

REVIEW ARTICLE

Modern management of phagocyte defects

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Abstract

Phagocytic neutrophil granulocytes are among the first immune cells active at sites of infection, forming an important first-line defense against invading microorganisms. Congenital immune defects concerning these phagocytes may be due to reduced neutrophil numbers or function. Management of affected patients depends on the type and severity of disease. Here, we provide an overview of causes and treatment of diseases associated with congenital neutropenia, as well as defects of the phagocytic respiratory burst.

Introduction

Neutrophil granulocytes constitute 50–70% of human blood leukocytes. They are produced in bone marrow from myeloid progenitor cells at 10^9 cells/kg/day (1). Neutrophil maturation in bone marrow is regulated through coordinated expression of cytokines like granulocyte-macrophage colony-stimulating factor (GM-CSF) or granulocyte colony-stimulating factor (G-CSF) (2). Mediated by cytokines and selectin/integrin, they migrate from peripheral blood to sites of infection where they play a key role in first-line defense against bacteria and fungi by phagocytosis, respiratory burst, and generation of neutrophil extracellular traps (NETs) (3) (Fig. 1).

Inherited quantitative neutrophil disorders are termed congenital neutropenia (CN), classified by absolute neutrophil count (ANC) in peripheral blood into mild ($1.0\text{--}1.5 \times 10^9/l$), moderate ($0.5\text{--}1.0 \times 10^9/l$), or severe ($<0.5 \times 10^9/l$) neutropenia, which may be constant, intermittent, or periodic (Table S1). Additional immunologic and non-hematologic manifestations characterize distinct subtypes of CN. The estimated overall incidence is 10–15/1 Mio births and the estimated prevalence $>10/1$ Mio inhabitants (4). Patients with CN suffer from recurrent infections with bacteria and fungi, causing cellulitis, pneumonia or sepsis, and oral aphtosis, paradontosis, or tooth loss (5). In some cases, CN may predispose to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (6, 7).

Inherited qualitative neutrophil disorders can be caused by defects of leukocyte adhesion, phagocytosis, or the respiratory burst. This review will only focus on latter defects, termed chronic granulomatous diseases (CGD).

Quantitative neutrophil disorders**CN without extra-hematopoietic manifestations****Benign ethnic neutropenia**

This is the most common (autosomal-dominant) type of neutropenia occurring in 25–50% of persons with African or West Indian descent and some Middle East ethnic groups (8). Neutropenia may range from mild to severe. Affected individuals generally do not have an increased susceptibility to infections. A polymorphism in *DARC* has been identified in some individuals associated with a protective effect against malaria (9).

ELANE deficiency

Autosomal-dominant heterozygous mutations of *ELANE*, encoding neutrophil elastase, are the most frequent cause of CN in Caucasians. The prevalence is 1/300,000–400,000 (calculated from (5)), accounting for 50% of all severe CN cases. *ELANE* deficiency is responsible for cyclic neutropenia

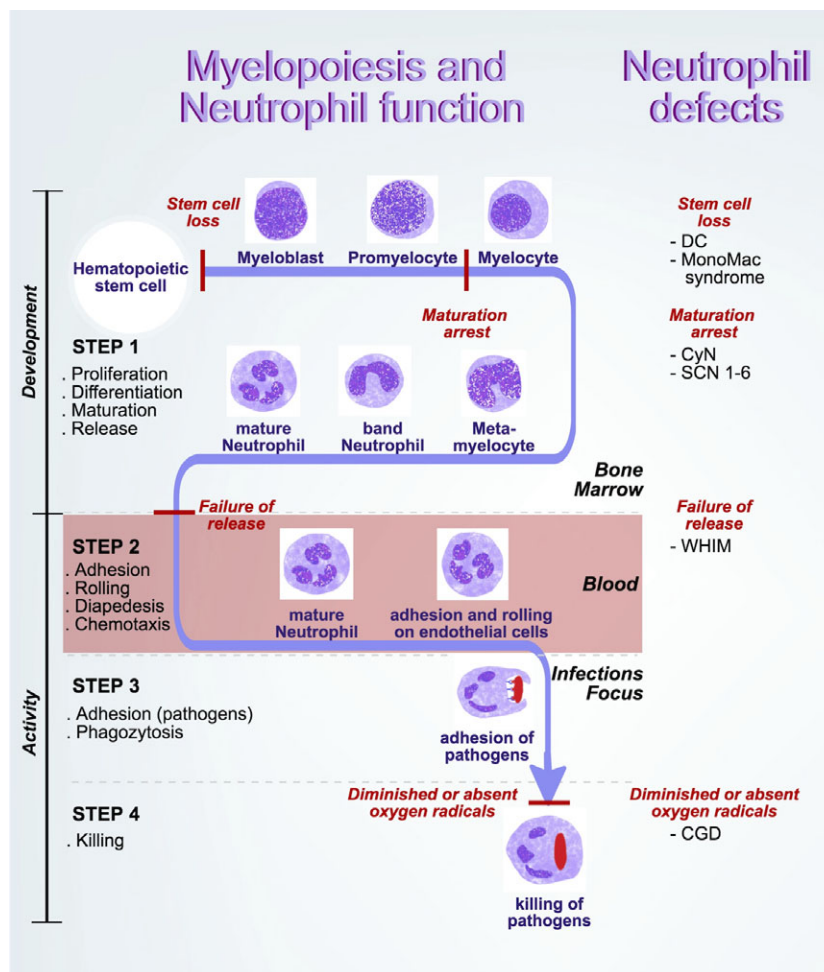


Figure 1 Myelopoiesis, neutrophil function, and neutrophil defects. CGD, chronic granulomatous disease; CyN, cyclic neutropenia; DC, dyskeratosis congenita; SCN, severe congenital neutropenia; WHIM, warts, hypogammaglobulinemia, infections, and myelokathexis.

(CyN) and severe CN type 1 (SCN1), which leads to more severe infections. In CyN, neutrophil counts typically fluctuate between normal and levels close to zero with approximately 21-day periodicity, associated with inverse monocyte cycling, sometimes together with variations in reticulocyte, eosinophil, lymphocyte, and platelet counts (10).

Patients with SCN1 have chronic profound neutropenia with a characteristic bone marrow maturation arrest at the promyelocyte stage, likely caused by apoptosis of neutrophil precursors (11).

CN with other immune defects

GFI1 deficiency

Autosomal-dominant mutations in *GFI1* cause SCN2 (prevalence < 1/22 Mio, calculated from (5)). *GFI1* is a transcription factor determining development of myeloid progenitors into granulocytes or monocytes/macrophages (12). SCN2 patients therefore have neutropenia and monocytosis caused by maturation arrest at promyelocyte and/or myelocyte stage in bone marrow.

WHIM syndrome

Autosomal-dominant gain-of-function *CXCR4* mutations lead to WHIM syndrome, combining warts, hypogammaglobulinemia, bacterial infections, and myelokathexis. Estimated prevalence is 1/2.2 Mio (calculated from (5)). *CXCR4* deficiency leads to retention of neutrophils in the bone marrow (13). In addition to recurrent otitis media, respiratory and skin infections or septic arthritis, patients may develop human papilloma virus (HPV)-associated warts (13), cervical cancer, or oral squamous cell carcinoma. Prophylaxis consists of immunoglobulin substitution, antibiotics, and HPV vaccine. *CXCR4* antagonist mozobil (Plerixafor ©) seems superior to G-CSF and granulocyte-macrophage (GM)-CSF to mobilize neutrophils to peripheral blood (14).

Wiskott–Aldrich syndrome and X-linked neutropenia

X-linked Wiskott–Aldrich syndrome (WAS), caused by mutations in *WASP*, manifests with thrombocytopenia with reduced platelet volume, eczema, susceptibility to infection, and an increased risk of autoimmunity and lymphomas (for review:

(15)). Autoimmunity can cause neutropenia, hemolytic anemia, vasculitis, inflammatory bowel disease, depressed IgM levels, and elevated IgA and IgE or Henoch–Schönlein-like purpura (15). WAS affects 1–9/1 Mio males and has an estimated prevalence of 1/2.2 Mio (calculated from (5)).

Loss-of-function mutations in *WASP* lead to WAS, whereas X-linked neutropenia (XLN) is caused by gain-of-function mutations in *WASP*. Hyperactivation and abnormal localization of actin polymerization in XLN result in impaired myelopoiesis and neutropenia (15).

Prevention of infections in patients with classical WAS consists of *Pneumocystis jirovecii* (*PCJ*) prophylaxis, immunoglobulin substitution, avoidance of live vaccines, and administration of CMV-negative irradiated blood products. Autoimmune manifestations may require aggressive immunosuppression (15). Hematopoietic stem cell transplantation (HSCT), or in selected cases gene therapy (16), may be required as curative therapy. The milder phenotypic variants, X-linked thrombocytopenia and XLN, are managed by antibiotic prophylaxis or symptomatic treatment.

CN with extra-hematopoietic manifestations

Barth syndrome

X-linked Barth syndrome is a mitochondrial disease caused by mutations in *TAZ* (17). The estimated prevalence is 1/300,000–400,000 live births (Barth foundation). Patients present in infancy with hypertrophic cardiomyopathy, skeletal myopathy, neutropenia, and delayed growth and frequently die prematurely due to heart failure or sepsis. In 20% of cases, patients first present with infections (18). Neutropenia is caused by maturation arrest at the myelocyte stage.

Cartilage–hair Hypoplasia

Autosomal-recessive *RMRP* mutations cause cartilage–hair hypoplasia (CHH) with a birth prevalence of 1/23,000 (19). *RMRP* is a mitochondrial RNA processing endoribonuclease involved in ribosomal assembly and cell-cycle regulation (20). Patients with CHH have metaphyseal chondrodysplasia, light colored hypoplastic hair and skin, onychodysplasia, microdontia, and Hirschsprung disease. In total, 27% have chronic neutropenia (21). The additional immunodeficiency is variable, ranging from SCID to less severe humoral defects. CHH patients may develop hematologic malignancies, especially non-Hodgkin's lymphoma (20). HSCT is required for patients SCID phenotype.

CD40 ligand deficiency

X-linked hyper-IgM syndrome (HIGM1) is caused by mutations of *CD40L*, causing impaired immunoglobulin isotype switch. In addition to impaired IgG, IgA and IgE generation, some patients have neutropenia, likely due to reduced G-CSF and GM-CSF production (22). They present with bacterial respiratory infections, *PCJ* pneumonia, *Cryptosporidium parvum* infections, seronegative arthritis and lymphoid

hyperplasia. Treatment consists of immunoglobulin substitution, *PCJ* prophylaxis, and eventually G-CSF in case of severe persistent neutropenia (23). Curative HSCT should be performed early in life.

Chédiak–Higashi syndrome

Autosomal-recessive *CHS1* mutations lead to defects in lysosomal trafficking with enlarged vesicles in neutrophils, cytotoxic T cells, and melanocytes (24). In total, 500 patients have been described in the literature (19). They present with light skin and silvery hair, photosensitivity, progressive neurologic abnormalities, bleeding, and recurrent pyogenic infections. The latter are due to neutropenia (caused by intramedullary destruction of neutrophils (24)) and defective cytotoxicity. Typical is infantile-onset hemophagocytic lymphohistiocytosis (HLH) requiring allogeneic HSCT (25).

Clericuzio-type poikiloderma with neutropenia syndrome

Autosomal-recessive *C16orf57* mutations cause this genodermatosis described in 50 cases (19). The majority of affected patients appear normal at birth and then develop an erythematous rash resolving with poikiloderma during first year of life. In addition, they present hyperkeratotic nails, palms and soles, neutropenia, recurrent pulmonary infections, and short stature (26). Half of the patients have transient thrombocytopenia/anemia. Bone marrow is hypocellular with increased myeloid precursors and delayed neutrophil maturation. Patients are prone to bone marrow failure, which may progress into MDS or AML. Long-term treatment with G-CSF is not required, as many patients have adequate rise of ANC during infections (26).

Cohen syndrome

Autosomal-recessive *VPS13B* mutations cause Cohen syndrome. In total, 200 cases have been described (19) in a variety of ethnic groups (27). Patients present with intermittent neutropenia, microcephaly, psychomotor retardation, muscular hypotonia, obesity, retinochoroidal dystrophy, and following facial features: high nasal bridge, prominent central incisors, down slanting and wave-shaped palpebral fissures, and short philtrum (28).

Dyskeratosis congenita

Dyskeratosis congenita (DC), a bone marrow failure syndrome, is caused by X-linked (most common), autosomal-dominant or autosomal-recessive mutations in at least 10 genes (Table S1) leading to premature telomere shortening. The estimated prevalence is 1/1 Mio (19). Patients have abnormal skin pigmentation, mucosal leukoplakia, nail dystrophy, bone marrow failure, and cancer predisposition (29). In total, 80–90% of the <30-year-old patients suffer from anemia, leukopenia, or thrombocytopenia (29). HSCT may cure bone marrow failure, but cannot improve other tissues affected by DC (30).

G6PC3 deficiency

Autosomal-recessive glucose-6-phosphatase- β (G6PC3) deficiency (SCN4) has been described in 57 patients (19) with neutropenia due to increased endoplasmic reticulum stress causing apoptosis (31). Phenotypic variability is broad, ranging from isolated SCN to SCN with thrombocytopenia and syndromic features (congenital heart and urogenital defects, failure to thrive, endocrine disorders, facial dysmorphism, inner ear hearing loss, hyperelastic skin, decreased subcutaneous fat tissue, and livedo reticularis) (32). SCN4 manifests in the first months of life with recurrent bacterial infections of the respiratory or urinary tract, skin abscesses, or sepsis.

Glycogen storage disease type 1b (GSD-1b)

Autosomal-recessive SLC37A4 mutations cause GSD-1b described in 50 cases (5). This metabolic disorder is characterized by neutropenia due to increased apoptosis, together with early-onset hepatosplenomegaly, growth retardation, osteopenia, kidney enlargement, hypoglycemia, hyperlactacidemia, hyperlipidemia, and hyperuricemia (33). G-CSF and vitamin E (34) have been used successfully to improve ANC.

Griscelli syndrome type II

Autosomal-recessive *RAB27A* mutations cause Griscelli syndrome type II, characterized by partial albinism (hypopigmentation of hair and skin) and immunodeficiency, including transient neutropenia and HLH (35). Impaired vesicular trafficking causes pigment clumping in melanocytes and impaired cytotoxicity in T cells and NK cells (36). Patients suffer from recurrent pyogenic infections and may present with early-onset HLH, requiring HSCT. Gene therapy is in preclinical development (36).

HAX1 deficiency

Autosomal-recessive *HAX1* mutations cause SCN3 (37), accounting for about 15% of SCN, estimated prevalence 1/1.5–2 Mio calculated from (5)). Neutropenia is caused by maturation arrest in bone marrow at the promyelocyte stage, accompanied by monocytosis and eosinophilia. During the first weeks of life, patients present with severe bacterial infections. Some suffer from mental retardation and epilepsy (38).

Hermansky–Pudlak syndrome type II

Autosomal-recessive *AP3B1* mutations cause Hermansky–Pudlak syndrome (HPS) type II, described in 8 patients (19). This lysosomal disease leads to impaired organelle biogenesis in neutrophils, platelets, T cells, melanocytes, and lung type II epithelial cells. Patients may present with bleeding, oculocutaneous albinism and progressive pulmonary fibrosis (24), neutropenia with maturation arrest at promyelocyte/myelocyte stage, defective cytotoxicity, and HLH. Symptomatic treatment includes vitamin E and DDAVP for chronic hemorrhages (39).

JAGN1 deficiency

Autosomal-recessive *JAGN1* mutations have been described to cause SCN6 in 14 patients (40). Patients may present with recurrent respiratory tract infections, sepsis, skin abscesses, and pancolitis. Other manifestations include short stature, scoliosis, hip dysplasia, osteoporosis, pyloric stenosis, non-epileptic seizures, pancreatic insufficiency, amelogenesis imperfecta, coarctation of aorta, mild facial dysmorphism, urogenital anomalies, and hypothyroidism. *JAGN1*-defective neutrophils have abnormal endoplasmic reticulum structure, absence of granules, abnormal N-glycosylation of proteins, and increased susceptibility to apoptosis (40). As *JAGN1* is required for G-CSF receptor signaling, there is poor response to G-CSF. GM-CSF might be a therapeutic option, as it restores fungal killing activity in *JAGN1*-deficient granulocytes (41).

GATA2 deficiency (MonoMAC syndrome)

Autosomal-dominant *GATA2* mutations cause monocytopenia and mycobacterial infection (MonoMAC) syndrome, with a prevalence of <1/1 Mio (5). Patients may have decreased or absent monocytes, B cells, NK cells, neutrophils, or dendritic cells. Patients mostly present in early adulthood with severe and/or recurrent non-tuberculous mycobacterial infections, lymphedema, and HPV-associated warts (42). There is an increased risk for myelodysplastic syndrome (MDS, 84%), acute myeloid leukemia (AML, 14%), or chronic myelomonocytic leukemia (CMML, 8%) (43). Therefore, regular blood count monitoring and annual bone marrow analysis are recommended to evaluate necessity of HSCT.

STK4 deficiency

Autosomal-recessive *STK4* mutations described in seven patients (19) lead to intermittent neutropenia and T- and B-cell lymphopenia. Patients have atrial septal defects, mucocutaneous candidiasis, cutaneous warts, and skin abscesses (44). *STK4* kinase deficiency leads to impaired leukocyte adhesion and chemotaxis (45).

P14 deficiency

Autosomal-recessive mutations in *LAMTOR2* encoding the endosomal adaptor protein p14 cause severe neutropenia, cytotoxic T-cell and B-cell deficiency, partial albinism, and short stature (46). Four related patients have been reported (19). Prompt and aggressive treatment of acute infections, as well as prophylactic antimicrobials, is required.

Pearson marrow pancreas syndrome

X-linked mitochondrial DNA deletions cause Pearson marrow pancreas syndrome in 60 described patients (19). Patients display macrocytic anemia, variable neutropenia, and thrombocytopenia, hypoplastic bone marrow with vacuolation of hematopoietic precursors and multiorgan involvement including exocrine pancreas, liver and renal tubular defects leading to

early death (47). In addition to symptomatic treatment and supplementation with carnitine, riboflavin, and coenzyme Q, HSCT may be beneficial to treat the hematologic aspects of the disease (48).

Reticular dysgenesis

Autosomal-recessive *AK2* mutations cause reticular dysgenesis (RD) in 1/3–5 Mio newborns, characterized by a complete absence of myelo- and lymphopoiesis due to apoptosis of precursors, combined with sensorineural deafness (49). RD is the most serious form of severe combined immunodeficiency (SCID), combining neutropenia, hypogammaglobulinemia, T- and NK-cell lymphopenia, which may cause fatal infections in the first days to year of life. Antimicrobial prophylaxis, immunoglobulin substitution, and G-CSF are used to bridge time to HSCT.

Shwachman–Diamond syndrome

Autosomal-recessive *SDBS* mutations cause Shwachman–Diamond syndrome (50), a multisystem disorder combining exocrine pancreatic dysfunction, metaphyseal chondrodysplasia, neutropenia with impaired chemotaxis, and bone marrow failure with predisposition to MDS and leukemia. The estimated incidence is 1/300,000–400,000 (19). Most patients with SDS need regular doses of pancreatic enzymes, multivitamins and fat-soluble vitamins as well as a low-fat diet (51). As neutropenia is typically mild to moderate or intermittent, regular G-CSF treatment is not required. SDS patients developing severe aplastic anemia, MDS or AML, require HSCT.

TWEAK deficiency

Autosomal-dominant *TWEAK* mutations account for rare cases of hypogammaglobulinemia, neutropenia, and thrombocytopenia (52). *TWEAK* mediates apoptosis and regulates angiogenesis. Patients suffer from recurrent infections, such as warts, otitis media, pneumonia, osteomyelitis, and pneumococcal meningitis (52).

VPS45 deficiency

Autosomal-recessive *VSP45* mutations have been described in eight patients with *SCN* (*SCN5*) and bone marrow fibrosis, causing thrombocytopenia, anemia, and extramedullary hematopoiesis with consecutive hepatosplenomegaly and nephromegaly (53). *VPS45* controls endosomal membrane trafficking and protein sorting/recycling. Patients with *SCN5* do not respond to G-CSF therapy.

Diagnostics CN

Clinical evaluation

Common infections in CN patients are oral ulcers, gingivitis, periodontitis, tooth loss, pharyngitis, otitis media, sinusitis,

pneumonia, cellulitis, colitis, perirectal infections, deep tissue infections, and sepsis (Fig. 2).

Careful medical history includes type/frequency of infections, degree of fever and type/duration of previous antibiotic therapy, constitutional symptoms suggesting immunodeficiency or metabolic defects, growth/development. Family history should include infectious deaths, hematologic malignancy, unexplained infant deaths, and consanguinity.

As neutropenia is associated with reduced inflammatory response with frequently less pain or redness, careful physical examination is crucial. Acute abdominal pain and fever is an alarm sign for inflammatory bowel disease or sepsis with abdominal origin.

Laboratory evaluation

Complete blood count (CBC) including differential is diagnostic. At least three CBCs over three months are required for evaluation of any neutropenic patient (11). To determine severity and duration of neutropenia, past CBCs, especially from early childhood, should be tracked. In patients with mild

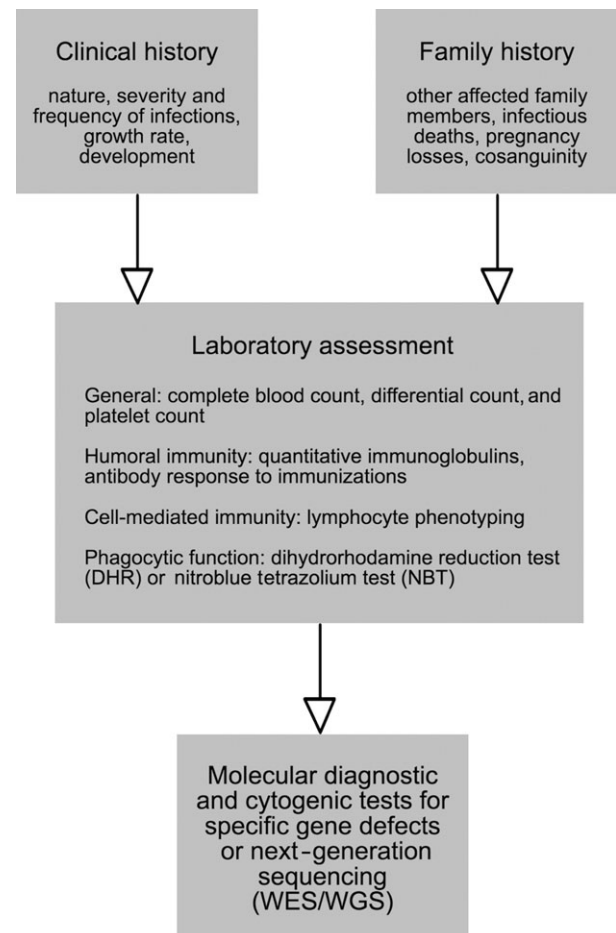


Figure 2 Diagnostic steps in patients with suspected congenital neutropenia.

neutropenia without recurrent infections or other health/hematologic problems, follow-up CBC and differential may be sufficient. In patients with recurrent fever or oral ulcers, CyN has to be sought by CBCs and differentials, three times/week for 6 weeks.

In newborns with isolated neutropenia and suspected maternofetal allo-immunization, and in patients with permanent profound neutropenia, anti-HNA1a-c IgG antibodies should be sought. Repeated analysis is often required, as only 75% are positive on first examination (10). The likelihood of retrieving autoantibodies against neutrophils is higher if neutrophil counts are $<500/\mu\text{l}$ in laboratories with considerable experience in performing and interpreting the assay.

Based on clinical findings and patient/family history, further tests may be required: evaluation for nutritional deficiencies (vitamin B12, folate, copper), immune deficiencies (IgG, IgA, IgM, lymphocyte phenotyping), rheumatologic disorders (e.g., antinuclear antibody), etc.

Genetic testing by candidate gene analysis or whole exome/genome sequencing may help support the diagnosis, indicate a risk for MDS/AML, and provide basis for genetic counseling.

Bone marrow examination

In patients with severe neutropenia and other hematologic anomalies or presence of organomegaly, bone marrow cytology should be performed immediately to rule out malignancy.

In case of persisting or recurrent neutropenia, bone marrow cytology and cytogenetics, as well as peripheral blood smears, should be obtained before patients are treated with G-CSF. Patients with SCN due to ELANE or HAX1 mutations have high numbers of promyelocytes but only few mature myeloid cells. Patients with myelokathexis have abundant hypermature neutrophils in bone marrow together with neutropenia in peripheral blood, due to impaired exiting the marrow. In patients with CyN, marrow cellularity depends on timing of the sample in the neutrophil cycle: Mature neutrophils accumulate in marrow preceding recovery of neutrophil counts in peripheral blood. Specific morphologic findings are large cytoplasmic granules in Chediak-Higashi syndrome, hemophagocytosis in dibasic protein intolerance and vacuolization of myeloid and erythroid precursors in Pearson syndrome (10).

Patients with SCN require annual bone marrow examinations with cytogenetics and analysis of G-CSF receptor or *RUNX1* mutations to recognize transition to MDS/AML as early as possible (54).

Treatment CN

Prophylaxis

Hand washing and cleaning of living spaces with soap and water are common sense measures to avoid infectious organisms. No dietary restrictions are necessary. Most vaccines may be administered, apart from BCG and oral live typhoid vaccine; pneumococcal and influenza vaccines are

recommended (10). Neutropenic patients are frequently infected by bacteria from their own skin and gut flora, mandating antibacterial prophylaxis: Oral sulfamethoxazole/trimethoprim at 36 mg/kg/day (trimethoprim part) divided in two doses is most often used. Rectal temperature measurement may be harmful and should be avoided. Good dental hygiene and regular dentist visits may prevent chronic periodontal disease and tooth loss. Prevention of fungal infections includes avoidance of sources with high spore load such as mulch, construction sites, or animal waste.

Acute infections

In moderate neutropenia, ENT or local infections may be treated with oral antibiotics and close ambulatory monitoring.

Patients with $\text{ANC} \leq 0.2 \times 10^9/\text{l}$ and fever require immediate hospitalization. Empirical IV broad-spectrum antibiotics, depending on local flora, should be given until identification and antibiotic sensitivity testing of the causative microbe. In case of fever and abdominal pain, anaerobic coverage should be included (11). Vancomycin may be used for methicillin-resistant *Staphylococcus aureus* or *Corynebacterium* species. If fever persists >48 h, antifungal treatment should be added (10).

G-CSF

Myeloid-specific G-CSF produced by genetic engineering is used to correct neutropenia, significantly improving clinical course and prognosis of SCN patients (55). G-CSF leads to differentiation of granulocytic precursors, increases release of mature granulocytes from bone marrow, and prolongs neutrophil survival by inhibition of apoptosis (56). It is available in three forms: filgrastim, its pegylated form pegfilgrastim, and the glycosylated form lenograstim, which share biologic effects (57). Pegfilgrastim has a higher half-life (15–80 h) and can therefore be given less frequently, but has a risk of overdose and potentially severe adverse effects (10). It is currently not licensed for use in SCN patients.

Long-term G-CSF treatment includes induction and maintenance phase: During 10–15-day induction, the individual response to G-CSF is evaluated in terms of increase in neutrophil count $>1.5 \text{ G/l}$; by serial CBCs and clinical improvement. Recommended initial dose should be 5 $\mu\text{g/kg/day}$ subcutaneously. In case of absent response, doses are increased in steps of 2–5 $\mu\text{g/kg}$. In case of rapid or excessive ($>5.0 \text{ G/l}$) response, the daily dose should be halved. In the maintenance phase, minimal dose and rhythm of injections will be determined (10). In many patients, especially in idiopathic, autoimmune, or CyN, administration every other day or three times per week are sufficient (11). Once the optimal dose has been identified, CBCs may be monitored every 4–6 months.

There are few local acute side effects of G-CSF. Flu-like symptoms are also rare. Headaches or bone pain may affect 2–5% of patients. They rapidly resolve after treatment cessation and generally do not recur upon lower doses of G-CSF (10). Hematologic side effects are usually benign, including

monocytosis, eosinophilia, thrombocytopenia, or hypersplenism. Spleen rupture has been observed occasionally (58). Acute febrile neutrophilic dermatosis (Sweet's syndrome) has been observed in patients with high neutrophil counts. Osteoporosis, which may lead to pathologic fractures, has been observed in nearly a quarter of patients with SCN on chronic G-CSF, but may be disease-inherent (59).

G-CSF has minimized the risk of infections during/after pregnancy in SCN patients (60). There are no formal recommendations concerning use and dosage of G-CSF during pregnancy. Mothers who received G-CSF for two trimesters had a higher rate of live births than untreated mothers (61). Importantly, there were no significant adverse (62).

As SCN is often an inherited condition, G-CSF is able to cross the placental barrier. Therefore, G-CSF treatment of SCN-affected mothers may prevent perinatal infections in the fetus (63) and it seems adequate to continue G-CSF treatment throughout pregnancy.

Leukemic transformation has been observed in patients with SCN due to mutations in *ELANE*, *HAX1*, *WASP*, *SBDS*, *G6PC3* (64), and *GATA2*. SCN patients with poor response to G-CSF, that is, requiring >8–10 µg/kg/day, have a greater risk of AML than good responders (7, 65). Patients with CyN, idiopathic, or autoimmune neutropenia seem to have no risk of developing MDS or AML (65). Due to the risk of malignant transformation, patients should be closely monitored, especially when high doses of G-CSF are necessary. Bone marrow analysis, including morphology, biopsy, cytogenetics, and search for G-CSF receptor mutations, which correlate with the appearance of leukemic clones, should be done once a year. The natural soy isoflavone genistein inhibits G-CSF-induced proliferation of HSC and DNA damage in mice, while preserving G-CSF-induced neutrophil production (66). It has not yet been tested in patients with SCN and chronic G-CSF treatment.

HSCT

Prior to the introduction of G-CSF, HSCT has been the only treatment for SCN. Today it is considered in patients failing to respond to G-CSF (requiring >20 µg/kg/day), in patients with high probability of evolving MDS/AML and G-CSF receptor mutations or abnormal cytogenetic clones in bone marrow or in patients with MDS/AML (7, 11). HSCT should be considered early to accelerate search for a suitable donor. In SCN patients without malignant transformation, standard HSCT procedures, including either myeloablative (MA) or reduced-intensity conditioning (RIC) regimens, may be followed. Patients with malignant transformation should not receive chemotherapy before HSCT (10), except for patients with frank leukemia in which cytoreductive chemotherapy prior to HSCT should include the least toxic regimen (67).

Except for patients with Shwachman's syndrome, survival after HSCT is good, exceeding 70% even in patients with malignant transformation (10). In patients with Shwachman–Diamond syndrome, outcome is favorable in >80% if HSCT was performed for pancytopenia without malignant clone, and poor with <35% survival in case of myelodysplasia/leukemic transformation (10).

Potential future treatment

STAT5 inhibition

STAT5 has an essential role in G-CSF-driven granulopoiesis in mice. It is hyperactivated in human CN and in AML (68), causing impaired myelopoiesis. The proteasome inhibitor bortezomib (Velcade® Millennium Pharmaceuticals, Inc. 40 Landsdowne Street Cambridge, MA 02139, USA) inhibits hyperactivated STAT5 and promotes myelopoiesis. It might thus offer therapeutic alternatives to patients with G-CSF-resistant SCN.

Wnt3a

Induced pluripotent stem cells from SCN1 patients were shown to lack Wnt3a (69). Administration of Wnt3a to iPSC cell cultures together with neutrophil differentiation medium induced the maturation of neutrophils in SCN1 iPSCs. It may offer novel treatment options for patients with *ELANE* mutation.

Qualitative neutrophil disorders

Chronic granulomatous diseases comprises a group of phagocyte defects, characterized by impaired intra- and extracellular pathogen elimination and granulomatous hyperinflammation linked to impaired inflammasome activity (3, 70, 71). It affects 1/120 000 in Europe (72). Defects of any of the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits gp91phox (*CYBB*, X-linked, 60% of cases) p47phox (*NCF1*, 7q11.23, 25% of cases), p67 (*NCF2*, 1q25, 5% of cases), p22 (*CYBA*, 16q24, 5% of cases), or p40 (*NCF4*, 22q13.1, one family) lead to impaired generation of reactive oxygen species (ROS) and NETs, while phagocytosis is intact (3, 71, 73).

Chronic granulomatous diseases patients have early-onset, recurrent severe infections with bacteria and fungi, causing abscesses of skin, lymph nodes or liver, fungal pneumonia, or bacterial osteomyelitis (74). In North America and Europe, most frequently isolated infectious organisms in CGD are *S. aureus* (lymphadenitis/liver abscess), *Burkholderia* complex (necrotizing pneumonia/sepsis), *Serratia marcescens* (sepsis/skin ulcers/osteomyelitis), *Nocardia*, and *Aspergillus* species (most frequently *A. fumigatus* subacute pneumonia; *A. nidulans* typically causes more severe disease) (75). Emerging pathogens in CGD were *Granulibacter bethesdaensis* (lymphadenitis) (76), *Actinomyces species* (severe pulmonary/abdominal/cervicofacial actinomycosis) (77), *Leishmania* (visceral leishmaniasis with hemophagocytosis) (78), and mycobacterium tuberculosis (severe localized disease) (79).

Patients with massive exposure to aerosolized organic decay containing *Aspergillus* spores may develop fulminant mulch pneumonitis, often requiring mechanical ventilation on ICU (80).

Chronic granulomatous hyperinflammation may cause granulomatous colitis, often misdiagnosed as Crohn's disease in the absence of overt infection, granulomatous cystitis, or recurrent infectious lesions (81). Patients have delayed wound

healing, fistula, and excessive granulation may develop on operative sites (82).

Female X-CGD carriers may develop photosensitive skin rashes (discoid lupus) in 60%, recurrent aphthous stomatitis in 40% (83), and chorioretinal lesions in 10% (82).

Diagnostics CGD

Standard diagnostics is quantification of ROS by dihydrorhodamine oxidation (DHR) or nitroblue tetrazolium reduction (NBT) test (84). To evaluate the degree of susceptibility to infections, O₂⁻ quantification, immunoblotting of the NADPH oxidase subunits or functional assays such as NETosis test should complement diagnostics, in particular in patients or X-CGD carrier mothers with residual ROS production (3) (71). Genetic diagnosis should be performed in every CGD patient, as it is important for genetic counseling, stem cell gene therapy, and exclusion of X-CGD carriers with unfavorably skewed X-inactivation as HSC donors for HSCT (82). X-CGD patients sometimes carry microdeletions at Xp21.1, leading to contiguous gene syndromes such as McLeod neuroacanthosis syndrome (85), retinitis pigmentosa, Duchenne muscular dystrophy, and ornithine transcarbamylase deficiency (82). Therefore, sequence analysis should involve multiplex probe amplification or array comparative hybridization of the relevant gene/chromosome segment (86).

Treatment CGD

Prophylaxis

Chronic granulomatous patients require lifelong daily prophylaxis with intracellular active agents. Trimethoprim/sulfamethoxazole is broadly effective against Gram-negative bacteria and staphylococci (87), while for antifungal prophylaxis, itraconazole is the drug of choice, as it has very high action against *Aspergillus* (88).

Interferon-gamma prophylaxis may reduce the frequency of severe bacterial infections (89), but only results in production of small amounts of functional gp91phox protein in patients with X-CGD due to splice site-mutations where it increases splicing efficiency (90).

Chronic granulomatous patients should receive all routine immunizations besides BCG, as it may cause BCGitis or rarely disseminated BCG-osis (91). Although CGD patients are generally not susceptible to viral infections, annual influenza vaccine is recommended, to prevent risk from bacterial superinfections.

Acute infections

Any febrile illness should be treated prompt with empirical broad-spectrum (often IV) antibiotics, covering Gram-positive and Gram-negative organisms. In case of non-response within 24–48 h, broad-spectrum antimycotic treatment such as voriconazole has to be added (82). The causative agent should be vigorously sought, if possible by culture from infected sites and subjected to antimicrobial resistance testing. Broad-

spectrum IV antimicrobials followed by oral therapy are often required for a prolonged period of time (until CRP normalizes) (72). Posaconazole has been used as salvage therapy against invasive (e.g., CNS) fungal infections (92).

Use of granulocyte transfusions from allogeneic donors as salvage therapy for life-threatening infections remains controversial (82). The risk of alloimmunization to HLA-antigens and CMV infection is an issue concerning subsequent allogeneic HSCT and therefore mandates careful consideration of risks and benefits.

Inflammatory complications

Mulch pneumonitis and liver abscesses often require concomitant anti-inflammatory treatment by corticosteroids to manage hyperinflammation. Gastric outlet or ureteral obstruction by granulomata may be cautiously treated by corticosteroids, followed by gradual tapering (82). Severe inflammatory bowel disease is first treated by prednisolone 1 mg/kg/day for 2 weeks, followed by tapering over 2 months to 0.1–0.25 mg/kg/day (93). Alternatively, use of biologics such as the gut-selective $\alpha\beta7$ -integrin antagonist vedolizumab may be considered (94) and should be preferred over TNF- α blocking agents which are very effective but increase risk of infection in CGD.

HSCT

Reduced-intensity conditioning (RIC) with targeted drug monitoring has been proven to be efficient and safe, resulting in an overall 2y survival after HSCT of 96% and an event-free survival of 91% in 56 CGD patients (95). A low incidence of graft-versus-host disease (GvHD) and excellent donor chimerism (>90%) were shown in 93% of the surviving patients, irrespective of use of matched sibling donors (MSD) or matched unrelated donors (MUDs). HSCT from alternative donor sources such as cord-blood (96) or donors with an HLA-match below 9/10 (97) remains experimental and usually require myeloablation.

Present consensus concerning HSCT indication is based on residual NADPH oxidase function and individual clinical course (82). Donor search should be performed at diagnosis of CGD. In case, a MSD, matched family donor (MFD), or MUD is available and the patient has no residual ROS production, RIC-based HSCT should be performed early. In case of residual ROS production, and a matched donor, HSCT should be performed if the patient has had a life-threatening or therapy-refractory infection, chronic hyperinflammation, or progressive organ dysfunction. In case, MSD, MFD, or MUD is unavailable, mismatched-unrelated donor (mMUD) or haploidentical myeloablative HSCT may be performed as salvage therapy in autosomal-recessive CGD (indication: therapy-refractory infections, refractory inflammation, or progressive organ dysfunction) or submyeloablative genotherapy may be performed in case of X-CGD.

Gene therapy

Experimental gene therapy has been performed in phase I/II clinical trials in 12 very severely affected X-CGD patients (98)

using first-generation LTR-driven gammaretroviral vectors. Submyeloablative chemotherapy resulted in transitory benefit on pre-existing infections in 9 of 12 patients. In 4 of 12 patients, engraftment of gene-corrected cells was successful, most likely due to MDS1/EVI-1 transactivation causing immortalization of myeloid progenitors (99). This event resulted in MDS in 3 of these 4 patients 13–16 months after appearance of clonal dominance of MDS1/EVI-1 clones: Two adults subsequently died from MDS or AML, respectively; one child survived after elimination of the malignant clone by two sequential HSCTs (99). This highlights the deleterious nature of MDS1/EVI-1 transactivation and interdicts further use of strong complete LTRs. Therefore, current gene therapy trials for CGD rely on next-generation self-inactivating lentiviral vectors, which lack

potent retroviral LTR-enhancers and show 2–3 log lower transactivation potential. Myelosppecific promoters are an additional safety measure to reduce probability of oncogene transactivation in HSCs (100). Such a vector is currently used in a European phase I/II clinical trial for X-CGD.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Types of congenital neutropenia or functional defects described in the review.