In The Name of God

Severe Neutropenia And Hematopoietic Stem Cell Transplant

A case Report

Dr. Ahmad Tamaddoni

Professor in Pediatric Hematology & Oncology

Non-Communicable Pediatric Diseases Research Center, Babol, I.R.Iran.

E-mail: drtamaddoni@yahoo.com

Neutropenia

- Neutropenia is defined as a decrease in the absolute neutrophil count (ANC).
- In Caucasians, neutropenia is defined as an ANC of less than 1000/mm3 in infants between 2 weeks and 1 year of age and less than 1500/mm3 beyond 1 year of age.
- African Americans may have lower counts, with ANC levels 200-600/mm3 less than in Caucasians.
- Neutropenia can be transient or chronic.

- Neutropenia is considered “chronic” when it persists beyond 3 months and is due to reduced production or increased destruction of neutrophils.

- It may be an inherited, intrinsic disorder or an acquired, extrinsic defect.

- In pseudoneutropenia, a normal neutrophil population may be shifted toward the marginating compartment, leaving fewer cells in the circulating compartment. The WBC count measures only the circulating cells and not the marginating pool; therefore, this represents a pseudoneutropenia. The bone marrow is normal in appearance. The neutrophils function normally and the leukocyte changes are usually found incidentally on blood count. Marginating neutrophils may be uncovered by the injection of epinephrine.

The severity of neutropenia is graded according to ANC as follows:

- Mild neutropenia: ANC 1000-1500/mm3
- Moderate neutropenia: ANC 500-1000/mm3
- Severe neutropenia: ANC less than 500/mm3

Causes of Neutropenia

1. **Decreased production or intrinisc defects**
   a. Neutropenia in various ethnic groups[a]
   b. Severe congenital neutropenia: sporadic (most common) or autosomal dominant or Kostmann disease—autosomal recessive
   c. Familial benign chronic neutropenia—autosomal dominant
   d. Cyclic neutropenia
   e. Reticular dysgenesis
   f. Pancreatic insufficiency syndromes (Shwachman–Diamond syndrome and Pearson syndrome)
   g. Neutropenia associated with metabolic disease
      i. Glycogen storage disease (type IB)
      ii. Barth syndrome
      iii. Idiopathic hyperglycinemia
      iv. Isovaleric acidemia
      v. Methylmalonic acidemia
      vi. Propionic acidemia
      vii. Thiamine-responsive anemia in DIDMOAD syndrome (Diabetes Insipidus, Diabetes Mellitus, Optic Atrophy, and Deafness)
   h. Neutropenia in. Bone marrow failure syndromes (Chapter 10)
      i. Fanconi anemia
      ii. Familial congenital aplastic anemia without anomalies
      iii. Dyskeratosis congenital

Causes of Neutropenia

2. **Increased destruction or extrinsic defects**
   a. *Congenital*
      i. Neutropenia associated with immunodeficiency disorders
         - XLA and dysgammaglobulinemia
         - Abnormal cellular immunity in cartilage hair hypoplasia
         - Common variable immune deficiencies
         - Hyperimmunoglobulin M syndrome
         - IgA deficiency
         - Dubowitz syndrome
         - Myelokathexis and WHIM syndrome
   b. *Acquired*
      i. Drug-induced
         - Idiosyncratic—antibiotics (sulfonamide, penicillin) antithyroid, antipsychotics
         - Toxic suppression—cytotoxic drugs, sulfasalazine, phenothiazines
         - Drug-hapten—penicillin, propylthiouracil
      ii. Infection
         - Viral infection (e.g., HIV, EBV, hepatitis A and B, respiratory syncytial virus, measles, rubella, varicella, influenza)
         - Bacterial infection (e.g., typhoid, paratyphoid, tuberculosis, brucellosis)
         - Rickettsial infection (e.g., ehrlichiosis)
      iii. Bone marrow aplasia
      iv. Chronic idiopathic neutropenia
      v. Secondary: chemicals, irradiation, immune reaction, malnutrition, copper deficiency, vitamin B_{12} deficiency, folate deficiency
      vi. Bone marrow infiltration, neoplastic
         - Primary: leukemia
         - Secondary: neuroblastoma, lymphoma, rhabdomyosarcoma
      vii. Bone marrow infiltration: non-neoplastic
         - Osteopetrosis, Cystinosis, Gaucher disease, Niemann–Pick disease
      viii. Immune
         - Drug-induced (e.g., anticonvulsants)
         - Alloimmune (isoimmune)
         - Maternofetal
         - Primary: autoimmune neutropenia
         - Secondary: autoimmune: systemic lupus erythematosus, lymphoma, leukemia, rheumatoid arthritis, HIV infection (in 20–44% of AIDS patients), infectious mononucleosis, associated with autoimmune thrombocytopenia and/or AIHA
      ix. Autoimmune lymphoproliferative syndrome
      x. Hypersplenism

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[a]*Chronic, mild with a benign course.*

Congenital Severe Neutropenia Incidence

The incidence of SCN is two per million population

Pathogenesis and Genetics

- In vitro bone marrow studies show a reduced number of granulocyte-macrophage colonies in SCN patients.
- There is also a reduced number of CD341/Kit1/G-CSFR1 myeloid progenitor cells in the bone marrow.
- Neutrophil elastase gene (ELANE) mutations: It has been hypothesized that mutations of ELANE in SCN result
- in a high rate of premature apoptosis in neutrophil precursors, which results in decreased myelopoiesis.
- Neutrophil elastase is a serine protease localized in the granules of neutrophils and monocytes.
- A mutant enzyme has a dominant negative effect on the normal wild-type elastase.
- This explains the defective proteolysis in the SCN neutrophils even though half of the normal amount of the elastase is present in the neutrophils of these patients.

Clinical Manifestations and Laboratory Investigations

- During the first year of life omphalitis, otitis media, upper respiratory tract infections, pneumonitis, skin abscesses, and liver abscesses occur commonly with positive cultures for staphylococci, streptococci, Pseudomonas, Peptostreptococcus, and fungi. Splenomegaly may be present. Other manifestations include the following:

- Blood counts reveal a normal WBC with an ANC less than 200/mm3 and a compensatory eosinophilia and monocytosis. Mild anemia and thrombocytosis may be present.

- Bone marrow examination shows a maturation arrest of myelopoiesis at the promyelocyte or myelocyte stage with marked paucity of mature neutrophils. There is an increase in monocytes, eosinophils, macrophages, and plasma cells.

Investigations of Patients with Neutropeniaa

1. History of drug ingestion, toxin exposure, infectious history
2. Physical examination—nature of infectious lesions, growth and development, presence of anomalies, presence of enlarged lymph nodes or hepatosplenomegaly
3. Familial: absolute neutrophil count in family members
4. Complete Blood Count (CBC)—with differential and platelet count, absolute neutrophil count and reticulocyte count; CBC and differential three times per week for 6–8 weeks (to exclude cyclic neutropenia)
5. Bone marrow
   a. Maturation characteristics of myeloid series; there is a reduction in mature neutrophils
   b. Maturation and number of megakaryocytes and erythroid precursors
   c. Karyotype (to identify myelodysplasia or acute myelocytic leukemia) and FISH studies for chromosome 7 and 5q
   d. Electron microscopy (subcellular morphology, congenital dysgranulopoiesis)
6. Detection of antineutrophil antibodies (see text for details)
   a. Granulocyte immunofluorescence test (GIFT)
   b. Granulocyte indirect immunofluorescence test (GIIFT)
   c. Granulocyte agglutination test (GAT)
   d. Enzyme-linked immunoassay (ELISA)
   e. Monoclonal antibody-specific immobilization of granulocyte antigens (MAIGA)
7. Immunologic tests
   a. Immune globulins (IgA, IgG, IgM, IgE)
   b. Cellular immunity (skin-test activity, purified protein derivative (PPD), lymphocyte subsets; suppressor T-cell assay)
   c. Antinuclear antibodies, C_3, C_4, CH_{50}
8. Evidence of metabolic disease
   a. Plasma and urine amino acid screening
   b. Serum vitamin B_{12}, folic acid, and copper
9. Evidence of pancreatic disease
   a. Exocrine pancreatic function: stool fat, pancreatic enzyme assays, CT scan of pancreas for pancreatic lipomatosis, serum levels of trypsinogen and isoamylase
10. Chromosomal breakage analysis (Fanconi anemia)
11. Radiographic bone survey (cartilage hair hypoplasia, Shwachman–Diamond syndrome, Fanconi anemia)
12. Serum muramidase (ineffective myelopoiesis)
13. Flow cytometry for CD59 (or other GPI linked protein) (paroxysmal nocturnal hemoglobinuria)
14. Bone density studies (14% of patients with chronic neutropenia show nonclinical osteoporosis or osteopenia)
15. Many gene mutation analyses are commercially available including: neutrophil elastase (*ELANE*) (SCN and cyclic neutropenia), *GFI-1* (SCN), *WAS* (X-linked neutropenia), *SBDS* (Shwachman–Diamond syndrome), *HAX 1*, *TAZ* (Barth syndrome), Fanconi family of genes, *CHS1* (Chediak Higashi syndrome) and others that are continually being discovered.

[a] *Absolute neutrophil count less than 1500/mm^3.*

Clinical Manifestations and Laboratory Investigations

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Treatment

- G-CSF: The initial dose of G-CSF employed is 5 μg/kg/day. Response occurs 710 days from the start of treatment.
- The goal of therapy is to achieve an ANC of approximately 1000-1500/mm3 and maintain the patient free of infections.
- More than 95% of patients with SCN will respond to G-CSF.
- After beginning G-CSF therapy, the dose should be adjusted up or down at 1- to 2-week intervals until the lowest effective dose is reached.

Treatment

- Baseline bone marrow cytogenetics should be obtained prior to G-CSF therapy. Initial cytogenetic studies at diagnosis are usually normal. However, during the course of the disease, clonal abnormalities may emerge, 50% of which are monosomy-7. Since 12% of patients with SCN develop MDS and/or acute myelogenous leukemia (AML), it is important to perform periodic bone marrow examinations for morphology and cytogenetic studies in the follow-up of these patients. Patients who require higher doses of G-CSF (more than 8 μg/kg/d) are at higher risk to develop MDS/AML than those who are more G-CSF responsive (40 vs 11% after 10 years of therapy).

Treatment

- The G-CSF receptor is normal in patients with SCN. However, patients with SCN are predisposed to develop acquired (somatic) mutations of the cytoplasmic domain of the G-CSF receptor. There is a good correlation between the development of leukemia/MDS and the acquisition of G-CSF receptor mutations in these patients. The time interval between these two events varies considerably.

- Hematopoietic Stem Cell Transplantation (HSCT) The following are the indications for HSCT:

- Patients who require greater than B8 μg/kg/day of G-CSF are statistically much more likely to succumb to
- infection or leukemia.

- Refractoriness to G-CSF treatment.

- Emergence of MDS/AML.

- HSCT can be considered as treatment for all patients with SCN who have a HLA-matched sibling donor available with matched unrelated transplant reserved for high-risk patients.

Complications associated with G-CSF

- Complications associated with the use of G-CSF include: bone pain, splenomegaly, hepatomegaly, thrombocytopenia, osteopenia/osteoporosis, HenochSchonlein purpura type of immune-complex-induced vasculitis of the skin, and/or glomerulonephritis.

Prognosis

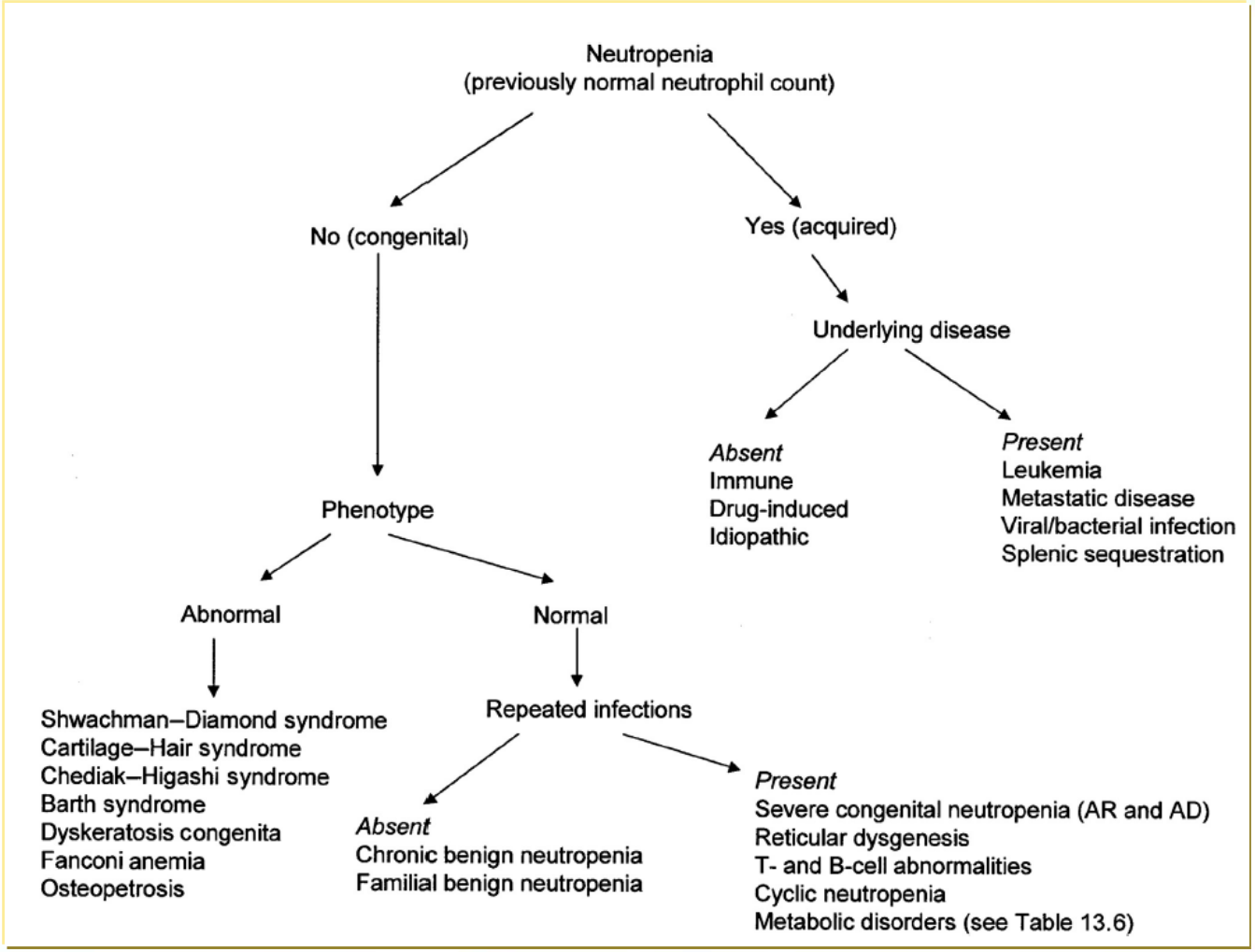
- The leading causes of death in SCN are infection and MDS/leukemia.

- Patients with severe chronic neutropenia should be registered with the Severe Chronic Neutropenia International Registry. This registry collects and maintains long-term outcome data as well as providing resources for physicians, patients, and families.

Reticular Dysgenesis

- Reticular dysgenesis is a disorder of stem cells in which maturation of both myeloid and lymphoid lineages is defective. Platelet and red cell production are normal. Affected individuals have severe neutropenia and moderate to severe lymphopenia. In addition, there is absence of peripheral lymphoid tissues, Peyer's patches, tonsils, and splenic follicles. The mortality rate is high from infection at an early age. Treatment: HSCT can be curative.

Approach to diagnosis of neutropenia



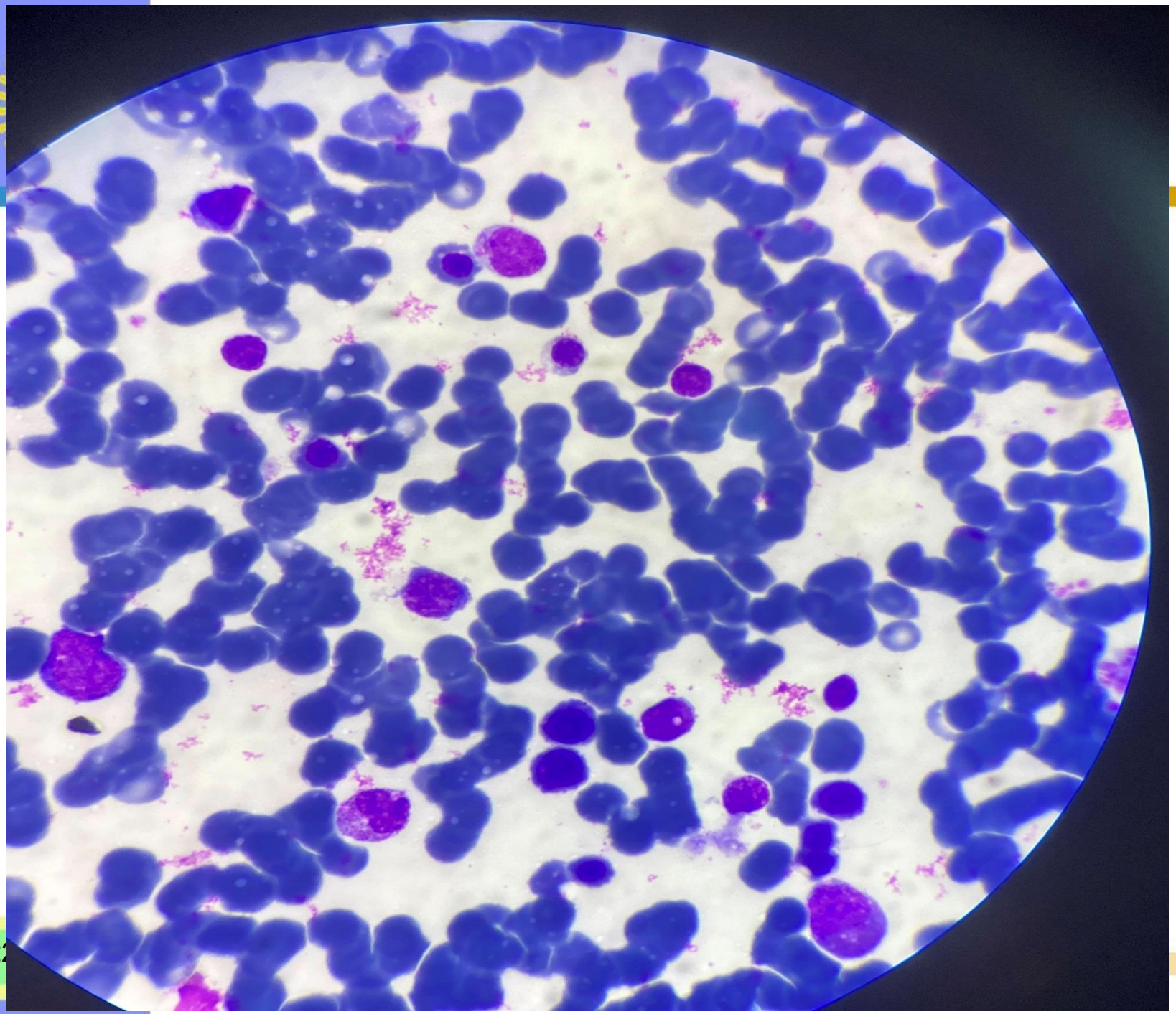
severe congenital neutropenia and kostmann syndrome

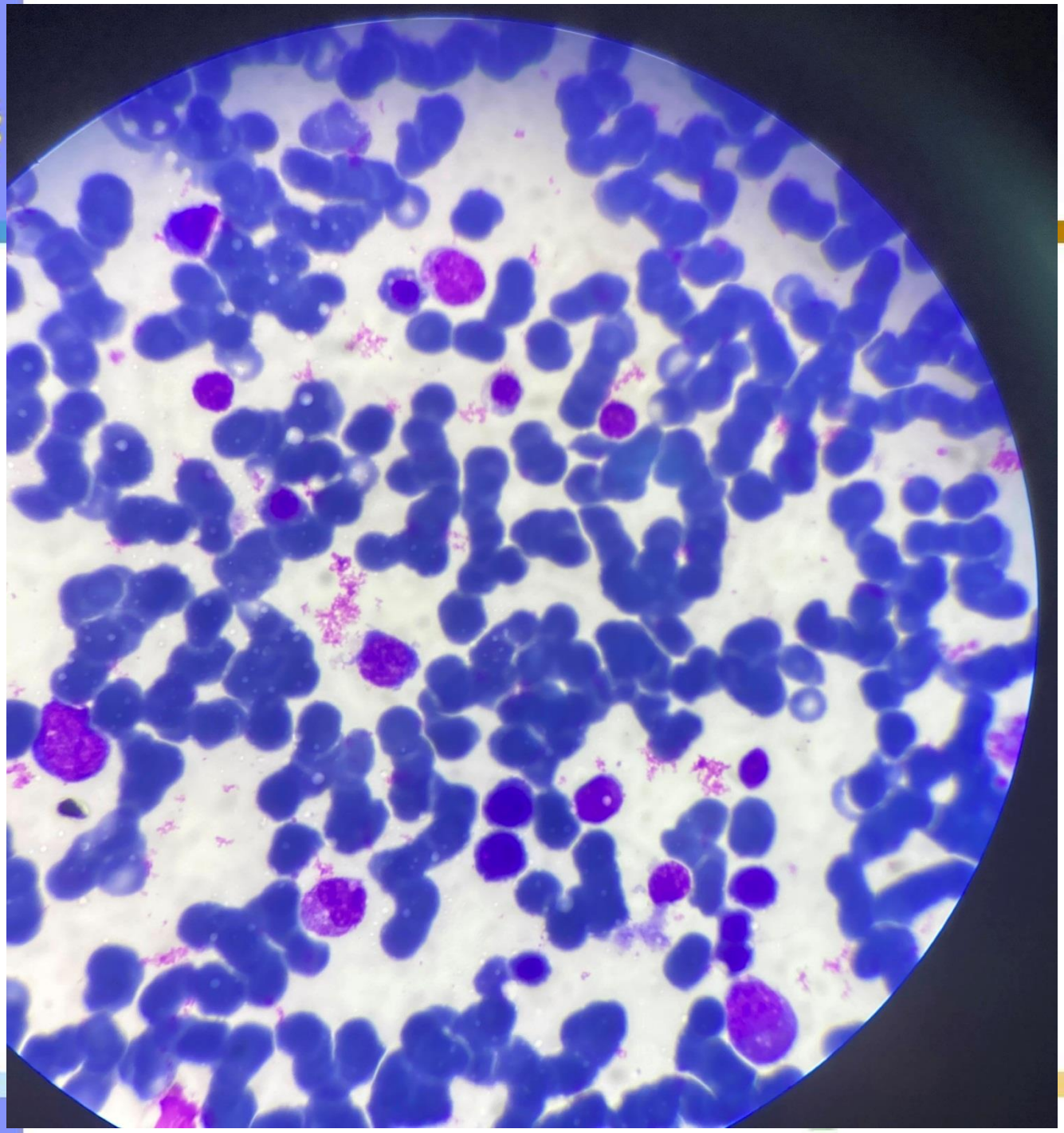
- Severe congenital neutropenia (SCN) includes a heterogeneous group of disorders with different patterns of inheritance.
- Kostmann syndrome (KS) follows an autosomal recessive pattern of inheritance.
- Its underlying genetic defect is due to homozygous mutations in the HAX1 gene on chromosome 1.
- Other SCN may follow autosomal dominant or sporadic patterns of inheritance.
- In this group of patients, ~ 60% have diverse mutations in the neutrophil elastase gene (ELANE).
- These mutations affect only one allele.
- The majority of patients present with a sporadic pattern, since autosomal dominant inheritance is relatively more lethal. In some patients, there may be germline mosaicism.

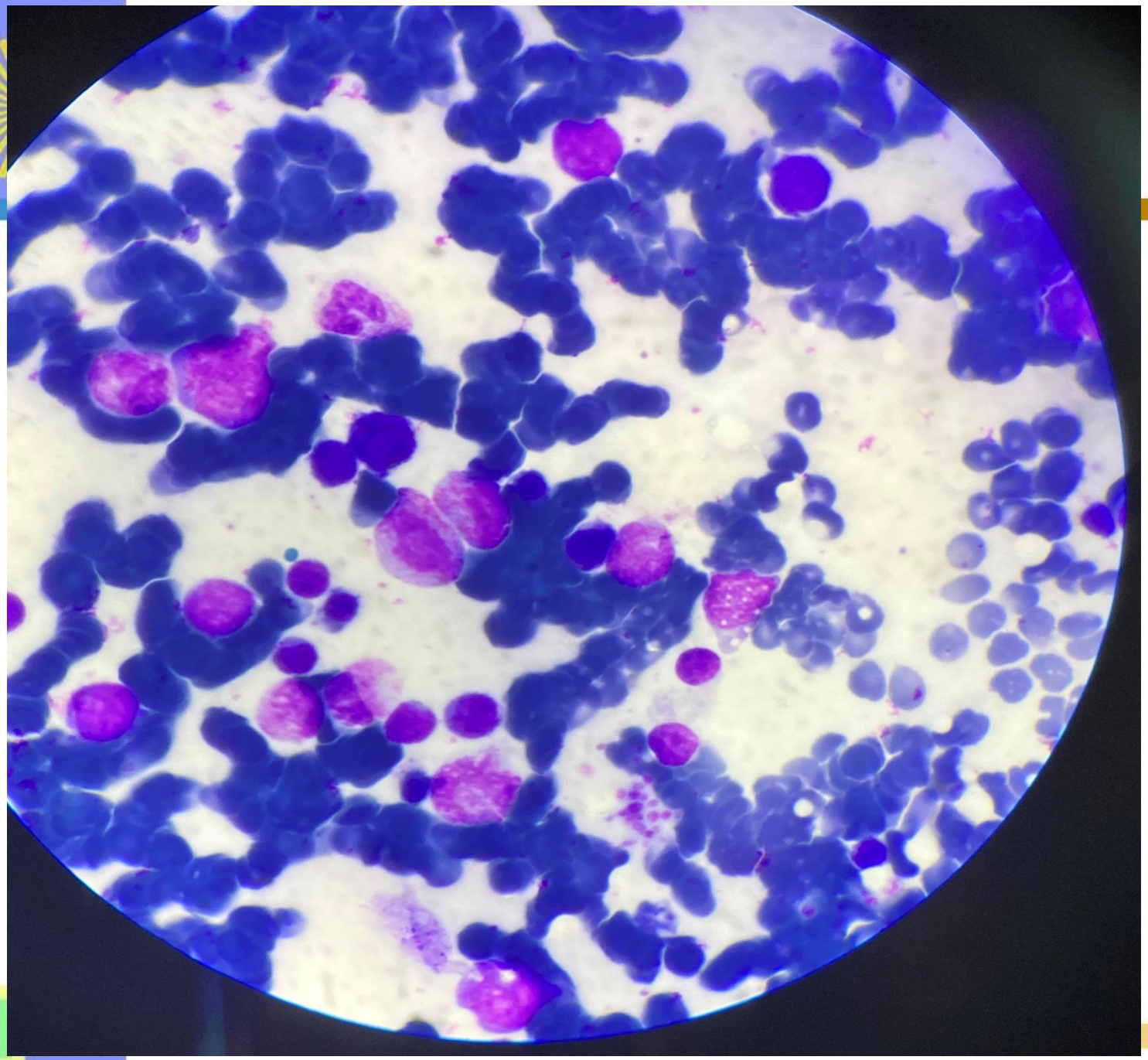
A Case Report of Severe Congenital Neutropenia

- بیمار جوان ۱۷ ساله ای است که جهت پیوند با سلول های بنیادی خونساز در بخش پیوند بیمارستان کودکان امیرکلا بستری شده است.

- تاریخچه:
  - بیمار از دوران شیرخوارگی پس از واکسیناسیون BCG دچار آبسه ناحیه تزریق شده بود که با آنتی بیوتیک تراپی طولانی بهبود یافت.
  - بیمار به طور مکرر دچار عفونت های دستگاه تنفس و گاستروانتریت می شده است که چندین نوبت سابقه بستری داشته است.
  - در سن ۴ سالگی بعلت تب طولانی بستری شد که علت آن نوتروپنی شدید بوده است که با آنتی بیوتیک تراپی و تزریق G-CSF بهبود یافت و از بیمارستان مرخص شد.
  - یک ماه پس از ترخیص جهت کنترل با CBC, diff انجام شده مجدداً نوتروپنی شدید داشت که جهت تشخیص قطعی تحت پونکسیون مغزاستخوان و بیوپسی مغزاستخوان قرار گرفت. که در پونکسیون مغزاستخوان تعداد نوتروفیل ها و باند و متاییلوسیت کاهش شدید داشته است.
  - بیمار تحت بررسی از نظر علل مختلف نوتروپنی از جمله بیماری های اختلالات دیگر سیستم ایمنی و بیماری های متابولیک ارثی و عوارض دارویی و عفونی و بیماری های کلاژن واسکولار و ... قرار گرفت که نهایتاً علل اتیولوژیک خاصی یافت نشد.
  - برای بیمار با تشخیص نوتروپنی شدید بدون علت خاصی G-CSF منظم و هفتگی شروع شد.







جناب آقای دکتر تمدنی ، استاد گرامی

در بازبینی لام آسپیره مغز استخوان آقای مهدی حسنجان تبار ،

در رده نوتروفیل، مقدار سگمنت و باند و تا حدودی متامیلوسیت، کاهش دیده می شود.

افزایش رده ائوزینوفیل به چشم می خورد.

افزایش رده بازوفیل نیز وجود دارد.

تغییر واضحی در رده لنفوئید دیده نشد.

ارادتمند- دکتر پورنصراله

- فاصله تزریق G-CSF وقتی به دو هفته تغییر یافت مجدداً بیمار دچار نوتروپنی شدید شد.
- بیمار با تزریق G-CSF هفتگی و سپس دو بار در هفته نسبتاً کنترل شد.
- تزریق G-CSF سال ها ادامه پیدا کرد تا نهایتاً تعداد تزریقات در هفته به سه بار افزایش یافت.
- درد استخوانها ناشی از تزریق G-CSF و شدت کاهش پلاکت نیز ایجاد شد.
- نهایتاً با توجه به عوارض درمان با G-CSF و خطر بروز بدخیمی های هماتولوژیک با توجه به داشتن دهنده مناسب پیوند که خواهر بیمار بود و از نظر HLA- typing کاملاً یکسان با بیمار بوده است تصمیم به Allogeneic H.S.C.T با رژیم ایندااکشن ATG + fludarabine + Busolfan انجام شد.
- بیمار سه هفته بعد از پیوند با کایمریسم بیش از ۹۰٪ و با حال عمومی خوب و سلولاریتی نرمال مغزاستخوان مرخص شد.
- بیمار مدت ۴ سال است که پس از انجام پیوند کاملاً سالم و هیچ مشکل هماتولوژیک ندارد.


Thanks for your attention