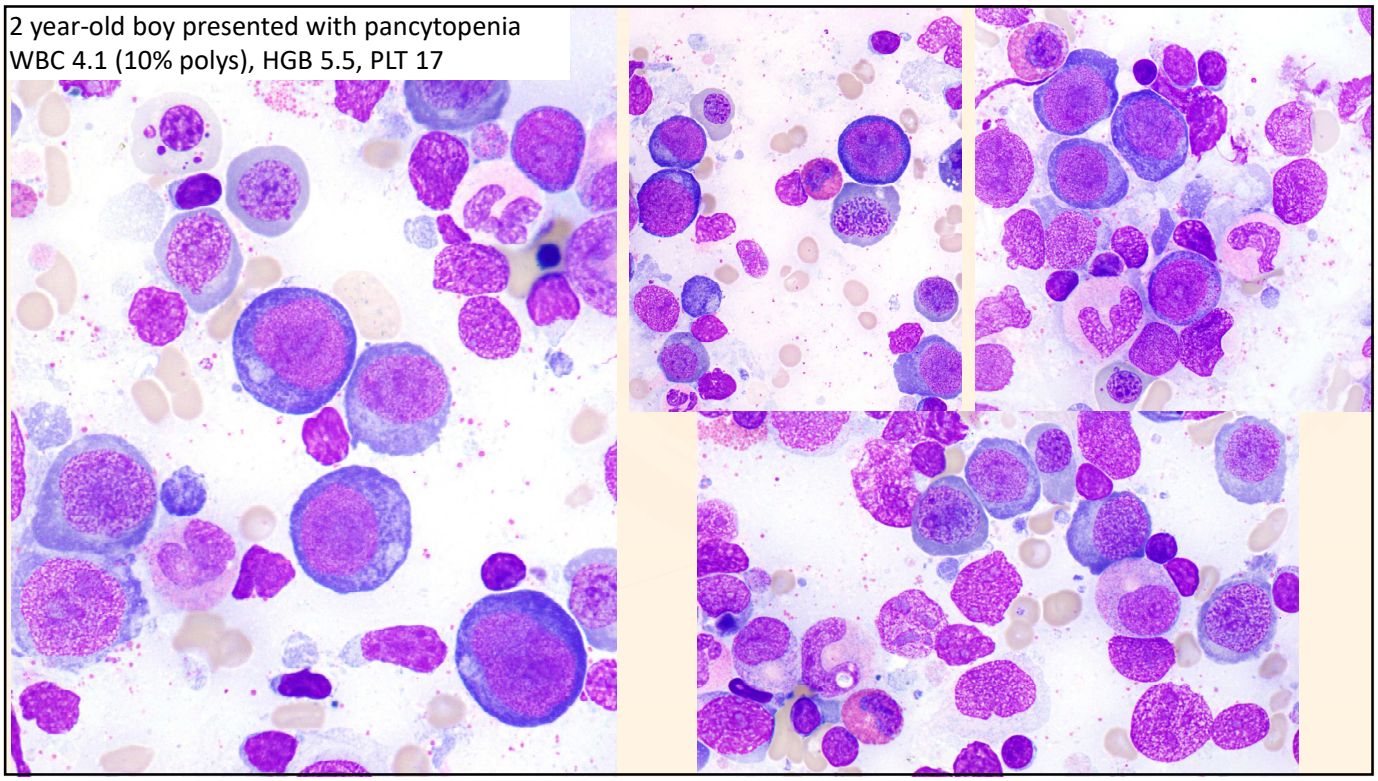
- Erythroid-predominant hypercellular marrow
  - Erythroid-rich MDS or erythroleukemia or florid reactive erythroid hyperplasia?
- Fibrotic marrow
  - MDS with fibrosis, myeloproliferative neoplasm, or reactive fibrosis?
- Hypoplastic marrow
  - Hypoplastic MDS or aplastic anemia?

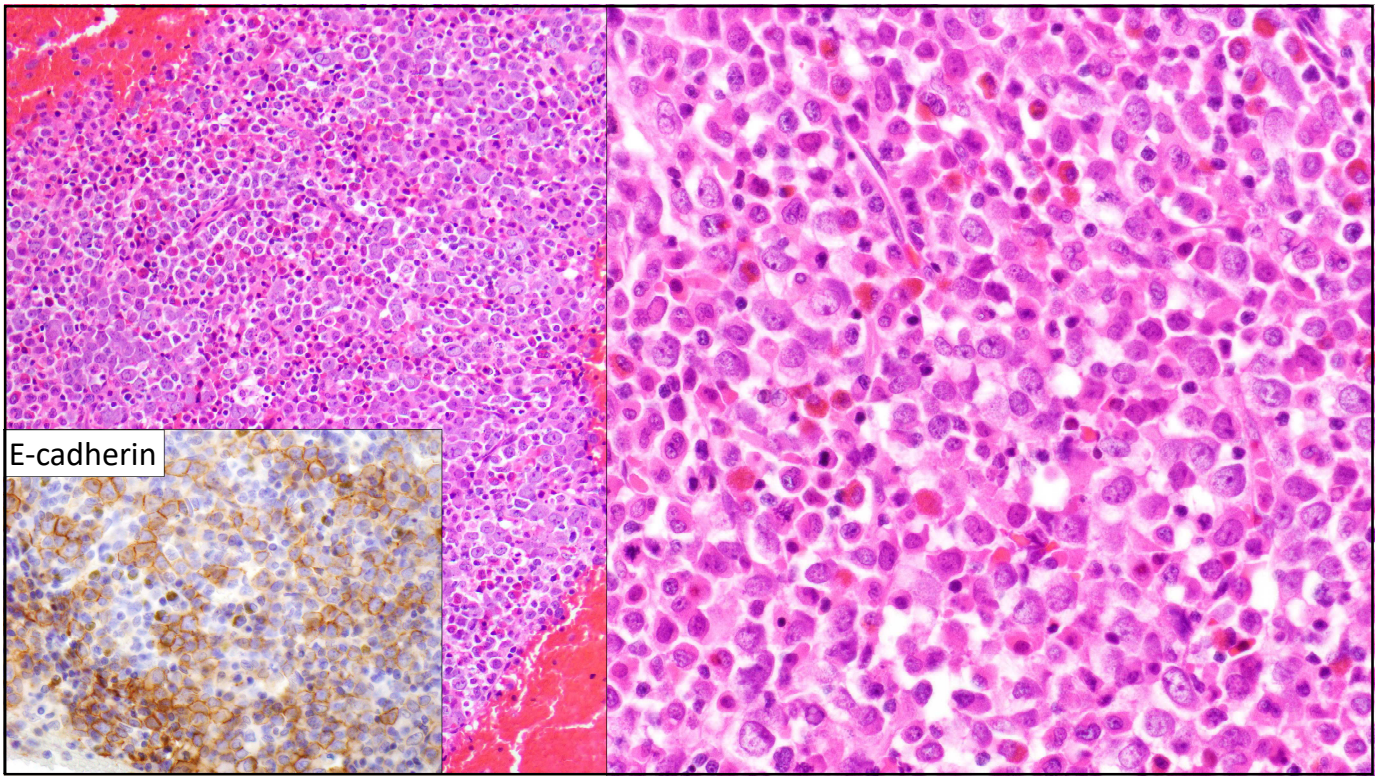
Challenges with erythroid predominant-bone marrow

- 15% of MDS cases have erythroid predominance (>50% erythroid elements)
- Blasts are always counted as a percentage of all cells, never non-erythroids
- MDS with erythroid predominance encompass two types of cases
  - MDS-EB (BM blasts ≥5%): Aggressive behavior, higher incidence of complex karyotype and *TP53* mutation
  - MDS-SLD and MDS-RS: Tend to exhibit indolent behavior
- Must exclude florid reactive erythroid proliferations and pure erythroid leukemia



Hasserjian RP Blood 2010;115:1985, Wang SA Mod Pathol 2016; 29:1221, Wang SA Mod Pathol 2008;21:1394, Bacher U Haematologica 96;1284


2 year-old boy presented with pancytopenia
WBC 4.1 (10% polys), HGB 5.5, PLT 17

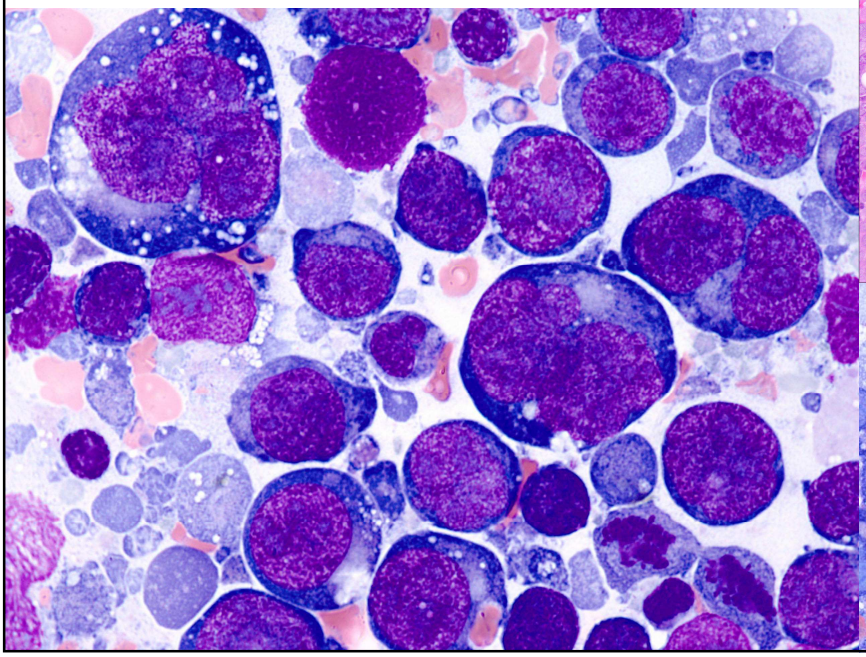

E-cadherin

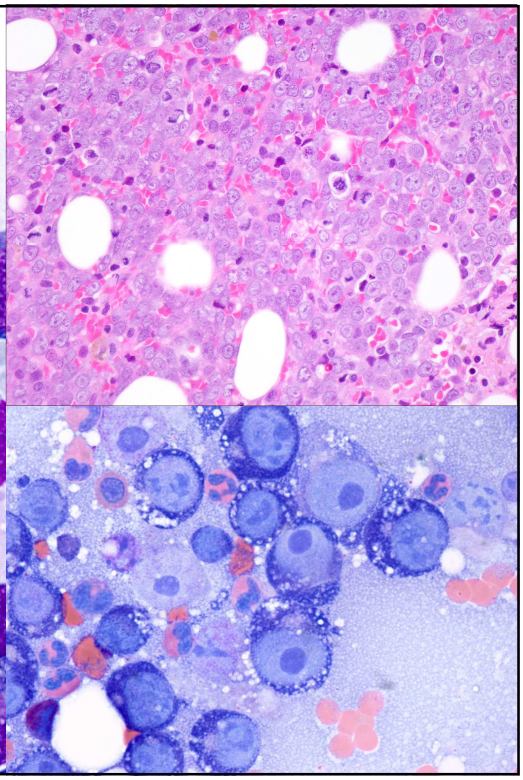
Clinical followup

- Found to be Vitamin B12 deficient
  - Methylmalonic acid level 10,700 nmol/L [normal 87-318 nmol/L]
- After Vitamin B12 supplementation, blood counts improved markedly
  - WBC 7.8, HGB 10.8, PLT 199 after 2 weeks

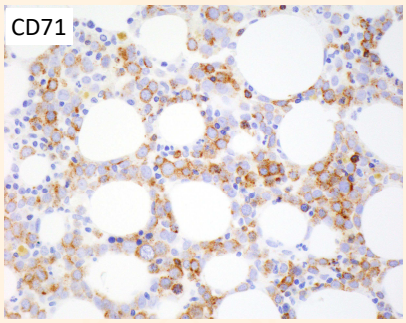
Diagnosis: Megaloblastic anemia

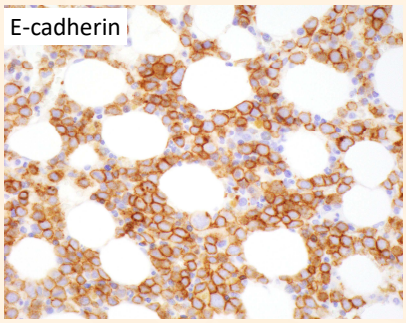
Pure erythroid leukemia

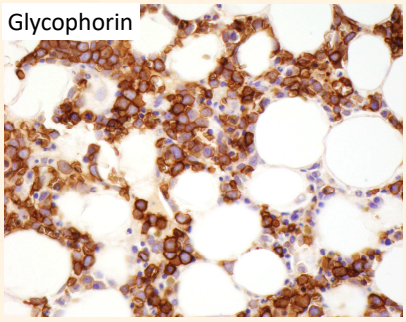


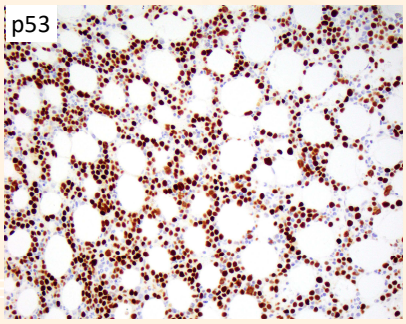


Immunoprofile and genetics of pure erythroid leukemia







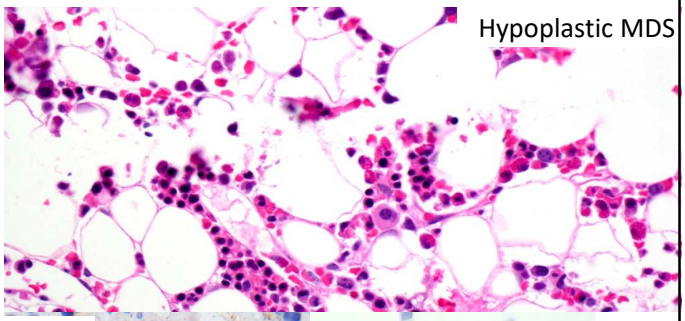


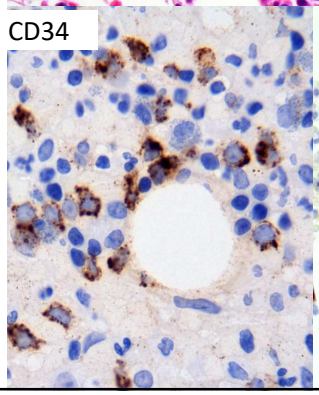
- Sheets of primitive erythroblasts with essential absence of maturing erythroids
  - Granulocytic maturation is intact
  - Current WHO criteria of >80% erythroids and >30% pronormoblasts may be too strict
- E-cadherin+, CD117+, CD71+, Glycophorin+/-
- Nearly all cases have complex karyotype and *TP53* mutations

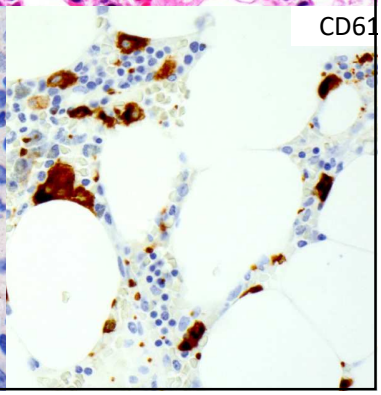
Liu et al. Mod Pathol 2011;24:375

Challenges with hypoplastic marrows

- Hypoplastic MDS (<30% cellular)
  - About 10% of cases
  - Differential diagnosis with aplastic anemia
  - CD34 and CD61 immunostains of biopsy can aid in diagnosis and prognosis
- Differential diagnosis
  - Aplastic anemia
  - Acute marrow injury

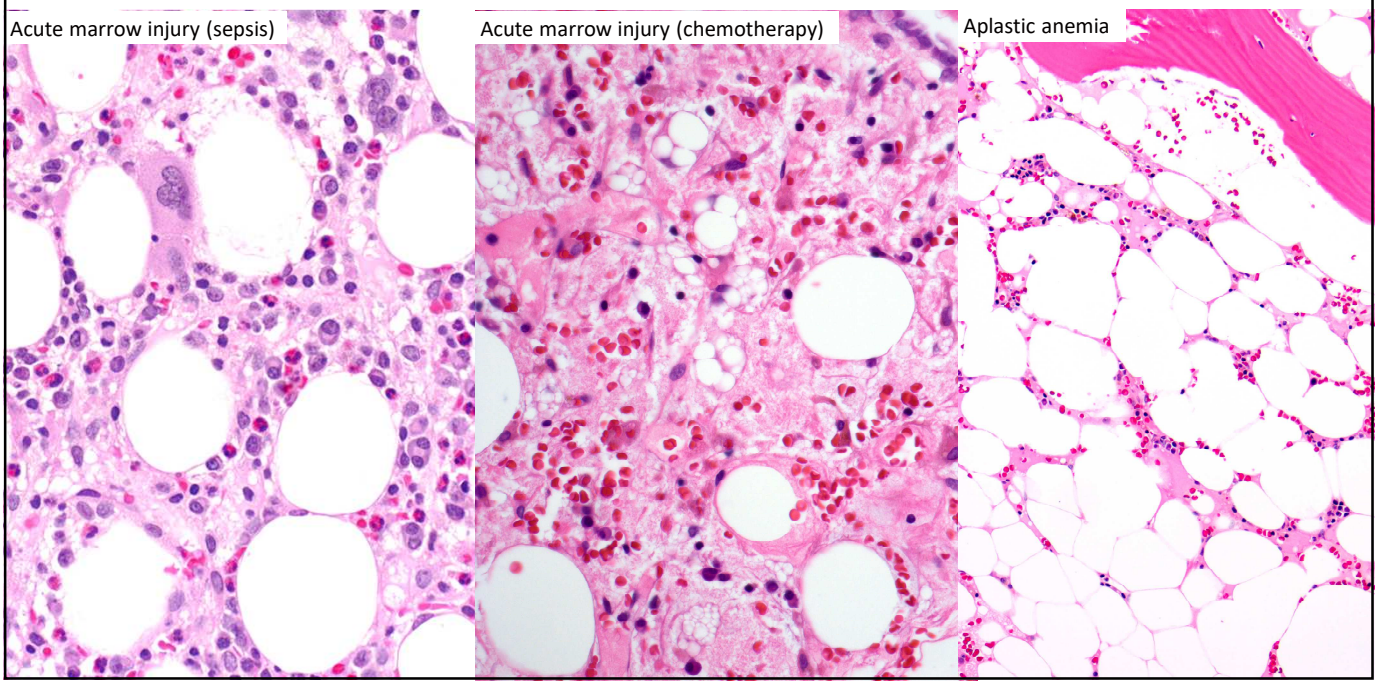






Yue G et al. Leuk Res 2008;32:553, Della Porta MG et al. Leukemia 2015;29:66

Non-neoplastic hypoplastic marrows



Hypoplastic MDS vs aplastic anemia

MDS

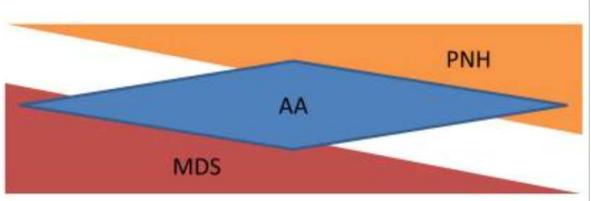
- Older (median 46-63)
- Blasts may be increased, aberrant antigen expression by flow cytometry
- Dysplasia is present, including micromegakaryocytes
- 40-48% have cytogenetic abnormalities

AA

- Younger (median 23-40)
- No increase in blasts, normal phenotype
- No dysplasia in residual hemopoietic elements, no ring sideroblasts
- 2-12% cytogenetically abnormal
  - 'Non-qualifying' abnormalities, e.g. +8

Lymphocytes and plasma cells may be increased in both
Both may have acquired somatic mutations (BCOR/BCORL, PIGA, DNMT3A, ASXL1 in AA)

Size of PNH Clone



Huang TC Leukemia 2008;22:544. Otawa M Leuk Res 2000;24:359. Calado RT. Semin Oncol 2011;38:667. Koh Y Leuk Res 2010;1344-1350. Yue G Leuk Res 2008;32:553. Orazi A and Bennett J Haematologica 2009:94:268, Yoshizato T NEJM 2015;373:35, Fatizzo B Leukemia 2021;35:3223

Hypoplastic MDS helpful features

Table 5. Calculation of the score for the differential diagnosis of myeloid disorders associated with reduced marrow cellularity

Variable	*Cohen's K-coefficient (inter-operator variability)*	*Score value*[a]
Cytological parameters		
Morphological erythroid dysplasia (according to morphological score)	0.81	1
Morphological granulocytic dysplasia (according to morphological score)	0.83	1
Histological parameters		
Hypolobulated, multinucleated megakaryocytes	0.82	1
CD34+ progenitor cells ⩾5%	0.91	2
Presence of CD34+ cell clusters	0.90	1
Molecular features		
Abnormal karyotype (excluding trisomy 8)	—	2

Abbreviations: MDS-Hypo, myelodysplastic syndromes with marrow hypocellularity. Most relevant histological features in patients affected with MDS-Hypo are reported in Supplementary Figure 4. [a]A score value ⩾3 is suggestive for a diagnosis of MDS.

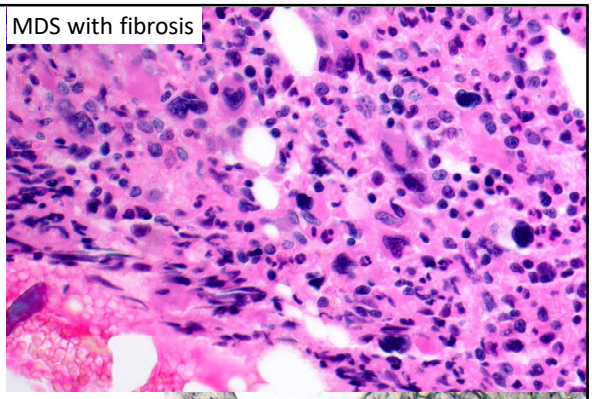
Della Porta MG et al. Leukemia 2015;29:66

Challenges with fibrotic marrow

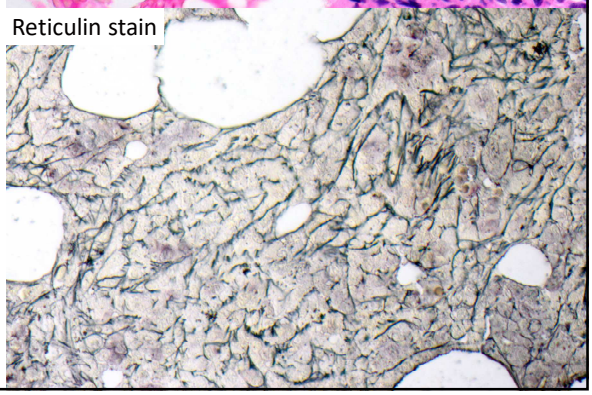
- Increased marrow reticulin fibrosis has a broad differential diagnosis
- Most causes of increased marrow reticulin (grade 2-3/3) are neoplastic (MPN and some MDS) but also consider non-neoplastic causes
  - Infections, especially HIV
  - Autoimmune disease
    - May present as cytopenia in a patient with no known autoimmune disease
- Grade 2-3 reticulin is a poor prognostic factor in MDS

Fu B Mod Pathol 2014;27:681, Della Porta MG JCO 2009;27:754

MDS with fibrosis

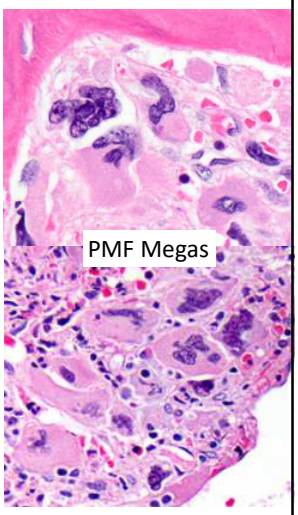


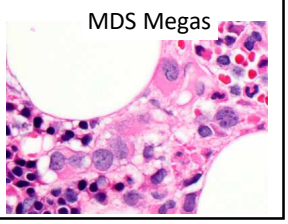
Reticulin stain



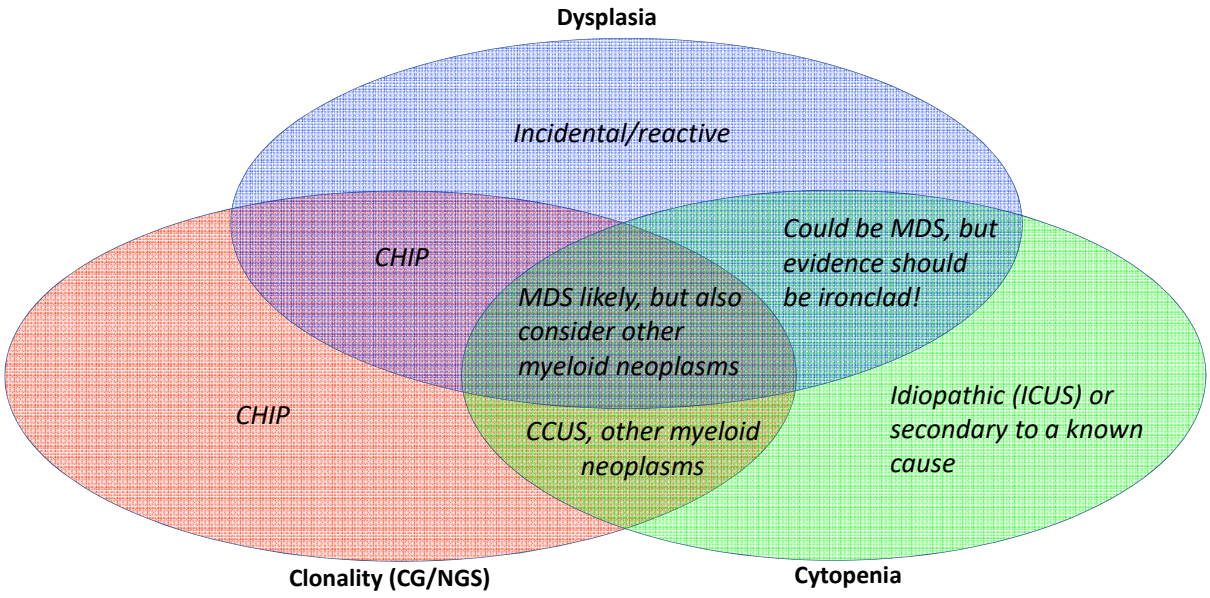
Differential diagnosis of the fibrotic marrow

Disease	Megakaryocyte appearance	Other features
Primary myelofibrosis	Enlarged, hyperchromatic, 'bulbous' nuclei	Splenomegaly, myeloid hyperplasia
Fibrotic phase of PCV or ET	Variably enlarged, often hyperchromatic nuclei	History of prior PCV or ET with progressive marrow fibrosis
MDS with fibrosis	Small, hypolobated nuclei	Morphologic dysplasia, often increased blasts
Hairy cell leukemia	Normal	Clonal B-cells with typical immunophenotype, monocytopenia
HIV	Normal or small, frequent naked nuclei	Plasmacytosis, lymphoid aggregates
Autoimmune myelofibrosis	Normal	Lymphoid aggregates, history of autoimmune disease





Leverage *all* information to make the correct diagnosis when considering an MDS diagnosis



Conclusions

- A wide variety of reactive and neoplastic conditions may present with cytopenia (and sometimes dysplasia) potentially mimicking MDS
- Accurate diagnosis requires integrating information from multiple modalities and weighing the ‘strength of evidence’
- Always keep the clinical context in mind
  - Try to evaluate the tempo of cytopenias and rigorously seek secondary ‘excuses’ for cytopenia
- Genetic information provides important information and can aid in distinguishing MDS from reactive mimics