Neutropenia & Inherited Bone Marrow Failure Syndromes

Dr. Laura Wheaton

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Neutropenia

Worried or not worried?

Case 1

You see a female term neonate for her first well baby check on day 1 of life.

- Her mother is a healthy 30-year-old, gravida 1, para 1, whose first child was born without specific problems.
- Birth weight was 2.81 kg, and she was born via spontaneous vaginal delivery.
- The baby appears healthy and physical examination reveals no abnormalities.
- On routine lab exams her CBC showed: WBC 7.2 x 10^9 cells/L, and ANC 0.43 x 10^9 cells/L. Other hematological and biochemical profiles were within normal limits.

Case 2

A 2 year old girl presents to the emergency department because of “odd streaky and very stinky” stools and poor growth.

- On history it was revealed she has had frequent episodes of purulent otitis media and pneumonias.
- Since the stools started around one year of age, has not been growing as expected.
- There is no significant family history.
- Physical exam shows height and weight less than the third percentile. The remainder of the exam was unremarkable.
- Laboratory studies show hemoglobin 113 g/L, WBC 3.0 x 10^9 cells/L, ANC 0.6 x 10^9 cells/L and platelets of 152 x 10^9 cells/L.

Case 3

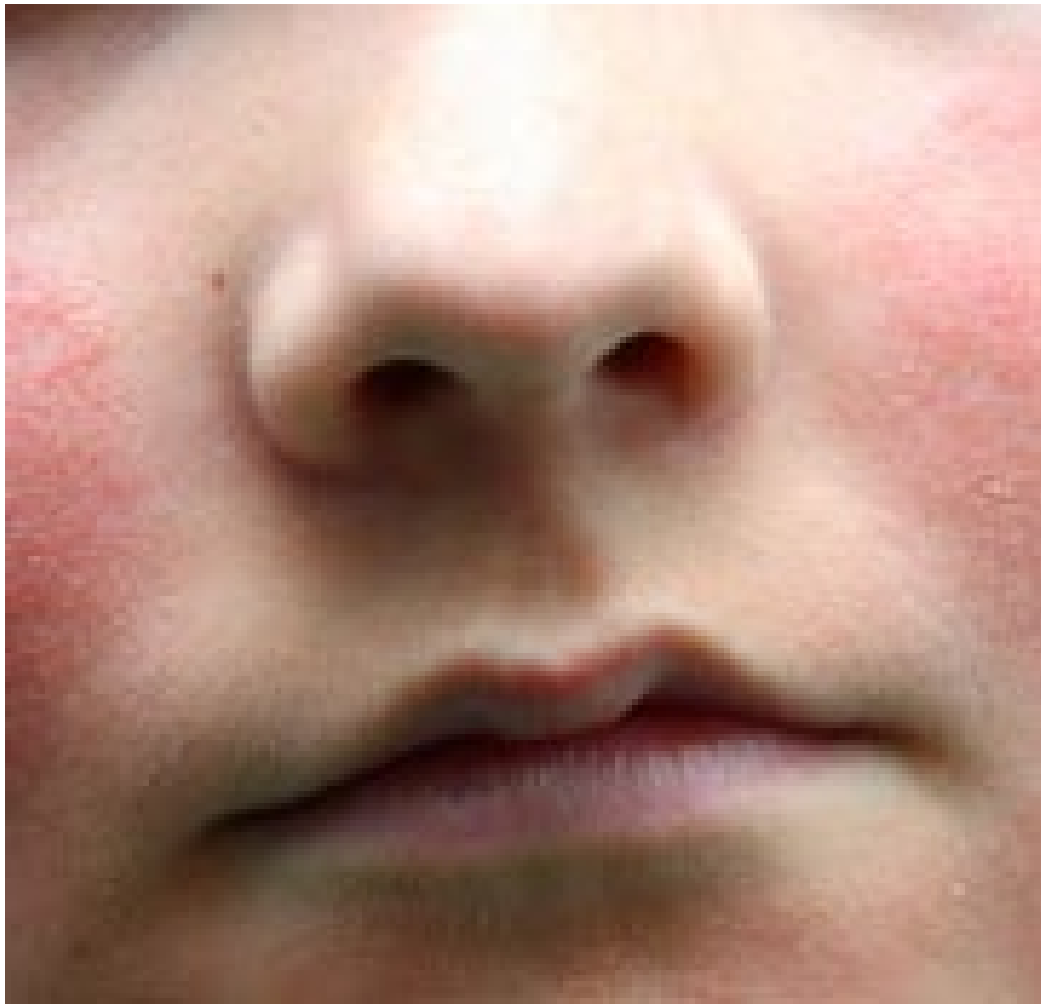
A 14 year old girl presents to the emergency department with:

- a one week history of increasing bilateral leg pain.
- three days of fever, pallor and anorexia.
- On physical examination she has diffuse lymphadenopathy and splenomegaly.
- Her CBC shows a WBC count of 4.0 x 10^9 cells/L, hemoglobin 56 g/L, platelets 20 x 10^9 cells/L and ANC 0.1 x 10^9 cells/L.

Case 4

A 3 year old boy presents to the pediatrician's office with:

- a history of fever three days ago, now resolved.
- Ongoing upper respiratory tract infectious symptoms, including rhinorrhea and cough.
- He also has a red rash (see picture) on his cheeks.
- A CBC shows a WBC count of 9.0 x 10^9 cells/L, with an ANC of 0.9 x 10^9 cells/L. The remainder of the blood work was unremarkable.



Case 5

A 4 month old baby boy presents to his general pediatrician's office with:

- a two day history of fever, T_{max} 39.4 axillary.
- Ulcerations on his lips and buccal mucosa which appeared within the last 24 hours.
- His mother mentions that he has been hospitalized twice in the past 2 months with fevers and diagnosed bacterial infections. Both times his neutrophils were less than 0.5 x 10^9 cells/L.
- A CBC shows a WBC count of 2.9 x 10^9 cells/L and ANC of 0.3 x 10^9 cells/L.

Case 6

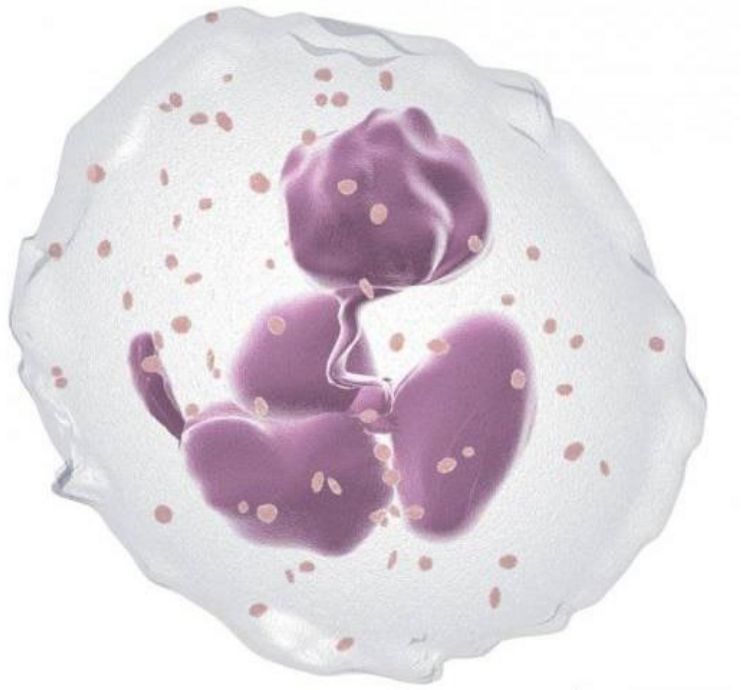
A 17-year old boy of African heritage visits his pediatrician for a routine yearly assessment prior to his departure for university.

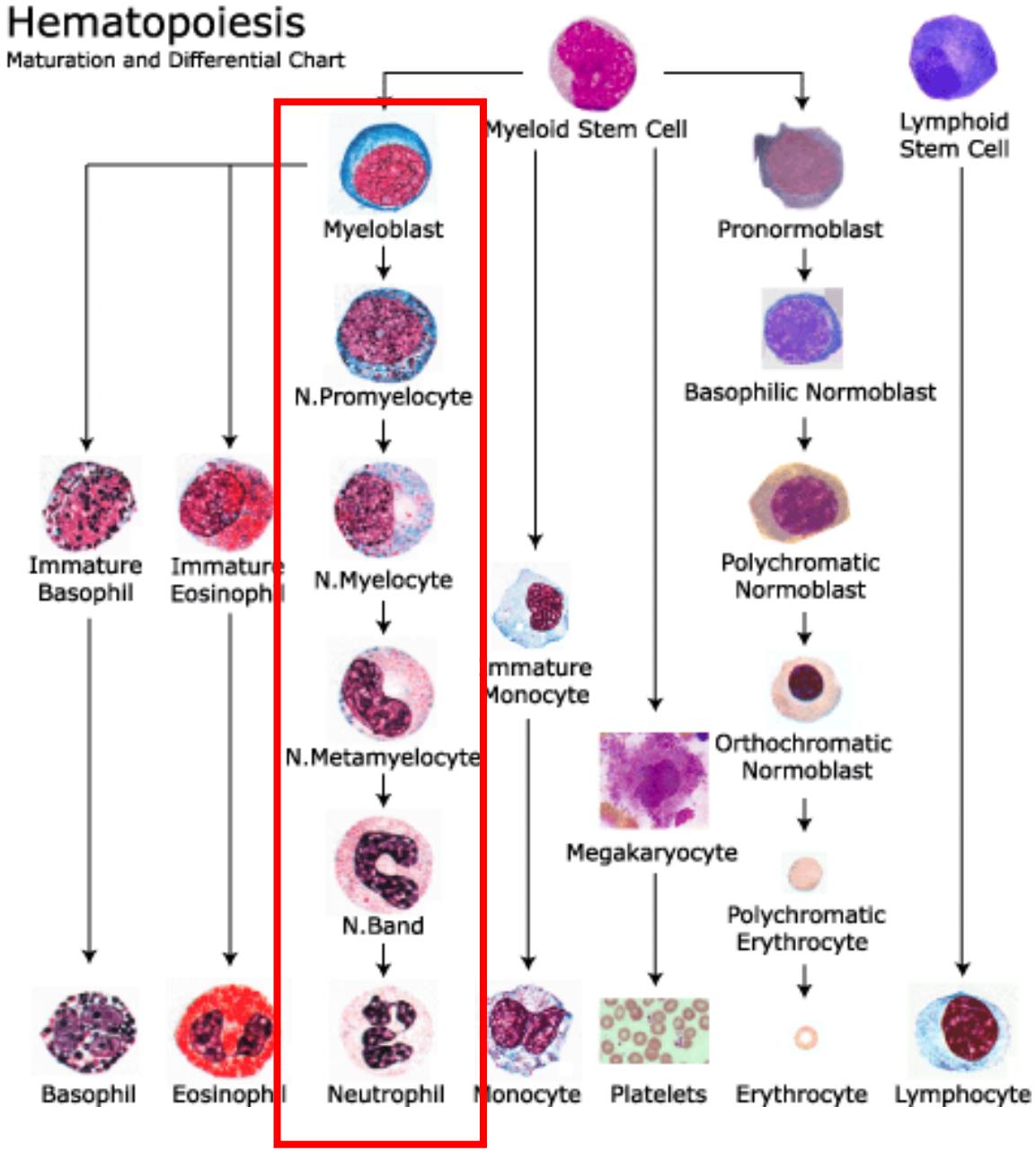
- His blood work returns showing a WBC count of 4.8 x 10^9 cells/L, hemoglobin of 180 g/L, platelets of 300 x 10^9 cells/L. Differential shows an ANC of 1.1 x 10^9 cells/L.
- He has previously been well. Physical examination is normal.

The Basics

Neutrophils

- Granulocyte

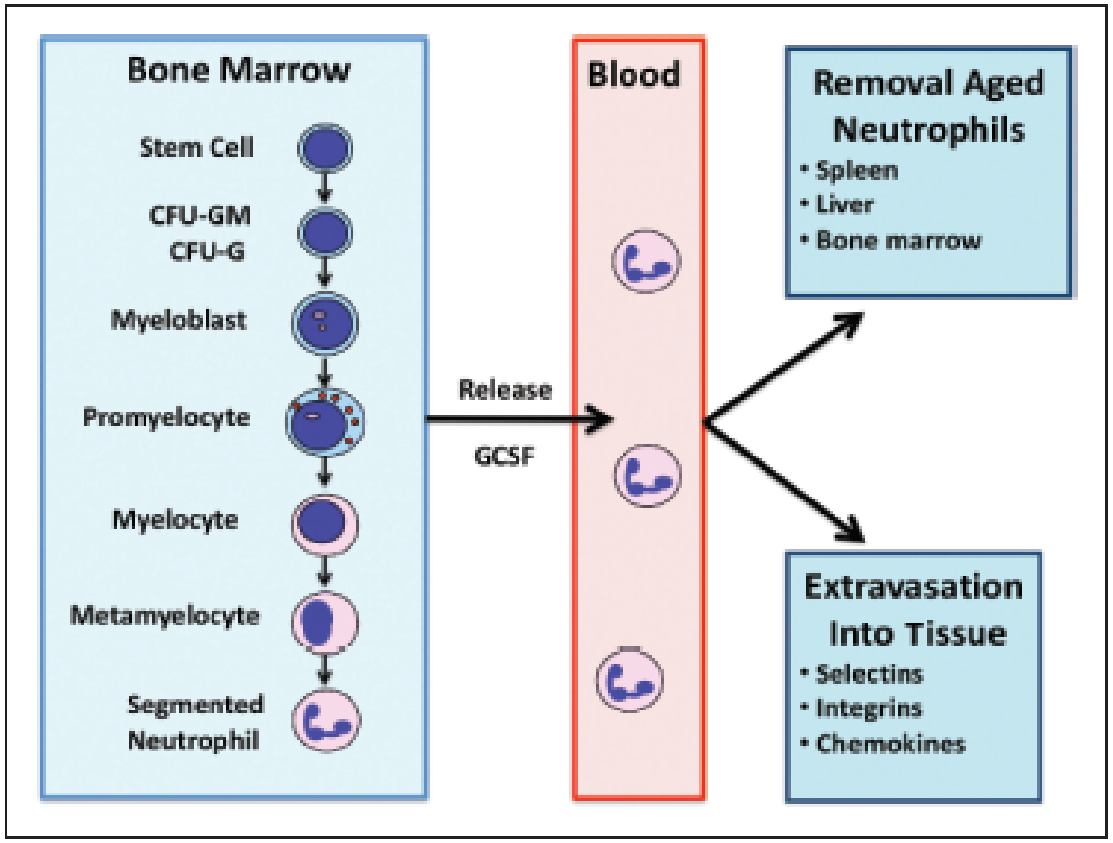




Taken from http://apbrwww5.apsu.edu/

Neutrophils

- Found in bone marrow (maturation, mitosis, storage), blood stream (circulating or marginated) and tissues.
- Mediators of innate immune function.
- Function dependent on adequate numbers of normally functioning neutrophils.



Neutrophil Counts by Age

Table 1. **Normal Blood Leukocyte Counts***

	Total Leukocytes		Neutrophils			Lymphocytes			Monocytes		Eosinophils	
Age	Mean	(Range)	Mean	(Range)	%	Mean	(Range)	%	Mean	%	Mean	%
Birth	18.1	(9.0 to 30.0	11.0	(6.0 to 26.0)	61	5.5	(2.0 to 11.0)	31	1.1	6	0.4	2
12 h	22.8	(13.0 to 38.0)	15.5	(6.0 to 28.0)	68	5.5	(2.0 to 11.0)	24	1.2	5	0.5	2
24 h	18.9	(9.4 to 34.0	11.5	(5.0 to 21.0)	61	5.8	(2.0 to 11.5)	31	1.1	6	0.5	2
1 wk	12.2	(5.0 to 21.0	5.5	(1.5 to 10.0)	45	5.0	(2.0 to 17.0)	41	1.1	9	0.5	4
2 wk	11.4	(5.0 to 20.0	4.5	(1.0 to 9.5)	40	5.5	(2.0 to 17.0)	48	1.0	9	0.4	3
1 mo	10.8	(5.0 to 19.5	3.8	(1.0 to 9.0)	35	6.0	(2.5 to 16.5)	56	0.7	7	0.3	3
6 mo	11.9	(6.0 to 17.5	3.8	(1.0 to 8.5)	32	7.3	(4.0 to 13.5)	61	0.6	5	0.3	3
1 y	11.4	(6.0 to 17.5	3.5	(1.5 to 8.5)	31	7.0	(4.0 to 10.5)	61	0.6	5	0.3	3
2 y	10.6	(6.0 to 17.0	3.5	(1.5 to 8.5)	33	6.3	(3.0 to 9.5)	59	0.5	5	0.3	3
4 y	9.1	(5.5 to 15.5	3.8	(1.5 to 8.5)	42	4.5	(2.0 to 8.0)	50	0.5	5	0.3	3
6 y	8.5	(5.0 to 14.5	4.3	(1.5 to 8.0)	51	3.5	(1.5 to 7.0)	42	0.4	5	0.2	3
8 y	8.3	(4.5 to 13.5	4.4	(1.5 to 8.0)	53	3.3	(1.5 to 6.8)	39	0.4	4	0.2	2
10 y	8.1	(4.5 to 13.5	4.4	(1.8 to 8.0)	54	3.1	(1.5 to 6.5)	38	0.4	4	0.2	2
16 y	7.8	(4.5 to 13.0	4.4	(1.8 to 8.0)	57	2.8	(1.2 to 5.2)	35	0.4	5	0.2	3
21 y	7.4	(4.5 to 11.0	4.4	(1.8 to 7.7)	59	2.5	(1.0 to 4.8)	34	0.3	4	0.2	3

*Numbers of leukocytes are in thousands/mcL ($\times 10^9$/L), ranges are estimates of 95% confidence limits, and percentages refer to differential counts. Neutrophils include band cells at all ages and a small number of metamyelocytes and myelocytes in the first few postnatal days.
From Dallman PR. Blood and blood-forming tissues. In: Rudolph AM, ed. *Rudolph's Pediatrics*. 16th ed. New York, NY: Appleton-Century-Crofts; 1977:1178, with permission.

Segel GB, Halterman S. Ped in Rev. 2008;29:12-24.

Neutropenia Definition

Neutropenia < 1.5 x10^9 cells/L

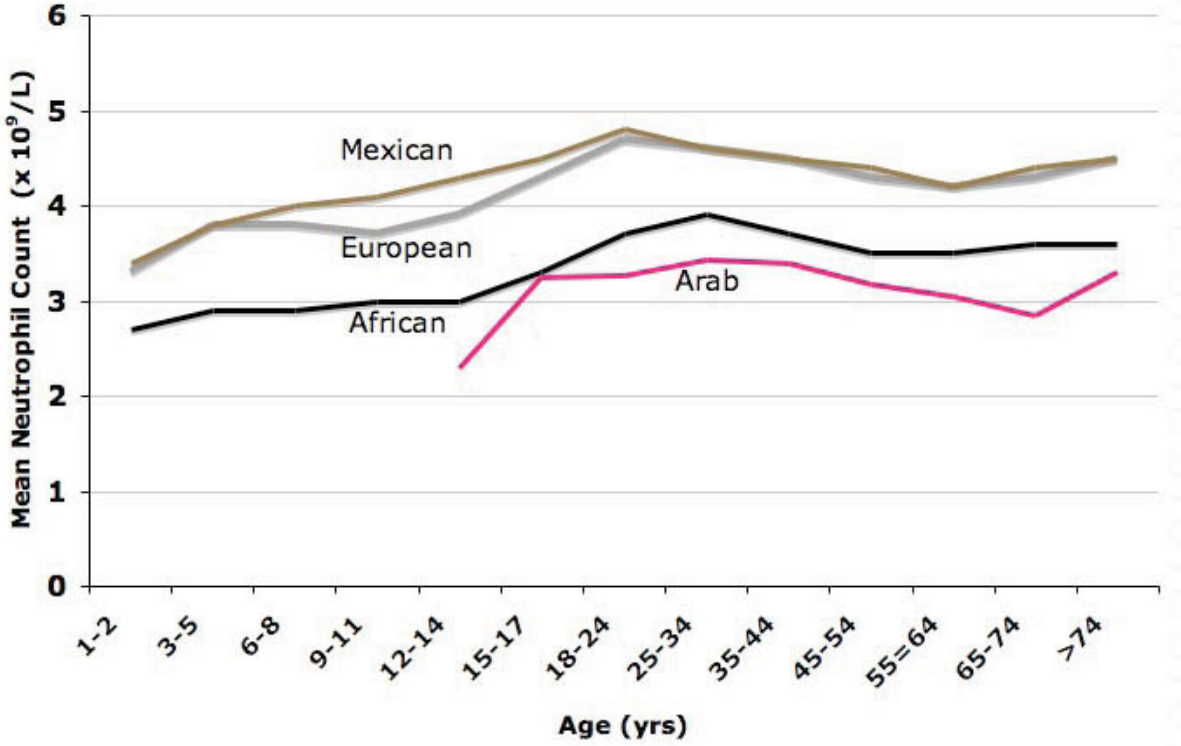
Severe Neutropenia < 0.5 x 10^9 cells/L

Moderate Neutropenia 0.5 – 1.0 x 10^9 cells/L

Mild Neutropenia 1.0 – 1.5 x 10^9 cells/L

Ethnic variation in Neutrophil counts

- Normal neutrophil counts will vary with ethnicity.
- Includes individuals of African and Middle Eastern descent.
- An ANC as low as 0.8 x 10^9 cells/L may be considered normal.
- Despite the neutropenia there is no increased risk of infection.

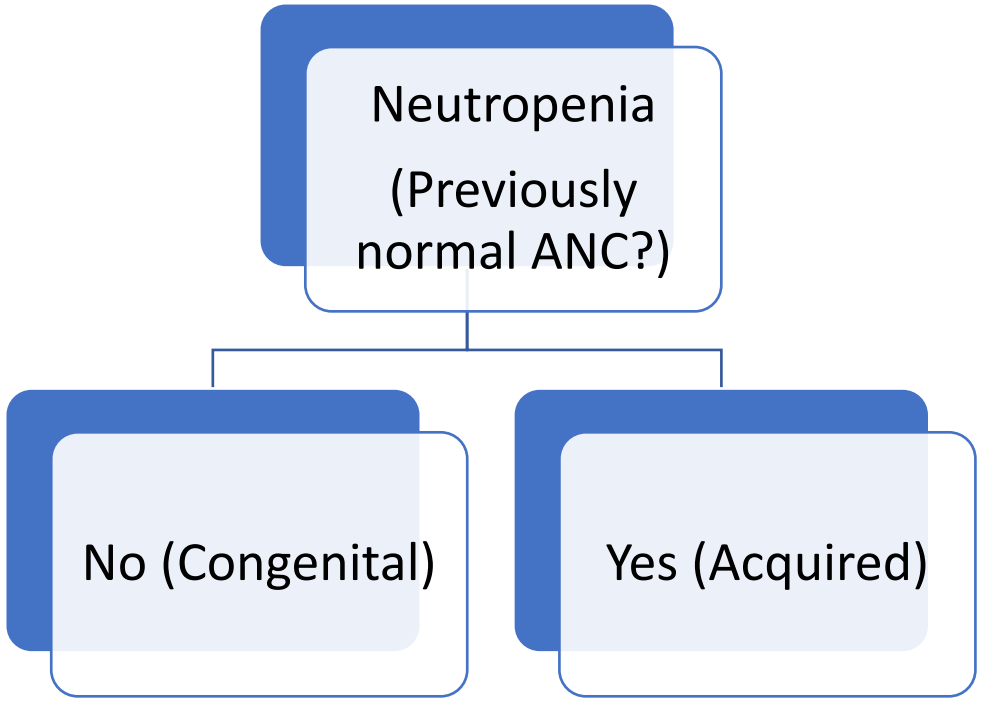


Why do we worry about neutropenia?

- Severity and duration = susceptibility to develop bacterial infection.
- Infection is usually due to endogenous flora (mouth, GI, skin).
- Could be a sign of a serious underlying condition.

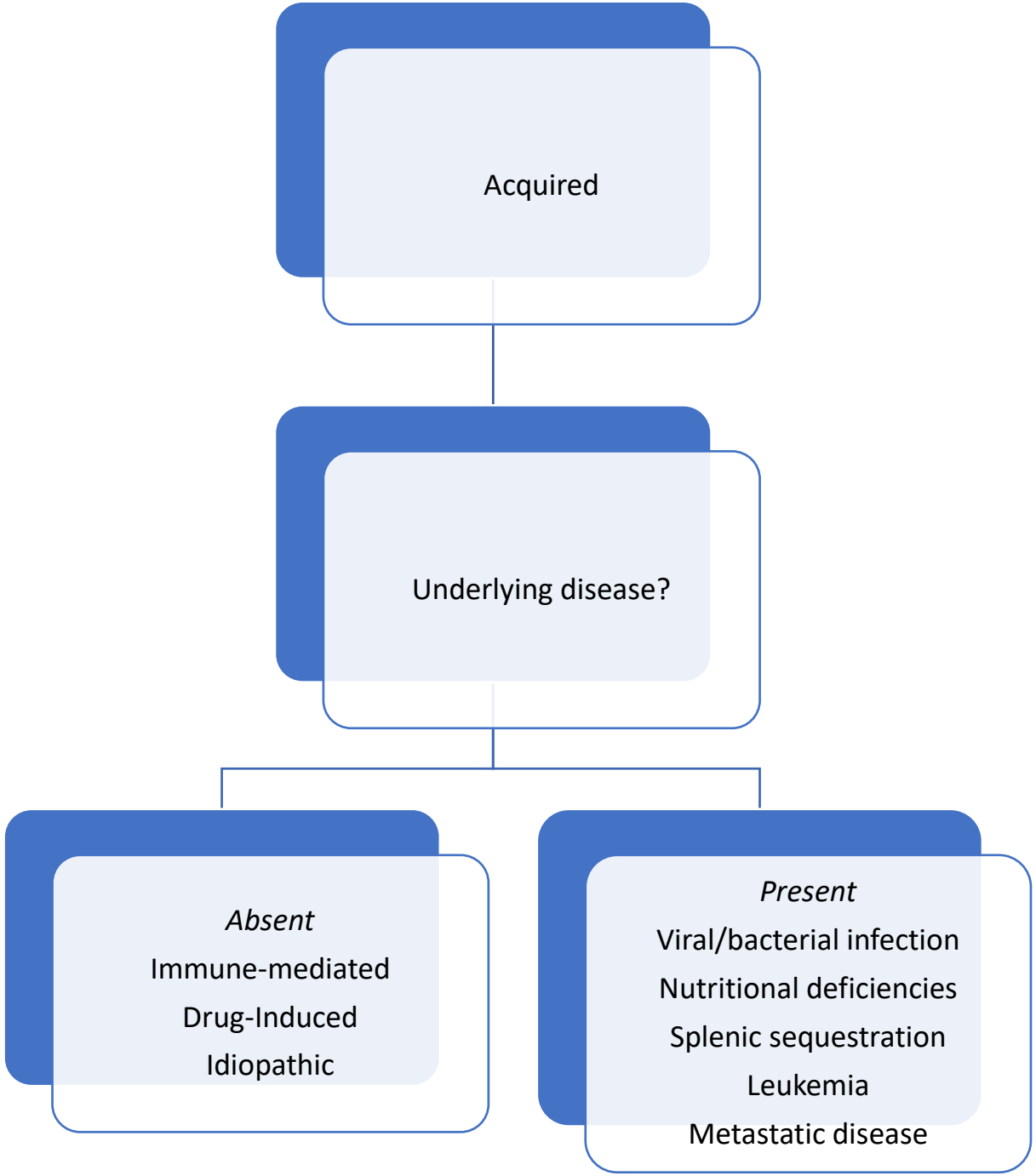
Neutropenia	ANC	Infection Risk	Infection Types
Mild	1.0-1.5 x 10^9 cells/L	Mild	-
Moderate	0.5-1.0 x 10^9 cells/L	Moderate	Stomatitis, gingivitis, cellulitis
Severe	< 0.5 x 10^9 cells/L	High	Perirectal abscess, LRTI, sepsis

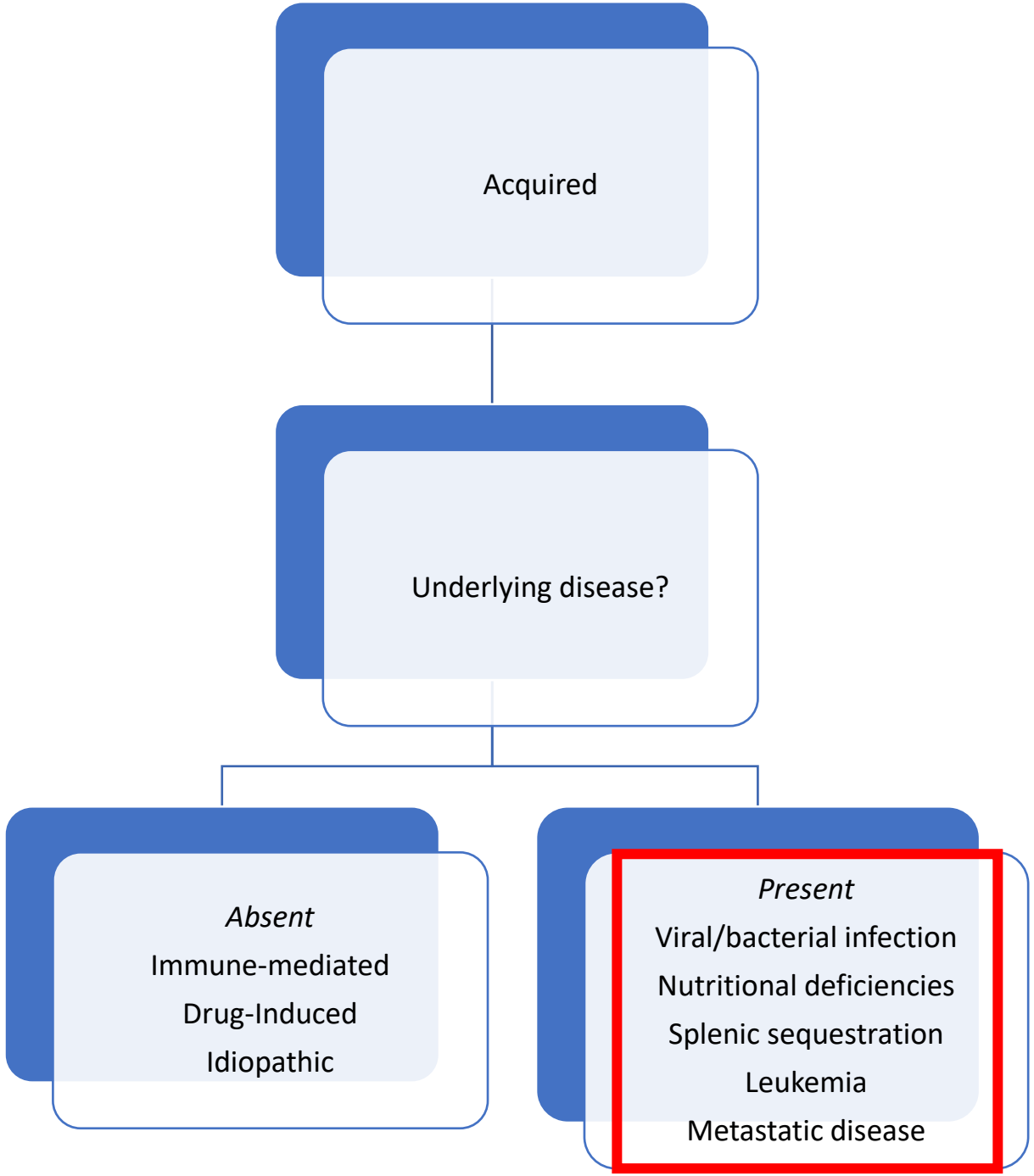
Causes of Neutropenia



Congenital	- **Intrinsically mediated + persistent** - **Rare and get severe infections**
Acquired	- Extrinsically mediated + transient - Infection, meds, immune disorders

Acquired Neutropenia


Acquired
Underlying disease?
Absent
Immune-mediated
Drug-Induced
Idiopathic
Present
Viral/bacterial infection
Nutritional deficiencies
Splenic sequestration
Leukemia
Metastatic disease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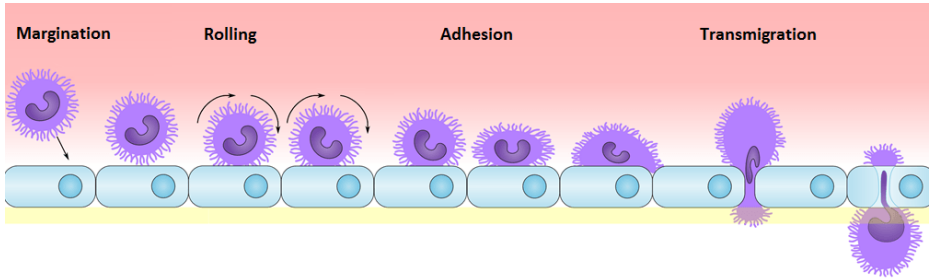
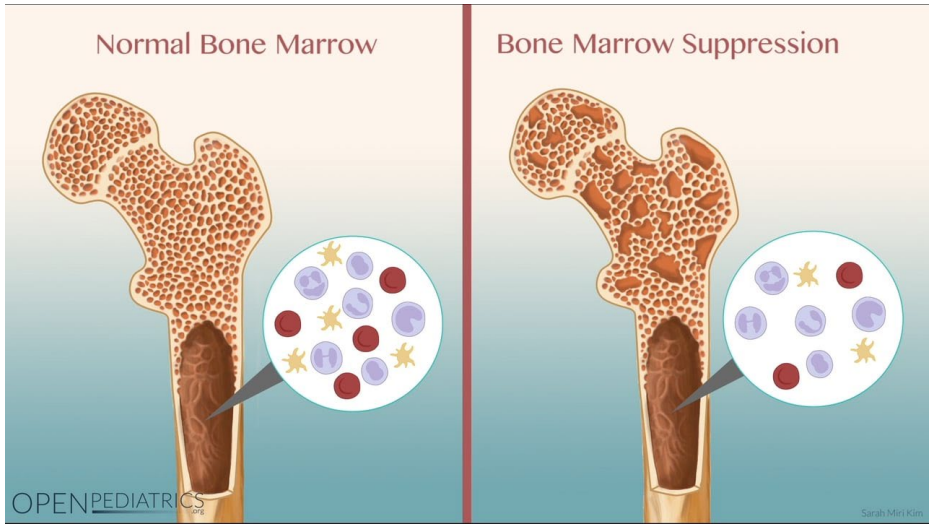
Infection-Associated Neutropenia

- Usually viral or bacterial infections.
- Rarely parasitic or fungal infection.
- Causative mechanisms:
  - Increased neutrophil margination
  - Direct pathogen-mediated destruction
  - Development of anti-neutrophil antibodies
  - Increased complement activation
  - Transient bone marrow suppression





Viral Induced Neutropenia

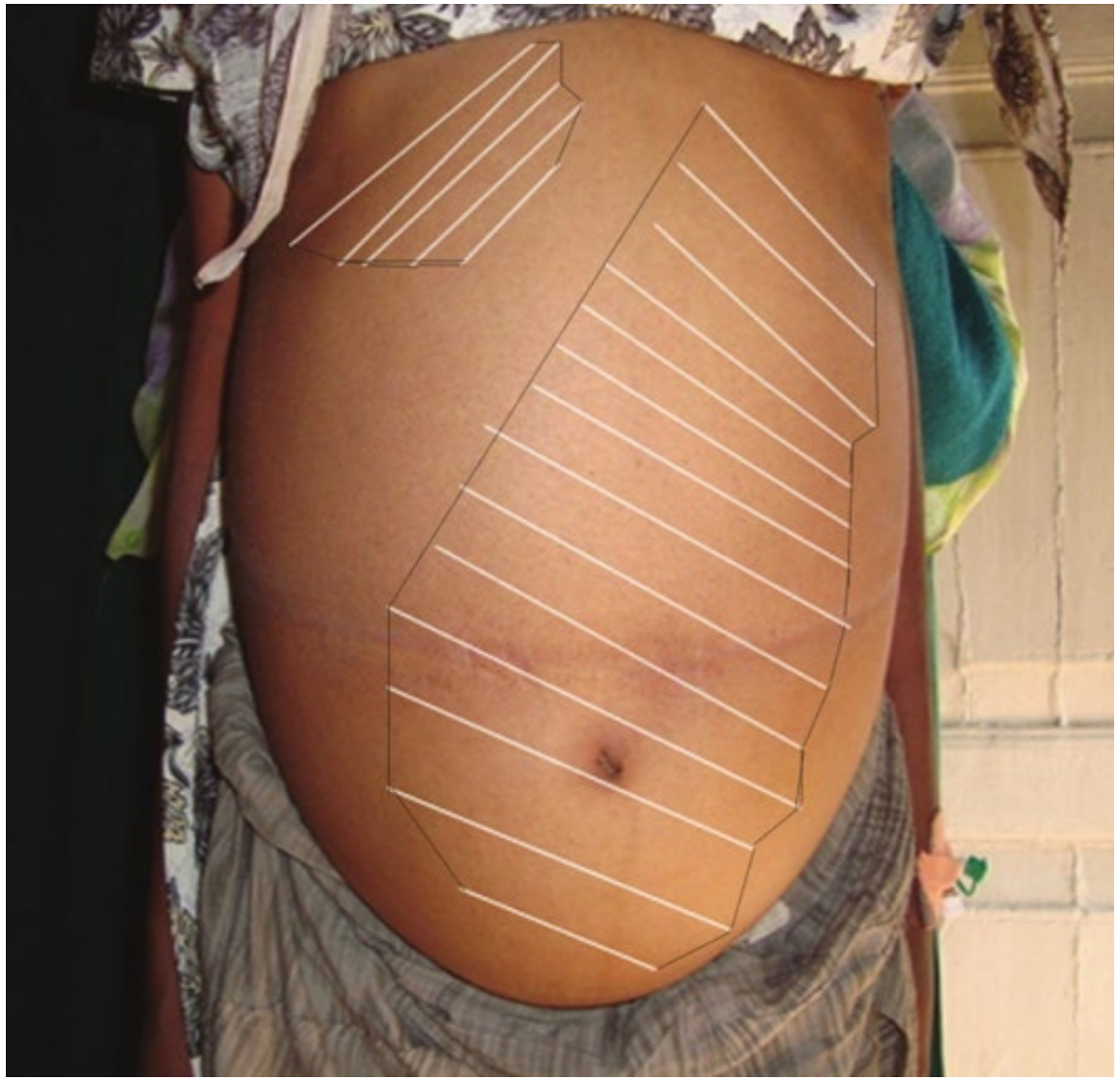
- **Most common cause of acquired neutropenia.**
- Multiple causative agents:
  - CMV, EBV, hepatitis A and B, HIV, influenza, measles, RSV, parvovirus B19, rubella and varicella.
- Develops during the first 24-48 hours of illness and may persist for 3-6 days.
  - Usually transient and not associated with bacteria superinfection.
- Management
  - Most commonly self-limited and does not require treatment.

Sepsis-Associated Neutropenia

- More serious cause of neutropenia.
  - Often associated with other cytopenias
- Multiple causative organisms:
  - *Brucella, Shigella, Tularemia, Salmonella* and Tuberculosis infections are often associated with neutropenia.
- Common with neonatal sepsis.
- Management:
  - Effective treatment of underlying infection.
  - Use of G-CSF is controversial.

Other Causes of Acquired Neutropenia

- Nutritional Deficiencies
  - Ineffective granulopoiesis is associated with severe copper, iron, vitamin B12, or folic acid deficiencies.
  - Does not cause isolated neutropenia.
- Reticuloendothelial sequestration
  - Splenomegaly from any cause can lead to sequestration and neutropenia.
  - Usually requires no treatment, but splenectomy may be indicated in severe cases.



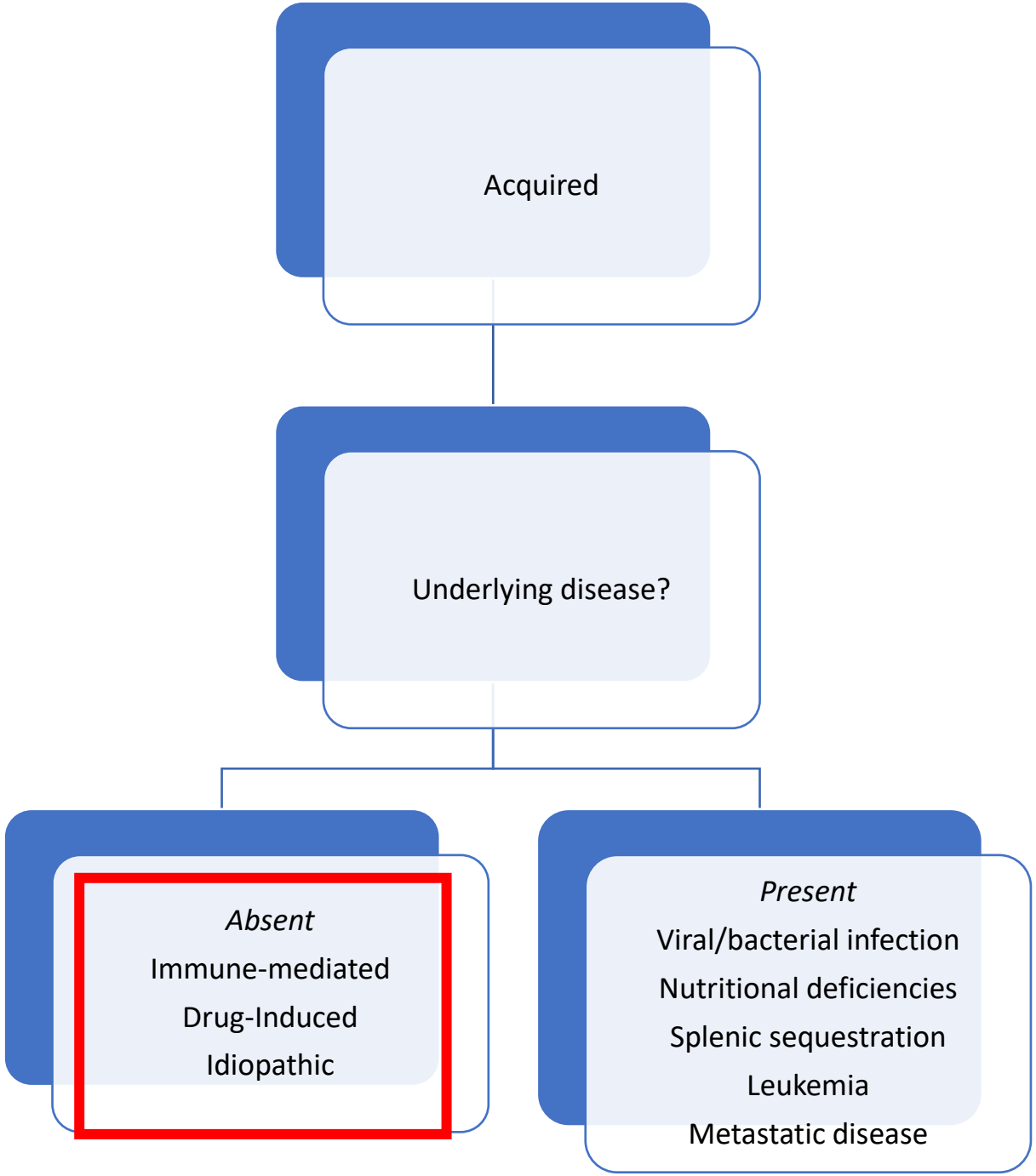
Bone Marrow Infiltration

Malignancy

- Leukemia and other metastasized malignancies.
- Fever and constitutional symptoms are common.
- Lymphadenopathy and hepatosplenomegaly may be seen on examination.

Metabolic Diseases

- GSD-IB, amino acidopathies, organic acidurias, mitochondrial disorders.
- May be associated with decreased neutrophil numbers and/or abnormal function.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Autoimmune Neutropenia (AIN)

- May be isolated or in association with autoimmune diseases, infection, drugs or malignancy.
- Associated with variable degrees of neutropenia.
  - Degree of neutropenia does not correlate with infection risk.
  - Associated with minor infections (i.e. otitis, skin, upper respiratory).
- Investigations:
  - Anti-neutrophil antibodies can be measured.
  - Bone marrow shows normal or hypercellular myeloid precursors.
  - Evaluation for immunodeficiency should be done.

Chronic Benign Neutropenia of Childhood

- May represent isolated auto-immune neutropenia in most cases.
- Predominantly in children younger than 3 years.
  - Median age at diagnosis is 8-11 months.
  - Clinically well.
- Management:
  - Oral hygiene
  - Remits spontaneously in most cases (median time 20 months).
  - G-CSF for prolonged severe neutropenia with recurrent infections.

Neonatal Alloimmune Neutropenia

- Maternal anti-neutrophil antibodies to paternal antigens
- 0.2% of pregnancies
- Delayed separation of cord, fever, mild skin infections, pneumonia within first 2 weeks of life
- CBC/diff on Mom- if normal ANC, send maternal neutrophil antigen typing and neutrophil antibody screen
- Self-resolves after 2-3 months (can take longer)
- Supportive care with prompt assessment and treatment with fever
- Consider use of G-CSF (minimal evidence)

Other Immune-mediated Neutropenias

- Neutropenia associated with immune dysregulation
  - i.e. hypogammaglobulinemia, dysgammaglobulinemia.
  - May also be associated with rheumatologic conditions (SLE, JIA) but this is less common in children.
  - May be due to the formation of autoantibodies.

Drug-Induced Neutropenia

- Caused by idiosyncratic reaction to offending medication.
- Severe infection is uncommon.
- Multiple medications have been implicated.
- Management:
  - Benefit of routine CBC monitoring with offending drugs is unclear.
  - Discontinuation of offending medication.
  - G-CSF for prolonged severe neutropenia in adult studies.

Drug-Induced Neutropenia

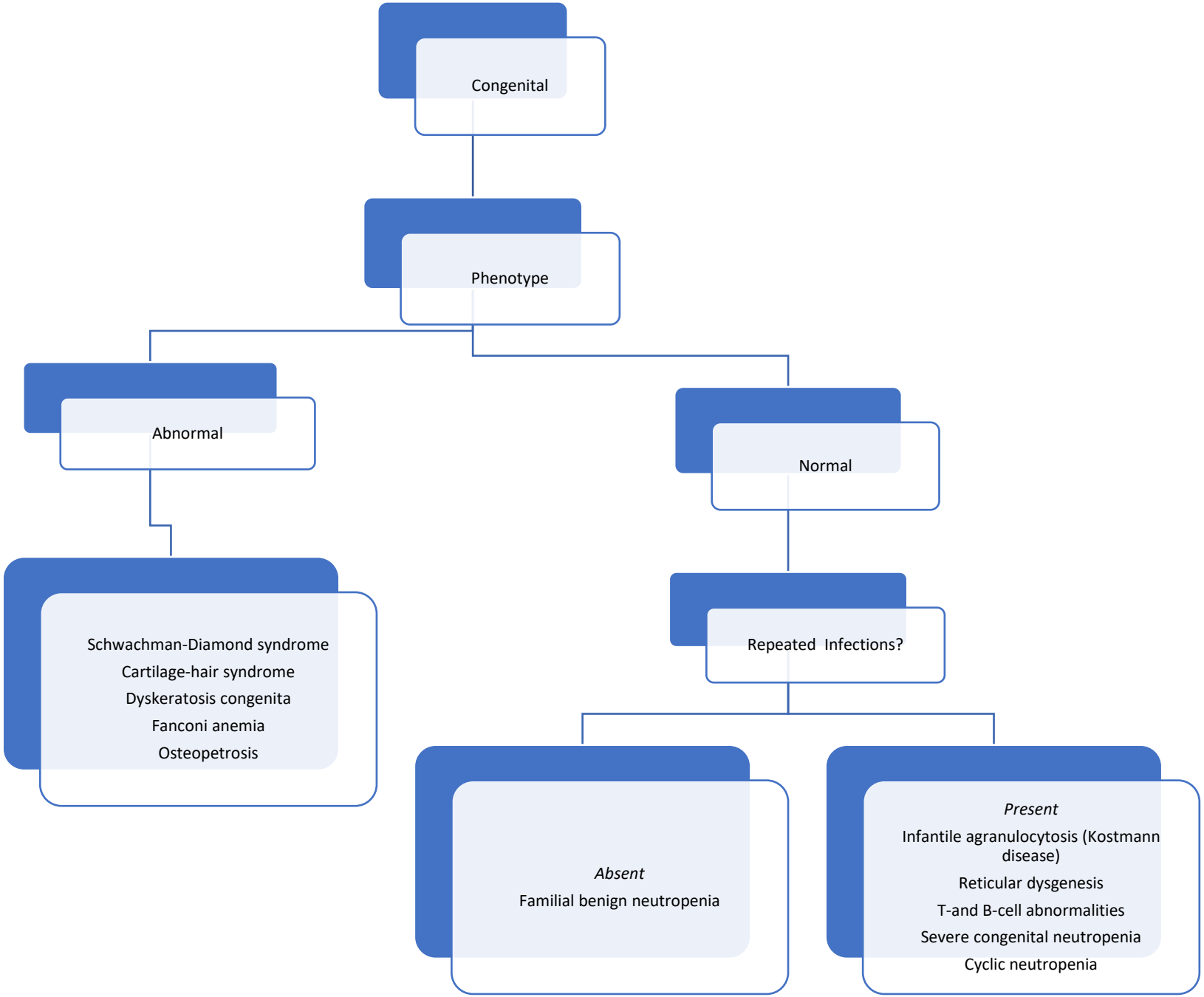
Table 5. **Partial List of Drugs Associated With Idiosyncratic Neutropenia**

Drug	Possible Mechanism		
	Direct Suppression	Metabolite Suppression	Immune Destruction
Analgesics/Anti-inflammatory Agents			
Aminopyrine			X
Ibuprofen			X
Indomethacin	X		
Phenylbutazone	X		
Antibiotics			
Chloramphenicol	X		
Penicillins	X		X
Sulfonamides	X		
Anticonvulsants			
Phenytoin			X
Carbamazepine		X	
Antithyroid Agents			
Propylthiouracil			X
Cardiovascular Agents			
Hydralazine			X
Procainamide			X
Quinidine			X
Hypoglycemic Agents			
Chlorpropamide			X
Tranquilizers			
Chlorpromazine	X		
Phenothiazines	X		
Other			
Cimetidine, ranitidine	X		
Levamisole			X

Reproduced from Dinauer MC. The phagocyte system and disorders of granulopoiesis and granulocyte function. In: Nathan DG, Orkin SH, Look AT, Ginsburg D, eds *Nathan and Oski's Hematology of Infancy and Childhood*. 6th ed. Philadelphia, Pa: WB Saunders Company; 2003:923–1010 with permission.

Segel GB, Halterman S. Ped in Rev. 2008;29:12-24.

Congenital Neutropenia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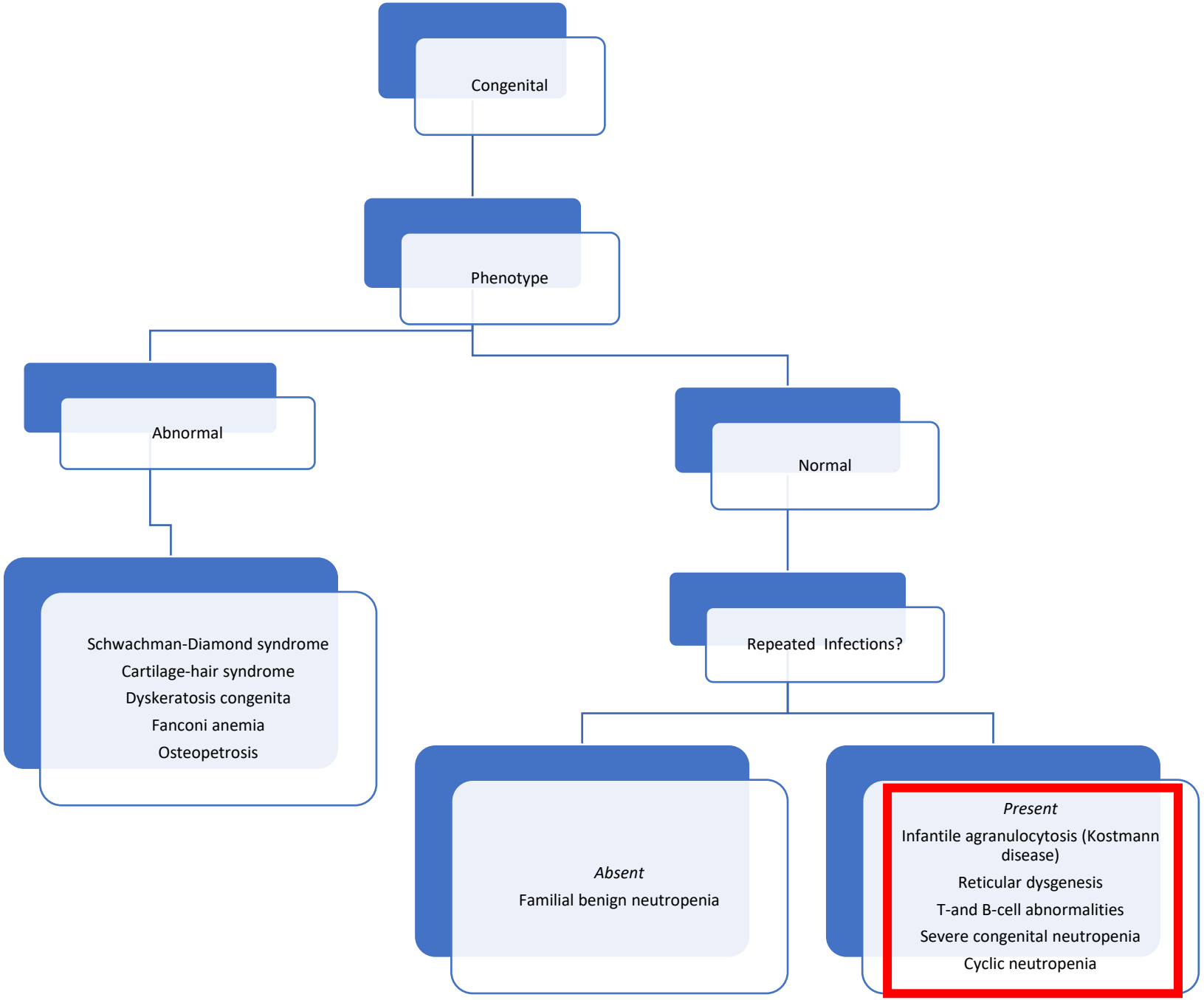
Cartilage-hair syndrome
Dyskeratosis congenita
Fanconi anemia
Osteopetrosis
Repeated Infections?
Absent
Familial benign neutropenia
Present
Infantile agranulocytosis (Kostmann disease)
Reticular dysgenesis
T-and B-cell abnormalities
Severe congenital neutropenia
Cyclic neutropenia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Cyclic Neutropenia

- Rare condition associated with neutrophil elastase (ELA-2) gene mutation
  - May be sporadic or with AD inheritance
  - Bone marrow shows maturational arrest of granulocytes during neutropenia episodes
- Neutrophil counts cycle every 21 +/- 3 days with a nadir $<0.2x10^9$ cells/L
  - Often fall to zero and remain $<0.2x10^9$ cells/L for 3 to 5 days
  - Associated monocytosis during neutropenic nadir
  - Infection risk correlates with depth of neutropenia
- *Diagnosis is made by repeated CBC and differential counts 2-3 times weekly for 6-8 weeks*
  - Genetic testing for ELA2 mutation is confirmatory

Cyclic Neutropenia

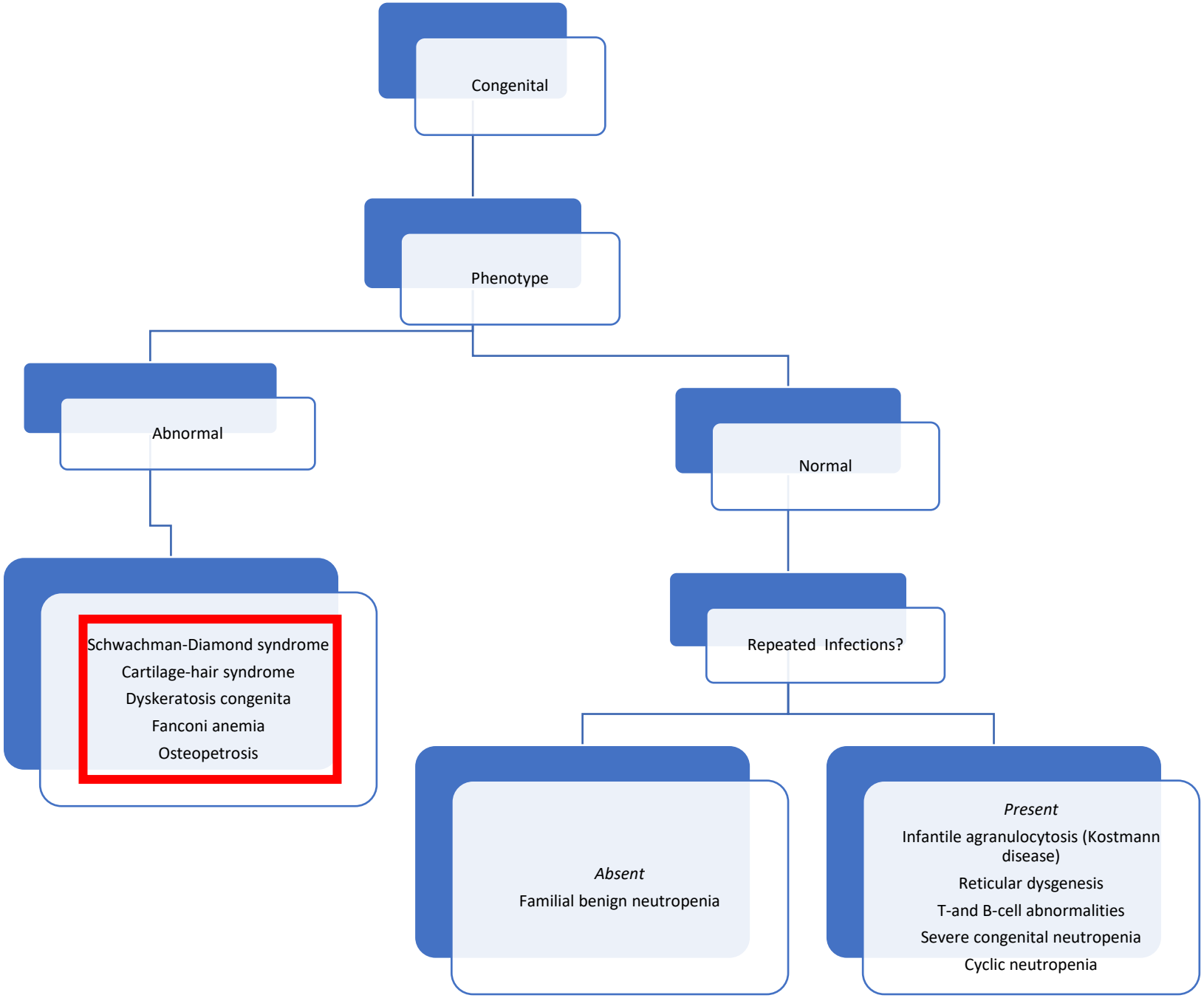
- Improves with age
  - Neutrophil counts continue to cycle at higher levels
- Management
  - Careful oral hygiene
  - G-CSF is indicated when ANC is persistently < 0.5 x 10^9/L

Severe Congenital Neutropenia

- Rare inherited conditions associated with neutrophil counts <0.5 x 10^9 cells/L.
- Often present in the neonatal period.
  - Umbilical infections, oral ulcers, pyoderma, pulmonary infections, otitis media, perineal infections
- Multiple genetic etiologies
  - 60-80% are associated with ELA2 mutation
- Affected patients have very high risk of developing myelodysplastic syndromes and/or AML.
- Management
  - Chronic G-CSF prophylaxis (5 µg/kg/day)

Reticular Dysgenesis

Reticular Dysgenesis	- Severe form of SCID. - Defective maturation of neutrophils and lymphoid lines. - Severe neutropenia and mod-severe lymphopenia. - Present with severe infections. - HSCT can be curative.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Evaluation of Childhood Neutropenia

History

- Age of presentation
- Infection history
  - Recent viral illness, fever, URI symptoms
  - Recurrent infections: sinopulmonary, gingivostomal, skin, perineal
  - Frequency, severity and type of infections
    - *S. aureus* and gram negative are most common
    - Unusual and severe infections: fungal, unusual bacteria
- Associated constitutional symptoms, signs of anemia, thrombocytopenia
- Past medical history (failure to thrive)
- Nutritional history
- Family history (ethnicity, consanguinity, unexplained infant deaths, history of neutropenia, severe infections, blood diseases)

Physical Examination

- Full physical examination with emphasis on:
  - Vital signs, height and weight
  - General appearance (well or unwell)
  - HEENT: dysmorphic features, albinism, adenopathy, oral ulceration, gingival inflammation, leucoplakia, tympanic membranes, sinuses
  - Respiratory: evidence of pneumonia
  - Abdomen: Hepatosplenomegaly, inguinal adenopathy
  - GI/GU: perineal inflammation/infection
  - Skin: skin folds, nail beds, cellulitis, pigmentation, dystrophic nails, eczema, bruising, petechiae

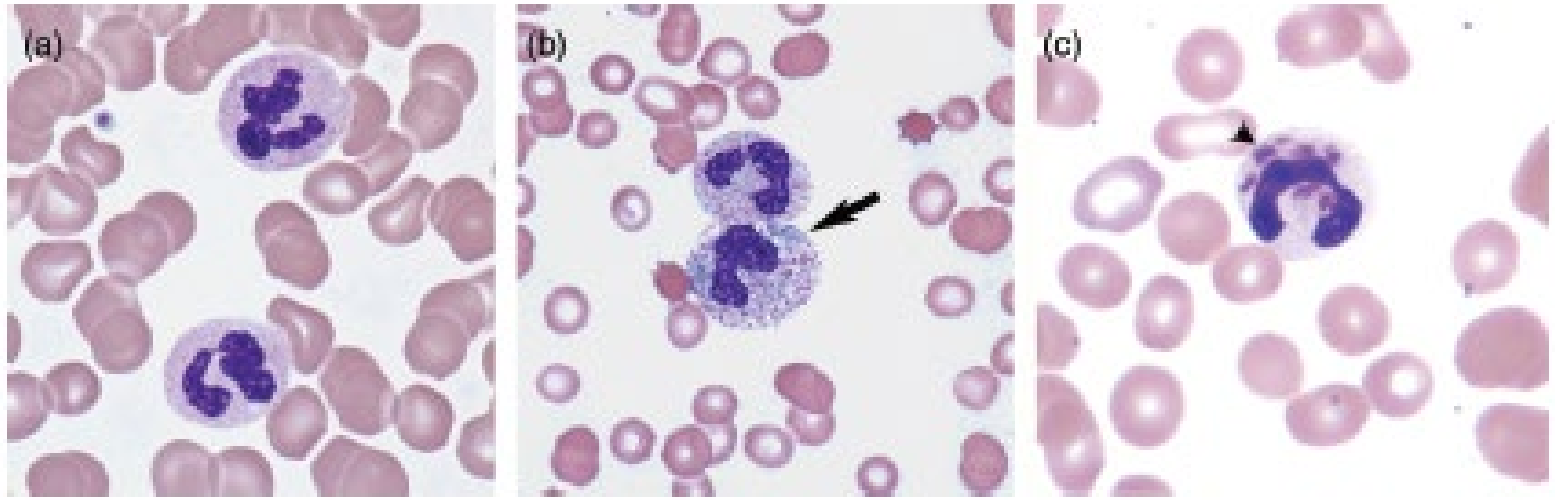

Figure 15-2 Photomicrographs of blood smears showing typical neutrophil morphology from (a) a healthy individual; (b) a patient with sepsis showing Döhle bodies (arrows) and toxic granulations; (c) a patient with Chédiak-Higashi syndrome showing the large cytoplasmic inclusions (arrowhead). From ASH Image Bank, #3780-3378-2979.

Laboratory Investigation

- CBC, reticulocyte count and differential
  - Confirm neutropenia, assess red cell production
  - Pancytopenia
- Peripheral blood film
  - To evaluate neutrophil and other cellular morphology

Other Investigations

- Immunologic investigations
  - ANA, Coombs', serum immunoglobulins, lymphocyte immunophenotyping
- **Bone Marrow aspiration and biopsy**
- Depending on clinical scenario:
  - Evaluation for autoimmune conditions, exocrine pancreatic function, vitamin B12, folate, copper and iron levels, metabolic screen, radiographic studies, response to single dose of corticosteroid

A brief word on management…

G-CSF

- Indicated when there is a history of recurrent severe infection in conjunction with severe neutropenia.
- Has been shown to result in fewer infections, reduced antibiotic use and reduced hospital admissions.
- Started at a dose of 5 µg/kg/day by subcutaneous injection into abdomen, thigh or upper arm.
- Response may be seen in the ANC within 1-2 days.
- Side effects may include: bone pain, and flu-like symptoms, but these resolve fairly quickly. Long term effects could include decreased BMD and transformation to MDS/AML (very rare).

Febrile Neutropenia

- In the case of severe neutropenia, this is a **medical emergency!**
- Evidence comes from the oncology literature.
- Risk of infection correlates with the degree and duration of neutropenia.

Table 9. **Bacterial Causes of Febrile Episodes in Neutropenic Patients**

Aerobic Bacteria (~90%)*
Gram-positive Cocci (~45%)
Staphylococcus
Coagulase-positive (*S aureus*)
Coagulase-negative (*S epidermidis* and others)
Streptococcus
S pneumoniae
S pyrogenes
viridans group
Enterococcus faecalis/faecium
Gram-positive bacilli (rare)
Corynebacterium sp
Gram-negative Bacilli (~45%)
Escherichia coli
Klebsiella sp
Pseudomonas aeruginosa
Anaerobic Bacteria (4% to 5%) (Often Polymicrobial)
Gram-positive Cocci (normal mouth flora)
Peptococci
Peptostreptococci
Gram-negative Bacilli
Bacteroides fragilis
Fusobacterium sp

*Percentages observed in patients receiving chemotherapy who were immunocompromised (Mathur P, Chaudry R, Kumar L, Kapil A, Dhawan B. A study of bacteremia in febrile neutropenic patients at a tertiary-care hospital with special reference to anaerobes. *Med Oncol.* 2002;19:267–272). Specific organisms were reported by Hughes WT, Armstrong D, Bodey GP, et al. 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. *Clin Infect Dis.* 1997;25:551–573 and Merck & Co, Inc, Whitehouse Station, NJ, USA: 1995–2007 (http://www.merck.com/mmpe/sec14/ch178/ch178j.html).

Febrile Neutropenia

Management

- Initial treatment relies on rapid initiation of broad-spectrum antibiotics (*Pipercillin-Tazobactam*) after blood cultures.
- Physical examination with close attention to oral mucosa, lungs, abdomen, skin and perineum
- No rectal temperatures, examination or medications
- No urinary catheterization

Febrile Neutropenia

Management

- Decision for inpatient vs outpatient management depends on:
    - age of patient
    - degree of neutropenia
    - clinical condition
    - proximity to medical centre
    - presence of underlying conditions.
- Anticipatory guidance is essential for parents of children with neutropenia
    - Clear definitions of fever
    - Importance of seeking prompt medical attention in the event of fever

Worried or not worried?

Case 1

You see a female term neonate for her first well baby check on day 1 of life.

- Her mother is a healthy 30-year-old, gravida 1, para 1, whose first child was born without specific problems.
- Birth weight was 2.81 kg, and she was born via spontaneous vaginal delivery.
- The baby appears healthy and physical examination reveals no abnormalities.
- On routine lab exams her CBC showed: WBC 7.2 x 10^9 cells/L, and ANC 0.43 x 10^9 cells/L. Other hematological and biochemical profiles were within normal limits.

Case 2

A 2 year old girl presents to the emergency department because of “odd streaky and very stinky” stools and poor growth.

- On history it was revealed she has had frequent episodes of purulent otitis media and pneumonias.
- Since the stools started around one year of age, has not been growing as expected.
- There is no significant family history.
- Physical exam shows height and weight less than the third percentile. The remainder of the exam was unremarkable.
- Laboratory studies show hemoglobin 113 g/L, WBC 3.0 x 10^9 cells/L, ANC 0.6 x 10^9 cells/L and platelets of 152 x 10^9 cells/L.

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Case 3

A 14 year old female presents to the emergency department with:

- a one week history of increasing bilateral leg pain.
- three days of fever, pallor and anorexia.
- On physical examination she has diffuse lymphadenopathy and splenomegaly.
- Her CBC shows a WBC count of 4.0 x 10^9 cells/L, hemoglobin 56 g/L, platelets 20 x 10^9 cells/L and ANC 0.1 x 10^9 cells/L.

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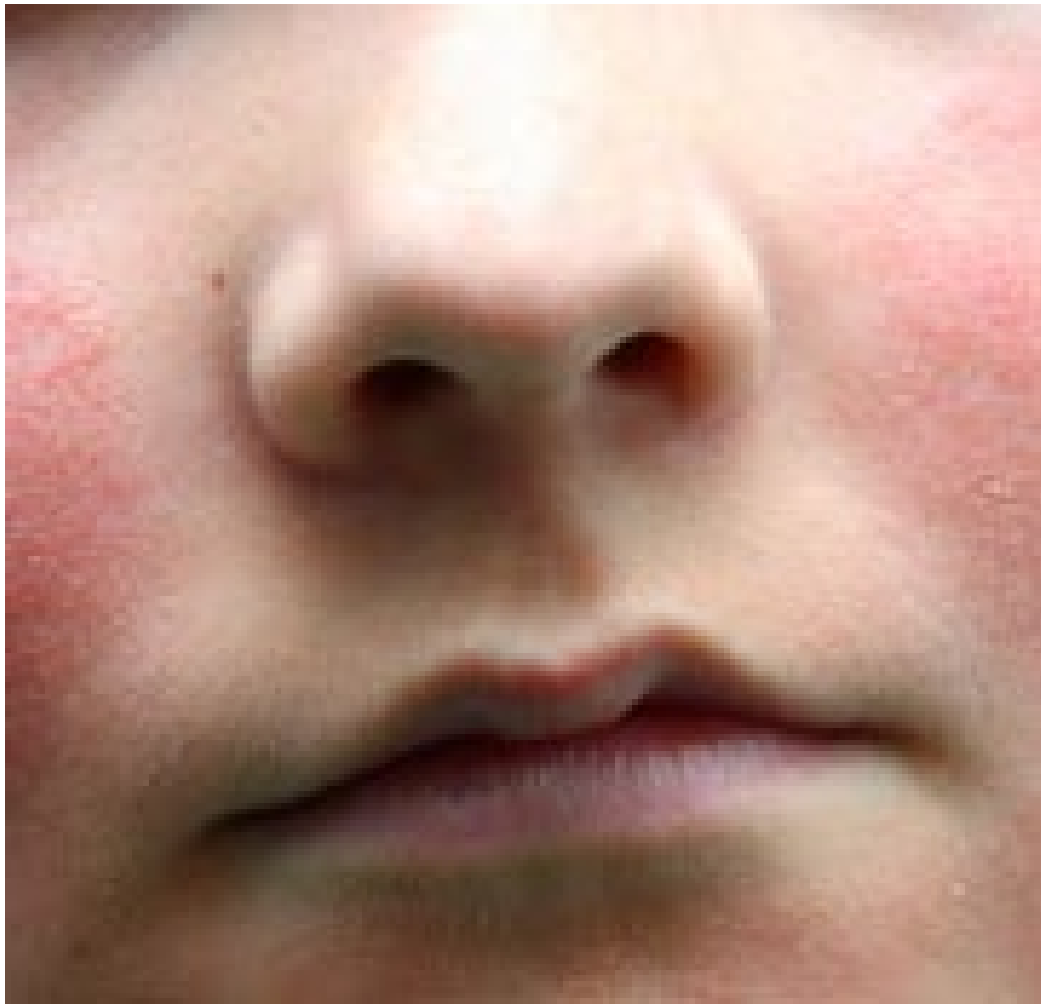
Case 4

A 3 year old boy presents to the pediatrician's office with:

- a history of fever three days ago, now resolved.
- Ongoing upper respiratory tract infectious symptoms, including rhinorrhea and cough.
- He also has a red rash (see picture) on his cheeks.
- A CBC shows a WBC count of 9.0 x 10^9 cells/L, with an ANC of 0.9 x 10^9 cells/L. The remainder of the blood work was unremarkable.



Case 5

A 4 month old baby boy presents to his general pediatrician's office with:

- a two day history of fever, T_{max} 39.4 axillary.
- Ulcerations on his lips and buccal mucosa which appeared within the last 24 hours.
- His mother mentions that he has been hospitalized twice in the past 2 months with fevers and diagnosed bacterial infections. Both times his neutrophils were less than 0.5 x 10^9 cells/L.
- A CBC shows a WBC count of 2.9 x 10^9 cells/L and ANC of 0.3 x 10^9 cells/L.

Case 5

A 4 month old baby boy presents to his general pediatrician's office with:

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- A CBC shows a WBC count of 2.9 x 10^9 cells/L and ANC of 0.3 x 10^9 cells/L.

Case 6

A 17-year old male of African heritage visits his pediatrician for a routine yearly assessment prior to his departure for university.

- His blood work returns showing a WBC count of 4.8 x 10^9 cells/L, hemoglobin of 180 g/L, platelets of 300 x 10^9 cells/L. Differential shows an ANC of 1.1 x 10^9 cells/L.
- He has previously been well. Physical examination is normal.

Red Flags

- Recurrent or persistent fevers
- Other constitution symptoms: night sweats, weight loss
- Past history of unusual or frequent major infections, chronic diarrhea, failure to thrive
- Family history
- Mucosal ulcers, gingivitis and abdominal pain
  - Could indicate significant chronic neutropenia
- Splenomegaly
- Dysmorphic features
- Pancytopenia

Summary

- The risk of infection associated with neutropenia depends on the duration and severity of the neutropenia.
- There is a broad differential for neutropenia, both congenital and acquired.
- It is important to differentiate when to worry and when not to worry through the history, physical exam and initial CBC and differential (see red flags).
- Febrile neutropenia is a **MEDICAL EMERGENCY**, and needs to be treated with IV antibiotics.

Pancytopenia

Aplastic Anemia

Aplastic Anemia

Presentation is often insidious with “inciting event” at least 6 to 8 weeks previously

Often presents with thrombocytopenia (bruising, petechiae)

Rarely presents with typical signs of malignancy

Aplastic Anemia

Definition of Severe Aplastic Anemia

- 2 of 3 peripheral blood criteria
  - ANC <0.5
  - Platelets <20
  - Retics <1%
- 1 of 2 bone marrow criteria
  - <25% cellularity on biopsy
  - 25-50% cellularity with <30% hematopoietic cells

Aplastic Anemia

- Cause is usually unknown.
  - Radiation
  - Drugs/Chemicals/Toxins
  - Viruses
    - EBV, CMV, hepatitis, HHV6, HIV
    - Others
  - Autoimmune Disease
  - Immune deficiency
  - Myelodysplasia
  - **An increasing number of genetic/bone marrow failure syndromes are being identified!**

Aplastic Anemia

- Diagnosis
  - Bone marrow aspirate and biopsy
  - RULE OUT inherited bone marrow failure syndromes/PNH
- Management
  - Immunosuppression (ATG, cyclosporine, eltrombopag)
  - Stem cell transplant
  - Higher rate of relapse with immunosuppression

Inherited Bone Marrow Failure Syndromes

Introduction to IBMFS

AKA constitutional or familial aplastic anemia

Frequently associated with physical abnormalities

- Radial ray, skeletal, short stature, renal
- Anomalies are not always obvious or present

Hematologic findings not usually present at birth

- May not present until adulthood

10-25% of pediatric aplastic anemia

Increased frequency of cancer

- Squamo-epidermal carcinoma, MDS/AML
- Presenting sign may be malignancy

Fanconi Anemia (FA)

FA Pathophysiology

- Defect in DNA repair
- Chromosomal fragility
- Increased sensitivity to cytotoxic therapies
- Predisposition to certain malignancies
- Loss of hematopoietic stem cells
  - ?mechanism

FA Genetics

- FA proteins: nuclear protein complex that repairs DNA
- 17 different FA genes
- *ALL* autosomal recessive EXCEPT *FANC-B* (X linked) and *FANCR(RAD51)* (AD)
- Heterozygotes are carriers
  - ?increased susceptibility to cancer
- Mutation analysis establishes definitive diagnosis
  - *FANC-A,* 16q24.3 (60-70%)
  - *FANC-C*, 9q22.3 (10-15%)
  - *FANC-G* (10%, 80% in black South African population)

FA Genetics

- Complementation group predicts clinical course
  - *FANC-A* later onset of BMF
  - *FANC-C* and *G* have a more severe course
  - *FANC-B/D1* very early onset MDS/AML
  - *FANC D1, N* – Wilms tumour, medulloblastoma

FA Epidemiology

Heterozygote frequency 1:300 (1:100 Ashkenazi, Afrikaans)

Homozygous frequency 1:100,000-250,000

Described in nearly all races and ethnic groups

	ANC	Platelet Count	Hemoglobin
Mild	➢1	➢50	➢100
Moderate	0.5-1	30-50	80-100
Severe	< 0.5	< 30	< 80

FA Hematologic Features

- Cytopenias
  - Mild at first
  - Usually diagnosis made based on other abnormalities
  - Macrocytosis
- Marrow failure
  - 90% by 40 years

FA Congenital Anomalies

- Not always present or may be subtle
- NOT required for diagnosis

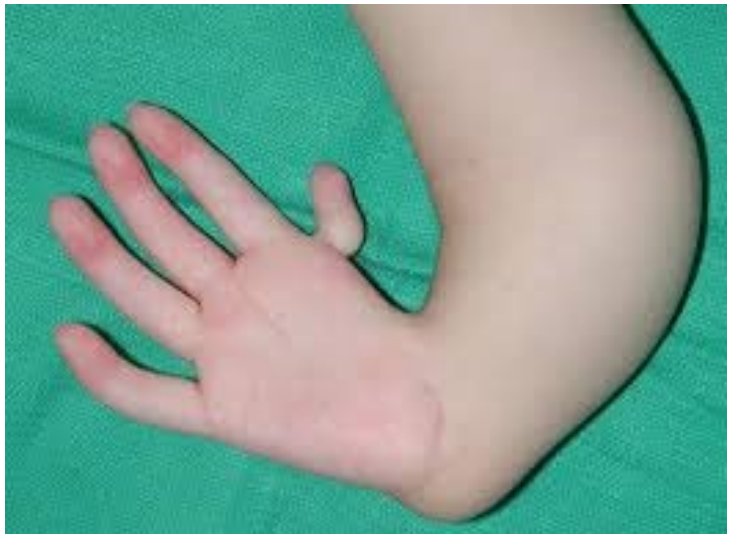
Skin – Generalized hyperpigmentation; hypopigmented areas; large freckles, café-au-lait spots	40 to 60%
Short stature, delicate features	40 to 60%
Upper limbs – Absent or hypoplastic thumb, supernumerary, bifid, clinodactyly	35 to 50%
Gonads – Hypogenitalia, undescended/absent testes, hypospadias; bicornuate or malpositioned uterus	25% (males) <5% (females)
Head – Microcephaly or hydrocephaly; birdlike face, mid-face hypoplasia, Sprengel's deformity of neck	20 to 25%
Eyes – Microphthalmia, ptosis, epicanthal folds, strabismus	20 to 40%
Kidney – Abnormal, ectopic, horseshoe, hypoplastic, or absent kidney; hydronephrosis	20 to 30%
Ears – Deafness (usually conductive); low set ears, abnormal shape, narrow canal	10 to 20%
Developmental delay	10%
Cardiopulmonary anomalies – Congenital heart disease (patent ductus arteriosus, atrial or ventricular septal defects, coarctation, situs inversus)	6%
Gastrointestinal anomalies – Atresias, imperforate anus, tracheoesophageal fistula, malrotation	5%

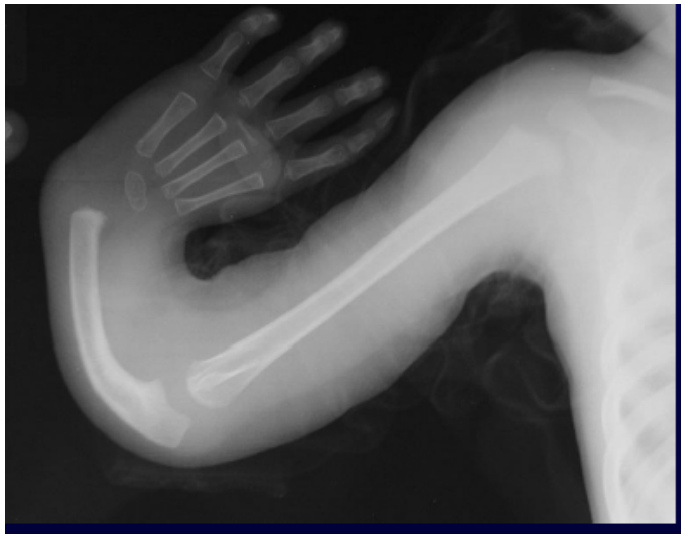
Refer to UpToDate for details. These frequencies were derived from studies of patients with classic presentations and may be an underestimate. Individuals with more subtle findings may be identified from genetic sequencing.

FA: Fanconi anemia.

FA Congenital Anomalies

- Classic hand anomaly
  - Partial or total absence of preaxial border of radius
  - Bilateral in 50% of cases
  - Ulna thickened, bowed toward absent radius
  - Hypoplastic thumb
  - Thenar eminence reduced in size





FA Cancer Predisposition

Risk of malignancy 1000x greater than normal

By adulthood about 30% develop malignancy

- Clonal abnormalities in 34-48% of patients
- 10% leukemia (AML>ALL), especially M4-M5
- 10% solid tumour: squamous cell head/neck
- 3% liver tumour: adenoma, hepatoma
- 6-8% female genital tract

Risk increased by HSCT

- Secondary squamous cell carcinoma risk 4x after BMT
- Shifts age of solid tumours 16 years earlier
- Solid tumour risk associated with inflammation of GVHD

FA Cancer Predisposition

Excessive toxicity with standard chemotherapy

- Standard chemotherapy and radiotherapy regimens may be lethal

FA often diagnosed after treatment for malignancy started due to increased toxicity

- Suspect underlying BMFS/FA if toxicity of leukemia therapy is early, unexpectedly severe

Surgical approach, especially to solid tumours is preferred

Early diagnosis of progression to MDS, AML allows for:

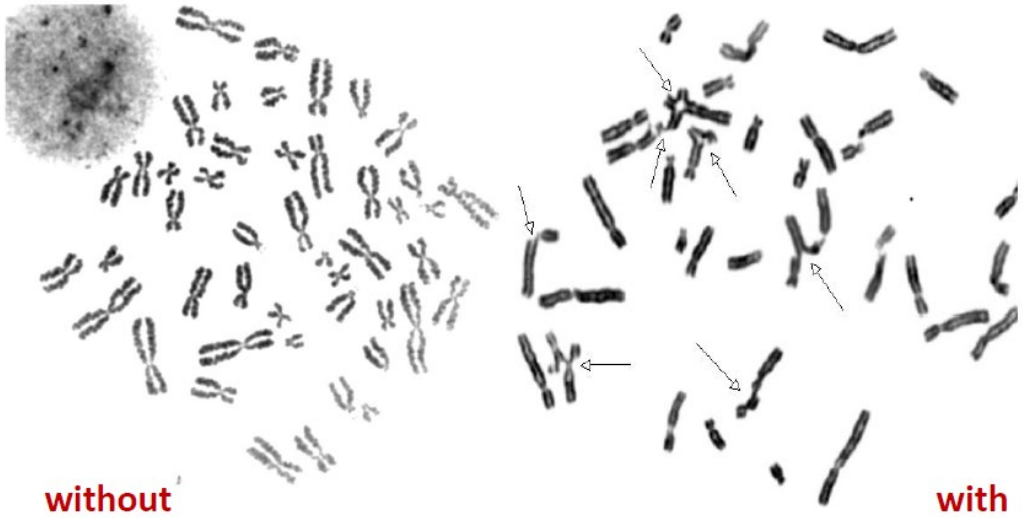
- Review of options for therapy
- Search for best URD for HSCT

FA Diagnosis

- Peripheral blood karyotype with and without exposure of patient cells to breakage inducing agent diepoxybutane (DEB) or mitomycin C (MMC)
  - Also done on skin fibroblasts
- Flow cytometry: clastogen induced G2/M arrest
- Specific mutation analysis

FA Diagnosis

FA Cells Incubated without/with diepoxybutane



Courtesy of Lisa Moreau, Dana Farber Cancer Institute

FA Management

- Supportive care as long as possible
- Monitor for MDS/AML
  - Yearly BMA and biopsy
- Oxymethalone (androgen) may slow count decline
  - Danazol less virilizing for females
- HSCT – reduced intensity conditioning
  - Increased toxicity due to DNA repair defect
  - Survival of URD approaching sibling donor

Dyskeratosis Congenita (DKC)

Short telomeres leading to DNA repair defect

DKC Pathophysiology

DNA repair problem

Telomeres are much shorter than normal

- Premature aging of DNA
- Premature cell death, senescence or genomic instability

DKC Genetics

- Three patterns of inheritance
- AD, recessive and X-linked
- Hallmark is VERY short telomeres (<3rd centile)
- All genes are in telomerase complex
- Telomeres are specialized protein:DNA complexes at the ends of chromosomes
- Stabilize chromosome ends and prevent premature shortening (aging)
- Prevent end-to-end fusions, translocations, breaks

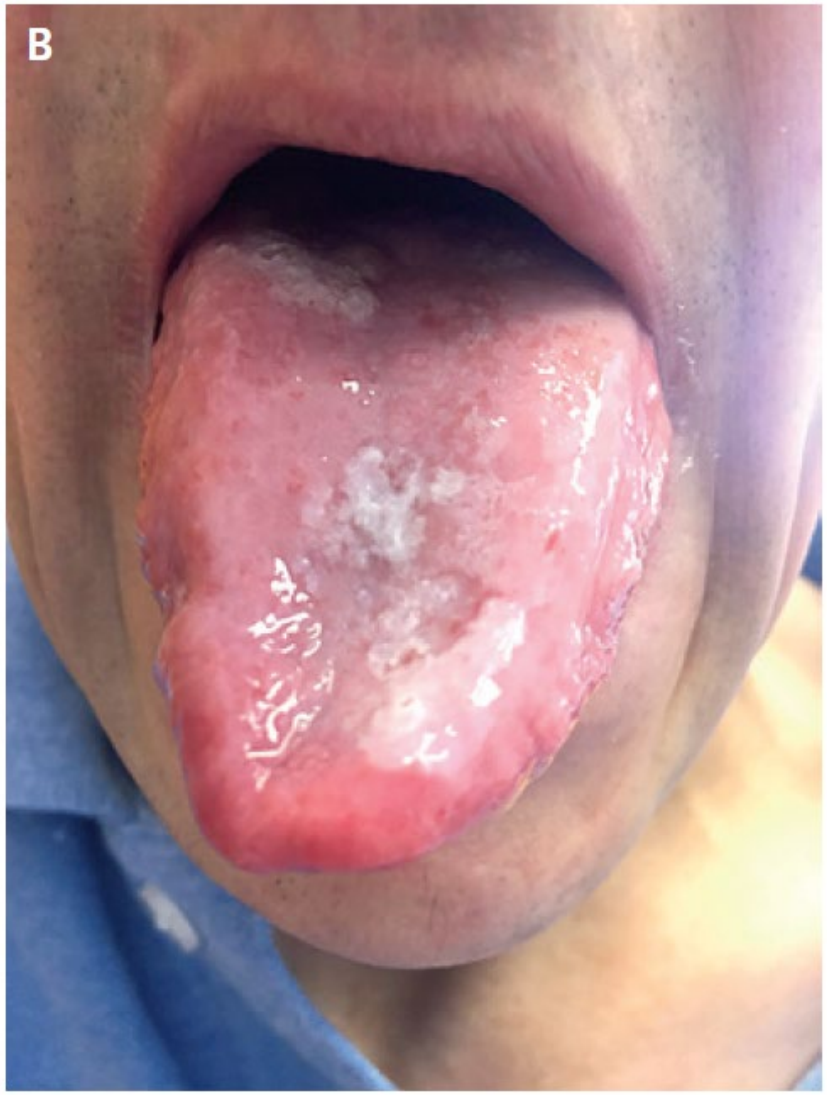
Gene	Frequency	Genetics	Gene Product
DKC1	17-36%	X-linked	Dyskerin
hTERC	6-10%	AD	Telomerase RNA
hTERT	1-7%	AD/AR	Telomerase Reverse Transcriptase
TINF2	11-24%	AD	Shelterin complex
NOP10	<1%	AR	H/ACA core protein
NHP2	<1%	AR	H/ACA core protein
WRAP53	3%	AR	TCAB1, Shelterin complex
CTC1	<2%	AR	Telomere maintenance

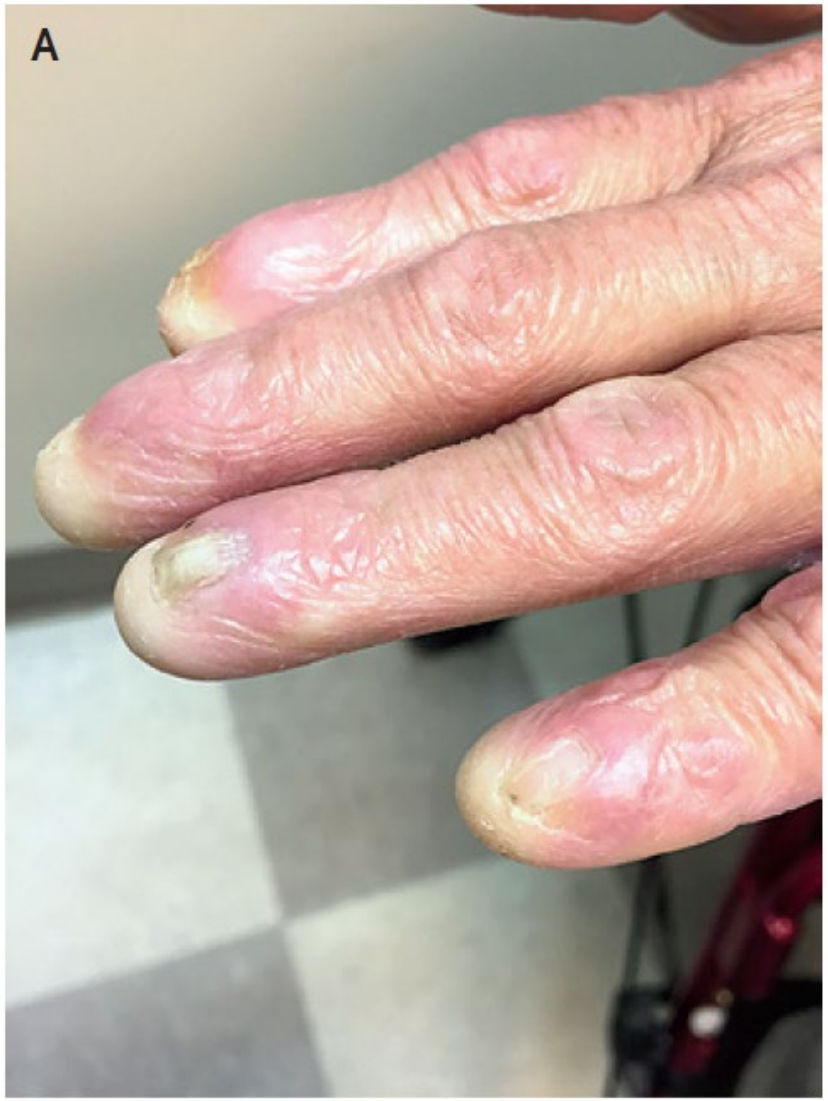
DKC Genetics

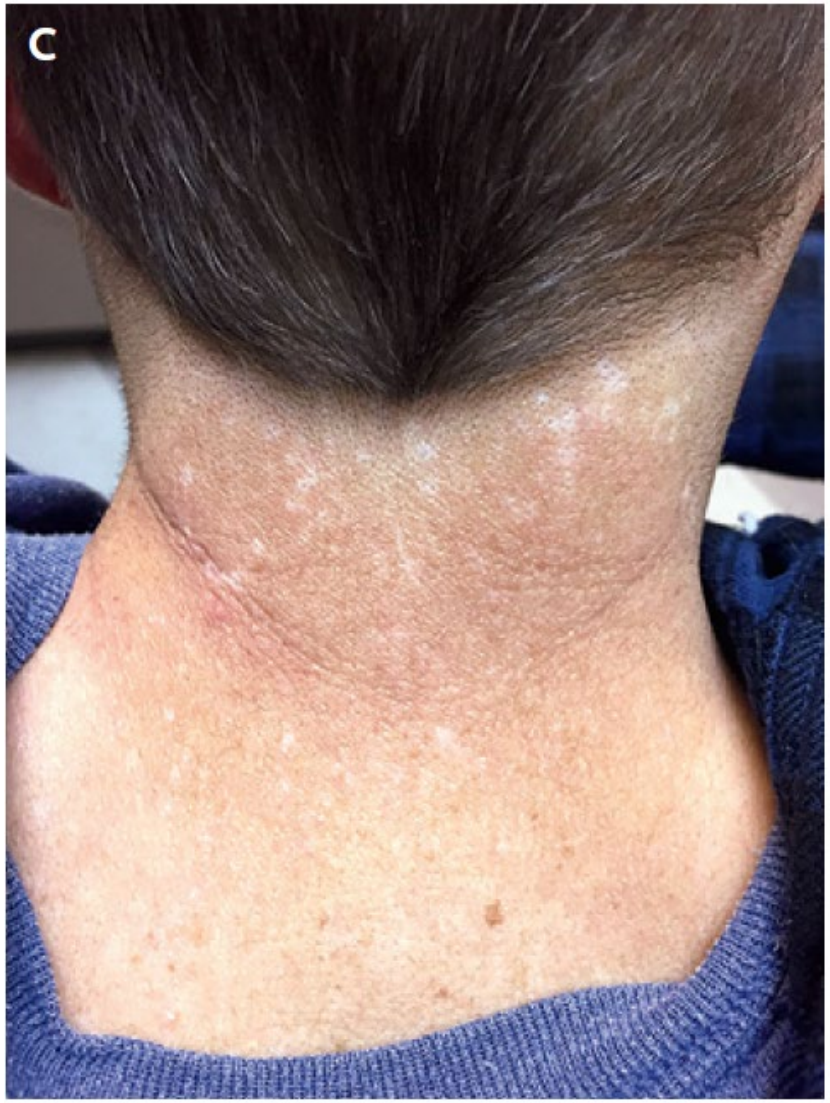
- Many patients with DKC lack mutations
- Likely additional genes yet to be identified

DKC Clinical Features

- Triad: reticulated skin hyperpigmentation, dystrophic nails, mucous membrane leukoplakia – develops with age
- Aplastic anemia develops in up to 50% in 2nd to 3rd decade
- Solid organ cancers (head, neck, GI) and leukemia at an early age in 3rd to 4th decades
  - AML
  - Carcinomas of bronchus, tongue, larynx, esophagus, pancreas, skin







DKC Clinical Features

DKC Clinical Features

DKC Diagnosis

- Clinical features and family history
- Do not need classical triad or physical stigmata
- Very short telomeres
- <1st centile for age in >/= 3 lymphocyte subsets
- Genetic testing
- Negative genetic testing does not rule out the diagnosis

DKC Treatment

Supportive care

Androgens and cytokines

- Caution about viscus rupture with androgen

HSCT

- Reduced intensity regimens
- Pulmonary toxicity (often delayed)
- Increased risk of veno-occlusive disease

Diamond Blackfan Anemia (DBA)

DBA Genetics

- Autosomal dominant
  - May be sporadic or inherited
- Mutations/deletions in ribosomal proteins:
  - *RPS19 (DBA 1*) 19q13.2 in 25% of patients
  - *RPL5, RPS10, RPL11, RPL25A, RPS26, RPS24, RPS 17, RPS 7, RPL 19, RPL 26*
  - 25-50% of patients with unknown mutations
  - Assemble proteins from amino acids
- Defective erythropoiesis from haploinsufficiency
- Special case: acquired haploinsufficiency of *RPS14* in 5q- MDS

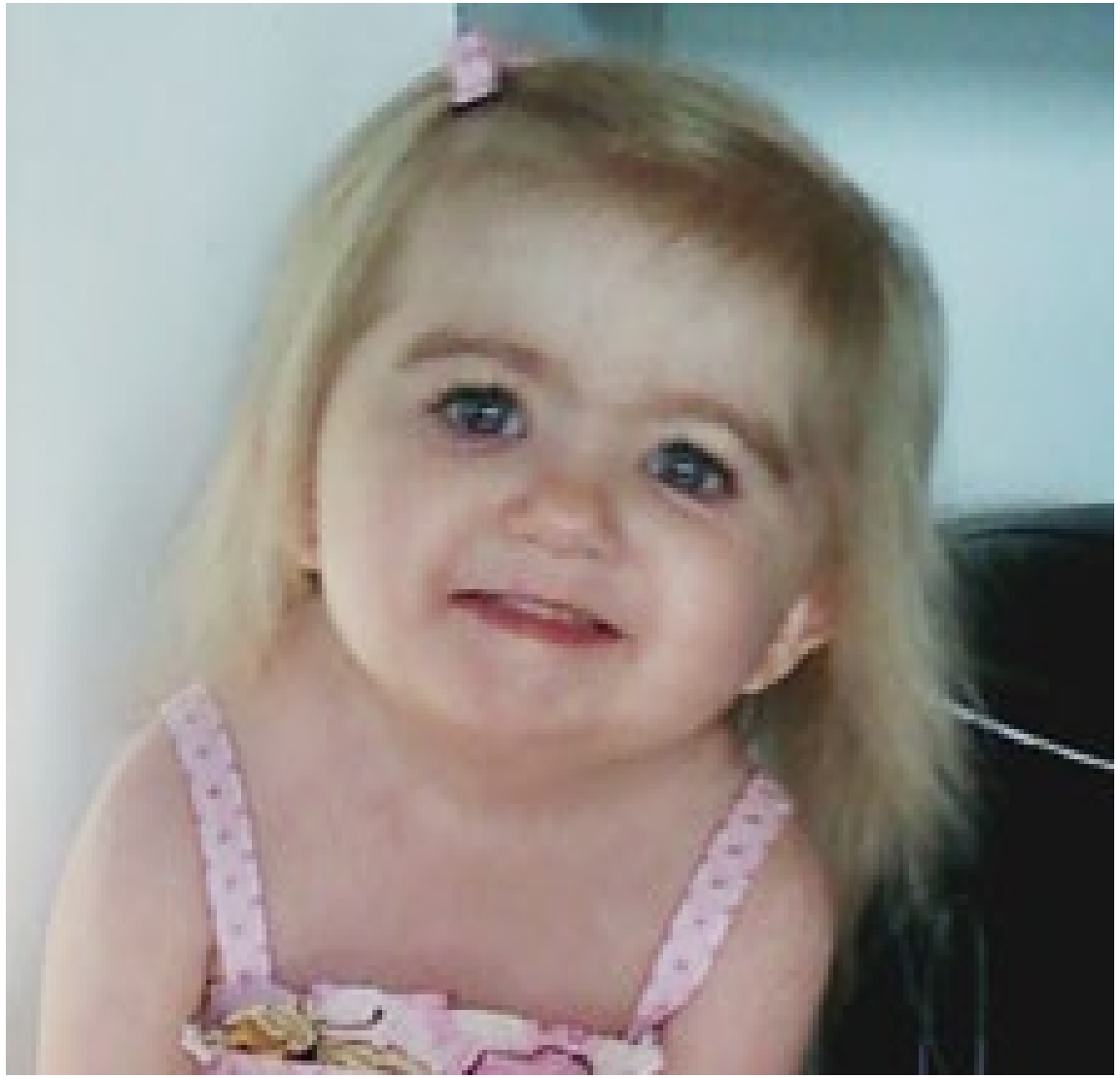
DBA Diagnostic Criteria

Diagnostic Criteria	Supporting Criteria
- Age < 1yo - Macrocytic anemia - Reticulocytopenia - Paucity of erythroid precursors in marrow	Major criteria - Pathogenic mutations - Positive family history Minor criteria - Elevated red cell ADA - Congenital anomalies - Elevated Hb F - No other bone marrow failure syndrome

- Classic DBA: all diagnostic criteria
- Non-classic DBA: various combinations

DBA Congenital Anomalies

- 50% cranio-orofacial (tow coloured hair, blue sclerae, glaucoma)
- 38% upper extremity (thumbs, may be subtle)
- 39% GU
- 30% cardiac
- Over 20% with more than one anomaly
- Short stature and bony abnormalities common and often overlooked
- Neutropenia and rarely thrombocytopenia also



30 Cases* Reported in the Literature (~5%

• AML/MDS	15
• ALL	1
• Osteosarcoma	6
• Hodgkin disease/NHL	3
• Breast carcinoma	2
• Hepatocellular carcinoma	2
• GI carcinoma	2
• Melanoma	1
• Malignant fibrous histiocytoma	1
• Soft tissue sarcoma	1
• Non-Hodgkin Lymphoma	1

From Alter, BP. In Shimamura and Alter, Blood Reviews, 2010

DBA Cancer Predisposition

DBA Treatment

Prednisone

- 2 mg/kg for up to 8-12 weeks before declaring failure
- Taper to minimum dose to maintain Hgb>90
- 79% steroid responsive (4% never treated)

For steroid refractory patients or those requiring high doses of steroids, consider chronic red cell transfusions

Red cell transfusions:

- Year 1 of life due to PJP risk
- Extended antigen typing of PRBCs, minimum volumes
- Iron overload & chelation

DBA Prognosis

Remission of anemia ~20% by age 25

- No predictive genetic or clinical features

HSCT for transfusion dependent patients, particularly those who are allo-immunized or have OTHER cytopenias (neutropenia)

- Does not cure solid tumour risk
- Sibling donor needs careful evaluation for DBA

Shwachman Diamond Syndrome (SDS)

SDS

Hematologic

- WBC: fluctuating neutropenia, impaired chemotaxis
- Anemia 1/3, thrombocytopenia 20%
- Aplasia in 10-25% leading to MDS/AML

Other findings:

- Exocrine pancreatic insufficiency, transaminitis
- Low trypsinogen, pancreatic isoamylase (for age), low fecal elastase, fatty pancreas by imaging
- Metaphyseal chondrodysplasia (bell shaped chest)
- Short stature, ichthyosis/eczema
- Cardiac, endocrine, developmental issues

SDS Differential Diagnosis

- Severe congenital neutropenia
- Kostmann syndrome
- Cyclic neutropenia
- Pearson syndrome

SDS Genetics

- Autosomal recessive – male predominance (1.7:1)
- 90% with mutation in *SBDS* gene (7 centromere:7p12-q11) or adjacent pseudogene *SBDSP*
- SBDS functions in:
  - Ribosome biogenesis (associated with 60S subunit, functions in promoting 40S:60S ribosome joining)
  - Mitotic spindle stabilization

SDS Treatment

- Pancreatic enzyme replacement, ADEK supplements
- Management of congenital anomalies
- G-CSF – least amount, shortest time
- Transfusions
- Monitoring for MDS/AML – periodic marrows
- HSCT (few)
  - Variable results due to conditioning regiment toxicity

Pearson Syndrome (PS)

Pearson Syndrome

- Refractory sideroblastic anemia by 6 months of age
- Exocrine pancreatic dysfunction (fat malabsorption)
- Associated usually mild neutropenia, thrombocytopenia
- Marrow: vacuolated precursors/ringed sideroblasts
- Death usually as a consequence of acidosis, sepsis, liver or renal failure related to tubular dysfunction
  - Median survival is age 3 years
- Genetics: mitochondrial DNA deletion
  - Pathognomonic, maternal inheritance

Amegakaryocytic Thrombocytopenia (AT)

Amegakaryocytic Thrombocytopenia

- Autosomal recessive
- Mutation involving gene for the TPO receptor c-MPL at *1p34*
- Decreased bone marrow megakaryocytes
- Thrombocytopenia at birth
- Classically red cells macrocytic, increased HbF
- Normal platelet size and morphology
- Hemoglobin normal early
- High risk of MDS to AML
- Two phenotypes early (80%) vs late thrombocytopenia and aplasia, correlated with specific mutations and c-MPL activity

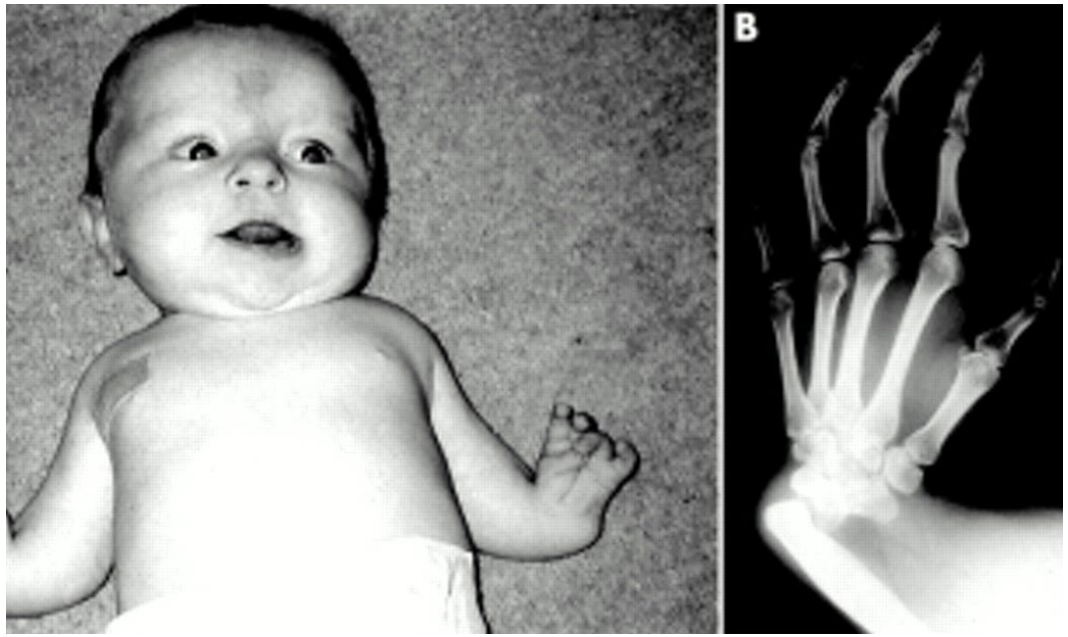
Amegakaryocytic Thrombocytopenia

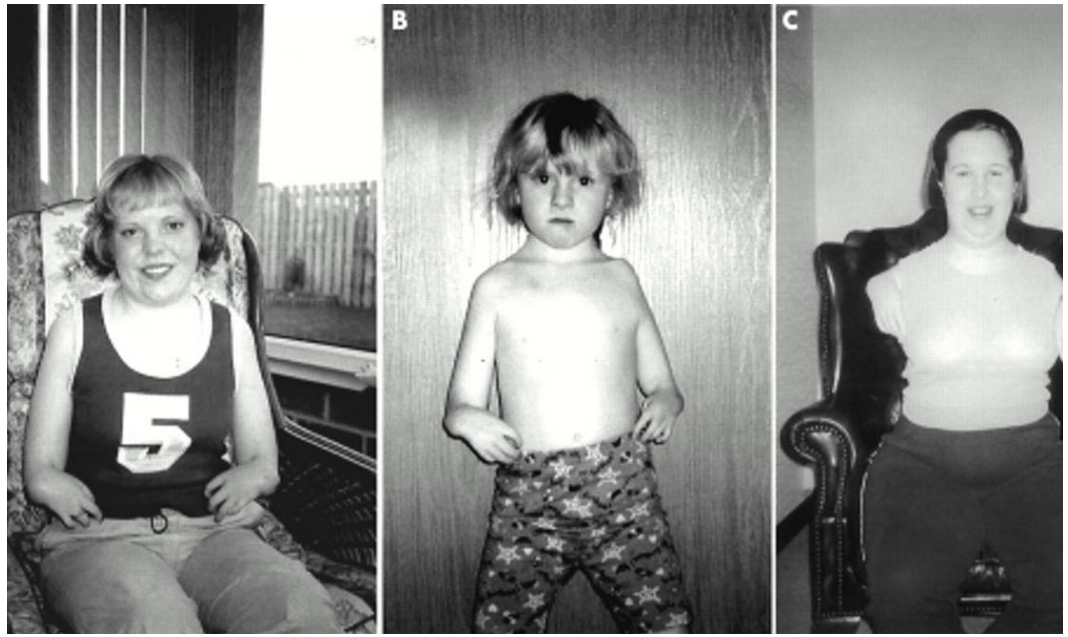
- Platelet count < 20
- Some degree of bleeding demonstrated in most children
- Approximately 50% have additional anomalies
- Progression to aplastic anemia within 5 years (high risk of MDS/AML)
- Management:
  - Supportive care with transfusions
  - Stem cell transplantation: should be performed prior to the development of severe pancytopenia or platelet allosensitization

Thrombocytopenia Absent Radius (TAR)

Thrombocytopenia Absent Radius Syndrome

- Autosomal recessive
- Due to *RBM8A* gene mutaitons (RNA-binding motif protein 8A) at *1q21.1*
- Typically one allele carries a deletion of 1q21.1 and the other a mutation in the remaining allele
- Thrombocytopenia presenting at birth
- Bilateral absence of radii with presence of thumbs (in FA the defect is terminal – thumbs are absent if the radii are absent; in TAR intercalary)





TAR Skeletal Features

TAR Syndrome

Other cytopenias

- Leukemoid reaction common >40
- Hypereosinophilia also

Other congenital anomalies

- Micrognathia, brachycephaly, hypertelorism
- Webbed neck, hypogonadism
- Various lower limb abnormalities 40%
- 10% congenital heart disease

2/3 outgrow severe thrombocytopenia by 1 year

- Eventual platelet count may not be normal

Transplantation is curative but usually not required

In Summary

IBMFS Diagnostic Suspicion**

- Presence of characteristic physical anomalies with hematologic abnormalities
- Unexplained macrocytosis in a patient with or without characteristic birth defects
- Children with aplastic anemia or myelodysplasia
- Patients with malignancy who are highly sensitive to chemotherapy or radiation
- Cancer in a patient at an atypically early age
  - Head/neck/esophageal cancer < 40 years of age
  - Vulvar cancer < 30 years of age
- Family members with any of the above

General Diagnostic Work Up

- CBC, differential, reticulocyte count, peripheral smear
- Bone marrow aspirate and biopsy
- Telomere length
- *SBDS* gene testing
- Chromosome fragility testing
- PNH testing (CD55 and CD59)
- ANA, dsDNA, C3, C4
- Immunoglobulins
- Infections: EBV, CMV, Hepatitis A/B/C, HIV, parvovirus, varicella
- B12 and folate
- Renal and hepatic function
- Skeletal survey, CXR
- Echocardiogram, abdominal U/S
- 72 hour fecal fat, fecal elastase, serum trypsinogen
- TSF, free T4

Management Rules of Thumb

- Genetic counselling
- Cancer surveillance
- Routine monitoring of blood counts with CBC, BMA & biopsy based on specific disease
- Routine monitoring and management of other comorbidities
- HSCT generally indicated when patients have progressive pancytopenia leading to severe aplastic anemia, patient is transfusion dependent, or with MDS/AML
- Supportive care: transfusions, infection prophylaxis etc

A Word On Prognosis

- Generally live to young adulthood
  - FA & SDS into 40s
  - DBA survival to age 40 (75%), sustained CR on steroids 80-100%
  - Pearson syndrome to 3 years
  - DC – AR survival to age 20, AD milder course and live longer
  - TAR improve after age 1

Questions?