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REVIEW

Congenital and Acquired Neutropenia Consensus Guidelines on Diagnosis From the Neutropenia Committee of the Marrow Failure Syndrome Group of the AIEOP (Associazione Italiana Emato-Oncologia Pediatrica)

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Congenital and acquired neutropenia are rare disorders whose frequency in pediatric age may be underestimated due to remarkable differences in definition or misdiagnosed because of the lack of common practice guidelines. Neutropenia Committee of the Marrow Failure Syndrome Group (MFSG) of the AIEOP (Associazione Italiana Emato-Oncologia Pediatrica) elaborated this document following design and methodology formerly approved by the AIEOP board. The panel of experts reviewed the literature on the topic and participated in a conference producing a document which includes a classification of neutropenia and a comprehensive guideline on diagnosis of neutropenia. Pediatr Blood Cancer 2011; 57:10–17. © 2011 Wiley-Liss, Inc.

Key words: acquired; congenital; diagnosis; neutropenia; severe

INTRODUCTION

Congenital and acquired neutropenia are rare disorders whose reported frequencies range from 1/100,000 for acquired autoimmune forms to 1/1,000,000 for congenital forms [1]. Both figures are probably underestimated partly because of the lack of fully comprehensive diagnostic tools. Nevertheless, neutropenias constitute a relevant proportion of the activity of many Pediatric Hematology Centers that are often faced with the issue of a correct diagnosis in the absence of a large support from literature. These reasons generated the need for shared practice guidelines on these rare diseases. These guidelines were explicitly intended not as standards or fixed rules but as an instrument to support Pediatricians in the decision making process of the diagnosis of patient with congenital and acquired neutropenia. In this view, the present document cannot guarantee a successful outcome in any case. The final choice has to be made by the patient's physician based on individual data and diagnostic options available. Finally, these guidelines can be the starting point of an evaluation process on how physician behavior and patients outcome can be influenced with the aim to collect new evidence for updating the original document.

DESIGN AND METHODS

Design and methodology were those adopted for the acute childhood idiopathic thrombocytopenia purpura: AIEOP Consensus Guidelines for diagnosis and treatment [2] whose procedures were validated by the AIEOP (Associazione Italiana Emato-Oncologia Pediatrica) board. In brief the Neutropenia Committee of the Marrow Failure Syndrome Group (MFSG) of the AIEOP elaborated between May and July 2004 a document on recommendations for management of children with congenital and acquired neutropenia. In April 2008, the MFSG of AIEOP decided to review and update this document and assigned this task to a group of experts who are member of the Neutropenia Committee of the MFSG. Each of the following topics: definition of the clinical entity and diagnosis, therapy and follow-up was initially revised by three experts of this committee who wrote two pre-guideline documents: one on definition of the clinical entity and diagnosis and another one on treatment and follow-up. The experts who worked on definition of the clinical entity and diagnosis also elaborated a comprehensive classification of neutropenia. Given the relevant amount of collected data, it was decided by the Neutropenia Committee to divide the work in two different papers: one dedicated to the definition of the clinical entity and the diagnosis and another one to the treatment and the follow-up of patients with congenital and acquired neutropenia. The present article therefore includes only the consensus guidelines on definition and diagnosis of congenital and acquired neutropenia.

Literature Review and Assessment of Evidence

Data source. For the pre-guideline documents, experts extracted evidence from literature searched for in the Medline database (from January 1971 to January 2009). Search terms included: neutropenia, congenital, acquired, severe, SCN, diagnosis, children. The Medline search sorted out a total of 107 articles of which 61 were suitable for the definition of the clinical entity and diagnosis. The search was also extended to hematology textbooks and proceedings of international hematology meetings.

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TABLE I. Levels of Evidence for Studies Evaluating Diagnosis of Neutropenia in Children

Level of evidence	Study design
I (strongest)	Prospective randomized trial with high statistical value
II	Prospective randomized trial with lower statistical value
III	Non-randomized study with concurrent control group
IV	Non-randomized study with historical control group
V (weakest)	Case report(s) with no control group
EO	Expert opinion either as derived from literature or from the panel of experts of the Neutropenia Committee of the MFSG of the AIEOP

Every collected evidence was attributed a strength that was scored using level of evidence criteria reported in Table I.

Consensus Conference

In keeping with the lack both of controlled and non-controlled studies and of case report series, for most issues levels of evidence from I to V were not available. These issues were regarded as experts opinions (EO) both in case they were contained within published literature or represented the opinion of the panel of experts of the Neutropenia Committee of the MFSG of the AIEOP. Then all the members of the Neutropenia Group met in three consensus conferences held in Milan 16th April 2008, Florence 26th May 2008, and Milan 16th June 2008 and expressed their consensus on each evidences and EO provided in the pre-guidelines document. The strength of this consensus was quantified on a 1–9 scale where 1 represented no consensus and 9 full consensus regarding the appropriateness and necessity of the practice. For each statement a mean score was calculated. Mean scores from 1 to 3 indicated an inappropriate practice; mean scores from 3.01 to 6.99 a practice of uncertain appropriateness; mean scores from 7 to 9 an appropriate/necessary practice. The level of unanimity of the opinions, indicating the level of consensus was evaluated as in Table II, based on this system in the text after each statement the following symbols will be found in brackets: Level of evidence in Roman numbers from I to V or EO if expert opinion; strength of consensus in Arabic numbers from 1 to 9; level of consensus in capital letters from A to D.

DEFINITION OF THE CLINICAL ENTITY

Neutropenia is a disorder characterized by a reduction of the absolute count of circulating neutrophils (ANC) below the lower limits which vary according to race and age. In Caucasians newborns and toddlers up to the age of 1 year the lower limit is 1.0×10^9/L whereas is 1.5×10^9/L from >1 year to adulthood [1] (V, 8.5, A). It has to be noted that black populations have lower normal inferior limits ($0.2–0.6 \times 10^9$/L circulating neutrophils) [3] than Caucasians (V, 8.5, A) which thing accounts for a different threshold for definition of neutropenia. In Caucasians after the first year of life neutropenia is defined as mild if circulating neutrophils are between 1.0 and 1.5×10^9/L, as moderate if between 0.5 and 1.0×10^9/L, and severe if below 0.5×10^9/L [1] (V, 8.5,A).

From 1980, the term severe congenital neutropenia has been introduced to define a heterogeneous group of disorders of myelopoiesis characterized by an ANC $<0.5 \times 10^9$/L [4,5] detected within the first months of life (V, 8.5, A). The panel elaborated a classification (Table III), which is based on the distinction of isolated neutropenia from those associated to other pathological conditions [6] (EO, 8.4, A).

TABLE II. Level of Consensus

(A) Strong agreement (variance was more than 1 SD below the mean variance)
(B) Moderate agreement (variance less than 1 SD below the mean variance)
(C) Moderate disagreement (variance less than 1 SD above the mean variance)
(D) Strong disagreement (variance more than 1 SD above the mean variance)

DIAGNOSTIC ITINERARY

The panel of experts outlined the importance of an initial evaluation including history and physical examination, particularly focused on elements illustrated in Table IV (EO, 8.7, A). Agreed on diagnostic itinerary to continue according to the initial evaluation level shown in Figure 1 and, if necessary, according to the advanced investigation steps represented in Figure 2 and herein discussed. In the presence of normal hemoglobin and platelet values, the diagnostic approach varies according to the absolute ANC [1] whose confirmation on microscopy by looking at the peripheral blood smear is recommended (V, 8.3, C). If initial ANC is $1.0–1.5 \times 10^9$/L (mild neutropenia), the count has to be re-checked after 4 weeks [7]. If the second count is between 1.0 and 1.5×10^9/L further ANCs are required and first level investigations (Table VI) can be performed. If the second ANC is $>1.5 \times 10^9$/L then this value has to be confirmed on two further occasions before letting the patient out of the algorithm (EO, 8.3, C). The C level of consensus was generated by the opinion of some expert who considered this procedure too tight. If initial ANC is $0.5–1.0 \times 10^9$/L (moderate neutropenia) three further counts at least seven days apart are recommended [8–10] and if initial values are confirmed, first level investigations (Table VI) need to be carried out (EO, 8.3, D). The low level of consensus was due the fact that not all experts considered this level of neutropenia worthy of investigating that promptly. If initial ANC is $<0.5 \times 10^9$/L (severe neutropenia) the panel unanimously agreed on the need to re-confirm this value in only two further counts at least seven days apart before proceeding to first level investigations (EO, 9, A). If the patient shows signs and symptoms suggestive of severe infections the panel agreed on establishing an accelerated diagnostic procedure including immediate first level investigations and bone marrow evaluation

TABLE III. Classification of Neutropenias (EO, 8.4, A)

- Isolated neutropenias
  - Severe congenital neutropenias (SCN)
    - with known genetic lesion
      - ELA2 (autos dom, sporadic)
      - HAX 1 (autos rec.) can be associated to neurologic symptoms
    - without known genetic lesion
  - Cyclic neutropenia (CyN) (ELA2, autos dom, sporadic)
  - Autoimmune neutropenia (AIN)
  - Neonatal allo-immune neutropenia
  - Post-infectious neutropenia
  - Drug-related neutropenia
  - Familial benign/ethnical neutropenia
  - Idiopathic neutropenia (IN)
- Neutropenias associated to other pathological condition
  - Associated to mitochondrial diseases:
    - Shwachman–Bodian–Diamond syndrome
    - Pearson syndrome
  - Associated to congenital organ malformations:
    - G6PC3 gene mutation
    - Blackfan–Diamond syndrome
  - Associated to metabolic diseases:
    - Glicogenosis Ib
    - Organic-acidosis
    - Tyrosinemia
    - Barth syndrome
    - Gaucher disease
  - Associated to immunodeficit:
    - Hyper IgM
    - Hypoagammaglobulinemia X-linked
    - Common variable immunodeficiency
    - Isolated IgA deficiency
    - Reticular dysgenesia
    - Dubowitz syndrome
    - WHIM's syndrome
    - Cohen syndrome
    - X-linked neutropenia
    - GFI1 deficiency
  - Associated to immunodeficit with hypopigmentation:
    - Griscelli syndrome (type 2)
    - Chediack–Higaschi syndrome
    - Hermansky–Pudlak syndrome (type 2)
    - P14 deficiency
  - Associated to autoimmune diseases:
    - SLE
    - Rheumatoid arthritis or Felty syndrome
    - Scleroderma
    - Sjogren syndrome
    - Autoimmune lymphoproliferative syndrome (ALPS)
    - Celiac disease
    - Primitive biliary cirrhosis
    - Crohn disease
    - Associated to activation of C5
  - Associated to nutritional deficiencies:
    - Vitamin B12 deficiency
    - Folate deficiency
    - Copper deficiency
  - Associated to intrinsic or extrinsic marrow failure:
    - Aplastic anemia
    - Myelodysplastic syndromes
    - Primitive or secondary macrophage activation
    - Fanconi anemia
    - Dyskeratosis congenita
    - Hair-cartilage hypoplasia
    - Myelofibrosis
    - Osteopetrosis
    - Marrow infiltration
  - Associated to myelo-lymphoproliferative disorders:
    - Acute myeloid leukemia
    - Acute lymphoblastic leukemia
    - Chronic myeloid leukemia
    - Juvenile myelo monocytic leukemia
    - Lymphomas
    - Chronic lymphoblastic leukemia
    - LGL syndrome
  - Associated to hypersplenism (±anemia, ±thrombocytopenia)
  - Associated to sequestration in infectious foci

followed by start of treatment. The same was considered appropriate also for patients with moderate neutropenia (EO, 9, A).

In case of a personal history of assumption of medications known to be associated with neutropenia or in case of recurrence of neutropenia after drug exposure and its regression after drug withdrawal [11,12] a diagnosis of drug-related neutropenia looked appropriate (V, EO, 8.1, B). The list of drugs associated with neutropenia reported in Table V was considered by the panel exhaustive (V, EO, 9, A).

In Blacks of South African extraction [13] (II), in American Mexicans [14] (I), in Afro Caribbean [15] (I), in Yemenite Jews ancestries and in some Arabic ethnicity [13–16] (II) an ANC between 0.5 and 1.0×10^9/L mainly if not associated to infections and found also in the parents, was considered to allow the diagnosis of the Ethnic neutropenia which is considered a variation from the normal [17] (V, EO, 8.7, B).

After confirmation of the neutropenia, the experts considered appropriate to perform the panel of first line investigations of Table VI (EO, 8.2, B), Figure 1. This panel was intended as a package aiming to confirm/exclude the commonest causes of neutropenia and to direct further diagnostic steps in case a firm diagnosis was not achieved. If history, clinical findings and first level investigations suggested a form associated to other pathological conditions (Table III), the panel reckoned appropriate to proceed to further more targeted analyses as indicated by patient's history and clinical-laboratory data (Fig. 2) (EO, 8.2, B).

If neutropenia was found to be associated to bone abnormalities of the chest and upper and lower limbs, hepatomegaly, diarrhea, anemia and/or thrombocytopenia and to consistently modified first level investigations (electrolytes changes and metabolic acidosis) diseases like Shwachman–Diamond, Pearson's syndrome and even Blackfan–Diamond syndrome should have been taken in account (Table III). In these cases the panel reckoned appropriate to proceed to further more targeted analyses (genomic DNA mutation study for Shwachman–Diamond, mitochondrial DNA analysis for Pearson's syndrome, erythrocyte ADA and mutation search for Blackfan–Diamond syndrome) [18–21] aiming to make a firm diagnosis of these diseases (Table VII) (EO, 8.2, B). In the case of signs of nutrition deficiency and consistent first level investigation (low IgG serum level and altered liver function tests), measurement of serum levels of Vit. B12, transcobalamin, folate, and copper was recommended [17] (EO, 8.4, A).

In the case of early, severe and recurrent infections associated to decreased Ig serum levels, increased CRP and positive markers

TABLE IV. Initial Evaluation for Patients WHO Have Neutropenia (EO, 8.7, A)

Family history
Ascertain ethnic origin, occurrence of other neutropenia cases, consanguinity
Personal history
Ask for occurrence of viral or bacterial infections and drug assumption during pregnancy and neonatal period
Investigate number, type, site, and recurrence of infections. Ask specifically for occurrence of gingivitis, periodontitis, skin infections, abscesses, otomastoiditis, and pneumonias and for type, administration way, duration of treatment and response to antibiotics
Drug history
Ask for type and duration of drug assumption, particularly those indicated as to be associated with occurrence of neutropenia (Table V)
Physical examination
Focus on weight, stature, psychomotor development, somatic dysmorphisms, signs of infections (skin, mouth), hearth function, liver, and spleen size, presence of enlarged lymphonodes, joints, neurological symptoms, symptoms compatible with autoimmune, metabolic, gastrointestinal, nutritional diseases

of infections, overall suggesting an immunodeficiency, peripheral blood immunophenotype, response to vaccines including polysaccharide antigens, lymphocyte proliferation to mytogens are indicated before referring the patient to an Immunodeficiency Reference Center where study of mutations of genes involved in neutropenia associated with immunodeficiency can be addressed [22–34] (Table VII) (EO, 9, A).

In the presence of symptoms suggestive for metabolic disorder (gastrointestinal, neurological involvement, failure to thrive, psychomotor delay, hepato-splenomegaly, cardiac anomalies) and with consistently altered first level investigation (abnormal liver function tests, hypoglycemia, metabolic acidosis) the patient needs to be referred to a Metabolic Disease Reference Center where specific biochemical (urinary organic acid and serum tyrosine) or molecular analyses can be performed (G6PT activity on liver tissue and TAZ gene analysis for Glicogenosis Ib and Barth Syndrome, respectively) [35–39] (Table VII) (EO, 9, A).

In case symptoms and first line investigations (i.e., altered liver function tests, abnormal Ig serum level, positive ANA, DAT, and/or IAT and possibly anti-neutrophil antibodies) were suggestive for an autoimmune disease, then serum anti-TG, EMA, anti-gliadin antibodies, ENA, C3, C4, CH 50, circulating IC, RA test, ds-DNA, p-ANCA, anti-phospholypid, and anti-cardiolypin antibodies are suggested before referring the patient to an Autoimmune Disease Reference Center. The panel suggested to consider amongst autoimmune diseases associated with neutropenia also the autoimmune lymphoproliferative syndrome (ALPS) whose diagnostic test are: increased percentages of peripheral blood double negative CD3 lymphocytes, T-lymphocyte reduced sensitivity to FAS-mediated apoptosis and mutation

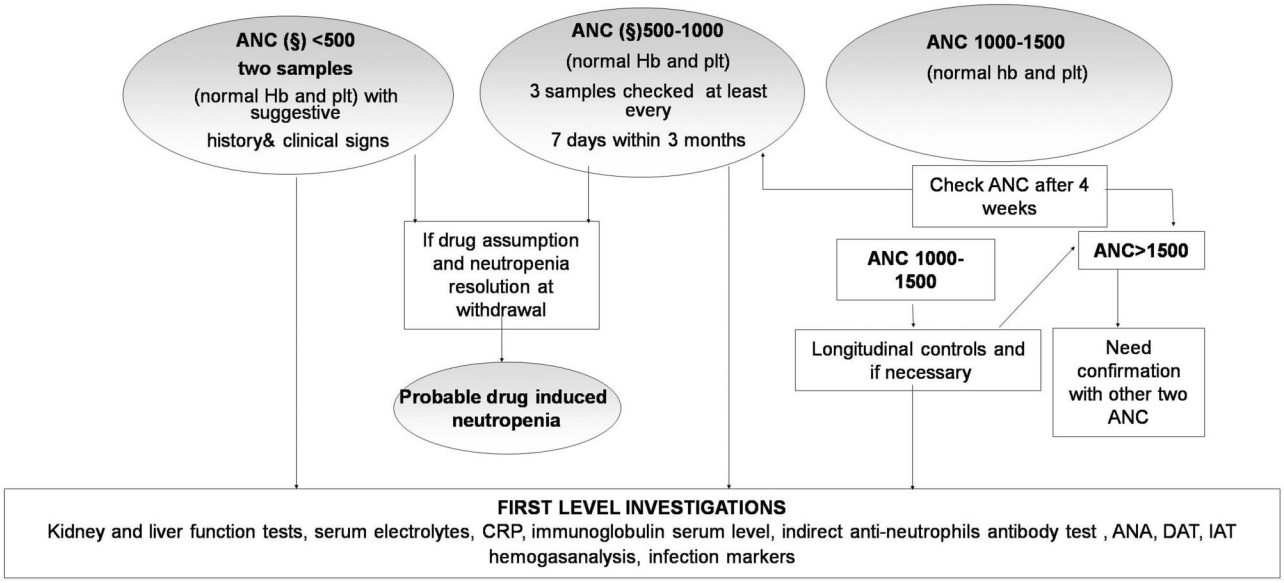

Fig. 1. Initial evaluation level, suggested before the consultation of the specialist. ANC, absolute neutrophils count; CRP, C-reactive protein; ANA, antinuclear antibodies; DAT, direct antiglobulin test; IAT, indirect antiglobulin test. § If the patient shows signs and symptoms suggestive of severe infections (in severe and moderate neutropenia), the panel agreed on establishing an accelerated diagnostic procedure including immediate first level investigation and bone marrow evaluation (EO, 9, A).

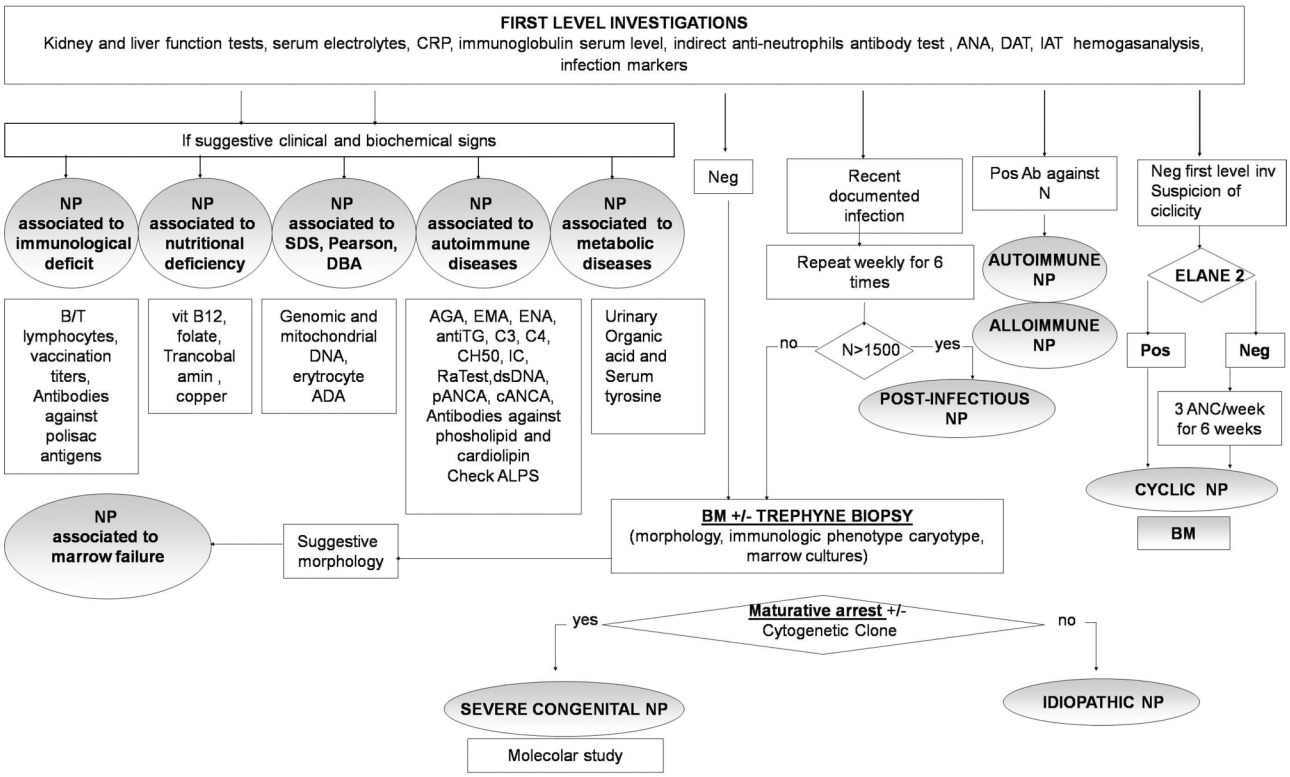

Fig. 2. Advanced level investigations. BM, bone marrow; ADA, serum adenosine deaminase; SDS, Shwachman–Diamond syndrome; DBA, Diamond–Blackfan anemia; AGA, antiglobulin antibodies; EMA, antiendomysium antibodies; ENA, antibodies against extractable nuclear antigen; antiTG, antibodies against thyroglobulin; ALPS, autoimmune lymphoproliferative syndrome.

detection of FAS, FAS-L, Caspase 8 and 10 genes [40,41] (EO, 9, A).

If a recent infection was documented, there has been agreement on repeating weekly full blood count for six weeks since the infection [1,7,17]. In this context the panel considered appropriate the following: (a) the search for the underlying cause immediately during an infection, (b) considering a bone marrow evaluation as potentially helpful, and (c) the start of treatment if any of the congenital neutropenia sub-diagnosis might have been plausible. A diagnosis of infection-related or post-infectious neutropenia

TABLE V. Drugs Implicated in Causing Neutropenia [11] (V, EO, 9, A)

Analgesics and non-steroidal antinfiammatory drugs	Acetaminophen, acetylsalicylic acid, aminopyrine, benoxaprofen, diclofenac, diflunisal, dipyrone, fenoprofen, indomethacin, ibuprofen, naproxen, phenylbutazone, piroxicam, sulindac, tenoxicam, tolmetin
Antipsychotics, hypnosedatives, and antidepressants	Amoxapine, chlomipramine, chlorpromazine, chlordiazepoxide, clozapine, diazepam, fluoxetine, haloperidolo, levopromazine, imipramine, indalpin, meprobamate, mianserin, olanzapine, phenothiazine, risperidone, tiapride, ziprasidone
Antiepileptic drugs	Carbamazepine, ethosuximide, phenitoin, trimethadione, valproate acid (valproate sodium)
Antithiroid drugs	Carbimazole, methimazole, potassium perchlorate, potassium thiocyanate di potassio, propylthiouracil
Cardiovascular drugs	Acetylsalicylic acid, amiodarone, aprindine, bepridil, captopril, coumarins, dipyrisamole, digoxin, flurbiprofen, furosemide, hydralazine, lisinopril, methyldopa, nifedipine, phenidione, procainamide, propafenone, propranonol, quinidina, ramipril, spironlactone, thiazide diuretics, ticlopidine, vesnarinone
Antinfective agents	Abacavir, acyclovir, amodiaquine, atovaquone, cephalosporins, chloramphenicol, chloroguanine, chloroquina, ciprofloxacin, clindamyicin, dapsone, ethambutol, flucytosine, fusidic acid, gentamicin, hydroxychloroquine, isoniazid, levamizole, linezolid, macrolids, mebendazole, mepacrine, metronizadole, minocycline, nitrofurantoin, norfloxacin, novobiocin, penicillins, pyrimethamine, quinine, rifampicin, streptomycin, terbinafine, tetracycline, thioacetazone, tinidazole, cotrimoxazole, vancomycin, zidovudine
Miscellaneous drugs	Acetazolamide, acetylcysteine, allopurinol, aminoglutethimide, arsenic compoounds, benzafibrate, brompheniramine, calcium dobesilate, chlorpheniramine, cimetidine, colchicine, dapsone, deferiprone, famotidine, flutamide, gold, glucocordicoids, hydroxychloroquine, mesalazine, metapyrilene, methazolamide, metoclopramide, levodopa, olanzapine, omeprazole, oral hyoglycemic agents (glibenclamide), mercurial diuretics, penicillamine, ranitidine, riluzole, sulfasalazine, sulfonamides, tamoxifene, thenalidine, tetinoid, tripelennamine

TABLE VI. First Level Investigations (EO, 8.2, B)

Kidney and liver function tests
Serum electrolytes
Venous blood pH
C-reactive protein (CRP)
Immunoglobulin serum level
Indirect anti-neutrophil antibodies (4 tests over 4–6 months) by flow cytometry analysis
Viral (serology or DNA/RNA) and bacterial investigations
Direct and indirect antiglobulin test
Antibodies against nucleus (ANA) test

was considered appropriate if after 6 weeks ANC returned to normal and no other causes has become apparent (V, EO, 7.8, B). If after six weeks ANC did not return to normal, bone marrow aspiration is recommended (EO, 7.8, B). Bone marrow trephine biopsy is not considered always appropriate but in the view of a potentially severe diagnosis of post-infectious aplastic anemia, the panel agreed on leaving this decision to the judgment of the treating physician (EO, 7.8, B). Performance of bone marrow aspiration and trephine biopsy in deep sedation was strongly encouraged.

Indirect anti-neutrophil antibodies detection by flow cytometry was agreed on to be the most useful and practical method for identifying the autoimmune neutropenia (AIN) [1,7,9,17,42–46]. The low sensitivity (74% at first assessment) [45] of this method was recognized by the panel but it was considered, in the respect of diagnostic power, less detrimental than the lower specificity (very high number of false positives) of the direct method [44] (III, V, EO, 9, A). If indirect anti-neutrophil antibody test is positive the diagnosis of AIN is appropriate (EO, 9, A). In case indirect anti-neutrophil antibodies are contemporarily positive in the newborn and the mother a diagnosis of allo-immune neutropenia is considered appropriate (EO, 9, A). Based on the limited sensitivity of the indirect test, the panel also agreed to consider as ''likely autoimmune neutropenia'' those cases with at least one border-line positivity of indirect anti-neutrophil antibodies coexisting with compatible ANC and clinical phenotype (EO, 9, A). However, in case the first test is negative or border-line, still in the presence of clinical suspicion of AIN, there was agreement on the need to repeat the test up to four times over a time-span of 4–6 months (EO, 9, A).

The panel agreed that the following steps need to diagnose the cyclic neutropenia (CyN). In case of negative first level investigations in the presence of ANC $<0.5 \times 10^9$/L on two consecutive occasions at least 21 days apart, with normal ANC in between, with or without recurrent aphthous stomatitis, it is justified to proceed to ELA2 mutation analysis (EO, 7.8, C). If mutation of ELA2 gene is found [1] then the diagnosis of CyN is established (EO, 9, A). If a mutation of ELA2 gene is not found then the occurrence of CyN needs to be proved by performing three ANCs/week for six weeks [1,4,7,17,47–49] (EO, 7.8, C). The panel agreed on outlining the following: (a) ELA2 mutations, in addition to CyN can occur also in severe congenital neutropenia. (b) Patients with CyN may not always reach normal blood counts.

TABLE VII. Known Genetic Alterations in Neutropenia Associated to Other Diseases

	Genetic defect	Inheritance
Neutropenias with immunodeficiency		
Severe congenital neutropenia + immunodeficit	WAS [54,55]	X-linked
Severe congenital neutropenia + immunodeficit	GFI1 [22]	AD
WHIM syndrome	CXCR4 [23]	AD
Cohen syndrome	COH [24]	AR
X-linked hypoagammaglobulinemia	BTK [25,26,27]	X-linked
Hyper IgM	CD40L [28,33]	X-linked
Neutropenias with immunodeficiency with hypopigmentation		
Chédiak Higashi syndrome (type 2)	LYST [29]	AR
Griscelli syndrome (type2)	RAB27A [30]	AR
Hermansky–Pudlak type 2 syndrome	AP3B1 [31]	AR
P14 deficiency	MAPBPIP [32]	AR
Neutropenias with metabolic, autoimmune diseases or organ malformations		
Glycogen storage disease 1B	G6PT [35–38]	AR
Barth syndrome	TAZ [39]	X-linked
Diamond–Blackfan anemia	RPS 19, RPS 24, RPS 17, RPL 35 A, RPL 5, RPL 11 [21]	AR
ALPS (autoimmune lymphoproliferative syndrome)	FAS, FAS-L, Caspase 8,10 [40,41]	AD
NCS with G6PC3 mutations (associated to genito-cardiac malformations)	G6PC3 [53]	AR
Neutropenias with mitochondrial diseases		
Shwachman–Bodian–Diamond syndrome	SBDS [18]	AR
Pearson syndrome	Mitochondrial genoma [19,20]	Behaves like an X-linked inheritance
Neutropenia with marrow failure		
Fanconi anemia	FANCA, B, C, D1, D2, E, F, G, H [58]	AD, X-linked
Dyskeratosis congenita	TERC, TERT, DKC1, TNF2, NPH2 [59,60]	AD, AR, X-linked
Cartilage hair hypoplasia	RMRP [61]	AR

AD, autosomal dominant; AR, autosomal recessive.

(c) The above recommendations reflected the shared experience of the panel who met a very poor compliance of the patients and their families to the program of three ANCs/week for six weeks resulting in a scarcely effective diagnostic tool leading to a potential underestimation of this clinical entity. (d) Given its faceted nature it is still possible that some atypical form of CyN can still be undiagnosed. The panel did not encourage the performance of bone marrow aspiration in an established diagnosis of CyN. On the contrary, bone marrow aspiration was recommended before the start of treatment with G-CSF (EO, 8.8, C).

In case of confirmed neutropenia with non-informative first level investigations and no signs/symptoms specific for associated forms, the panel agreed on the need to perform bone marrow aspiration for morphology [1,7,9,12,14,17,48] (EO, 9, A). The panel also agreed on the appropriateness of taking on this occasion marrow samples also for cytogenetic, immunophenotyping, cultures, and molecular analysis and to carry out these tests only in case morphological examination showed dysplastic or atypical cells or significantly reduced cellularity in the fragments of the marrow smear (EO, 8.8, B). The panel agreed on the need to obtain specific informed consent from the patients/parents for taking all marrow samples including also those that might not undergo subsequent analyses (EO, 9, A).

The experts did not consider as always appropriate the performance of bone marrow trephine biopsy and agreed on to leave the final decision to the treating physician in order to obtain baseline information on cellularity and dysplastic features (EO, 8.8, B). Performance of bone marrow aspiration and trephine biopsy in deep sedation was strongly encouraged. If marrow morphology shows maturation arrest at the stage of pro-myelocyte/myelocyte $\pm$ the presence of a cytogenetic clone, then the diagnosis of severe congenital neutropenia (SCN) is considered as appropriate (EO, 8.6, B) and the study of ELA2 and HAX-1 [12,50–52] mutations, as the most frequently involved genes, were recommended by the panel (EO, 8.6, B). In presence of genital-cardiac malformations the lesion of G6PC3 gene has to be considered [53]. Activating mutations in the WAS gene have been found to be responsible for some cases of X-linked-SNC [54,55] (EO, IV, V, 8.1, B). Finally as patients with digenic mutations have been found [56], mutation search in a second gene known to be involved in SCN should also be considered. In case these investigations fail to detect a genetic cause of SCN, the panel recommended to carefully re-evaluate history, clinical symptoms and specific laboratory tests. If also this re-evaluation process does not lead to identify a known genetic form of SCN then, based on the observation that forms of SCN exist without known genetic lesions [6,10,34,50,52,57], a diagnosis of SCN without known genetic lesion was reckoned as appropriate (EO, V, 8,1, B).

At this stage of the pathway, if no maturation block on marrow morphology is detected, and the indirect antibodies test against neutrophils are negative at least four times, the diagnosis of idiopathic neutropenia (IN) is considered appropriate [1,7–9,17,46] (EO, 9,A). Since IN still remains an exclusion diagnosis the panel agreed on the need to place IN patients on a monitoring program and to perform diagnostic re-evaluation in case new elements suggestive of other diagnosis arise (EO, 9, A).

The panel agreed on the role of maturation arrest in discriminating SCN from IN and AIN (E.O, V, 9, A). However the experts experienced cases in which the block was not clearly evident. This raised the issue that a standardization of this parameter is not presently available in literature and that also a threshold of promyelo/myelocytes number/percentage above which the block take place is lacking.

In conclusion, the panel outlines the need of a multicentre international study aiming to provide an objective and repeatable definition of maturation block.

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