
Cyclic Neutropenia

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Cyclic neutropenia is a rare hematologic disorder, characterized by repetitive episodes of fever, mouth ulcers, and infections attributable to recurrent severe neutropenia. Fluctuations in blood cells are due to oscillatory production of cells by the bone marrow. Recent genetic, molecular, and cellular studies have shown that autosomal-dominant cyclic neutropenia and sporadic cases of this disease are due to a mutation in the gene for neutrophil elastase (ELA2), located at 19p13.3. This enzyme is synthesized in neutrophil precursors early in the process of primary granule formation. It is currently presumed that the mutant neutrophil elastase functions aberrantly within the cells to accelerate apoptosis of the precursors, resulting in effective and oscillatory production. Cyclic neutropenia is effectively treated with granulocyte colony-stimulating factor (G-CSF), usually at doses of 1 to 5 $\mu\text{g}/\text{kg}/\text{d}$ (median dose, 2.5 $\mu\text{g}/\text{kg}/\text{d}$). Long-term, daily, or alternate-day administration reduces fever, mouth ulcers, and other inflammatory events associated with this disorder. Leukemic transformation is not a recognized risk for cyclic neutropenia, with or without treatment with G-CSF.

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CYCLIC NEUTROPENIA is a rare hematological disorder characterized by recurrent fevers, mouth ulcers, and infections attributable to regularly recurring severe neutropenia. Other synonyms are cyclic agranulocytosis, periodic neutropenia, periodic aphthous stomatitis, periadenitis mucosa necrotica recurrens, and Sutton's disease.³¹ Because blood monocytes, reticulocytes, platelets, and lymphocytes oscillate with the same periodicity as blood neutrophils, the disease is sometimes called periodic or cyclic hematopoiesis.⁹

The first case of cyclic neutropenia was reported in 1910 in a 3½-month-old boy with recurrent fever, furunculosis, and severe neutropenia.¹⁷ In 1930, the blood cell oscillations and continuing cyclic pattern of recurrent illness in this patient were reported again.²⁹ Important milestones in the understanding of this disorder were recognition of the autosomal-dominant pattern of transmission in 1949,²⁶ publication of five affected families with classic autosomal-dominant inheritance in 1967,²¹ description of the treatment response to granulocyte colony-stimulating factor (G-CSF) in 1989,¹⁰ discovery of mutations in the gene for neutrophil elastase (ELA2) as the molecular mechanism in 1999,¹⁵ and recognition of accelerated apoptosis as the cellular mechanism for cyclic neutropenia in 2001.¹

Clinical Features

Several previous reviews have described the clinical features of cyclic neutropenia.^{2,16,27,34} Usually, the diagnosis is suspected in a child, often less than 1 year of age, who presents with recurrent fever, pharyngitis, mouth ulcers, and lymphadenopathy, or with recurrent cellulitis. A few adult cases of acquired cyclic neutropenia have been described, with similar clinical and hematologic features.^{15,18,28,35} Characteristically, the mouth ulcers are deep and painful

and often last a week or more. They may be accompanied by painful cervical lymphadenopathy. Generalized lymphadenopathy is uncommon. Sinusitis, otitis, pharyngitis, and bronchitis are often present. Cellulitis, particularly from minor cuts on the hands and in the perianal areas, occurs frequently. Patients with cyclic neutropenia also present with acute peritonitis manifest by abdominal guarding, ileus, and septic shock. Often such severe infections have been fatal. In these patients, colonic ulcers may have occurred during their neutropenic period, similar to the patients' mouth ulcers, and resulted in bacteremias due to Clostridial species and gram-negative organisms.⁸ Between these periods of recurrent fever, mouth ulcers, and infections, patients are usually without symptoms and have normal physical examinations.

Hematologic Features

Typical cases of cyclic neutropenia have oscillations of neutrophils and monocytes with 21-day periodicity^{2,16,23,27,34,35} (Fig 1). During the neutropenic period, blood neutrophil levels fall to less than $0.2 \times 10^9/\text{L}$ for 3 to 5 days. The neutrophil count then usually increases to near the lower limit of normal, about $2.0 \times 10^9/\text{L}$, and remains at approximately this

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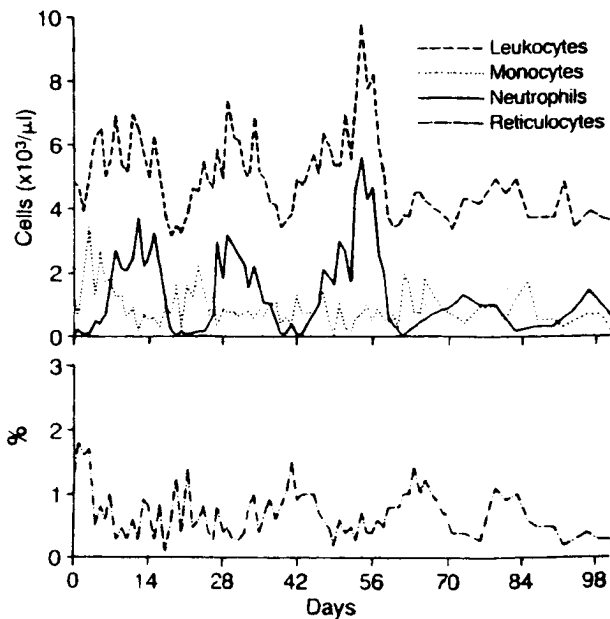


Figure 1. Typical cycle for neutrophils, monocytes, leukocytes, and reticulocytes in a patient with cyclic neutropenia. (Reprinted with permission.²)

level until the next neutropenic period. In most, but not all patients, there are detectable oscillations of reticulocytes and platelets; some have fluctuations of eosinophils and lymphocytes.^{9,35}

Families with several affected members usually have similar oscillations of neutrophil counts within and across generations (Fig 2). Commonly, however, the affected parent of an affected child has not been recognized to have neutropenia, primarily because of milder manifestations or the lack of blood count data. Other relatives of the proband may also have neutropenia but with even milder symptoms.

The diagnosis of cyclic neutropenia is often applied to patients with neutropenia of varying severity, with cycles varying in length from 14 to 40 days. In many of these cases, there are insufficient data to measure a cycle length precisely. In more than 90% of well studied patients, the cycle length is very close to 21 days, and the characteristic oscillatory pattern for individual patients remains relatively constant for many years.

Pathophysiological Studies

Cyclic neutropenia is attributable to periodic variation in the production of neutrophils by the bone marrow. Serial bone marrow aspirates at 2- to 4-day intervals show waves of activity. At the onset of neutropenia, the postmitotic neutrophil cells are absent from the marrow, but there are usually recognizable early myeloid precursors. Typically, recovery occurs rapidly, with the appearance of promyelocytes, myelocytes, metamyelocytes, and band neutrophils over the next 3 to 5 days. Electron microscopic

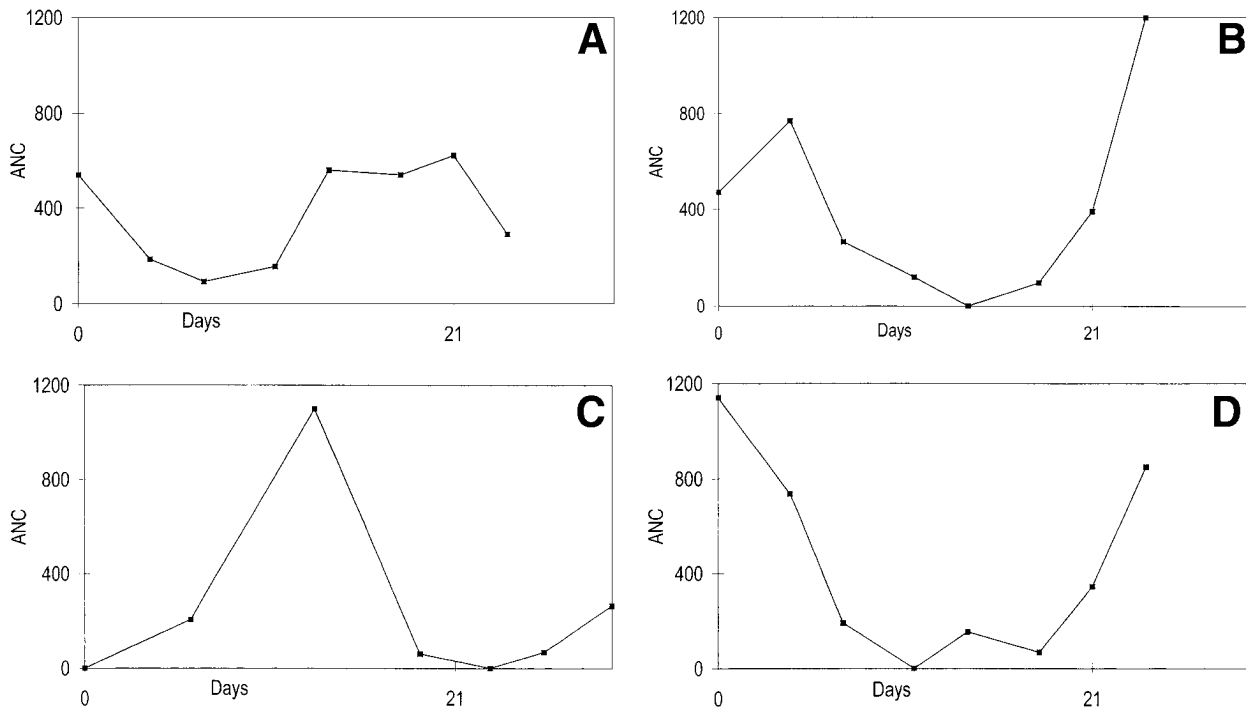


Figure 2. Neutrophil cycles for a mother (A) and her three children (B, C, D). The mother was not recognized to have cyclic neutropenia until the diagnosis was made in her children. ANC, absolute neutrophil count.

studies performed at all phases of the neutrophil cycle show that developing cells have membrane blebbing and nuclear condensations typical for cells undergoing apoptosis.¹ Macrophages containing debris of degenerating cells can also be seen readily. Recent studies using flow cytometric analysis for myeloid precursors have demonstrated increased numbers of annexin V-stained cells in patients compared to controls, indicating that selective apoptotic death of the neutrophil precursors with their removal by marrow macrophages is the primary cellular abnormality causing this disease.¹ The marrow also shows oscillatory variations in erythroid precursors,⁹ presumably attributable to variations in erythropoietin levels and a secondary feature of cyclic neutropenia.

Periodic oscillations in marrow myeloid cells first suggested that cyclic neutropenia occurs because of regularly recurring interruptions in cell production.^{2,16,25,34} Investigations using *in vivo* injection of tritiated thymidine at different phases of the cycle supported this concept.⁹ During the proliferative phase, thymidine incorporation was normal, whereas at the onset of neutropenia very little of the injected thymidine was incorporated into blood neutrophils. *In vitro* studies of colony-forming cells demonstrated that granulocyte-macrophage colony-forming units (CFU-GM) and erythroid colony-forming cells (CFU-E) are present at all phases of the cycle, although the responsiveness of these cells to growth factors was generally less than normal.^{11,36} Several studies have shown that colony-forming cells of various types fluctuate with the same periodicity as the blood neutrophil counts.^{11,20,32,36}

Recent investigations utilizing purified marrow progenitor cells have added to the description of the marrow defect in cyclic neutropenia. Compared to CD34 cells from normal subjects, CD34 cells from

patients with cyclic neutropenia tend to form a higher proportion of large colonies, cells described as high proliferative potential colony-forming cells (HPPC).¹ At all phases of the cycle, however, the patient's CD34 cells had much reduced formation of more differentiated colonies, similar to previous reports of decreased cluster-forming cells with maximum G-CSF stimulation.³² These data suggest that the very early hematopoietic progenitor cells may proliferate normally. However, as these cells differentiate to the neutrophil lineage, they lose their proliferative capacity as they undergo accelerated apoptosis.

Genetic and Molecular Studies

Cyclic neutropenia is inherited as an autosomal-dominant disorder with full penetrance but varying severity of clinical manifestations. Using linkage analysis and DNA samples from 13 families with at least one member with well-characterized typical clinical features and 21-day periodicity in the oscillation of blood neutrophils, the genetic defect in this disorder recently was localized to chromosome 19p13.3.¹⁵ The genes for three serine proteases found in neutrophil granules all localized to this region of chromosome 19. Gene sequencing revealed that all of the families with cyclic neutropenia had mutations in the gene for neutrophil elastase (*ELA2*).¹⁵ The mutations occurred predominantly in exon 4 or 5, or at the junction of exon 4 with intron 4 (Fig 3). Patients with congenital neutropenia also have mutations in the gene for neutrophil elastase but the pattern of these mutations appears to be different (Fig 3). Further studies are needed to clarify whether the two clinical disorders have distinctive genotype-phenotype patterns. In all of the cyclic neutropenia patients studied to date, the mutations occur in only one allele of the neutrophil elastase gene. It is presumed that this

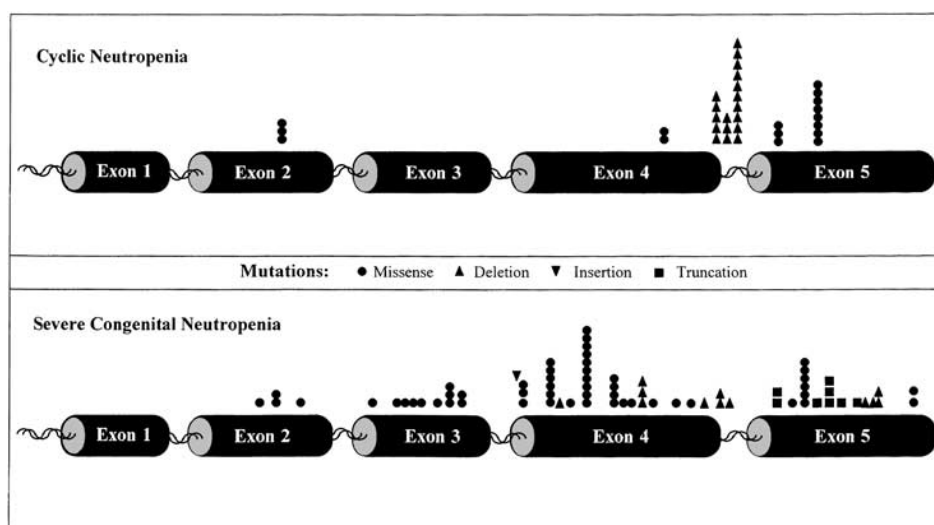


Figure 3. Loci for mutations in the neutrophil elastase gene associated with cyclic and congenital neutropenia.

mutation results in a gain of function or aberrant function, so that the product of the mutant gene in some way damages the developing myeloid precursor, resulting in cell loss and neutropenia.

Mathematical Investigations

The strikingly regular periodic oscillations of blood counts in cyclic neutropenia have led to many mathematical investigations. In 1966, it was proposed that cyclic neutropenia represented an accentuation of normal cyclic variations in blood neutrophils.²² This concept, however, was not supported by subsequent serial studies of normal individuals.³ In 1978, Mackey hypothesized that oscillations in the hematopoietic system would occur if there were an increased rate of irreversible cell loss in the stem cell compartment in the hematopoietic system, assuming that the level of cells is being regulated by feedback control.¹⁹ Other mathematical models have suggested that cycling will occur if there is a stronger than normal feedback signal regulating production,³³ if the transit time through the marrow is increased,³⁰ or if a combination of these abnormalities is present.⁶ At present, it appears that molecular and cellular data are most consistent with Mackey's model.¹³

Complications

Patients with cyclic neutropenia grow and develop normally; there are no associated congenital abnormalities and there is no recognized risk for myeloid or

other malignancies. The recurrent mouth ulcers and gingivitis are chronic problems, often causing accelerated dental decay. Extractions are subsequently required in young adults. Sepsis and death from bacteremias are uncommon, but they are a source of fear and concern for patients and their parents. The regularly recurring infection results in poor school performance, with a lifelong impact on the patient's work, social, and psychological status. Women with cyclic neutropenia have an increased rate of spontaneous abortions, and approximately 50% of their children will have cyclic neutropenia.²⁴ There is no recognized effect on male fertility.

Treatment Responses

Before the availability of the hematopoietic growth factors, there was no predictably effective treatment for cyclic neutropenia. Splenectomy, androgens, glucocorticosteroids, and lithium were used, but overall were not effective therapies. Most patients were managed with intermittent antibiotics for fever and signs of infections during neutropenic periods.

The availability of recombinant human G-CSF has greatly changed the management of cyclic neutropenia. A series of trials clearly established that G-CSF treatment (2 to 5 $\mu\text{g}/\text{kg}/\text{d}$) increased the amplitude of neutrophil oscillations, shortened the duration of neutropenia, and changed the cycle length from 21 to about 14 days^{4,7,10,14} (Fig 4). Clinical improvement was probably attributable largely to the shortened duration of severe neutropenia. Concomitant with

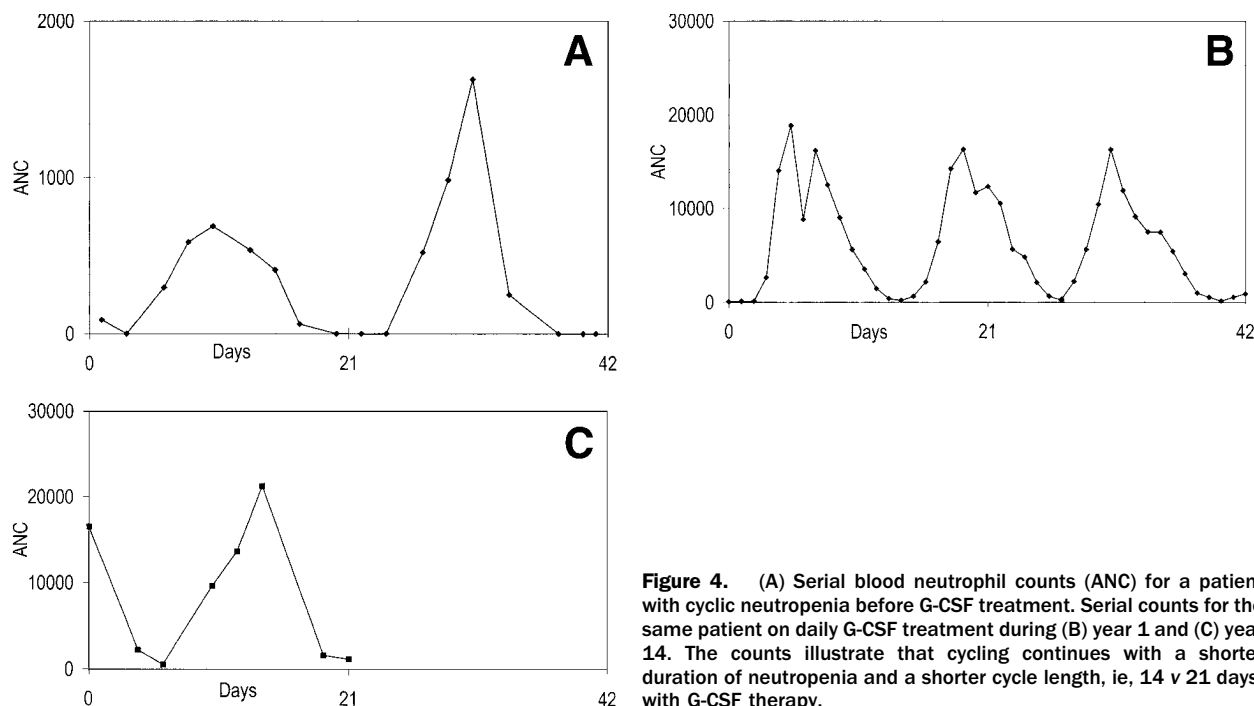


Figure 4. (A) Serial blood neutrophil counts (ANC) for a patient with cyclic neutropenia before G-CSF treatment. Serial counts for the same patient on daily G-CSF treatment during (B) year 1 and (C) year 14. The counts illustrate that cycling continues with a shorter duration of neutropenia and a shorter cycle length, ie, 14 v 21 days, with G-CSF therapy.

this response, patients predictably have a reduction in recurrent fevers, mouth ulcers, and all other disease manifestations. Granulocyte-macrophage colony-stimulating factor (GM-CSF) treatment has been used far less often. It appears to have different effects on the cycle, reducing the nadir but elevating counts far less than G-CSF.³⁷ Long-term treatment is generally not given due to the cytokine's side effects.

The Severe Chronic Neutropenia International Registry now maintains data on more than 160 patients, approximately 82% currently receiving G-CSF, at a median dose of 2.5 $\mu\text{g}/\text{kg}/\text{d}$ for a median of 5.4 years (range, 0.15 to 12.5 years). Prior to G-CSF treatment, all patients who had sufficient data available for review had cycle lengths of approximately 3 weeks. All of the patients with these typical cycles have responded to G-CSF treatment and more than 90% are on daily or alternate-day G-CSF at doses of 5 $\mu\text{g}/\text{kg}/\text{d}$ or less. The pattern of continuing neutrophil oscillations in one of these patients now treated for more than 12 years with G-CSF is shown in Fig 4.

Complications of G-CSF Treatment

Initiation of G-CSF is frequently associated with mild bone pain and headaches. The occurrence and severity of these symptoms vary considerably from patient to patient and are probably dose-dependent. Starting with a low dose (1 $\mu\text{g}/\text{kg}/\text{d}$) and increasing it if necessary (if fever, mouth ulcers, or evidence of infection persist) appears to reduce the severity of these symptoms. Otherwise, the complaints of bone pain and headache tend to decrease with the duration of treatment, usually after about 1 to 2 months. An increase in spleen size detectable either by physical examination, ultrasound, or magnetic resonance imaging, probably occurs commonly, but in most patients the change in spleen size is modest and is of no known clinical consequence. Evolution to myeloid leukemia is not a recognized complication for cyclic neutropenia with or without treatment with G-CSF. As described by Cottle et al elsewhere in this issue, there is increasing concern about the risk of osteoporosis developing in all patients on long-term G-CSF treatment.

Cyclic Neutropenia in Grey Collie Dogs

Collie dogs with a grey coat color have autosomal-recessive cyclic neutropenia with a cycle length of 13 days.⁵ The dogs respond to treatment with daily injections of endotoxins and lithium, as well as to canine G-CSF.¹² The molecular basis for canine cyclic neutropenia is not yet known.

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