CHAPTER 90
FEBRILE NEUTROPENIA

Melissa A. Claus, DVM, DACVECC

KEY POINTS

- Neutropenia is defined broadly as less than 2900 cells/μl in dogs and less than 2000 cells/μl in cats.
- Three primary mechanisms by which febrile neutropenia can develop include increased tissue use of neutrophils, decreased egress of neutrophils from the bone marrow, and immune-mediated destruction of neutrophils.
- Diagnostic tests to consider performing in a patient with febrile neutropenia include blood cultures, a urine culture, chest radiographs, abdominal ultrasound, cardiac ultrasound, and bone marrow aspiration or core biopsy.
- Animals with febrile neutropenia should receive broad-spectrum antibiotics using an antipseudomonal β-lactam. Neutropenic patients in septic shock should receive an antipseudomonal β-lactam coupled with an aminoglycoside. These regimens should be de-escalated with receipt of the susceptibility profiles of cultured organisms.
- Recombinant human and canine granulocyte-colony stimulating factors (rhG-CSF and rcG-CSF) should not be used in Parvovirus infection–induced neutropenia because of poor efficacy (rh-GCSF) and lack of safety data (rcG-CSF).

- Recombinant canine G-CSF has been demonstrated effective at generating myelopoiesis in dogs with chemotherapy-induced neutropenia and cyclic hematopoiesis.
- Meticulous hand washing before and after handling neutropenic patients and using alcohol-based hand sanitizer and donning gloves before handling any indwelling devices is imperative to decrease incidence of nosocomial infection in these patients.

Febrile neutropenia in dogs and cats has multiple etiologies. Regardless of the underlying cause, insufficient numbers of circulating neutrophils can affect significantly patient morbidity and mortality. Without these vital cells of the innate immune system, patients with febrile neutropenia have little protection against invading pathogens and are even at risk of developing life-threatening infections from their own commensal microflora. This chapter discusses the normal processes and production of neutrophils, the etiologies of febrile neutropenia, and diagnostic tests and recommended treatments for patients with this condition.

NEUTROPHIL PHYSIOLOGY

Neutrophil Function

Neutrophils are the most abundant leukocyte in dogs and cats. They are a crucial part of the innate immune system because they are often the first phagocytes to recognize and destroy invading pathogens, including bacteria and fungi. Recognition of invading pathogens occurs when pattern recognition receptors (PRRs) present on the neutrophil membrane bind pathogen-associated molecular patterns (PAMPs) on the cell wall of pathogens. Binding of PRRs activates the neutrophil. Neutrophils also become activated when PRRs bind damage-associated molecular patterns (DAMPs), released from necrotic and apoptotic tissues. Via this mechanism, the neutrophilic response that occurs with sterile inflammation can mimic that occurring with infection.

Neutrophils kill invading pathogens with two main mechanisms: phagocytosis followed by degranulation or formation of neutrophil extracellular traps (NETs). When an organism expressing PAMPs is phagocytosed, cytoplasmic granules containing lethal proteases and peptides fuse with an intracytoplasmic phagosome in a process known as degranulation. These cytotoxic molecules coupled with the production of reactive oxygen species via an intraphagosomal oxidative burst destroy the organism and trigger apoptosis of the neutrophil. The other mechanism by which neutrophils help eradicate foreign organisms is a newly recognized process called *NET formation*. During this process, nuclear material including DNA and histones combine with cytotoxic molecules from cytoplasmic granules and are expelled from the cell into the extracellular space. This web of deadly material ensnares and kills microorganisms while limiting the spread of cytotoxic molecules to prevent damage to regional tissues. The entire process leads to death of the neutrophil via a process termed NETosis, a programmed cell death that appears to have different characteristics from necrosis and apoptosis.[1-3]

Neutrophil Production

The driving factors behind myelopoiesis and neutrophil homeostasis are not understood completely. The production of neutrophils depends in part on the presence of the cytokine granulocyte-colony stimulating factor (G-CSF). G-CSF is produced primarily by bone marrow stromal cells but also is secreted from a variety of other cells, including macrophages, monocytes, endothelial cells, and fibroblasts.[4,5] It is the most important cytokine responsible for maintaining neutrophil homeostasis. It promotes progenitor differentiation into committed production of neutrophils, increases cell division, decreases the time to maturation, and increases release of neutrophils from the bone marrow.[4,5] One stimulus for G-CSF release by bone marrow stromal cells is an elevated level of cytokine IL-17.[6] This cytokine is produced by specific lymphocytes under the stimulation of cytokine IL-23, a cytokine released by tissue macrophages.[7] This pathway of G-CSF production is the underpinning of the main theory for the homeostatic maintenance of neutrophils. When neutrophils reach the end of their life span in the tissues, they undergo apoptosis and are phagocytized by IL-23–producing tissue macrophages. The act of ingesting apoptotic neutrophils decreases the secretion of IL-23 by the macrophage, thus in turn decreasing the secretion of IL-17 by lymphocytes and downregulating the secretion of G-CSF by the bone marrow stromal cells.[8] Once the neutrophil population is depleted and fewer neutrophils are phagocytized in the tissues, the secretion of IL-23 increases. This, in turn, increases IL-17 by lymphocytes and then increases G-CSF concentrations in the plasma to promote further neutrophil production.

Emergency myelopoiesis is a term used to describe the ramping up of leukocyte production that occurs outside of a more steady-state production of neutrophils. It is driven by cytokine stimulation and PAMP/DAMP binding to PRRs on hematopoietic stem cells. These molecules promote proliferation of progenitor cells and directed differentiation into the granulocyte cell line. Recent research suggests that myelopoiesis more likely exists along a continuum, rather than in a switched on (emergency) or switched off (steady-state) form. This is due to the constant presence of PRR signaling in hematopoietic stem cells and progenitors stimulated by commensal microflora. This idea was exemplified in a study of germ-free mice living in a pathogen-free environment. These mice were healthy but demonstrated a markedly attenuated neutrophil steady state with 10% of normal circulating neutrophil counts along with a low serum G-CSF concentration, demonstrating no stimulation to increase myelopoiesis.[9]

Neutrophils are present in several different locations in the body, including the bone marrow, the blood vessels, marginated in the microcirculation, and in the tissues. Neutrophils are produced by progenitor cells in the bone marrow. It is here that they mature into segmented neutrophils. In dogs and cats, the bone marrow houses a fairly large reserve pool of mature neutrophils. Under the stimulation of growth factors and cytokines including G-CSF, GM-CSF, TNF-α, TNF-β, and complement 5a, neutrophils are released from the bone marrow.[10] Once they enter the circulation, they are found in one of two pools: the circulating pool or the marginated pool. The circulating pool neutrophils travel rapidly through the center of larger vessels along with the red blood cells. These neutrophils are the ones that are sampled and counted in a complete blood count. The marginated pool neutrophils roll slowly along the endothelium of smaller vessels and capillaries and tend to stagnate in postcapillary venules. In dogs, about half of the neutrophils in circulation are in the circulating pool and half are in the marginated pool. In cats, only about a quarter of neutrophils are in the circulating pool, whereas three quarters are in the marginated pool. This distinction affects the definition of neutropenia in dogs versus cats. Neutropenia is defined broadly as less than 2900 cells/μl.[11] However, it may be more appropriate to consider a different definition for neutropenia in cats because the majority of neutrophils in circulation are marginated and thus are not actually within the blood collected when assessing a complete blood count. For this reason, neutropenia in cats may be better defined as less than 2000 cells/μl.[12]

PATHOPHYSIOLOGY OF NEUTROPENIA

Neutropenia places a patient at high risk of developing overwhelming infections and thus can contribute significantly to patient morbidity.

Severe neutropenia usually is accompanied by a fever because of the systemic inflammatory response elicited by opportunistic invading pathogens. Febrile neutropenia can occur because of a multitude of different disease processes. Animals may become neutropenic during hospitalization, or severe febrile neutropenia may be the reason they are admitted to the ICU. Febrile neutropenia develops by three main mechanisms, including increased use of neutrophils, decreased egress from bone marrow, and immune-mediated destruction.

Increased Utilization

Infectious microorganisms in the tissues as well as DAMP-expressing endogenous tissues trigger a host inflammatory cascade. Cytokines and chemokines are generated by tissue macrophages and neutrophils. These molecules promote the margination and then extravasation of circulating neutrophils into the tissues, where they depopulate the invading pathogens or necrotic tissue via the two mechanisms described above, phagocytosis with degranulation or NETosis. The larger the population of infectious organisms or the more extensive the quantity of necrotic tissue, the stronger the inflammatory response and the higher the concentration of secreted cytokines. Acutely, the tissue recruitment of neutrophils depletes the neutrophils in circulation. Low levels of circulating neutrophils decrease the concentration of specific chemokines in the bone marrow that retain neutrophils. This allows the stored mature neutrophils to egress into circulation.[9] Ultimately, this can deplete the reserve pool of mature neutrophils in the bone marrow. If the inflammation is overwhelming and persistent, it can exceed the ability of the bone marrow to generate new neutrophils, thus leading to neutropenia.

The severity of neutropenia may not be due entirely to increased extravasation but also may be due to decreased bone marrow production. A 2009 study in a septic mouse model demonstrated the ability of the bone marrow to replete the population of circulating neutrophils is compromised during sepsis by precluding the hematopoietic stem and progenitor cells from differentiating into committed myeloid progenitor cells.[13] Decreasing the differentiating capacity of the bone marrow maximally affects the neutrophil concentration because this cell line has the shortest half-life of the blood cells, especially during a period of increased extravasation. Other studies have shown similar effects on granulopoiesis in the presence of the inflammatory cytokine IFN-γ, as well as in murine models of sepsis and thermal injury.[14-16]

Decreased Egress from the Bone Marrow

Depletion of granulocyte progenitor cells and ineffective granulopoiesis are two bone marrow-centric causes of circulating neutropenia. Generalized bone marrow hypoplasia reduces quantities of granulocyte progenitor cells along with the other hematopoietic cell lines. Bone marrow hypoplasia can occur because of a variety of processes, including infectious diseases, exposure to some drugs and toxicants, radiation, myelophthisis, and cyclic hematopoiesis (gray collie syndrome). Ineffective granulopoiesis is the term used to describe the presence of adequate granulocyte precursors in the bone marrow coupled with a peripheral neutropenia. This can be due to maturational arrest of the neutrophil cell line or retention and/or destruction of mature neutrophils in the bone marrow. Ineffective granulopoiesis can occur with infectious diseases (feline leukemia virus, feline immunodeficiency virus), myelodysplasia, lithium administration in cats, acute myeloid leukemia, and trapped neutrophil syndrome of Border Collies.[17,18]

Depletion of granulocyte progenitor cells

Infectious diseases

Parvovirus in dogs and cats infects rapidly dividing cell populations, including hematopoietic precursor cells. This leads to apoptosis of the cells, depopulation of the bone marrow, and severe leukopenia results.[19] Neutrophils are affected early and severely because of the short half-life of this cell population. This is especially significant in the presence of increased extravasation and use of neutrophils in the gut, where Parvovirus causes severe compromise to the gut mucosal barrier, allowing invasion from gut flora.[20,21]

Although not consistently present, neutropenia may be seen with different rickettsial infections. It is reported more consistently with *Ehrlichia canis*, a monocyte-infecting bacterium, than with *Anaplasma phagocytophilum* and *Ehrlichia ewingii*, granulocytic ehrlichioses. The mechanism by which neutropenia is induced in acute infections is unknown, although severe generalized bone marrow hypoplasia with secondary pancytopenia is described to occur with chronic infections.[22]

Cats infected with one of the retroviruses, feline leukemia virus or feline immunodeficiency virus, have an increased risk of developing neutropenia, although neither disease routinely leads to neutropenia.[23] Underlying causes for the development of neutropenia are varied and depend on the virus involved. Cats with feline leukemia virus tend to develop myelophthisis and myelodysplastic disorders secondary to round cell neoplasms infiltrating the bone marrow.[23] One mechanism for the development of neutropenia in cats with feline immunodeficiency virus is that infected bone marrow stromal cells secrete myelosuppressing factors that depress granulopoiesis.[24] Neutropenia also may develop in feline immunodeficiency virus–infected cats as a result of myelodysplasia occurring with infection of bone marrow and stromal cells.[25]

Medications, toxicants, and radiation

Several drugs, including antiinfective agents, antiepileptics, colchicine, captopril, methimazole, and phenylbutazone, have been reported to induce neutropenia idiosyncratically.[10,26-28] The mechanisms by which neutropenia develops vary between different drugs and often are understood incompletely. Potential causes may include bone marrow necrosis or fibrosis, suppression of granulopoiesis, immune-mediated destruction of granulocytic precursors or mature granulocytes, or a combination of these effects.[28-30]

Chemotherapeutic drugs are the most common drugs associated with the development of severe neutropenia. In one retrospective observational assessment of the causes of neutropenia in dogs and cats, two thirds of cases of dogs with suspected drug-induced neutropenia and the only case of a cat with suspected drug-induced neutropenia were due to antineoplastic agents.[12] These drugs are effective in the treatment of neoplasia because they primarily decimate colonies of rapidly dividing cells. Thus myelotoxicity is a common side effect of administration of these agents.[10] Likewise, radiation as a treatment for cancer can induce mitotic failure and apoptosis of hematopoietic progenitor cells, leading to bone marrow failure and severe neutropenia.[31]

Estrogens have been demonstrated to be myelotoxic in mice, rats, ferrets, and dogs.[32-35] Dogs are exposed to this steroid hormone by ingestion of an estrogen analog, or from estrogen-secreting tumors (Sertoli cell tumors). Doses at which depressed granulopoiesis is seen are variable in dogs, but significant neutropenia is not seen typically until the dose ingested exceeds the recommended therapeutic dose. Studies in dogs and mice demonstrate no direct effect of estrogen on the hematopoietic progenitor cells. Instead, in both species some evidence suggests that a myelopoiesis inhibitory factor is produced by thymic stromal cells exposed to estrogen.[34,36-38]

Myelophthisis

Myelophthisis is the failure of bone marrow to continue normal hematopoiesis because of its decimation by infiltrating abnormal tissue, typically neoplastic cells or collagen (myelofibrosis), and rarely osteoid (osteosclerosis) or diffuse intramedullary inflammation (e.g., fungal osteomyelitis).[39] Neoplasms associated with

myelophthisis are usually round cell neoplasms, including leukemias, lymphomas, multiple myeloma, and histiocytic sarcoma.[12,32] Myelofibrosis has been found in dogs with a variety of diseases, including immune-mediated hemolytic anemia, medullary lymphoma, and extramedullary neoplasia. It has also been documented in dogs receiving chronic treatment with different medications. Of 19 dogs reported to have myelofibrosis in one retrospective study, only two were reported to be neutropenic, despite all dogs displaying a poorly regenerative anemia.[28] The main mechanism by which neutropenia develops with myelophthisis is due to a loss of granulocytic progenitor cells coupled with a loss of the nurturing marrow microenvironment that occurs as a result of destruction of the bone marrow stromal cells.[40,41]

Cyclic hematopoiesis

Canine cyclic hematopoiesis is an autosomal recessive genetic disorder also known as gray collie syndrome because it is found in Collies with a diluted (gray) coat color. The disease is characterized by severe neutropenia developing every 10 to 14 days. Assessment of the bone marrow before a neutropenic episode shows a drastic decline in the myeloid lines, whereas myeloid hyperplasia prefaces the recovery of circulating neutrophil numbers.[42] The disease in gray Collies is associated with a genetic mutation that decreases the neutrophil elastase activity within the neutrophil. How this mutation specifically leads to the clinical presentation of the disease is unclear, but it is postulated that neutrophil elastase must be involved with normal feedback inhibition during granulopoiesis.[41,43]

Ineffective granulopoiesis despite normal to excessive quantities of progenitor cells

Dysgranulopoiesis describes the presence of dysplastic granulocyte progenitor cells that lead to a peripheral circulating neutropenia. This neutropenia occurs in the presence of normal to excessive quantities of progenitor cells in the bone marrow. Dysmyelopoiesis is a more general term used to describe the presence of dysplastic hematopoietic cells, not just granulocyte precursors. Three major classifications of dysmyelopoiesis include myelodysplastic syndrome (MDS), secondary dysmyelopoiesis, and congenital dysmyelopoiesis (which will not be discussed further).[44]

Myelodysplastic syndrome ultimately arises as a result of clonal expansion of a mutated hematopoietic progenitor cell. The cells arising from the mutant cell do not follow the normal maturation pathway and ultimately undergo apoptosis before they are released from the marrow. Thus the bone marrow appears hyperplastic and contains an abnormally high number of blasts, but there are insufficient cells in circulation.[44] Myelodysplastic syndrome can be one cause of neutropenia in cats with feline leukemia virus.[45]

Secondary dysmyelopoiesis is similar to MDS with the exception that the number of blasts present in the marrow is not increased from normal. Secondary dysmyelopoiesis can occur secondary to different diseases, including immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, and lymphoma. Secondary dysmyelopoiesis also can be seen with the administration of some drugs, including antineoplastic drugs, estrogen, phenobarbital, cephalosporins, chloramphenicol, and colchicine, as well as lithium in cats.[17,44,46] Many of these drugs also lead to hypoplastic bone marrow, as described above.

Separate to dysmyelopoiesis, a heritable disease has been described in Border Collies, in which the bone marrow displays hyperplastic granulopoiesis and no evidence of maturation arrest or dysplasia but a severe circulating neutropenia.[47] This disease is known as trapped neutrophil syndrome (TNS). Although the gene mutation that causes TNS has been identified, the underlying mechanism by which this mutation leads to decreased release of segmented neutrophils into circulation remains unknown.[48]

Immune-Mediated Destruction

Applicable to small animal veterinary patients are two main types of immune-mediated destruction of neutrophils: (1) primary or idiopathic immune-mediated neutropenia and (2) immune-mediated neutropenia that occurs secondary to an underlying trigger including infection, drugs, or neoplasia. Idiopathic immune-mediated neutropenia occurs when antibodies are produced against neutrophil surface proteins. These antibodies bind to the surface proteins and either activate complement-mediated death of the neutrophil or opsonize the cell for phagocytosis by macrophages.[49] The veterinary literature includes few reports of patients with confirmed immune-mediated neutropenia: the standard for definitive diagnosis is to demonstrate the presence of antineutrophil antibodies in the serum of the patient. Although successful detection of antineutrophil antibodies has been reported in dogs with immune-mediated neutropenia using flow cytometry, this test is not readily and widely available.[50-52] Instead, diagnosis often is based on exclusion of the other causes of neutropenia discussed above, in conjunction with improvement in neutrophil counts when treated with immune-suppressive agents.[12,53-58]

CLINICAL PRESENTATION AND DIAGNOSTIC TESTS

The clinical signs exhibited by the patient with febrile neutropenia ultimately depend on the underlying cause for neutropenia. Animals with an increased tissue demand and extravasation of neutrophils often demonstrate signs of severe sepsis or septic shock, typically with a marked suppurative exudate at the focus of infection or inflammation. In addition, these patients would likely display a marked degenerative left shift on a complete blood count. If neutropenia is secondary to decreased egress from bone marrow or immune-mediated destruction, patients may have opportunist infections of their skin (e.g., IV catheter site) or in their lungs or urinary tract but show minimal pathologic changes on visual inspection, thoracic radiographs, or urinalysis because of marked suppression of the normal inflammatory response.[59,60] Some animals, despite being profoundly neutropenic, may not have a fever on presentation. For example, many septic cats and very ill septic dogs are hypothermic on presentation to the emergency clinic. In addition, neutropenic patients that have received glucocorticoids or nonsteroidal antiinflammatory drugs may have suppression of their fever because of inhibition of prostaglandin formation.

Determining the underlying cause of neutropenia helps to develop an appropriate treatment plan. In many cases, the underlying cause for neutropenia may be clear: an obvious septic focus or a recent history of chemotherapy administration. In other cases, exhaustive diagnostics may be necessary to determine the underlying cause. Diagnostic tests to consider in a patient with neutropenia include blood cultures, urinalysis and culture, radiographs of the thorax and abdomen, ultrasound of the abdomen, and a bone marrow evaluation.

Blood cultures should be performed in febrile neutropenic patients as early as possible and ideally before administering antimicrobials. The 2012 Surviving Sepsis Guidelines recommend taking at least two 10- to 20-ml blood samples from different sites before starting antimicrobial therapy. Blood cultures allow for conclusive diagnosis of sepsis, and the susceptibility profile allows for appropriate de-escalation of antimicrobial therapy.[61] A urinalysis may have benign sediment in the face of a severe infectious process because of lack of the patient's ability to mount an immune response. A urine culture, like a blood culture, is important to evaluate to determine if antimicrobial therapy is appropriate and can allow de-escalation of these drugs.

Imaging of the thorax and abdomen using radiographs and ultrasound can help determine if there is a septic focus that has led to neutropenia. It also can help assess for other causes of neutropenia or for the presence of multiple organ dysfunction as a result of neutropenia (e.g., acute respiratory distress syndrome). Imaging of the cardiac valves also may be indicated in a neutropenic patient with a new murmur because vegetative endocarditis may be the septic source.

Bone marrow aspiration and/or a core biopsy are required to diagnose many of the causes of neutropenia, including infiltrative neoplasia, myelofibrosis, osteosclerosis, and dysmyelopoiesis. Evidence of maturation arrest in a bone marrow aspirate also may help support a presumptive diagnosis of immune-mediated neutropenia.[10] Bone marrow aspiration is a test that may help a clinician determine if the patient is responding to therapy and may help assess the likelihood of recovery from neutropenia.

TREATMENT AND SUPPORTIVE CARE

Most of the literature assessing treatment for febrile neutropenic patients consists of studies assessing care of oncology patients that are febrile secondary to their chemotherapy or radiation treatments. This group of neutropenic patients is different than patients that develop neutropenia for any of the other reasons mentioned previously, thus not all recommendations apply to all neutropenic patients.

Broad-spectrum antimicrobial therapy should be initiated in all febrile neutropenic patients. Decisions as to which antimicrobial therapy to choose should be based on the individual patient's characteristics, including previous antimicrobial exposure, previous culture results, suspected pathogen based on clinical signs, and regional microbe infection patterns. In people, monotherapy using an antipseudomonal β-lactam (e.g., ceftazidime, piperacillin-tazobactam, ticarcillin-clavulanate) has been shown to be efficacious in stable febrile neutropenic patients. In neutropenic people exhibiting signs of septic shock, the recommendation is to combine a β-lactam with an aminoglycoside (e.g., amikacin).[62] If infection with a fungal organism is suspected, antifungals also are recommended.[61] Once the susceptibility results of blood culture and urine culture are available, the antimicrobial therapy is changed to target specifically the pathogen(s) cultured. This can decrease the risk for the development of secondary infections with microorganisms that are multidrug resistant.[61] It also can help decrease the risk of organ damage that can develop with the use of some antimicrobials.

Any patient that is neutropenic and has clinical signs of sepsis, severe sepsis, or septic shock should be resuscitated aggressively using intravenous crystalloids and vasopressors as needed, and broad-spectrum antimicrobial therapy should be initiated as soon as possible.[61,63] If present and identified, source control of the septic focus should occur. Intensive monitoring for the development of multiple organ dysfunction syndrome and meticulous supportive care has to be provided to these patients. See Chapters 5, 6, and 7 for further information on this topic.

Another medication recommended for the treatment of febrile neutropenia is recombinant G-CSF. G-CSF increases the differentiation of progenitor cells into neutrophils and acts on mature neutrophils to increase chemotaxis, enhance the respiratory burst, and improve IgA-mediated phagocytosis.[64] In specific situations, recombinant human G-CSF (rhG-CSF) is recommended as a prophylactic treatment to people receiving chemotherapy because it has been shown to decrease the incidence of febrile neutropenia and secondary infection in this population.[65-68] Recombinant human G-CSF has been reported effective at stimulating granulopoiesis in dogs and cats.[69,70] However, it was shown to be ineffective in treating dogs with neutropenia secondary to Parvovirus.[71,72] In addition, administration of this human protein to dogs may lead to the development of antibodies against rhG-CSF. These antibodies cross-react with and neutralize canine G-CSF, which causes a chronic neutropenia.[73]

Recombinant canine G-CSF (rcG-CSF) was developed and has been used effectively to increase granulopoiesis in dogs and cats.[74-78] It has been demonstrated effective in dogs to accelerate recovery from neutropenia resulting from Parvovirus, chemotherapeutics, and cyclic hematopoiesis.[64,76,77,79] Some questions remain regarding its safety, however. In one study, dogs with Parvovirus treated with rcG-CSF had a higher mortality rate than dogs with Parvovirus that were not treated with this drug.[64] In addition, no studies have been performed in cats to determine if long-term or repeat rcG-CSF use may induce antibody formation and lead to chronic neutropenia as seen with rhG-CSF use in dogs. Evidence suggests that this outcome does occur in rabbits receiving rcG-CSF.[80] Use of this medication to treat dogs with neutropenia should be at the discretion of the clinician. Given lack of safety data, it is not recommended to use rcG-CSF in dogs with Parvovirus infections, or in alternate species.

Frequently recommended nursing practices include isolating severely neutropenic patients from other hospitalized animals and practicing barrier nursing, in which the carer dons a gown, gloves, and a mask before handling the patient. No literature reviews the efficacy of these practices in veterinary medicine, and the studies available regarding people tend to be small and uncontrolled with multiple interventions occurring simultaneously, making their results difficult to interpret.[81] Arguments against going to these lengths include the cost associated with maintaining a clean isolation ward and providing disposable barrier clothing, in addition to the potential for a decreased level of nursing care given the increased work required to prepare to care for these patients.[82] The current recommendation is to focus on meticulous hand hygiene, ensuring thorough washing of the hands before and after handling a neutropenic patient, in addition to using alcohol-based hand sanitizer and donning gloves before handling any indwelling devices including intravenous catheters, urinary catheters, feeding tubes, or tracheostomy tubes. Because these patients are at risk of developing infections secondary to their own commensal flora, keeping these patients clean and dry is critical. Preventing fecal and urine contamination of skin and indwelling devices is imperative.

REFERENCES

1. Seeley EJ, Matthay MA, Wolters PJ: Inflection points in sepsis biology: from local defense to systemic organ injury, Am J Physiol Lung Cell Mol Physiol 303:L355-363, 2012.
2. Borregaard N: Neutrophils, from marrow to microbes, Immunity 33:657-670, 2010.
3. Hostetter SJ: Neutrophil function in small animals, Vet Clin North Am Small Anim Pract 42:157-171, 2012.
4. Bugl S, Wirths S, Muller MR, et al: Current insights into neutrophil homeostasis, Ann N Y Acad Sci 1266:171-178, 2012.
5. Panopoulos AD, Watowich SS: Granulocyte colony-stimulating factor: molecular mechanisms of action during steady state and "emergency" hematopoiesis, Cytokine 42:277-288, 2008.
6. Schwarzenberger P, Huang W, Ye P, et al: Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis, J Immunol 164:4783-4789, 2000.
7. Aggarwal S, Ghilardi N, Xie MH, et al: Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17, J Biol Chem 278:1910-1914, 2003.
8. Stark MA, Huo Y, Burcin TL, et al: Phagocytosis of apoptotic neutrophils regulates granulopoiesis via IL-23 and IL-17, Immunity 22:285-294, 2005.
9. Bugl S, Wirths S, Radsak MP, et al: Steady-state neutrophil homeostasis is dependent on TLR4/TRIF signaling, Blood 121:723-733, 2013.
10. Schnelle AN, Barger AM: Neutropenia in dogs and cats: causes and consequences, Vet Clin North Am Small Anim Pract 2012;42:111-122.

11. Schultze AE: Interpretation of canine leukocyte responses. In Weiss DJ, Wardrop KJ, editor: Schalm's veterinary hematology, ed 6, Ames, Iowa, 2010, Blackwell, pp 321-334.
12. Brown MR, Rogers KS: Neutropenia in dogs and cats: a retrospective study of 261 cases, J Am Anim Hosp Assoc 37:131-139, 2001.
13. Rodriguez S, Chora A, Goumnerov B, et al: Dysfunctional expansion of hematopoietic stem cells and block of myeloid differentiation in lethal sepsis, Blood 114:4064-4076, 2009.
14. de Bruin AM, Libregts SF, Valkhof M, et al: IFNgamma induces monopoiesis and inhibits neutrophil development during inflammation, Blood 119:1543-1554, 2012.
15. Santangelo S, Gamelli RL, Shankar R: Myeloid commitment shifts toward monocytopoiesis after thermal injury and sepsis, Ann Surg 233:97-106, 2001.
16. Shoup M, Weisenberger JM, Wang JL, et al: Mechanisms of neutropenia involving myeloid maturation arrest in burn sepsis, Ann Surg 228:112-122, 1998.
17. Dieringer TM, Brown SA, Rogers KS, et al: Effects of lithium carbonate administration to healthy cats, Am J Vet Res 53:721-726, 1992.
18. Tvedten HaRRE: Leukocytic disorders. In Small animal clinical diagnosis by laboratory methods, ed 5, St Louis, 2012, Elsevier Saunders, pp 63-91.
19. Breuer W, Stahr K, Majzoub M, et al: Bone-marrow changes in infectious diseases and lymphohaemopoietic neoplasias in dogs and cats—a retrospective study, J Comp Pathol 119:57-66, 1998.
20. Goddard A, Leisewitz AL, Christopher MM, et al: Prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis, J Vet Intern Med 22:309-316, 2008.
21. Kruse BD, Unterer S, Horlacher K, et al: Prognostic factors in cats with feline panleukopenia, J Vet Intern Med 24:1271-1276, 2010.
22. Little SE: Ehrlichiosis and anaplasmosis in dogs and cats, Vet Clin North Am Small Anim Pract 40:1121-1140, 2010.
23. Gleich S, Hartmann K: Hematology and serum biochemistry of feline immunodeficiency virus-infected and feline leukemia virus-infected cats, J Vet Intern Med 23:552-558, 2009.
24. Tanabe T, Yamamoto JK: Phenotypic and functional characteristics of FIV infection in the bone marrow stroma, Virology 282:113-122, 2001.
25. Fujino Y, Horiuchi H, Mizukoshi F, et al: Prevalence of hematological abnormalities and detection of infected bone marrow cells in asymptomatic cats with feline immunodeficiency virus infection, Vet Microbiol 136:217-225, 2009.
26. Jacobs G, Calvert C, Kaufman A: Neutropenia and thrombocytopenia in three dogs treated with anticonvulsants, J Am Vet Med Assoc 212:681-684, 1998.
27. Brazzell JL, Weiss DJ: A retrospective study of aplastic pancytopenia in the dog: 9 cases (1996-2003), Vet Clin Pathol 35:413-417, 2006.
28. Weiss DJ, Smith SA: A retrospective study of 19 cases of canine myelofibrosis, J Vet Intern Med 16:174-178, 2002.
29. Weiss DJ: Bone marrow necrosis in dogs: 34 cases (1996-2004), J Am Vet Med Assoc 227:263-267, 2005.
30. Garbe E: Non-chemotherapy drug-induced agranulocytosis, Expert Opin Drug Saf 6:323-335, 2007.
31. Kulkarni S, Ghosh SP, Hauer-Jensen M, et al: Hematological targets of radiation damage, Curr Drug Targets 11:1375-1385, 2010.
32. Weiss DJ, Evanson OA, Sykes J: A retrospective study of canine pancytopenia, Vet Clin Pathol 28:83-88, 1999.
33. Hart JE: Endocrine pathology of estrogens: species differences, Pharmacol Ther 47:203-218, 1990.
34. Crandall TL, Joyce RA, Boggs DR: Estrogens and hematopoiesis: characterization and studies on the mechanism of neutropenia, J Lab Clin Med 95:857-867, 1980.
35. Sontas HB, Dokuzeylu B, Turna O, et al: Estrogen-induced myelotoxicity in dogs: a review, Can Vet J 50:1054-1058, 2009.
36. Luster MI, Boorman GA, Korach KS, et al: Mechanisms of estrogen-induced myelotoxicity: evidence of thymic regulation, Int J Immunopharmacol 6:287-297, 1984.
37. Farris GM, Benjamin SA: Inhibition of myelopoiesis by serum from dogs exposed to estrogen, Am J Vet Res 54:1374-1379, 1993.
38. Farris GM, Benjamin SA: Inhibition of myelopoiesis by conditioned medium from cultured canine thymic cells exposed to estrogen, Am J Vet Res 54:1366-1373, 1993.
39. Topper MJ: Hemostasis. In Latimer KS, Mahaffey EA, Prasse KW, editors: Duncan & Prasse's veterinary laboratory medicine clinical pathology, ed 4, Ames, Iowa, 2003, Blackwell Publishing Company, pp 99-135.
40. Makoni SN, Laber DA: Clinical spectrum of myelophthisis in cancer patients, Am J Hematol 76:92-93, 2004.
41. Harvey JW: Evaluation of leukocytic disorders. In Veterinary hematology: a diagnostic guide and color atlas, St Louis, 2012, Elsevier Saunders, pp 122-176.
42. Dale DC, Ward SB, Kimball HR, et al: Studies of neutrophil production and turnover in grey collie dogs with cyclic neutropenia, J Clin Invest 51:2190-2196, 1972.
43. Horwitz MS, Corey SJ, Grimes HL, et al: ELANE mutations in cyclic and severe congenital neutropenia: genetics and pathophysiology, Hematol Oncol Clin North Am 27:19-41, 2013.
44. Weiss DJ: Recognition and classification of dysmyelopoiesis in the dog: a review, J Am Vet Med Assoc Am Coll Vet Int Med 19:147-154, 2005.
45. Weiss DJ: Evaluation of dysmyelopoiesis in cats: 34 cases (1996-2005), J Am Vet Med Assoc 228:893-897, 2006.
46. Weiss DJ, Aird B: Cytologic evaluation of primary and secondary myelodysplastic syndromes in the dog, Vet Clin Pathol 30:67-75, 2001.
47. Allan FJ, Thompson KG, Jones BR, et al: Neutropenia with a probable hereditary basis in Border Collies, New Z Vet J 44:67-72, 1996.
48. Shearman JR, Wilton AN: A canine model of Cohen syndrome: trapped neutrophil syndrome, BMC Genomics 12:258, 2011.
49. Chickering WR, Prasse KW: Immune mediated neutropenia in man and animals: a review, Vet Clin Pathol 10:6-16, 1981.
50. Weiss DJ: An indirect flow cytometric test for detection of anti-neutrophil antibodies in dogs, Am J Vet Res 68:464-467, 2007.
51. Weiss DJ: Evaluation of antineutrophil IgG antibodies in persistently neutropenic dogs, J Vet Int Med 21:440-444, 2007.
52. Weiss DJ, Henson M: Pure white cell aplasia in a dog. Veterinary clinical pathology, Am Soc Vet Clin Pathol 36:373-375, 2007.
53. Brown CD, Parnell NK, Schulman RL, et al: Evaluation of clinicopathologic features, response to treatment, and risk factors associated with idiopathic neutropenia in dogs: 11 cases (1990-2002), J Am Vet Med Assoc 229:87-91, 2006.
54. Snead E, Vargo C, Myers S: Glucocorticoid-dependent hypoadrenocorticism with thrombocytopenia and neutropenia mimicking sepsis in a Labrador retriever dog, Can Vet J 52:1129-1134, 2011.
55. Fidel JL, Pargass IS, Dark MJ, et al: Granulocytopenia associated with thymoma in a domestic shorthaired cat, J Am Anim Hosp Assoc 44:210-217, 2008.
56. McManus PM, Litwin C, Barber L: Immune-mediated neutropenia in 2 dogs, J Vet Intern Med 1999;13:372-374.
57. Vargo CL, Taylor SM, Haines DM: Immune mediated neutropenia and thrombocytopenia in 3 giant schnauzers, Can Vet J 48:1159-1163, 2007.
58. Perkins MC, Canfield P, Churcher RK, et al: Immune-mediated neutropenia suspected in five dogs, Aus Vet J 82:52-57, 2004.
59. Bodey GP: Unusual presentations of infection in neutropenic patients, Int J Antimicrob Agents 16:93-95, 2000.
60. Sipsas NV, Bodey GP, Kontoyiannis DP: Perspectives for the management of febrile neutropenic patients with cancer in the 21st century, Cancer 103:1103-1113, 2005.
61. Dellinger RP, Levy MM, Rhodes A, et al: Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012, Intens Care Med 39:165-228, 2013.
62. Tam CS, O'Reilly M, Andresen D, et al: Use of empiric antimicrobial therapy in neutropenic fever. Australian Consensus Guidelines 2011 Steering Committee, Intern Med J 41:90-101, 2011.
63. Kumar A, Roberts D, Wood KE, et al: Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock, Crit Care Med 34:1589-1596, 2006.
64. Duffy A, Dow S, Ogilvie G, et al: Hematologic improvement in dogs with parvovirus infection treated with recombinant canine granulocyte-colony stimulating factor, J Vet Pharmacol Ther 33:352-356, 2010.
65. Aapro MS, Bohlius J, Cameron DA, et al: 2010 update of EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphoproliferative disorders and solid tumours, Eur J Cancer 47:8-32, 2011.

66. Liang DC: The role of colony-stimulating factors and granulocyte transfusion in treatment options for neutropenia in children with cancer, Paediatr Drugs 5:673-684, 2003.
67. Sung L, Nathan PC, Lange B, et al: Prophylactic granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor decrease febrile neutropenia after chemotherapy in children with cancer: a meta-analysis of randomized controlled trials, J Clin Oncol 22:3350-3356, 2004.
68. Sung L, Nathan PC, Alibhai SM, et al: Meta-analysis: effect of prophylactic hematopoietic colony-stimulating factors on mortality and outcomes of infection, Ann Intern Med 147:400-411, 2007.
69. Fulton R, Gasper PW, Ogilvie GK, et al: Effect of recombinant human granulocyte colony-stimulating factor on hematopoiesis in normal cats, Exper Hematol 19:759-767, 1991.
70. Lothrop CD Jr, Warren DJ, Souza LM, et al: Correction of canine cyclic hematopoiesis with recombinant human granulocyte colony-stimulating factor, Blood 72:1324-1328, 1988.
71. Rewerts JM, McCaw DL, Cohn LA, et al: Recombinant human granulocyte colony-stimulating factor for treatment of puppies with neutropenia secondary to canine parvovirus infection, J Am Vet Med Assoc 213:991-992, 1998.
72. Mischke R, Barth T, Wohlsein P, et al: Effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on leukocyte count and survival rate of dogs with parvoviral enteritis, Res Vet Sci 70:221-225, 2001.
73. Hammond WP, Csiba E, Canin A, et al: Chronic neutropenia. A new canine model induced by human granulocyte colony-stimulating factor, J Clin Invest 87:704-710, 1991.
74. Obradovich JE, Ogilvie GK, Powers BE, et al: Evaluation of recombinant canine granulocyte colony-stimulating factor as an inducer of granulopoiesis. A pilot study, J Vet Intern Med 5:75-79, 1991.
75. Zinkl JG, Cain G, Jain NC, et al: Haematological response of dogs to canine recombinant granulocyte colony stimulating factor (rcG-CSF), Comp Haematol Int 2:151-156, 1992.
76. Ogilvie GK, Obradovich JE, Cooper MF, et al: Use of recombinant canine granulocyte colony-stimulating factor to decrease myelosuppression associated with the administration of mitoxantrone in the dog, J Vet Intern Med 6:44-47, 1992.
77. Mishu L, Callahan G, Allebban Z, et al: Effects of recombinant canine granulocyte colony-stimulating factor on white blood cell production in clinically normal and neutropenic dogs, J Am Vet Med Assoc 200:1957-1964, 1992.
78. Obradovich JE, Ogilvie GK, Stadler-Morris S, et al: Effect of recombinant canine granulocyte colony-stimulating factor on peripheral blood neutrophil counts in normal cats, J Vet Intern Med 7:65-67, 1993.
79. Yamamoto A, Fujino M, Tsuchiya T, et al: Recombinant canine granulocyte colony-stimulating factor accelerates recovery from cyclophosphamide-induced neutropenia in dogs, Vet Immunol Immunopathol 142:271-275, 2011.
80. Reagan WJ, Murphy D, Battaglino M, et al: Antibodies to canine granulocyte colony-stimulating factor induce persistent neutropenia, Vet Pathol 32:374-378, 1995.
81. Larson E, Nirenberg A: Evidence-based nursing practice to prevent infection in hospitalized neutropenic patients with cancer, Oncol Nurs Forum 31:717-725, 2004.
82. Mank A, van der Lelie H: Is there still an indication for nursing patients with prolonged neutropenia in protective isolation? An evidence-based nursing and medical study of 4 years experience for nursing patients with neutropenia without isolation, Eur J Oncol Nurs 7:17-23, 2003.