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HOW I APPROACH

Diagnosis and management of neutropenia in children: The approach of the Study Group on Neutropenia and Marrow Failure Syndromes of the Pediatric Italian Hemato-Oncology Association (Associazione Italiana Emato-Oncologia Pediatrica - AIEOP)

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Abstract

Neutropenia refers to a group of diseases characterized by a reduction in neutrophil levels below the recommended age threshold. The present study aimed to review the diagnosis and management of neutropenia, including a diagnostic toolkit and candidate

Abbreviations: AIEOP, Associazione Italiana Ematologia ed Oncologia Pediatrica; AML, acute myeloid leukemia; ANC, absolute neutrophil count; CyN, cyclic neutropenia; EBMT, European Bone Marrow Transplantation Study Group; G-CSF, granulocyte colony-stimulating factor; HSCT, hematopoietic stem cell transplantation; I-GIFT, indirect granulocyte immunofluorescence test; MDS/AL, myelodysplastic syndrome/acute leukemia; NGS, next-generation sequencing; SCN, severe congenital neutropenia; SCNIR, Severe Chronic Neutropenia International Registry; SNFR, Severe Neutropenia French Registry.

underlying genes. This study also aimed to review the progress toward the definition of autoimmune and idiopathic neutropenia rising in infancy or in late childhood but without remission, and provide suggestions for efficient diagnostics, including indications for the bone marrow and genetic testing. The management and treatment protocols for common and unique presentations are also reviewed, providing evidence tailored to a single patient.

KEYWORDS
children, granulocyte colony-stimulating factor, neutropenia, neutropenia diagnosis, neutropenia therapy

1 | INTRODUCTION

Neutropenia is defined as a reduction of the absolute number of neutrophils (ANC) in the blood and includes several disorders, which are primarily classified as inherited or acquired.[1–3] Its congenital form, known as the Kostman disease after the physician who first described it, is caused by a mutation in *ELANE* gene, which regulates the maturation of neutrophil precursors.[4–6] Many other genes have been found to cause congenital neutropenia with mechanisms different from the accelerated apoptosis of precursors within the bone marrow.[7] Acquired neutropenia, mainly occurring in early infancy, is usually considered a benign and transitory disorder; however, the types of acquired neutropenia that develop later in life can be severe and long lasting.[8–11]

Despite major advances, driven among others by the North American and European branches of the Severe Chronic Neutropenia International Registry (SCNIR),[3] accurately classifying cases as inherited or acquired neutropenia remains challenging and requires specialist diagnostics. In addition, evidence-based management is difficult due to the paucity of data.

While international guidelines, under the auspices of EHA-Innochron Eunet task force are awaited, in this study we present an update of the diagnosis and treatment protocols in pediatric age, including a follow-up schedule, based on patients' history and clinical characteristics that are in use in the Centers of the Pediatric Italian Hemato-Oncology Association (Associazione Italiana Emato-Oncologia Pediatrica [AIEOP]).

2 | METHODS

The Neutropenia Committee that included a panel of experts from the Marrow Failure Syndrome Study Group of the AIEOP aimed to review and update the guidelines on diagnosis, treatment, and follow-up for neutropenia in children. The search was performed in the Medline database (1971 to September 2021) and included keywords such as neutropenia, congenital, acquired, autoimmune, secondary, severe, severe congenital neutropenia (SCN), granulocyte colony-stimulating factor (G-CSF), filgrastim, lenograstim, pegfilgrastim, bone marrow transplantation, myelodysplasia, growth factor receptor, children, leukemia, and infections. This search yielded a total of 300 articles, of which 91 were relevant to all topics. The search was also extended to hematology textbooks and proceedings of international hematology meetings.

3 | DEFINITION AND CLASSIFICATION

In Caucasian newborns and toddlers up to the age of 1 year, the lower limit of neutrophil count is 1.0×10^9/L, whereas from the age of >1 year to adulthood, this limit is 1.5×10^9/L.[1–3] In neonates and in preterm (28–36 weeks) infants during the first week of life (72–240 hours), the lowest ANC threshold is 2.5×10^9/L, while in early-preterm (<28 weeks) infants, neutropenia is defined as neutrophil values of $<1.0 \times 10^9$/L.[2] Neutropenia is defined mild (<1.5–1.0×10^9/L), moderate (<1.0 to $\geq 0.5 \times 10^9$/L), severe ($<0.5 \times 10^9$/L), or very severe ($<0.2 \times 10^9$/L) according to ANC values.[3] Neutropenia may be defined as acute (triggered by an infection and remitting within a few weeks) or chronic (duration of >3 months without resolution).[3]

Congenital neutropenia, when severe is defined as SCN, is a heterogeneous group of constitutional diseases associated with mutations in genes coding for proteins involved in maturation, trafficking, and homeostasis of the myeloid lineage.[12–16] SCN patients show different phenotypes that can be divided into isolated, where neutropenia represents the whole or most of the phenotype, or associated with other conditions (Table 1).[4,12] Affected patients are at an increased risk of life-threatening infections and may progress toward myelodysplastic syndrome/acute leukemia (MDS/AL), usually the myeloid type.[17,18]

Acquired neutropenia in childhood, either defined as autoimmune (AIN) or idiopathic (IN), usually has a milder phenotype than the constitutional forms.[8–10]

4 | DIAGNOSTIC ROUTE

Extensive study of history and detailed physical examinations are recommended in patients with suspected neutropenia before specialist

TABLE 1 Classification of neutropenia

		Type of disease	Further description/responsible genes
Constitutional neutropenia	**Isolated**	Severe congenital neutropenia	ELANE, CSF3R, CXCR2, WAS
		Cyclic neutropenia	
	Possibly associated with various extra hematological signs	Nonisolated severe congenital neutropenia	HAX1, G6PC3, GFI1, TAZ, USB1, VPS13, VPS45, SMARCAL1, JAG1, DNM2, SBDS, SRP54, GATA1
		Kostman	
		Barth disease	
		Poikiloderma Clericuzio	
		Schimke immuno-osseous dysplasia	
		Cohen syndrome	
		Charcot Marie tooth disease	
		Shwachman–Diamond syndrome	
		Pearson syndrome	
	Associated with congenital bone marrow failure[a]	Fanconi anemia	FANC-genes, DKC1, NHP2, NOP10, RTEL1, TERC, TERT, TINF2, WRAP53, RPS-RPL genes
		Telomere disease	
		Blackfan–Diamond anemia	
	Associated with immune deficiency/immune dysregulation	Reticular dysgenesis, Iper IgM syndrome	AK2, CD40
		Hermansky–Pudlack type 2, Griscelli type 2	AP3B1, RAB27A
		Hemaophagocytic lymphohistiocytosis (HLH), Chediak–Higashi disease, WHIM syndrome	PRF1-UNC13D, STX11, STXBP2, LYST, CXCR4
		Hypoplasia cartilagine-capelli,	RMRP
		Autoimmune lymphoproliferative syndrome (ALPS)	FAS, FASL, CASP10
	Associated to metabolic disorders and nutritional deficiency	Transcobalamin 2 defect	TCN2
		Glycogenosis 1b	SLC37A
		Propionic aciduria	PCCA-PCCB
		Metilmalonic aciduria	MMUT-MMAA-MMAB-MCEE-MMADHC
		Isovaleric aciduria	IVD
		Gaucher disease type 1	GBA
		Tyrosinemia	
		Vitamin B12 deficiency	
		Copper deficiency	
Acquired neutropenia	**Autoimmune neutropenia**	Primary autoimmune neutropenia	Adults and children
		Secondary autoimmune neutropenia	Others immune disease (autoimmune disorders and immunodeficiency) neoplasm/BMT

(Continues)

TABLE 1 (Continued)

	Type of disease	Further description/responsible genes
Alloimmune neutropenia	Primary alloimmune neutropenia	Neonatal alloimmune neutropenia
		Iso-immune
	Secondary alloimmune neutropenia	TRALI (transfusion-related acute lung injury) Transfusion-related allo-immune neutropenia Febrile transfusion reactions
Pregnancy or delivery related neutropenia	Pregnancy- or delivery-related neutropenia Perinatal asphyxia, hypertension, pre-eclampsia, Rh and AB0 incompatibility, feto-fetal transfusion syndrome, maternal viral infections, maternal drugs	
Neutropenia associated to myeloproliferative disorders	Acute myeloid leukemia, myeloproliferative disorder lymphomas, acute lymphoblastic leukemia, chronic myeloproliferative diseases, juvenile myelomonocytic leukemia, myelodysplasia	
Neutropenia associated to acquired BMF	Aplastic anemia/secondary HLH	

[a]Only in BMF cases, in which neutropenia is among the main features.

TABLE 2 Initial evaluation of neutropenic patients for preliminary diagnosis

Family history	Assess consanguinity, occurrence of additional cases of neutropenia, ethnic origin, neoplasm, miscarriages, cytopenia, and autoimmunity
Personal history	Collect any ANC findings both recently and previously obtained
	Ascertain history of pre- and neonatal infections and exposure to drugs
	Investigate the number, type, site, and frequency of infections, including omphalitis, gingivitis, periodontitis, skin infections, abscesses, otomastoiditis, mycobacterial or HPV infections, and pneumonia, as well as duration of treatment, administration method, and response to antibiotics, symptoms consistent with autoimmune, metabolic, gastrointestinal, and nutritional diseases. Drug exposure history may indicate an increased risk of neutropenia, in particular, exposure to antibiotics and anticonvulsants
Physical examination	Focus on weight, stature, psychomotor development, skin/nails disturbances, somatic dysmorphisms, hearth function, liver, and spleen size, presence of enlarged lymph nodes, joint swelling and neurological abnormalities.

tests can be performed (Table 2). Patients presenting with a history of drug use or those presently treated with medication associated with neutropenia should be evaluated for drug-related neutropenia, which may be a suitable diagnosis. Any drug could potentially cause neutropenia; an extensive list of drugs associated with neutropenia is presented in Table S1.[19,20]

Some ethnic groups, including those of African, Afro-Caribbean, and Middle Eastern descent, have an ANC in the range of $0.5–1.5 \times 10^9$/L, which is not associated with an increased risk of infection or progression to MDS/acute myeloid leukemia (AML). These groups present with a specific single-nucleotide polymorphism (SNP), rs 2814778, located at the promoter of the *ACKR1* gene (previously known as *DARC*), which also confers resistance to malaria.[21,22] Consequently, these ANC values are considered normal in patients presenting with the Duffy null phenotype.[23,24] These groups should be assessed for the presence of SNP rs 2814778, the promoter of *ACKR1* gene, to prevent incorrect diagnosis and treatment.

The recommended approach to diagnosis in the general population is presented in Figure 1, which captures neutropenia severity from highest to lowest (A–C).

The first step is to confirm that neutropenia persists by repeating a blood test within 7 days or 3 months of the first test, as suitable. More frequent testing may be required based on the patient's neutrophil levels and clinical course. Procedures should be accelerated in patients presenting with severe disease or experiencing disease progression over time.

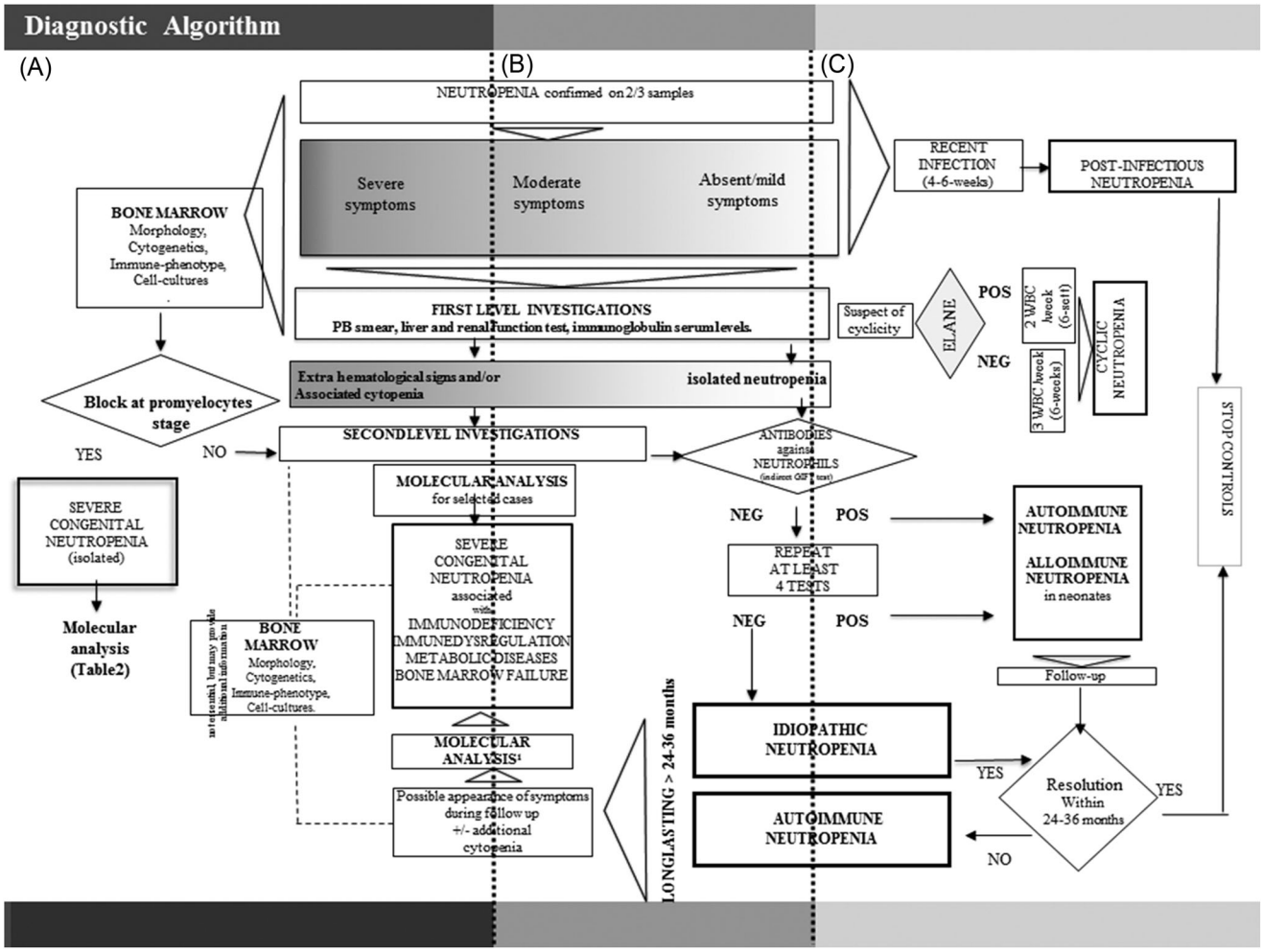

FIGURE 1 Neutropenia severity is marked by the degree of gray shading from section A (highest) to C (lowest). Diagnosis should be confirmed according to the following steps: (A) When neutropenia (ANC of <500/cm), either isolated or associated with complex syndromes, is accompanied by severe (sepsis, meningitis, osteomyelitis, deep abscesses, phlegmons, or pneumonia) or recurrent/torpid infections (otitis, mastoiditis, skin abscesses, urinary tract infections, enteritis), and appears in early infancy (<1 year of age), ANC count should be confirmed (two or three times, depending on suspected severity), and first-level and bone marrow investigations should be performed. If a block at the promyelocyte level is identified, targeted gene analysis is recommended. (B) When neutropenia is confirmed and associated with signs or symptoms (deficiency of lymphocyte subset[s]), a targeted route must be followed. When the phenotype indicates associated conditions, molecular analysis may be performed even before the bone marrow examination. (C) When neutropenia is confirmed in the absence of any clinical signs or symptoms or history of infections, the diagnosis should be based on findings from at least three samples obtained at least 1 week apart. First-level investigations should be performed, followed by tests for antibodies against neutrophils. The probable diagnosis is autoimmune or idiopathic neutropenia. Re-evaluations are periodically required in cases of long-lasting neutropenia (>24–36 months). "Cyclic neutropenia" is a mild phenotype, in which the ANC levels oscillate within 21–28 days. Mutations in the *ELANE2* gene were present in 60% of the cases. Cycling has to be proven by repeat ANC tests (three ANC/week for 4–6 weeks) in patients without genetic lesions and in those with *ELANE2* gene mutation (two times/week for 4–6 weeks). Gene names are presented in Table 2. *All procedures should be accelerated in cases of clinical progression

The most severe phenotypes (Figure 1, pathway A) tended to be associated with severe or recurrent/torpid infections appearing in early infancy. Such cases should be approached with immediate first-level investigations that include the bone marrow tests and genetic analysis, provided typical features are found (e.g., block at the promyelocyte stage). This approach enables the diagnosis of classical SCN, which can be either an isolated or associated disorder. The latter group of disorders presents with different phenotypes based on the organ/system involved.

The second level of investigations is aimed at confirming the underlying causes of neutropenia, including metabolic or auto-immune or immune dysfunction problems, bone marrow failure, and other hematological and nonhematological conditions. Table S2 details disease names and the corresponding genetic variants in both associated and isolated SCN.

Neutropenia of intermediate severity (Figure 1, pathway B) tends to allow more time to explore possible associated conditions. Second-level investigations are not specified because of the diversity of

diagnostic areas (immunology and metabolic) involved; this approach may help achieve a diagnosis before bone marrow examination. In cases of isolated neutropenia, the direction leads to the anti-neutrophil-antibodies box, aiming to define an autoimmune or idiopathic type.

The least severe neutropenia type (Figure 1, pathway C) allows more time to reach a final diagnosis. Mild/moderate neutropenia can be postinfection neutropenia that tends to resolve within 4–6 weeks.

Cyclic neutropenia (CyN) is defined as an oscillation in ANC values, with typical nadir values presenting every 21–28 days, and confirmed by an ANC test two to three times per week over a period of 4–6 weeks. *ELANE2* gene mutations are associated with CyN in approximately 60% of cases.

Neutropenia that persists for >36 months may be an epiphenomenon of another disorder. In some cases, rheumatological, immunological symptoms/signs, or other types of cytopenia appear over time, thus driving the diagnosis of specific disorders. In other cases, neutropenia remains isolated without a relevant increase in the frequency of infectious episodes. In both cases, careful periodic re-evaluations of immunological/autoimmune markers have been suggested. This workup should also include extended genetic panels (germline variants of immunodeficiency, immune dysregulation, or bone marrow failure disorders) that may help identify immune disorders associated with autoimmune types of cytopenia or mildly phenotypically expressed bone marrow failures.[11]

4.1 | Special issues

4.1.1 | Antineutrophil antibodies

The presence of antibodies against neutrophils may indicate autoimmune neutropenia, in the absence of clear elements of inherited forms.

The detection of free antibodies in the serum (indirect antineutrophil antibody testing [indirect granulocyte immunofluorescence test, I-GIFT]) combined with the agglutination technique is considered the most powerful method for identifying autoimmune neutropenia[4,8,9,25–29] by the Fourth International Workshop on Granulocytes.[30] In a multicenter study, I-GIFT with flow cytometry had sensitivity, specificity, and positive predictive values of 62.5%, 85%, and 91.8%, respectively.[29] Evidence from other studies has shown that on repeated testing, this sensitivity progressively increases up to 82%.[8,9]

Techniques other than I-GIFT (e.g., microbeads assay for granulocyte antibody detection and microbead assay, among others) are available; however, they remain technically challenging, time consuming, and cumbersome in daily clinical practice.[25]

In contrast, the direct test is associated with a high rate of false-positive findings, particularly, when performed in laboratories with little experience.[31] The characterization of antibodies against neutrophils, if available, may be useful to better define the antigenic profile; however, it is not required for diagnostic purposes.[26]

Overall, despite reduced specificity, sensitivity, and need for repeated testing, which are inherent in the indirect antineutrophil antibody test, AIEOP experts reckon that in the absence of a better assay, this screening tool remains useful in the context of autoimmune neutropenia. If the first test is negative, despite the clinical suspicion of AIN, the test should be repeated at least four times over 6–12 months to increase sensitivity. If no serum antineutrophil antibodies are detected, the patient may have idiopathic neutropenia, which is an exclusion diagnosis[4,32] that may either resolve spontaneously or evolve into a longer lasting disease, possibly unveiling an underlying immune dysregulation/deficiency that may be identified by a specific next-generation sequencing (NGS) panel.

Overall, given the limitations of the indirect test, the positivity of serum antineutrophil antibodies must be evaluated within the biochemical, clinical, and other relevant context,[33] which may include the age of onset that tends to be 4–5 months for the diagnosis of a primary autoimmune disease.[9]

4.1.2 | Bone marrow examination and additional analysis

Bone marrow testing is not always recommended as a first-line treatment. The panel recommends that bone marrow aspiration should be performed when neutropenia is associated with severe, very severe, or torpid infections, or recurrent stomatitis or gingivitis, irrespective of the neutrophil count values. New and recurrent infections tend to be associated with congenital neutropenia, and the detection of bone marrow blocks may be helpful for diagnosis in this context.

Moreover, bone marrow exam is reckoned appropriate when neutropenia is associated with any other type of cytopenia (anemia, thrombocytopenia, and lymphopenia) with or without lymphoproliferation to exclude marrow failures, MDS, and leukemias.

Bone marrow is also indicated in chronic neutropenia in adolescents/young adults, in the diagnostic workup or infants affected with idiopathic or autoimmune neutropenia lasting >24 months in patients aged >5 years to exclude any possible dysplastic features that may accompany isolated neutropenia.

Assessment of the bone marrow mature neutrophil reserve and marginated vessel compartment can help predict an increase in ANC in cases of infection. It can be performed either with glucocorticoids, which were commonly used in the past, or G-CSF stimulation tests, which have recently been adopted in Italian centers. G-CSF acts in a manner similar to steroids, and the test results help anticipate the effect of drugs that may be required during treatment, for example, to treat infection or perform surgery.

4.2 | Molecular testing

If the bone marrow morphology presents with maturation arrest at the promyelocyte/myelocyte stage, SCN diagnosis may be suitable. Mutations in *ELANE/HAX-1* genes are associated with most SCN cases.[5–7,34–36] If no pathogenic variants of *ELANE/HAX1* genes are

detected, other genes responsible for congenital neutropenia should be examined (Table S2).

When peculiar clinical signs are present alongside neutropenia, other genes should be examined (i.e., SDS genes in case of congenital-cardiac malformations or pancreatic insufficiency)[37–41] (Table S2).

Sanger sequencing can be a cost-effective strategy in confirmatory diagnostics when clinical features strongly indicate a specific type of SCN. In cases where the clinical phenotype does not suggest any specific disease, multiplex targeted analysis with NGS panels for multiple neutropenia genes is indicated.[42] In cases where these tests yield negative findings, whole-exome sequencing or whole-genome sequencing can be used. In cases where a known genetic cause of SCN remains unclear despite careful re-evaluation,[5–7,34] a diagnosis of SCN without known genetic lesions is appropriate.[13,14]

In cases of idiopathic neutropenia and autoimmune neutropenia, which last more than 24–36 months, immunological status must be investigated and a dedicated NGS panel may be applied, as per the center policy, to detect a possible dysregulation of the immune system or other related disorders, including SCN.[11] This is because neutropenia, as an epiphenomenon of primary immune dysregulation syndrome, may be misdiagnosed, and constitutional neutropenia may have an atypical clinical phenotype.[43]

5 | NEUTROPENIA MANAGEMENT

5.1 | Treatment

5.1.1 | General issues

G-CSF is a cytokine that enhances the division of myeloid precursors and accelerates neutrophil maturation. Previous phase I–III clinical trials have shown that G-CSF increases ANC in SCN and other acquired neutropenias.[44] The introduction of G-CSF into clinical practice radically changed the prognosis of SCN patients, with rates of survival significantly increasing.[45–47] Glycosylated (lenograstim) and nonglycosylated (filgrastim) forms of G-CSF have comparable efficacy.[48,49]

More recently, a form of filgrastim linked to polyethylene glycol, named pegfilgrastim, was introduced in clinical practice, mostly in oncological settings and for use in stem cell mobilization of stem cells.[50–52] Data on pegfilgrastim in noncancer-related neutropenia are scarce; however, it appears promising in some contexts, such as SCN, in which standard G-CSF is not effective or tolerated.[50] However, larger studies with longer follow-up are required to understand the impact of pegfilgrastim on leukemogenesis and other late effects.

Biosimilars of G-CSF and pegfilgrastim are considered noninferior to their original preparations by the European Medical Agency and the Food and Drug Administration. These products are officially licensed for use in chemotherapy-induced neutropenia, after bone marrow transplantation, mobilization of peripheral blood stem cells, congenital and CyN, and neutropenia associated with human immunodeficiency virus.[53–56]

5.1.2 | G-CSF treatment indication and type of treatment

G-CSF treatment is indicated in patients who are unable to control infectious episodes, irrespective of their type of neutropenia.[57,58,63] In everyday clinical practice, G-CSF can be administered on-demand or over a long term. The latter includes administrations in the presence and absence of infections that can be over a lifetime, and it can be delivered daily or as frequently as required (every other day, every other third day, etc.). Long-term therapy is considered appropriate in patients with SCN, CyN, and in some neutropenia types associated with other pathological conditions, such as glycogen storage disease 1b.[57–63]

Continuous administration is also indicated in idiopathic or autoimmune neutropenia when severe infections occur or in cases in which recurrent episodes may negatively impact patients' quality of life.[58,61]

On-demand therapy tends to be short term (i.e., prophylaxis before surgery). It is indicated in autoimmune and idiopathic neutropenia, specifically, in cases of isolated and prolonged fever (>3 days at 38°C) and/or fever associated with signs and/or symptoms of severe infection and/or rapid deterioration or septic shock.[58,61,62] However, G-CSF administration may rarely be administered in cases of severe infection, regardless of the presence of fever.

Moreover, the suggestion is that for a patient with neutropenia who is rapidly deteriorating, or experiencing septic shock or a life-threatening infection (e.g., typhlitis, fungal pneumonia, etc.), G-CSF should be initiated on the first day of fever[58,63] and interrupted when infection has resolved, despite the low number of neutrophils.[58,59,62]

5.1.3 | Goal of G-CSF therapy and schedule according to diagnosis

The main goal of G-CSF therapy is to achieve good infection control that is usually obtained when the ANC is 1.0×10^9/L–5.0×10^9/L).[17,33,45,58,63]

The G-CSF standard dose is 5 γ/kg/day, but the optimal choice is the minimal effective dose that may achieve treatment goals, without major side effects or stem cell overstimulation.[17,45,58] The dose and schedule of G-CSF may be initiated and adjusted, depending on the type of neutropenia (Table 3).

Severe congenital neutropenia

The standard dose of G-CSF to achieve the therapeutic target (ANC >1.0×10^9/L to <5.0×10^9/L) is 5 γ/kg/day; however, individual responses may require subsequent modifications.[58,63]

Approximately 90% of SCN patients can reach the therapeutic target with a median G-CSF dose of 7.3 γ/kg/day (range of 3–10 γ/kg/day),[9] whereas data from the Severe Neutropenia French Registry (SNFR) show that the required median daily dose of G-CSF is 9 γ/kg.[60] Patients treated with doses greater than 10 and 20 γ/kg/day who did not reach the goal of 1.5×10^9/L ANC were defined as poor responders and nonresponders, respectively. Some authors suggest

TABLE 3 G-CSF therapy and doses according to neutropenia (Np) diagnosis

Np type	Starting dose(γ/kg/day)	Primary recommended ANC between 1.0×10^9/L and 5.0×10^9/L	Increasing dose if ANC of $<1.0\times10^9$/L	Lowering dose if ANC $>5.0\times10^9$/L	Special situation
SCN	5	If reached within 5–7 days, maintain starting dose	After 7 days, increase by 2.5 γ/kg/day every 5–7 days	G-CSF should be lowered gradually in any type of Np with ANC of $>3.0–5.0\times10^9$	In case of torpid or severe infections in any type of Np, the increase of G-CSF depends on patient's clinical condition
Cyclic neutropenia	2	If reached within 2–4 Np phases, maintain starting dose	After the next Np phase, increase of 2 γ/kg/day, every 2–4 Np phases		
GSD-1b	1–3	If reached within 5–7 days, maintain starting dose	After 7 days, increase by 2 γ/kg/day every 5–7 days		
SDS	1–3	If reached within 3–5 days, maintain starting dose	After 5 days, increase by 2 γ/kg/day		
IN and AIN	1–3	If reached within 5–7 days, maintain starting dose	After 7 days, increase by 1–2 γ/g/day		

that nonresponders are patients treated with >20 γ/kg/day without an increase in ANC above 0.5×10^9/L.[13]

A study from the Italian Registry of Neutropenia suggested that in SCN patients who poorly respond or are scarcely compliant with daily G-CSF treatment, pegfilgrastim increases ANC values and decreases infection rate,[50–52] with an acceptable side effects profile, thus representing an alternative option in these patients.[50]

Cyclic neutropenia

In CyN, G-CSF shortens the degree and duration of neutropenia, improving patients' quality of life and lowering the incidence of severe infections.[58,59]

In CyN patients, G-CSF treatment may begin at doses of <5 γ/kg/day (Table 4).

G-CSF schedule and frequency of injections should be tailored to the type of cycling and recurrence of infections (daily/every other day/only during neutropenia phases).[13,58,59]

Glycogen storage disease 1b

A recent SCNIR study on approximately 100 patients affected by glycogen storage disease 1b reported that a dose of <5 γ/kg/day may protect this group from infections.[60]

The panel agrees on the use of G-CSF starting from 1 to 3 γ/kg per day or every other day.

Progression to myelodysplasia and leukemia is very rare; eight patients with neutropenia transformed into MDS/AML have been reported to date and some of these patients experience disease transformation before and without G-CSF treatment.[60]

Shwachman–Diamond syndrome

G-CSF in patients with Shwachman–Diamond syndrome is generally recommended for a limited period and only in cases of severe or recurrent infections.[64] Long-term G-CSF treatment is not recommended by the panel because of the intrinsic predisposition to leukemic transformation. Median doses of G-CSF in the range from 3.84 to 6.9 mg/kg/day have been used in patients enrolled in the SNFR and SCNIR trials, respectively.[17,18] The experts suggest that on-demand treatment should be considered in patients with severe or recurrent infections, and administered at a starting dose of 3 γ/kg/day and with subsequent adjustments by 2 γ/kg/day, based on the increase in neutrophil count (Table 3).

Idiopathic neutropenia and autoimmune neutropenia

In autoimmune neutropenia, specifically in secondary forms or long-lasting forms associated with severe and/or recurrent infections, if an on-demand schedule is not effective at controlling the infection, long-term G-CSF administration may be considered after performing bone marrow examination with cytogenetic analysis, as suitable. A starting dose of 1–3 γ/kg/day is suitable for achieving the target ANC values. Further dose adjustments are shown in Table 4.[58,63]

5.2 | Follow-up

Patients with congenital neutropenia have an intrinsic risk of developing MDS/AML, which increases with the degree (dose and duration) of G-CSF exposure. Thorough follow-up assessments are required to survey and intercept possible transforming events[4,17,65–67] and side effects associated with long-term exposure to G-CSF, including splenomegaly and osteoporosis.

The sequential rise of *CSFR3* and *RUNX 1* mutated clones have been described as a "cooperative model" of leukemogenesis in SCN patients. Additional acquired driver mutations of the *CXXC4* gene were recently shown to reduce the expression of TET2 protein, thus promoting

TABLE 4 Follow-up schedule, according to the different types of neutropenia, including during G-CSF treatment

	Full blood count	Biochemical[a] parameters	Bone marrow[b]	Abdomen ultrasound scan	Bone density tests	Further consideration
SCN G-CSF	At least every 4 months	At least every 6 months	Every 12–18 months[c]	12 months	12–24 months	If morphological dysplasia or any abnormal cytogenetic clone during the follow-up period occurs, repeat bone marrow tests more frequently[d] If an isolated mutation of G-CSF-r without RUNX1-associated mutation occurs, repeat bone marrow analysis every 12 months[e] If CSFR3 clone is associated to RUNX1 mutation, follow-up assessments should be tailored to each case. HSCT may be recommended
Cyclic neutropenia G-CSF	At least every 4 months	At least every 12 months	Not routinely indicated (to be reconsidered in young adults)	12–24 months	24 months	—
AIN/IN G-CSF treated continuously	In G-CSF-treated every 4 months	At least every 4–6 months	At least once (AIN) Suggested periodically (IN)	12–24 months	24 months	If neutropenia is persistent and strongly indicative of AIN, repeat indirect antibodies against neutrophils tests to define an ID (at least 4 times). If a spontaneous resolution of neutropenia does not occur within the expected timeframe, consider performing an expanded autoimmunity panel.[f] In both AIN and ID lasting more than 36 months or with a delayed onset (>5 years), consider immune deficiency/dysimmunity and perform genetic tests for PID, as per site policy and availability
AIN/IN/Cy without G-CSF therapy	At least every 4 months	Not indicated except cyclic neutropenia at least every 12 months	Not indicated routinely	—	—	

[a]Biochemical parameters (hepatic and renal function), ALP, LDH, uricemia, hemoglobin F (neutropenia at risk of transformation to MDS/AML), and urine analysis.
[b]Bone marrow analysis for morphology, cytogenetics, FISH cr 7 and 8, immunophenotype, and bone marrow biopsy (as per hospital policy).
[c]G-CSF receptor mutation and RUNX 1 analysis on bone marrow aspirate every 12 months.
[d]Bone marrow aspiration for morphology, cytogenetics, FISH cr 8 and 9, hematopoietic stem cell transplantation (HSCT) procedures should be initiated.
[e]Bone marrow aspiration: morphology, cytogenetics, FISH cr 8 and 9, and immunophenotype analysis. In this case, if a sibling donor is available, HSCT may be considered.
[f]Enlarged panel of autoimmunity should be performed periodically and include tests for thyroiditis, celiac disease, and autoimmune lymphoproliferative syndrome (ALPS).

multiple proinflammatory pathways that are critical for the malignant transformation of SCN.[68,69]

The cumulative incidence of MDS/AL has been estimated by the SCNIR as 22% after 15 years of treatment. This rate was estimated at 15% among patients treated with G-CSF at a dose of <8 mg/kg/day and increased to 34% among those treated with G-CSF at a dose of >8 mg/kg/day.[17]

The incidence of transformation to MDS/leukemia has been reported by the French, Swedish, and Italian groups as 10.8%, 31%, and 6% after 20, 15, and 4 years of treatment, respectively.[18,70,71] These discrepancies may be due to differences in sample sizes and composition, follow-up duration, and study period (1990, 2000, and 2010).

For example, SDS and GATA2 subjects are more likely to develop MDS and AML than other SCN categories (the risk is almost double according to the French Registry and Canadian data), requiring careful monitoring of peripheral blood counts and bone marrow function.[65,67,72]

In SCN, the experts suggested a thorough monitoring of peripheral blood counts (at least three times per year) and an annual bone marrow function evaluation.

In glycogen storage disease 1b, although transformation to MDS/leukemia is very rare, isolated cases have been reported.[60] Bone marrow tests are not required, except in cases in which clinical and/or biochemical findings (i.e., changes in peripheral blood counts) indicate clonal evolution.

In acquired neutropenia, most pediatric autoimmune and idiopathic forms resolve within 24–36 months.[8–10] Follow-up assessments are required to ensure stable ANC values. In contrast, in patients with idiopathic and autoimmune neutropenia, which rarely spontaneously remits, follow-up may help identify evidence required to redefine the diagnosis (Table 4).[11]

5.3 | Hematopoietic stem cell transplantation (HSCT) in severe congenital neutropenia

A few case reports and case series,[73–78] and a single large study from the European Bone Marrow Transplantation (EBMT) cohort[79] have been reported on HSCT in SCN patients. The overall survival of 136 SCN patients from the EBMT study was 82% after 3 years of follow-up; this finding was consistent with that of a previous study.[79] HSCT in SCN yielded better outcomes in patients younger than 10 years, treated after the year 2000. Matched sibling and related donors are associated with better outcomes than mismatched donors, and bone marrow is considered a better source of cells than cord blood or peripheral blood. Despite previous reports of SCN patients transplanted with overt leukemia at the time of HSCT, the EBMT study did not confirm this risk. Nevertheless, this risk remains subject to debate, as the lack of relevant data precludes definitive conclusions.[75,77,78] Prior to HSCT, leukemia is generally treated in a manner similar to that of other constitutional diseases.[80]

Transplant-related mortality rate was estimated to be 17% in the EBMT cohort, suggesting that HSCT may not be suitable for patients who could be otherwise managed with G-CSF at standard/low doses.[79,80]

HSCT is absolutely recommended in disease transformed to MDS/AL, or presenting with dysplastic features carrying high-risk karyotype abnormalities (monosomy 7 and trisomy 8), or with a combination of acquired *RUNX1* and *CSFR3* mutations, and in neutropenia due to mutations bearing a high intrinsic risk of leukemic transformation (i.e., *GATA2*).[81–83]

In all other cases, the advantages and disadvantages of HSCT should be carefully considered, and patients and their families should be presented with all the information required for decision-making.

In this respect, elements to be weighted in favor of HSCT include scarce response or nonresponse to G-CSF (failure to reach ANC levels of 1.0×10^9/L with G-CSF doses in the range of 10–20 γ/kg/day or higher), poor infection control, irrespective of the G-CSF doses, and the availability of an identical sibling match or of a 10/10 HLA identical matched unrelated donor.[82,83]

In contrast, elements to be weighed against HSCT are G-CSF response at doses of <10 γ/kg/day, good tolerability, and compliance with daily subcutaneous injections, satisfactory infection control, and lack of an HLA-matched donor.

Contextual factors, such as access to an HSCT and the experience of the administering center, are unfortunately not uniformly distributed in the world, which should also be considered when managing patients with SCN.

5.4 | Pregnancy

The availability of G-CSF treatment allowed women with SCN to reach childbearing age and pregnancy. However, data on G-CSF schedules/indications in pregnancy are scarce.

According to findings from two large studies, G-CSF administration during pregnancy is safe and well tolerated.[83,84] The rate of infection was lower in women treated with G-CSF than in those that did not receive this treatment; meanwhile, there was no difference in pregnancy or infant outcomes between these groups.[83,84] As G-CSF crosses the placental barrier, this treatment in late pregnancy may help prevent perinatal infections in newborns with inherited neutropenia.[84–86] The panel of experts recommends that G-CSF treatment may be continued throughout pregnancy, even in the first trimester, in women with SCN, helping prevent maternal infections and newborn complications. The goal of treatment is to maintain ANC levels at 1.0×10^9/L with infection control. G-CSF can be used in breastfeeding women.

5.5 | Vaccinations

Neutropenia comprises heterogeneous disorders with different degrees of immune impairment and susceptibility to infections; consequently, recommendations on immunization vary and are determined by the type of disorder of each patient.[87,88]

In general, inactivated vaccines are safe and well tolerated in patients with neutropenia; in contrast, live attenuated viral and bacterial vaccines may require the evaluation of immunological status for safe administration. However, in cases of immunodeficiency, a lack of efficacy is expected. Patients with autoimmune and idiopathic neutropenia, in which an underlying immunodeficiency has been ruled out, may receive all types of vaccines. Live vaccines should be cautiously avoided, until after any suspected immunodeficiency has been diagnosed. In these cases, only inactivated vaccines may be safely administered.

Patients with classical SCN may respond to the antigenic stimulation of live virus vaccines. Given the increased risk of bacterial complications caused by viral infections, these patients should receive all inactivated viral vaccines. BGC and vaccines against *Salmonella thyphi*, even in countries where these infections are endemic, are contraindicated, whereas mRNA vaccines are considered safe.[89]

Finally, immunization against all vaccine-preventable diseases is recommended for close contacts of patients with neutropenia, with or without immune suppression.

5.6 | Infections, antibiotic treatment, and prophylaxis

SCN patients are susceptible to bacterial and fungal infections and are at risk of developing sepsis, which may be lethal, at a cumulative incidence ranging from 6% to 34% according to findings from the largest European registries (SCNIR, French Registry, Swedish Cohort, and Italian Registry).[17,18,70,71]

The respiratory tract, skin, and soft tissue (mainly in the arms, face, and perineal region, but also at the umbilical cord at birth) are common sites of infection. However, deeply seeded abscesses may occur.[57] Recurrent oral stomatitis is present in most cases of CyN.[57]

Compared to SCN, autoimmune and idiopathic neutropenia are characterized by a lower rate (10%–15%) of nonlethal infection.[8–11]

Data on the use of antibiotic prophylaxis are not available for patients with neutropenia, and prophylaxis is generally adopted based on each center's policy and practice. In addition, the potential advantages of antibiotic prophylaxis in patients with neutropenia (outside the transplant setting) must be balanced against the risk of generating antibiotic-resistant bacteria.

The shared idea of the experts is that any major advantage of antibiotic prophylaxis over treating infection once it has occurred remains unproven in this patient population.

The treatment of infectious episodes is largely derived from the approach adopted in patients with cancer and leukemia. In cases of first infection in patients without a specific neutropenia diagnosis (autoimmune, post infection, SCN), broad-spectrum antibiotics, as defined by each center's policy, are recommended, irrespective of ANC levels and G-CSF treatment.

Targeted antibiotic treatment is mandatory when a bacterial agent has been identified.[90,91]

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

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