Literature Review

Diagnosis and Management of Children with Chronic Neutropenia

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Background

Single cytopenia, a bone marrow failure that apparently involves only one cell line, is often observed in pediatric patients. An absolute decrease in the number of peripheral mature segmented polymorphonuclear and band form of neutrophils defined as "Neutropenia" which could be due to a disturbance of bone marrow productivity, pooling of neutrophils in the tissue, utilization or destruction of circulating neutrophils, or from combined causes. Normal neutrophil levels should be stratified for age group. The lower limit for absolute neutrophil count (ANC) including mature segmented polymorphonuclear and band cells is 1,000/μL during infancy and an average of 1,500/μL after 1 year of age.[1] Severity of neutropenia may be classified as mild, moderate and severe with neutrophil counts of 1,000 to 1,500/μL, 500 to 1,000/μL, and fewer than 500/μL, respectively. Chronic neutropenia is only reserved for patients with persistent neutropenia for more than 6 months. Endogenous bacteria are the most common cause of infections presenting as cellulitis, abscess, stomatitis, otitis media, peri-rectal inflammation, pneumonia and septicemia, which are increased mostly in patients with severe chronic neutropenia (SCN). Through discussion in this review, the intrinsic etiologies of neutropenia will be focused. The acquired conditions resulting from exogenous factors such as drugs, infections, or immune processes are also listed in table 1. Among these acquired causes, only chronic idiopathic neutropenia and autoimmune neutropenia will be discussed in detail.

Table 1. Common etiologies of chronic neutropenia in children[1]

Neutropenia caused by intrinsic defects in granulocytes or their progenitors
○ Cyclic neutropenia
○ Severe congenital neutropenia (SCN or Kostmann disease)
○ Shwachman-Diamond syndrome (SDS)
○ Chediak-Higashi syndrome (CHS)
Neutropenia caused by extrinsic factors
○ Infection
○ Drug-induced neutropenia
○ Autoimmune neutropenia (AIN)
○ Chronic idiopathic neutropenia (CIN)
○ Metabolic diseases
○ Nutritional deficiencies
○ Bone marrow infiltration

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Etiologies of neutropenia

Various intrinsic defects of stem cells committed to myeloid development can lead to several conditions associated with severe neutropenia. Common etiologies of isolated neutropenia are shown in table 1. Most recent discoveries in this decade have highlighted mechanisms, clinical consequences, courses and managements of chronic idiopathic and severe congenital neutropenia. Furthermore, for cyclic neutropenia, ultrastructural studies have been conducted to investigate the apoptotic mechanism and survival of neutrophil precursors for a better understanding of its pathogenesis.

Cyclic neutropenia

Cyclic neutropenia is an autosomal dominant disorder characterized by periodic oscillations in the number of circulating neutrophil counts by approximately every 21 days.[2-3] To ascertain the diagnosis of cyclic neutropenia, two or three times a week of neutrophil counts have to be monitored. During neutropenic period, local or systemic bacterial infections can be observed as well as non-specific clinical features of neutropenia, for instance, fever, malaise, oral ulcers, and lymph node enlargement. Severity of infections occurs in parallel with severity of neutropenia. However, older children tend to be less susceptible to severe infection compare to a younger age group. From literature reviews, pneumonia and sepsis, as well as peritonitis, are the most common causes of death in cyclic neutropenia. Bone marrow aspirations and trephine biopsies in cyclic neutropenia patients reveal varying degrees of maturation arrest depending on the time when the tests have been performed. For example, during nadir period, mature segmented neutrophils are sparsely detected in the bone marrow. But only few days later, neutrophil precursors recover spontaneously with higher peripheral neutrophil counts.[4-6]

Recent studies have demonstrated that apoptosis is a regulatory mechanism in hematopoietic derangement, marrow oscillation and progression to myelodysplastic syndrome (MDS).[7-9] Survival of the bone marrow-derived progenitor cells has been documented to be severely shortened in cyclic neutropenia patients. However, this abnormality is partially corrected by *in vivo* administration of granulocyte colony-stimulating factor (G-CSF).[10] G-CSF that acts as an anti-apoptotic factor may take some roles to improve neutrophil progenitor cell survival.

From positional cloning studies, the specific locus of cyclic neutropenia was mapped to chromosome 19p13.3. This region contains the genes for three neutrophil proteases including azurcidin, proteinase 3, and neutrophil elastase.[11] Mutations of neutrophil elastase (*ELA2*), a serine protease, have been identified as a basic genetic abnormalities causing ineffective neutrophil production. *ELA2* mutations are speculated as the reason for premature death of neutrophil progenitors.

Prevention of prolonged and severe neutropenia with G-CSF and treatments of infections are two major approaches of cyclic neutropenia management. An initial recommended G-CSF dose in cyclic neutropenia is 1-3 μg/kg/day to maintain an average ANC of 1,500/μL. However, an oscillation of ANC in cyclic neutropenia is continued to be observed despite G-CSF therapy. Therefore, the Severe Chronic Neutropenia International Registry (SCNIR) experts suggested multiple checking of neutrophil counts to make G-CSF dose adjustment at 2-4 week intervals rather than only single measurement. Due to the lower doses of G-CSF are required in cyclic neutropenia patients comparing with severe congenital neutropenia (SCN) or Shwachman-Diamond syndrome (SDS), the risk of hematologic malignancies is smaller as of 1% from a 10-year follow-up report by SCNIR.[12]

Severe congenital neutropenia (SCN or Kostmann disease)

SCN is characterized as a life-long severe neutropenia with neutrophil counts under 500/μL and usually below 200/μL. Before G-CSF treatment era, most of affected patients died from recurrent and severe bacterial infections during infancy and early childhood. Bone marrow examination in SCN shows a typical arrest of neutrophil maturation at the promyelocyte or myelocyte stage with a remarkable depletion of band and mature neutrophils.

SCN is a heterogeneous genetic disorder. It can manifest as both a recessive or dominant inheritance. *ELA2* mutations are also found in approximately 60% of cases, particularly those with autosomal dominant form and sporadic SCN. In the remaining 30% of cases, which are autosomal recessive SCN, HS-1-associated protein X (*HAX1*) gene mutations are responsible.[13-16] *HAX1* is a mitochondria-targeted protein, which is acting as an inner mitochondrial membrane potential keeper. Without *HAX1*, apoptosis in myeloid cells is enhanced.[17-18] More recent data also described antibacterial protein including lactoferrin, cathepsin G, myeloperoxidase, elastase and alpha-defensin deficiencies in SCN patients.[19] In peripheral blood-derived neutrophils of *ELA2*-mutated patients who have been treated with G-CSF, neutrophil elastase is nearly absent. This suggests that the treatment with G-CSF is not sufficient to correct all the functional deficiencies of neutrophils.[20]

The empiric use of G-CSF for the treatment of SCN has changed clinical outcomes of this condition. Infection episodes have been reduced dramatically by means of increasing ANC in the majority of patients.[21-22] Unfortunately, MDS or acute myeloid leukemia (AML) is observed in SCN patients who receive G-CSF. Particularly, children with SCN who require large doses of G-CSF (> 8 mg/kg/day) to achieve optimal ANC values are reported as a higher risk group to develop MDS/ AML. A report of Severe Chronic Neutropenia International Registry (SCNIR) showed that the risk of hematologic malignancies was approximately 13% during a follow-up period of 8 years.[23-24] In spite of dismal outcome of malignant transformation, G-CSF is currently used as a standard treatment for SCN which has improved survival and quality of life of patients.[25-26] From SCNIR recommendation, G-CSF should be started at a dose of 3-5 μg/kg/day and gradually increased 5-10 μg/kg every 2 weeks until achieving ANC of 1,000-2,000/μL. While on treatment, peripheral blood count should be monitored regularly as well as annual bone marrow examination. In SCN patients who require G-CSF doses of over 100 μg/kg/day or refractory cases, hematopoietic stem cell transplant (HSCT) should be considered.

Shwachman-Diamond syndrome (SDS)

Neutropenia is the most common hematological presentation in SDS. Other features include skeletal defects (metaphyseal dysostosis and epiphyseal dysplasia) and pancreatic insufficiency.[27-30] A recent report also shows learning difficulties and impaired psychological development in affected children. In addition to neutropenia, which is seen in more than 80% of patients, impaired neutrophil motility, migration, and chemotaxis were reported in patients with SDS resulting in severe recurrent bacterial infections. Mild normochromic normocytic anemia and thrombocytopenia are usually observed. Similar to other bone marrow failure syndrome, MDS/AML have also been reported with the incidence of AML transformation of 5% in children and 25% when reaching adulthood.[31-32] Furthermore, severe aplastic anemia has been observed during follow-up.

Exocrine pancreatic failure due to severe lacking of acini with fatty replacement was detected in SDS.[33-34] Steatorrhea is noted in 50% and 90% of affected infants at the age of 6 months and 1 year, respectively. In contrast to cystic fibrosis which is the most common cause of exocrine pancreatic insufficiency in children, pancreatic function of SDS often improves with age. Seventy-two hours stool fat collection is a helpful test apart from screening microscopic stool examination for fat globules. Other screening tests for pancreatic insufficiency, such as fecal chymotrypsin may be unreliable in the presence of diarrhea. In that circumstance, fecal elastase is claimed to be a better screening test. For imaging studies, CT or ultrasonography reveals an abnormally small size of pancreas that composes mainly of fat without evidence of pancreatic endocrine dysfunction.[35] Obviously high liver transaminases were found in 60% of SDS cases with 15% of hepatomegaly probably from fatty liver. In SDS patients with untreated pancreatic insufficiency, abnormalities of fat soluble vitamins and vitamin K dependent clotting factors are detectable.

The primary skeletal defects of SDS are associated with

an impaired growth plates and metaphysis development. Most patients display delayed bone age and progressive bone deformities particularly vertebral kyphosis and scoliosis. In approximately 40%-80% of SDS patients, metaphyseal chondrodysplasia is observed by plain x-ray but this is often asymptomatic.[36] Other bone abnormalities include thoracic cage defects, syndactyly, and clinodactyly. Incidences of osteoporosis, osteomalacia and pathological fractures are increased in patients with SDS secondary to impaired vitamin D and K absorption from exocrine pancreatic dysfunction.[37]

SBDS (Shwachman-Bodian-Diamond syndrome) gene alteration on chromosome 7 (7q11) was identified in more than 90% of SDS patients.[38-39] This *SBDS* gene encodes a 250 amino acid protein, which is present in pancreas, bone marrow, and leucocytes. Although function of this protein remains unknown, studies in yeast homologues suggest its possible role in ribosomal RNA processing.[40-41]

Management of SDS patients should be under multidisciplinary concept including broad-spectrum antibiotics for infections, annual bone marrow examination for MDS/AML surveillance, pancreatic enzyme supplements, high calorie diet for catching up growth, and supplementation of fat soluble vitamins. In previous studies, required enzyme supplements usually decline with age and, thus, 50% of SDS children can achieve near normal fat balance by 4 years of age. The most concerned sequelae of SDS are MDS/AML transformation because SDS leukemia has a poor prognosis.[42] HSCT is considered as the only definitive treatment to prevent clonal progression to MDS/AML. Although overall outcomes of HSCT in SDS patients are similar to other bone marrow failure syndrome, transplant related toxicity among SDS patients is more commonly observed.[43]

Chediak-Higashi syndrome (CHS)

CHS is a rare autosomal recessive disease in children characterized by manifestations of abnormal granules in most leukocytes.[44] Other clinical features include pigmentary dilution resulting in pseudoalbinism, increased susceptibility to bacterial infection, an accelerated phase accompanied by pancytopenia, recurrent severe bacterial infection, lymphadenopathy, hepatosplenomegaly and a lymphoreticular infiltrative disease resembling hematologic malignancy.[45-46] Peripheral and cranial neuropathies which associated with decussation defects at the optic chiasm were also reported in patients with CHS.

Most patients with CHS have moderate neutropenia due to ineffective granulopoiesis. Notably, specific systemic disorders and complications during the accelerated phase can distinguish CHS from other causes of neutropenia. Demonstration of giant granules in peripheral blood mature granulocytes and myeloid progenitors in bone marrow are the key findings in CHS leading to *CHS1* gene mutation study.[47-50]

In the stable phase, treatment basis of CHS focuses on prophylactic administration of co-trimoxazole. During infectious complications, empirical intravenous antibiotics should be commenced without delay. Again HSCT is the only curative treatment and should be considered before or at the beginning of accelerated phase.[51] Without HSCT, most patients with CHS decease from bleeding diathesis, infection or complications of the accelerated phase during the first or second decade of life. HSCT prevents recurrence of accelerated phase in a report of 10 CHS cases post-HSCT. On the other hand, ocular and cutaneous albinism is not corrected after HSCT.[52]

Autoimmune neutropenia (AIN)

Autoimmune neutropenia may be associated with collagen vascular disorders, drug ingestion, viral infection, or malignancy. These are usually referred to as secondary immune neutropenia. The remaining patients without underlying factors are recognized as chronic benign neutropenia. Due to the peak incidence of AIN presents during infancy, this is so called autoimmune neutropenia of infancy.[53-54] Most of the children with AIN experience minor infections for example otitis media, gingivitis, and respiratory tract or skin infections. The median age of

diagnosis was 8 months and the ANC was usually less than 500/μL with normal total white blood cell count.[55] A longitudinal study showed that 95% of infants with AIN recovered from neutropenia by 4 years of age.[56] A transient increase of ANC from less than 500/μL to more than 1,500/μL was observed during an infectious event.

To exclude other causes of neutropenia, bone marrow examination should be performed. An active picture of increased myeloid to erythroid ratio with no abnormalities of cell morphology is the major finding in AIN. Granulocyte-specific antibodies detected in AIN cases are often the IgG class but may occur together with IgM. If the first investigation is negative, repeated antibody testing with additional blood samples should be carried on up to 3 times at 2-4 weeks interval. The battery of granulocyte-specific antibodies testing comprises granulocyte immunofluorescence (GIFT) and granulocyte agglutination test (GAT) with the 4 panel cells typed for NA1, NA2, NB1, and 5b antigens. Furthermore, nongranulocyte-specific antibodies such as HLA antibodies should also be excluded using lymphocyte immunofluorescence or lymphocytotoxicity to minimize its interference.[57-58]

Ninety percent of AIN cases with infections are well controlled with antibiotics without late complications. Prophylactic cotrimoxazole is usually initiated after an infectious episode. Corticosteroids and G-CSF given during infections or before elective surgery can increase ANC for a longer period than high-dose intravenous immunoglobulin infusion. However, because AIN is a benign and self-limiting condition, corticosteroids or G-CSF should be cautiously used only after infections occur.

Chronic idiopathic neutropenia (CIN)

Chronic idiopathic neutropenia (CIN) was defined as persistent unexplained reduction in ANC values below 1,800/μL for Caucasians and 1,500/μL for African-American for more than 3 months.[59-61] This condition has been considered as an acquired and benign with mildly or moderately low ANC when compared with SCN. After ruling out any associated underlying disease that can cause neutropenia, history of radiation, chemotherapy or drug exposure also has to be excluded. However, lymphopenia, mild anemia, or thrombocytopenia occasionally found in 37%, 14%, and 10%, respectively in patients with CIN. This is mainly explained by an increased production of TGF-beta1 in bone marrow.[62-63] On the other hand, normal bone marrow examination and negative results of antineutrophil antibodies tests are the prerequisites in most cases.[64] At least two antineutrophil antibodies test methods consisting of GAT and GIFT should be performed repetitively to exclude AIN. The differentiating feature of CIN from cyclic neutropenia and SCN is the absence of *ELA2* gene mutation.

Inflammation of bone marrow milieu has shown to have a major contribution to pathophysiology of CIN. Both myelosuppressive properties of activated T-lymphocytes and pro-apoptotic mediators including IFN-gamma, TNF-alpha, fas-ligand, TGF-beta1 lead to early apoptosis of granulocytic progenitor cells.[65-67] Nonetheless, IL-10 reduction can also disturb the balance between survival and pro-apoptotic mediators in CIN.[68]

CIN shares pathophysiology with other acquired bone marrow failure syndromes but presents with mild to moderate neutropenia in the majority of cases. Therefore, severe infection is unlikely to be observed. Accordingly, antibiotics and G-CSF are seldom required in CIN patients with the estimated utilization rate of 1.9% per year.[69] Unlike SCN, development of MDS/ AML is rare in CIN. From the large cohort, the overall incidence of AML transformation was 1.6%.

Summary

Complete history taking and physical examination to evaluate underlying conditions, drug exposure, phenotypic abnormalities, and current infections should be performed in patients with neutropenia. Furthermore, family history of an unexplained death in young children should be asked. The major features of each disorder with chronic

neutropenia discussed in this chapter are summarized in table 2.[70] In patients with asymptomatic isolated neutropenia, the follow-up for clinical evaluation and repeated ANC are the recommended approaches. On the other hand, appropriate culture and antibiotics should be started promptly in case of infections. In patients with persistent neutropenia, three times weekly complete blood count for 6-8 weeks should be observed for oscillation pattern in cyclic neutropenia, as well as antineutrophil antibody tests for autoimmune neutropenia. To rule out intrinsic bone marrow failure syndrome and hematologic malignancy, bone marrow aspiration and trephine biopsy with cytogenetics and flow cytometry should be performed in patients with persistent neutropenia particularly when other cell lineages also reveals abnormalities. Other relevant investigations such as radiographic study of bone, stool fat, and mutation study should be considered when particular disorders are suspected.

Patients with severe neutropenia have a very high risk for developing life-threatening infections. Parenteral broad-spectrum antibiotics as in-patients are usually required together with close monitoring. For prolonged febrile neutropenic patients (fever more than 7 days) after antibiotics, fungal culture and intravenous amphotericin

Table 2. Major features of hematologic disorders presenting with chronic neutropenia

Features	Cyclic neutropenia	SCN	SDS	CHS	AIN	CIN
Median age at diagnosis (y)	1.5	3	1	2.6	0.7	0.9
Male (M) or female (F) predominance	F	M	M	F	F	F
Co-physical morbidities	No	Hoyeraal-Hreidarsson syndrome *	Short stature, metaphyseal dysostosis, thoracic deformity, pancreatic insufficiency	Oculocutaneous albinism, cranial and peripheral neuropathies	No	No
Aplastic anemia	No	No	Yes	No	No	No
MDS/AML risk	Low	High	High	High	No	No
Median age of cancer (y)	-	14	18	5	-	-
Projected median survival age (y)	Normal	50	35	15	Normal	Normal
Gene Mutation	*ELA2*	*ELA2, HAX1*	*SBDS*	*CHS1*	-	-
Special tests and positive findings	-	-	Malabsorption, pancreatic exocrine insufficiency, bone radiography	Giant granules in peripheral blood and bone marrow granulocytes	GIFT/ GAT	-

* *Hoyeraal-Hreidarsson syndrome: Prenatal growth retardation, progressive pancytopenia, cerebellar hypoplasia, microcephaly and developmental delay*

B are also suggested. G-CSF was discussed previously with the benefit of increasing ANC during infections in AIN and CIN. As prophylactic use, G-CSF is highly recommended in SCN and cyclic neutropenia with excellent results in reducing morbidity and mortality. However, the incidence of MDS/AML has been escalated since G-CSF era.

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