

Neutrophil Abnormalities

Laurence A. Boxer, MD*

Objectives After completing this article, readers should be able to:

1. Name the most common cause of neutropenia in a 5-year-old child.
2. Characterize cyclic neutropenia.
3. Name the most common presenting feature of a patient who has severe chronic neutropenia.
4. Explain the risks of viral-induced neutropenia.
5. Describe the infections to which patients who have chronic granulomatous disease are susceptible.

Introduction

The differential diagnosis for a patient presenting with recurrent infections is challenging, given the complexity of the immune system. Similarities in the clinical presentation of neutrophil, antibody, and complement disorders can prove difficult for the physician attempting to establish a diagnosis. Infants and children who are brought to the pediatrician for “repeated infections” must be evaluated carefully. Often these patients ultimately have no identifiable underlying disease, but frequently they have respiratory allergy or other risks for recurrent infection (Table 1). Most patients who have recurrent infections do not have an identifiable phagocyte defect or immunodeficiency. Given the low probability of identifying a discreet immune defect, the physician faces the difficult decision of which patients merit a complete evaluation.

In general, evaluations should be initiated for those who have had at least one of the following clinical features within a 1-year period: 1) more than two systemic bacterial infections (eg, sepsis, meningitis, osteomyelitis); 2) serious respiratory infections (eg, pneumonia, sinusitis); 3) bacterial infections (eg, cellulitis, draining otitis media, lymphadenitis); 4) the presence of an infection at an unusual site (eg, hepatic or brain abscess); 5) infections caused by unusual pathogens (eg, *Aspergillus* pneumonia, disseminated candidiasis, infection with *Serratia marcescens*, *Nocardia* sp, *Burkholderia cepacia*); 6) infections of unusual severity; and 7) chronic gingivitis and recurring aphthous ulcers.

Once the decision is reached that a phagocyte evaluation is warranted, a thorough clinical history, physical examination, and laboratory testing for immunodeficiency (Table 2) should provide the diagnosis and help to formulate an appropriate therapeutic plan.

Quantitative Disorders of Neutrophils

Absolute neutrophil counts (ANCs) vary widely in healthy individuals. The relative proportion of neutrophils and lymphocytes in the blood changes with age. Neutrophils predominate at birth, but decrease rapidly in the first few days of life. During infancy, they constitute 20% to 30% of the circulating leukocyte populations. Approximately equal numbers of neutrophils and lymphocytes are found in the

Abbreviations

AIN:	autoimmune neutropenia
AML:	acute myelogenous leukemia
ANC:	absolute neutrophil count
CBC:	complete blood count
CGD:	chronic granulomatous disease
DHR:	dihydrorhodamine
HLA:	human leukocyte antigen
Ig:	immunoglobulin
MDS:	myelodysplasia
NBT:	nitroblue tetrazolium
O₂⁻:	superoxide anion
rhG-CSF:	recombinant human granulocyte colony-stimulating factor
SCN:	severe congenital neutropenia

*Director, Pediatric Hematology/Oncology, University of Michigan and Women's Hospital, Ann Arbor, MI. Dr. Boxer receives industrial funding from Amgen.

Table 1. Causes of Infection in Patients Who Have No Primary Immunodeficiency Syndromes

- Alteration of mucocutaneous barrier
- Aspirated pulmonary foreign body
- Inhalation therapy
- Surgical wounds
- Fistula-sinus communications
- Intravenous drug abuse
- Prosthetic devices
- Chronic disease (eg, malnutrition, cystic fibrosis, diabetes mellitus, nephrotic syndrome, uremia, cirrhosis, prolonged broad-spectrum antibiotic therapy, spinal cord injury, sickle cell anemia, congenital heart disease, urinary tract anomaly, dysmotile cilia syndrome, eczema, protein-losing enteropathy, periodontitis, chronic blood product transfusion of transmitted viral and parasitic disease)

peripheral circulation by the time the child reaches about 5 years of age, and the characteristic 70% predominance of neutrophils that occurs in adulthood usually is attained at puberty. In healthy children, therefore, 20% to 70% of the total circulating white blood cells may be neutrophils.

Neutropenia is defined as a decrease in the absolute numbers of circulating segmented neutrophils and bands in the blood. Obtaining a complete blood count (CBC) and differential count identifies this condition. The ANC is determined by multiplying the total white blood cell count by the percentage of segmented and band forms. The ANC for the general population normally ranges

between 1.5 and $8.0 \times 10^3/\text{mcL}$ (1.5 and $8.0 \times 10^9/\text{L}$) for Caucasian children older than 6 years of age. As much as 30% of the African-American population may have ANC levels as low as $1.0 \times 10^3/\text{mcL}$ ($1.0 \times 10^9/\text{L}$). Individual patients may be characterized as having mild neutropenia when the ANC is 1.0 to $1.5 \times 10^3/\text{mcL}$ (1.0 to $1.5 \times 10^9/\text{L}$), moderate neutropenia when the ANC is 0.5 to $1.0 \times 10^3/\text{mcL}$ (0.5 to $1.0 \times 10^9/\text{L}$), and severe neutropenia when the ANC is less than $0.5 \times 10^3/\text{mcL}$ ($0.5 \times 10^9/\text{L}$). This classification is useful for predicting the risk associated with pyogenic infections among patients who have sustained neutropenia over 2 to 3 months. It also is useful in counseling families because generally only those patients who have chronic severe neutropenia are likely to develop a life-threatening illness.

Patients who have neutropenia are infected most frequently with endogenous flora. Colonization with various nosocomial organisms often is observed. Susceptibility to bacterial infections, even in the presence of severe neutropenia, varies. Some patients who have chronic neutropenia and an ANC of less than $0.2 \times 10^3/\text{mcL}$ ($0.2 \times 10^9/\text{L}$) do not experience serious infection, but they commonly experience gingivitis, probably because other parts of their immune system remain intact. In contrast, patients receiving immunosuppressive drugs, particularly in conjunction with malignancies, who develop neutropenia are more likely to develop serious bacterial infections than those whose neutropenia is isolated because immune suppression compromises both function and numbers of lymphocyte and monocytes.

The types of pyogenic infections occurring most frequently among patients who have profound neutropenia

Table 2. Causes and Mechanism of Recurrent Infection in Primary Immune Deficiency States

Disorders	Deficiency
Humoral immunodeficiency (predominantly B-cell defects)	Impaired opsonization; failure of lysis and agglutination of bacteria; failure to neutralize bacterial toxins
Cellular immunodeficiency states (predominately T-cell defects)	Absent T-cell cooperation for B-cell synthesis of antibodies to T-cell-specific antigens
Severe combined immunodeficiency	Absent T- and B-cell response
Wiskott-Aldrich syndrome	Decreased antibody response to carbohydrate antigens
Ataxia-telangiectasia	T-helper cell deficiency; immunoglobulin deficiency
Splenic insufficiency or absence	Defective opsonization; defective clearing of encapsulated organisms
Complement deficiencies	Defective opsonization
Neutrophil dysfunction syndromes, including chronic granulomatous disease	Impaired neutrophil bactericidal activity arising from failure to generate H_2O_2 by phagocytes
Neutropenia ($<0.5 \times 10^3/\text{mcL}$ [$0.5 \times 10^9/\text{L}$])	Inadequate numbers of phagocytes

are cutaneous cellulitis and abscesses or furunculosis, pneumonia, and septicemia. Stomatitis, gingivitis, perirectal inflammation, and otitis media are also common. In contrast, isolated neutropenia does not predispose patients to parasitic, viral, or fungal infections or to bacterial meningitis. The most common pathogens isolated from patients who have neutropenia are *Staphylococcus* and gram-negative organisms. Often, the signs and symptoms of local infections and inflammations, such as exudate, abscess formation, and regional lymphopathy, are less evident in patients who have neutropenia than in those who do not because there is a paucity of neutrophils to mediate the inflammatory response. Other signs and symptoms, such as redness, pain, tenderness, and warmth accompanied by fever, generally are present.

Neutropenias Associated With Factors Extrinsic to Bone Marrow Myeloid Cells

Infection

A large number of acquired conditions may be associated with neutropenia (Table 3). Infectious diseases are among the most common causes of neutropenia in children. Viral infection is the major cause of acute neutropenia in childhood. Viruses commonly causing neutropenia include respiratory syncytial virus, varicella, influenza A and B, measles, and rubella. Neutropenia often occurs during the first 24 to 48 hours of illness and usually persists for 3 to 8 days, which corresponds to the period of acute viremia. Significant neutropenias also may be associated with bacterial, protozoal, rickettsial, and severe fungal infections.

The mechanisms responsible for neutropenia in acute bacterial and viral infections include: 1) redistribution of neutrophils from the peripheral blood circulating pool to the marginating pool following release of cytokines that increase expression of the protein's intracellular adhesion molecule (ICAM)-1 and -2 on endothelium, 2) increased use of neutrophils at sites of infection, and in some cases, 3) decreased production of neutrophils. Sepsis is a particularly serious cause of neutropenia, especially among young infants and children. Neonates are especially prone to exhausting their marrow reserve pool of segmented neutrophils and bands and succumbing rapidly to bacterial sepsis. In contrast, marrow reserves in older children and adults can increase by a log during infection.

Drug-induced Neutropenia

Drugs can induce severe neutropenia by immunologic, toxic, and hypersensitivity-mediated mechanisms (Table 4). This form of neutropenia must be distinguished from

Table 3. Classification of Neutropenia

Secondary Neutropenia Caused by Factor Extrinsic to Bone Marrow Myeloid Cells (Common Disorders)

- Infection (eg, viral, bacterial, protozoal, rickettsial, fungal)
- Drug-induced (eg, phenothiazine, sulfonamides, anticonvulsants, penicillin)
- Immune-induced (eg, alloimmune and autoimmune)
- Bone marrow replacement and failure (eg, malignancy, aplastic anemia)
- Ineffective myelopoiesis (eg, vitamin B12 and folate deficiency)
- Hypersplenism (eg, Gaucher disease, chronic hemolysis, malaria)

Neutropenia Arising from Intrinsic Defects in Myeloid Cells or Their Precursors (Rare Disorders)

- Severe chronic neutropenia (eg, cyclic neutropenia)
- Severe congenital neutropenia
- Idiopathic neutropenia
- Neutropenia associated with dysgammaglobulinemia or hyper IgM syndrome
- Syndrome-associated neutropenias (eg, Shwachman-Diamond syndrome, cartilage hair syndrome, dyskeratosis congenita, glycogen storage disease type 1b)
- Myelodysplasia

Used with permission and modified from Boxer LA. Approach to the patient with neutropenia. In: Humes HD, ed. *Kelley's Textbook of Internal Medicine*. 4th ed. New York, NY: Lippincott Williams and Wilkins; 2000:1576.

that seen with viral infections and from the severe neutropenia that accompanies administration of large doses of cytotoxic drugs or following radiation therapy. Once neutropenia occurs, the most effective therapeutic measure is withdrawal of all drugs that are not essential, particularly drugs suspected of being myeloid-toxic. Bacterial infections should be treated with antibiotics. Often the neutropenia will respond to withdrawal of the offending drug. If the neutropenia fails to respond to drug withdrawal and the patient subsequently experiences signs and symptoms related to severe neutropenia, subcutaneous administration of 5 mcg/kg recombinant human granulocyte-colony stimulating factor (rhG-CSF) should be considered.

Immune Neutropenia

Immune neutropenias are associated with the presence of circulating antineutrophil antibodies. The antibodies are directed against specific neutrophil antigens that are ge-

Table 4. Immune, Toxic, and Hypersensitivity-mediated Neutropenia

Characteristic	Immunologic Form	Toxic Form	Hypersensitivity Form
Paradigm drugs	Aminopyrine, propylthiouracil, penicillin	Phenothiazine	Phenytoin, phenobarbital
Time to onset	Days to weeks	Weeks to months	Weeks to months
Clinical appearance	Acute, often explosive symptoms	Often asymptomatic or insidious onset	May be associated with fever, rash, lymphadenopathy, hepatitis, nephritis, pneumonitis, or aplastic anemia
Rechallenge	Prompt recurrence with small test dose	Latent period; high doses required	Latent period; high doses required
Laboratory findings	Antibody test results positive	Evidence of direct toxicity to cells	Evidence of metabolite-mediated damage to cells
Used with permission from Boxer LA. Approach to the patient with neutropenia. In: Humes HD, ed. <i>Kelley's Textbook of Internal Medicine</i> . 4th ed. New York, NY: Lippincott, Williams and Wilkins; 2000:1579.			

netically controlled independent of the human leukocyte antigen (HLA) system. The antibodies mediate neutrophil destruction by complement-mediated lysis or splenic phagocytosis of opsonized neutrophils. The assays employed most commonly to detect neutrophil antibodies are indirect or direct immunofluorescence to identify surface antigens on the neutrophil and microcapillary agglutination assays, which evaluate the ability of the antibody to clump neutrophils. Usually a combination of immunofluorescence and microcapillary agglutination assays is employed along with a panel of neutrophils that have different known antigen specificity to assure identification of the antineutrophil antibodies.

Alloimmune Neonatal Neutropenia

This form of neonatal neutropenia occurs after transplacental transfer of maternal alloantibodies directed against an antigen of the infant's neutrophils. It is present in 0.3% of pregnancies. Prenatal sensitization induces maternal immunoglobulin G (IgG) antibodies to neutrophil antigens of the fetal cells. Symptomatic infants may present with delayed separation of the umbilical cord, mild skin infections, fever, and pneumonia within the first 2 weeks after birth; these resolve with antibiotic therapy. The neutropenia often is severe and associated with fever and infection due to the usual microbes that cause neonatal disease. By 7 weeks after birth, the infant's neutrophil count generally returns to normal, reflecting the duration of survival of the maternal antibody in the infant's circulation. Treatment consists of supportive care and appropriate antibiotics for clinical infections.

Autoimmune Neutropenia of Infancy

Primary autoimmune neutropenia (AIN) is observed most commonly in infants and is caused by granulocyte-specific autoantibodies. For many patients, AIN is diagnosed only after an expensive and burdening investigation and unnecessary treatment with rhG-CSF because AIN is not well known among physicians. Primary AIN typically is diagnosed in infants between the ages of 5 and 15 months. In 90% of infants, AIN is not associated with an increased risk of repeated pyogenic infections, even in the presence of severe neutropenia. In the past, many of these patients were categorized as having chronic benign neutropenia of childhood. Typically, 95% of infants undergo spontaneous remission within 7 to 24 months. Often screening must be repeated for antibodies several times until the antibodies are detected because they are not always observed in the serum. The bone marrow typically is normocellular or hypercellular and usually contains a reduced number of segmented neutrophils. Symptomatic treatment with antibiotics is satisfactory in most infants. Among patients treated for severe infection or for surgical preparation with rhG-CSF, neutrophil counts can be increased. When combined with the detection of neutrophil-specific antibodies, most patients can be diagnosed readily without burdening investigations, including a need for bone marrow aspiration.

Intrinsic Disorders of Proliferation and Maturation of Myeloid Cells

The isolated disorders of proliferation and maturation of myeloid stem cells are rare (Table 3). Affected patients frequently benefit from rhG-CSF therapy. Congenital disorders that have severe neutropenia as a clinical feature

include the severe combined immunodeficiency syndromes, hyper IgM syndrome, common variable immune deficiencies, glycogen storage disease type 1b, Shwachman-Diamond syndrome, cyclic neutropenia, and severe congenital neutropenia.

Cyclic Neutropenia

Cyclic neutropenia is a rare congenital granulopoietic disorder. The mode of inheritance is autosomal dominant, and it is characterized by regular, periodic oscillations, with the number of peripheral neutrophils ranging from normal to neutropenic values. The mean oscillatory period is 21 ± 4 days. The estimated frequency of this condition is approximately 1 per 1 million population. Clinically, patients may suffer from oral ulcers, stomatitis, or cutaneous infections associated with lymph node enlargement during the neutropenic phase. Serious infections occur occasionally and may lead to pneumonia or recurrent ulcerations in the oral, vaginal, and rectal mucosa. Approximately 10% of patients who had cyclic neutropenia prior to the availability of rhG-CSF developed fatal *Clostridium perfringens* infection, likely arising from dissemination of organisms from ulcers in the gastrointestinal tract. Cyclic neutropenia frequently is called cyclic hematopoiesis because of the cycling of other blood cells, such as platelets and reticulocytes. Monocytes also cycle, but in a reciprocal fashion to the neutrophils.

Cyclic neutropenia is diagnosed by obtaining blood counts two to three times per week for 2 months. The diagnosis can be confirmed with molecular genetic studies demonstrating mutations in the elastase gene. Once affected patients are treated with daily rhG-CSF, their cycle changes from a 21-day interval to a 9- to 11-day interval. Such patients no longer are at risk for fatal infections with clostridia, and antibiotic use associated with inflammatory disease is diminished.

Severe Congenital Neutropenia

Severe congenital neutropenia (SCN), also known as Kostmann disease, is characterized by an arrest in myeloid maturation at the promyelocyte stage of the bone marrow, resulting in an ANC of less than $0.2 \times 10^3/\text{mCL}$ ($0.2 \times 10^9/\text{L}$). This disorder occurs sporadically.

Patients who have SCN experience a predictable pattern of infection and inflammation. Mouth ulcers, gingivitis, otitis media, respiratory infections, cellulitis, and

skin abscesses are the most common conditions. Pneumonia and deep-tissue abscesses occur frequently and are life-threatening. The most common causes of infection are *S aureus* and *Streptococcus* present on the body surfaces.

The onset of mouth ulcers and gingivitis occurs in early childhood. Mild hepatosplenomegaly is common. Evidence for gingivitis is the most frequent finding. SCN is diagnosed by an ANC of less than $0.2 \times 10^3/\text{mCL}$ ($0.2 \times 10^9/\text{L}$) on at least three separate occasions over a 1-month period. Peripheral blood eosinophilia and monocytosis and a bone marrow reflecting arrest of promyelocyte cell maturation are associated with the

More than 90% of patients who have severe congenital neutropenia respond to therapy with rhG-CSF.

profound neutropenia. The platelet count often is mildly elevated, and patients have anemia associated with chronic inflammatory disease. In the past, two thirds of patients died from fatal infections before reaching adolescence. Prior to the availability of rhG-CSF, leukemic transformation occurred in patients who had SCN and Shwachman-Diamond syndrome. The use of rhG-CSF has had a major impact on the management of SCN. After 11 years of clinical use, it has been documented that approximately 10% of patients who are diagnosed with SCN convert to myelodysplasia/acute myelogenous leukemia (MDS/AML).

The onset of MDS can be insidious, with patients developing thrombocytopenia, anemia, or an increase or decrease in the dose of rhG-CSF required to maintain the target ANC. Cytogenetic analysis of unstimulated bone marrow cells frequently documents loss of entire chromosome homolog (monosomy 7) or a partial deletion involving the long arm. Some patients have trisomy 21. After development of MDS/AML, activating *ras* oncogene mutations have been identified retrospectively. In 80% of patients who have MDS/AML, point mutations in the gene for the G-CSF receptor occur, resulting in a truncated cytoplasmic region of the receptor. The mutations eliminate a crucial portion of the receptor involved in myeloid maturation signaling. The receptor mutations are acquired and are not the cause of congenital neutropenia.

In contrast, patients who have SCN may have existing mutations in the neutrophil elastase gene (60% to 80%). Three-dimensional molecular modeling has suggested that most, if not all, of the mutations associated with cyclic neutropenia occur in proximity to the active site of the elastase gene and binding pocket for the enzyme's natural inhibitors. The mutations responsible for SCN would be predicted to alter molecular folding. Potentially, these abnormalities affect the storage of neutrophil elastase in primary granules of the neutrophil and may contribute to accelerated apoptosis of myeloid precursors found in the bone marrow of both groups of patients.

Currently, more than 90% of patients respond to

physical examination should note growth and development; phenotypic abnormalities; and sites of bacterial infections, including mucous membranes, gingiva, skin, tympanic membranes, and rectum. Lymphadenopathy, hepatosplenomegaly, and signs of a possible underlying disease also should be noted. The presence of petechiae and purpura suggesting thrombocytopenia might indicate a more generalized disease process. Documentation of fever is not necessary, but rectal temperatures should be avoided in the neutropenic patient to prevent possible injury to the mucous membranes and subsequent spread of bacteria into the circulation.

The severity and duration of the neutropenia determines the extent of laboratory evaluation. If the child is neutropenic at the time of examination or shortly after a viral infection, a CBC should be performed 3 to 4 weeks later to evaluate recovery of the ANC. For the infant who remains asymptomatic clinically despite the persistence of neutropenia, studies should be initiated to determine whether the patient's serum contains antineutrophil antibody. Bone marrow

examination usually is not needed in the patient who has acute-onset neutropenia, is not experiencing more than the usual childhood bacterial infections, and does not have a history of chronic gingivitis or recurrent mouth ulcers.

In contrast, children who have a clinical history consistent with infections due to chronic neutropenia, such as gingivitis in infancy, require more extensive evaluation. CBCs should be obtained twice weekly for 6 weeks to establish whether there is a cycle of 21 ± 4 days, which differentiates cyclic neutropenia from severe congenital neutropenia. Bone marrow aspirate and bone marrow cytogenetics are required to evaluate the risk for MDS/AML as well as to assess cellular morphology and the extent of myeloid cell maturation. Children who present with a history consistent with malabsorption and neutropenia should be evaluated for Shwachman-Diamond syndrome. These patients require studies to evaluate pancreatic enzymes and skeletal evaluation to assess the possibility of metaphyseal chondrodysplasia. All children who have chronic neutropenia associated with recurrent infections should have growth curves plotted to evaluate the effect of recurrent infections on growth and development. Antinuclear antibody determination, red cell folate, and serum B12 levels are indicated for patients in whom collagen vascular disease and nutritional deficiencies, respectively, are suspected. More extensive immu-

If patients have an absolute neutrophil count below $1.0 \times 10^3/\text{mCL}$, a manual differential count should be requested to determine if a leukoerythroblastic response is responsible.

rhG-CSF administered at 10 mcg/kg per day in two divided doses at 12-hour intervals, resulting in a rise in their ANC. Only the 10% of patients who do not respond to G-CSF with an increase in ANC and require 100 mcg/kg per day or more of the cytokine should be considered as candidates for stem cell transplantation from an HLA-identical sibling. It is important to point out that no cases of MDS/AML have occurred in other cell groups of congenital neutropenia patients other than those who have SCN or Shwachman-Diamond syndrome. Stem cell transplantation has proven to be the only successful treatment once SCN patients convert to MDS/AML.

Evaluation of Children Who Have Neutropenia

Evaluation begins with confirmation of neutropenia according to standards of neutrophil counts per age. If patients have ANCs below $1.0 \times 10^3/\text{mCL}$ ($1.0 \times 10^9/\text{L}$), a manual differential count should be requested to determine whether blasts or immature neutrophils are present in the peripheral smear, which might indicate a leukoerythroblastic response. The examining physician should obtain a thorough history to establish the onset of neutropenia; the type, frequency, and severity of infections; drug history for toxic exposures; and family history of recurrent infection for unexplained infant deaths. The

Table 5. Disorders of Neutrophil Function

Disorder	Genetics	Functional Impairment Variable	Clinical Consequences
Chemotaxis Hyperimmunoglobulin E syndrome	AD	Variable chemotactic defect of neutrophil; high levels of antistaphylococcal IgE, leading to impaired IgG opsonization of <i>Staphylococcus aureus</i>	Severe dermatitis; recurrent skin and sinopulmonary infections
Adhesion Leukocyte adhesion deficiency	AR	Absence of CD11/CD18 surface B ₂ integrins on leukocyte plasma membranes, leading to impaired binding of C3bi to neutrophils and impaired adhesion of neutrophils to ICAM-1 and ICAM-2	Neutrophilia; recurrent bacterial infections without associated pus formation; delayed separation of the umbilical cord
Degranulation Chediak-Higashi syndrome	AR Responsible gene found at 1q42-q45	Disordered coalescence of lysosomal granules and melanophores, leading to decreased neutrophil chemotaxis, degranulation, and bactericidal activity and oculocutaneous phenotype	Mild neutropenia; recurrent bacterial infections; development of marked hepatosplenomegaly in the accelerated phase
Microbicidal Activity Chronic granulomatous disease	X-linked (gp91 ^{phox}) AR (p22 ^{phox} , p47 ^{phox} , or p67 ^{phox})	The absence of either gp91 ^{phox} , p22 ^{phox} , p47 ^{phox} , or p67 ^{phox} , leading to failure to activate the neutrophil respiratory burst and failure to kill catalase-positive microbes	Recurrent pyogenic infections with catalase-positive microorganisms
Myeloperoxidase deficiency	AR Responsible gene found at 17q22-q23	Defective posttranslational processing of an abnormal MPO precursor polypeptide arising from at least four genetic lesions. Partial or complete MPO deficiency leads to diminished production of HOC1 and HOC1-derived chloramines; MPO products are required to kill <i>Candida</i> rapidly	Usually of no clinical consequence except in diabetes mellitus where patients can experience candidiasis
AD = autosomal dominant; AR = autosomal recessive; CD = cluster designation; ICAM = intracellular adhesion molecule; MPO = myeloperoxidase; C3bi = fragment of the third component of complement Used with permission and modified from Boxer LA. Neutrophil disorders: qualitative abnormalities of the neutrophil. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U, eds. <i>Williams Hematology</i> . 6th ed. New York, NY: McGraw-Hill; 2001:836.			

nologic evaluation is indicated for selected patients suspected of having a concurrent immunodeficiency.

Children presenting with pancytopenia require a bone marrow aspiration and biopsy to aid in the diagnosis and to assess bone marrow cellularity. Additional marrow studies, including cytogenetic analysis, flow analysis, and special stains for detecting leukemia and other malignant disorders, are required in certain cases. Selection of other laboratory tests is determined by the duration and sever-

ity of the neutropenia and by the findings obtained on the physical examination.

Disorders of Neutrophil Function

Neutrophils are particularly important in protecting the skin, mucous membranes, and lining of the respiratory and gastrointestinal tracts. As such, they form the first line of defense against microbial evasion. During the critical 2- to 4-hour period after invasion by pathogenic

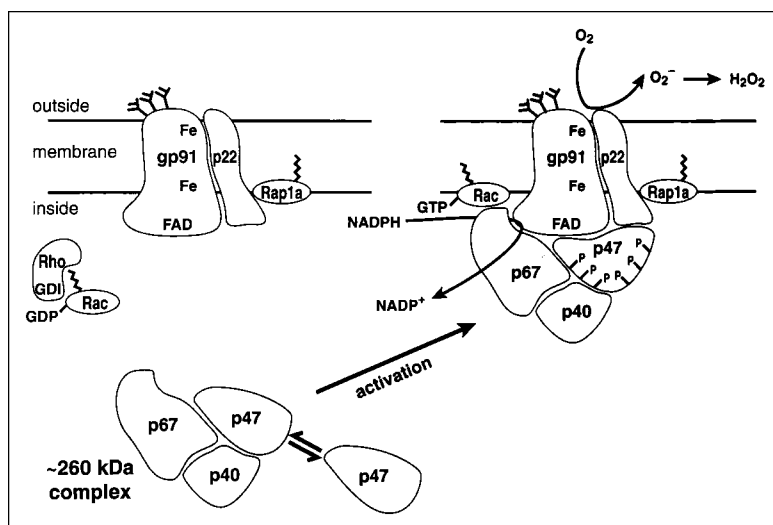


Figure 1. Current knowledge suggests that the oxidase in its dormant state (left) is composed of both membrane-bound and cytosolic components. The former includes the gp91^{phox} and p22^{phox} subunits of the flavocytochrome-b and the associated low-molecular weight GTP-binding protein Rap1a. The flavin and heme groups (Fe) that mediate the transfer of electrons from NADPH to molecular oxygen are localized in the cytochrome. The cytosolic components p47^{phox} and p67^{phox} may exist as a preformed complex of 260 kDa, which also includes a third protein, p40^{phox}. The small GTPase Rac2 is present in the cytosol in its inactive guanosine diphosphate-bound state (GDP). Following phagocyte activation (right), the cytosolic complex translocates to the membrane, which may be under the control of the active guanosine triphosphate (GTP)-bound form of Rac2 and further regulated by phosphorylation of p47^{phox}. By a mechanism that is not fully understood, binding of the cytosolic components activates the flavocytochrome to catalyze the transfer of electrons from NADPH to oxygen through the FAD and heme redox centers. Modified from Curnutte J, Dinanuer M. Genetic disorders of phagocyte killing. In: Stamatoypannopoulos G, Perlmutter RM, Varmus H, eds. *The Molecular Basis of Blood Diseases*. 3rd ed. Philadelphia, Pa: WB Saunders; 2001:543.

organisms, phagocytic cells must arrive at the site of invasion if infection is to be contained. If not, the resulting infection leads to a larger local lesion or dissemination throughout the host. To be effective and arrive at the site of inflammation, phagocytic cells must adhere to the vascular endothelium near the invasion site, engage in diapedesis through the vessel wall, move unidirectionally toward the site (chemotaxis), adhere to and ingest the offending organisms, and activate biochemical pathways important in intracellular microbial killing. Microbial killing is accomplished by two mechanisms: 1) de novo synthesis of highly toxic and often unstable derivatives of molecular oxygen by an enzyme known as the respiratory burst oxidase and 2) delivery into phagocytic vesicles containing the ingested microbes of preformed polypeptide antibiotics and proteases stored within several types of lysosomal granules.

Patients whose neutrophils have defects in adhesion

and motility generally develop cutaneous abscesses with common pathogens such as *S aureus* or have mucous membrane lesions caused by agents such as *Candida albicans* or oral anaerobic bacterial flora. If the defect in adhesion and chemotaxis is profound, lesions may contain few, if any, neutrophils. Disorders of phagocytic microbicidal activity, such as chronic granulomatous disease, are associated with cutaneous abscesses, lymphadenitis, pulmonary infections, and gastrointestinal problems such as antral obstruction. These patients tend to have more deep-seated and chronic infections involving the liver and lung (Table 5).

Chronic Granulomatous Disease

Chronic granulomatous disease (CGD) is a genetic phagocyte disorder in which neutrophils and monocytes are unable to kill certain bacteria and fungi after ingesting them. The estimated incidence of CGD in the United States is 1 in 250,000 live births. The other disorders of phagocyte function listed in Table 5 occur much less frequently. Only limited numbers of

patients who have these disorders of phagocyte dysfunction have been reported in the literature and account for a few hundred reported patients who have a predisposition to recurrent bacterial infections. The underlying defect in CGD is an inability of phagocytic cells to reduce molecular oxygen and create the active oxygen metabolites that are necessary for efficient intracellular microbicidal activity.

The inability of phagocytes to generate superoxide anion (O_2^-) is caused by the absence of one of the components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system (Fig. 1). Approximately 70% of affected patients lack the membrane-bound protein gp91^{phox}. The gene for this protein is located on the X chromosomes. All other patients lack one of the cytosol proteins: p47^{phox}, p67^{phox}, or rarely the membrane-bound p22^{phox}. Genes on different autosomal chromosomes encode these latter three proteins.

The increased susceptibility to infection among patients who have CGD is limited to bacteria and fungi that are catalase-positive and do not themselves have any production of reduced oxygen metabolites such as hydrogen peroxide. Catalase-positive organisms are not killed efficiently by the phagocytic cells in patients who have CGD, and they are responsible for most serious infections in these patients. In contrast, microorganisms that are catalase-negative and can produce hydrogen peroxide supply the necessary reactive oxygen metabolites when they are ingested, thereby contributing to their own demise (Fig. 2). Patients who have CGD also have an increased susceptibility to developing a variety of inflammatory or rheumatic diseases such as inflammatory bowel disease and lupuslike syndrome.

In 1993, a National Registry of patients who have CGD was established, providing a minimal estimate of the incidence of the disorder and characterizing some of its epidemiologic features. Although 76% of affected patients were diagnosed before the age of 5 years, 10% were not diagnosed until the second decade of life, and 4% were diagnosed in the third decade or later. Delayed diagnosis was common in autosomal recessive forms of the disease, where 33% of the patients were identified in the second decade of life or later.

Although the clinical presentation varies, several clinical features suggest the diagnosis of CGD. Any patient who has recurrent lymphadenitis should be considered as having CGD. Additionally, bacterial hepatic abscesses, osteomyelitis at multiple sites or in the small bones of the hands or feet, or a family history of recurrent infections of unusual catalase-positive microbial infections all point to the disorder. The severity and frequency of infections vary widely. The most common offending organism is *S aureus*, although any catalase-positive microorganism may be involved. Infection with *S marcescens*, *B cepacia*, *Nocardia* sp, and *Aspergillus* sp occurs most frequently. Pneumonia, lymphadenitis, cellulitis, osteomyelitis, and

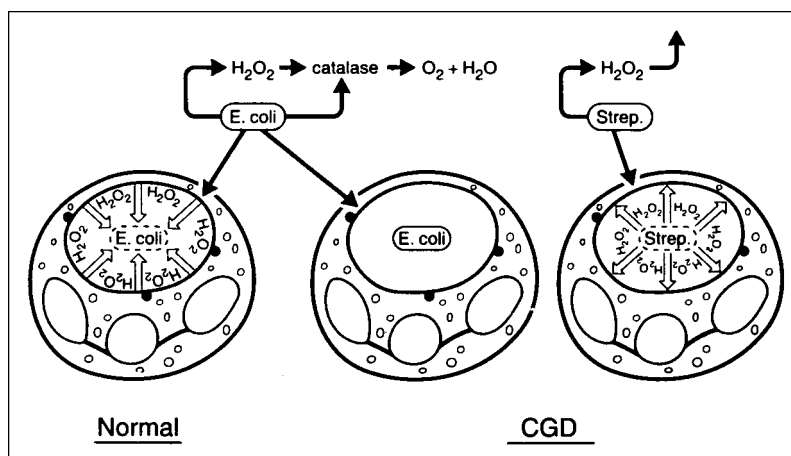


Figure 2. The pathogenesis of chronic granulomatous disease (CGD). The manner in which the metabolic deficiency of the CGD neutrophil predisposes the host to infection is shown schematically. Normal neutrophils accumulate hydrogen peroxide in the phagosome containing ingested *Escherichia coli* (left). Myeloperoxidase is delivered to the phagosome by degranulation, as indicated by the closed circles, and in this setting, hydrogen peroxide acts as a substrate for myeloperoxidase to oxidize halide to hypochlorous acid and chloramines that kill the microbes. The quantity of hydrogen peroxide produced by the normal neutrophils is sufficient to exceed the capacity of catalase, a hydrogen peroxide-catabolizing enzyme produced by many aerobic microorganisms, including most gram-negative enteric bacteria, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus* sp. When organisms such as a *E coli* gain entry into CGD neutrophils, they are not exposed to hydrogen peroxide because the neutrophils do not produce it, and the hydrogen peroxide generated by the microbes is destroyed by their own catalase. When CGD neutrophils ingest streptococci or pneumococci, these organisms, which lack catalase, generate sufficient hydrogen peroxide to result in a microbicidal effect (right). On the other hand, as indicated in the middle figure, catalase-positive microbes, such as *E coli*, can survive within the phagosome of the CGD neutrophil. Used with permission from Boxer LA. Neutrophil disorders: qualitative abnormalities of the neutrophil. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U, eds. *Williams Hematology*. 6th ed. New York, NY: McGraw-Hill; 2001:845.

sepsis remain the infections encountered most commonly. Often the infections are characterized by microabscesses and granuloma formation. Patients may suffer from the consequences of chronic infections, including obstructive lesions of the esophagus, gastrointestinal tract, and urinary tract as well as inflammatory bowel disease, hepatosplenomegaly, chronic dermatitis, or restrictive lung disease.

The diagnosis usually is suggested by using the nitroblue tetrazolium test (NBT), in which the yellow water-soluble tetrazolium dye is reduced to the blue insoluble forms in pigment by O_2^- generated from normal activated phagocytes. Phagocytes from patients who have CGD fail to reduce NBT because they cannot produce O_2^- . The diagnosis is confirmed by quantitative assays to determine hydrogen peroxide response to activating stimuli using flow cytometry in which the conver-

sion of dihydrorhodamine (DHR)¹²³ to rhodamine¹²³ is monitored. The DHR method not only diagnoses CGD, but it also suggests the CGD genotype because stimulated neutrophils from patients who have p47^{phox}-deficient cytochrome-positive CGD generate low levels of hydrogen peroxide by DHR, which is distinct from the total lack of hydrogen peroxide formation in the X-linked recessive form of CGD. Once CGD is diagnosed, the genotype can be determined. A mosaic population of oxidase-positive and -negative neutrophils in a male patient's mother or sister strongly supports the diagnosis of X-linked CGD. On the other hand, the lack of a mosaic pattern among female relatives does not exclude the X-linked mode of inheritance because the defect can arise spontaneously. Absence of the membrane-bound cytochrome b subunits by spectral or immunoblot analysis indicates a defect in either the gp91^{phox} or p22^{phox}. Definitive methods for establishing CGD genotype require immunoblotting for p47^{phox} and p67^{phox} or direct sequencing of the gp91^{phox} and p22^{phox} genes because the absence of either cytochrome protein generally leads to the loss of the other.

The prognosis of patients who have CGD has improved in recent years. More than 25% of living patients who have X-linked CGD and 42% of those who have autosomal recessive forms are 20 years or older. The estimated mortality is approximately 5% and 2% per year, respectively, for the two patient groups. Both *Aspergillus* and *B cepacia* account for more than 50% of the deaths.

CGD patients are managed by long-term antibiotic prophylaxis (trimethoprim-sulfamethoxazole), long-term gamma-interferon (50 mcg/m²) administered three times per week, vigorous treatment of acute infections with antibiotics in adequate doses, and surgery, if indicated. Granulocyte transfusions in CGD are supported by the observation that a small number of normal phagocytes may be able to compensate for the metabolic

defect in CGD phagocytes. Transfused granulocytes have respiratory burst activity and appear to function normally based on their recovery from sites of infection. The value of granulocyte transfusions in CGD has not been evaluated in prospective, controlled studies, but when used, they generally are well tolerated. Bone marrow transplantation has been used in at least 10 CGD patients. Because of the morbidity and mortality associated with bone marrow transplantation, its repeated use in CGD cannot be recommended at present. Bone marrow transplantation may be considered for patients who have recurrent serious infections despite antibiotic and interferon-gamma prophylaxis or those who have HLA-matched normal siblings. Finally, CGD is a prototype disorder amenable to gene therapy, and studies are underway to evaluate this possibility.

Suggested Reading

- Dale D, Bonilla M, Davis M, et al. A randomized controlled phase III trial of recombinant human G-CSF for treatment of severe chronic neutropenia. *Blood*. 1993;81:2496
- Dale D, Hammond W. Cyclic neutropenia: a clinical review. *Blood Rev*. 1988;2:178
- Dale DC, Person RE, Bolyard AA, et al. Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. *Blood*. 2000;96:2317
- Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. *Nature Genetics*. 1999;23:433
- Segal BH, Leto TL, Gallin JI, et al. Genetic, biochemical, and clinical features of chronic granulomatous disease. *Medicine*. 2000;79:190
- Welte K, Boxer LA. Severe chronic neutropenia: pathophysiology and therapy. *Semin Hematol*. 1997;34:267
- Winkelstein JA, Marino MD, Johnston RB, Jr, et al. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine*. 2000;79:155

PIR Quiz

Quiz also available online at www.pedsinreview.org.

8. A 4-year-old boy has a culture-positive staphylococcal skin infection. Absence of which one of the following signs would be *most* consistent with an underlying neutropenia?
 - A. Erythema.
 - B. Exudate.
 - C. Pain.
 - D. Tenderness.
 - E. Warmth.
9. You have diagnosed primary autoimmune neutropenia (AIN) in a 9-month-old girl. You are *most* likely to tell the parents that:
 - A. Periodic examination of the bone marrow is required to monitor the condition.
 - B. Repeated pyogenic infections will occur throughout life.
 - C. Severe neutropenia is not an associated finding.
 - D. Spontaneous remission occurs in 95% of patients.
 - E. Treatment with recombinant human granulocyte colony-stimulating factor is recommended for any infection.
10. A 2-year-old child has had recurrent gingivitis for more than 1 year. You have obtained a complete blood count (CBC) during three of the five episodes and have noted a white blood cell count of 1.5 to $2.0 \times 10^3/\text{mCL}$ (1.5 to $2.0 \times 10^9/\text{L}$) each time. The differential count typically revealed 30% to 40% neutrophils. You are considering the diagnosis of cyclic neutropenia and order twice-weekly CBCs for 8 weeks. Which of the following cycles of neutropenia would help establish the diagnosis?
 - A. 7 ± 3 days.
 - B. 10 ± 2 days.
 - C. 14 ± 4 days.
 - D. 21 ± 4 days.
 - E. 28 ± 3 days.
11. A 1-year-old boy has had recurrent lymphadenitis for most of his life. Testing of his phagocytes revealed inability to reduce tetrazolium and reduced conversion of dihydrorhodamine¹²³ to rhodamine¹²³. The *most* likely diagnosis in this patient is:
 - A. Hyperimmunoglobulin M syndrome.
 - B. p47^{phox} genotype chronic granulomatous disease.
 - C. Severe combined immunodeficiency syndrome.
 - D. Severe congenital neutropenia.
 - E. X-linked chronic granulomatous disease.