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REVIEW ARTICLE

Investigation of neutropenia in pediatric patients

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Abstract

Neutropenia is common in both adults and children. Defined as a count of peripheral blood neutrophils <1,500 cells/µl. The causes are variable, it is important to consider the different age groups and associated clinical conditions. Its consequences can be serious and may lead even to death. In the literature, mortality from neutropenia, when associated with fever, can reach up to 10%. In Oncohematological patients this percentage reaches 16%. In view of the above and the importance of theme for pediatricians and hematologists, we chose to formulate this work. The study will be presented in the form of a narrative review. It intends to review the main causes of neutropenia in childhood, propaedeutics and identification of severity indicators with the proposal application of a neutropenia initial investigation flowchart in pediatrics, in addition to guiding when refer these patients to specialized services.

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INTRODUCTION

Neutrophils are cells measuring 10 to 15 mm, with a multilobed polymorphic nucleus. They have highly condensed chromatin and a yellow-pink cytoplasm containing numerous granules as well as glycogen clusters. The neutrophil production rate is 109 cells/kg/day, with a lifespan in the bone marrow of 14 days. It is estimated that in the bloodstream the half-life is 6 to 10 hours[1].Neutrophils exit the circulation by diapedesis between/through endothelial cells to sites of inflammation or infection in tissues. In the latter, their lifespan is 1 to 2 days and then they undergo apoptosis and are absorbed by macrophages[2].

Neutropenia is defined as an absolute neutrophil count in peripheral blood <1,500 cells/μl in adults and children[3]. It is a common change in both adults and children. The causes vary, and it is important to consider different age groups and associated clinical conditions.

Neutropenia can often be found in asymptomatic children and clinical manifestations generally only occur when the count is <500 cells/μl. This is the point that defines neutropenia as severe, where the patient is at high risk of life-threatening infections, especially when counts are <200 cells/μl[4]. It is important to highlight that the severity of the manifestations depends not on only the absolute value of neutrophils, but also the onset time and duration of neutropenia. The relationship between value and severity occurs in neutropenias due to hypoproduction due to suppression or bone marrow failure. In general, neutropenia due to peripheral destruction is less severe, probably due to the ability to release the "pool" of neutrophils available in the bone marrow.

Neutropenia can be found isolated or associated with other cytopenias. This is a determining factor for the continuation of the investigation. When the patient presents more than one cytopenia, they should be referred for immediate evaluation by a hematologist, as we may be facing more serious diseases.

The causes of neutropenia are divided into chronic and acute or transient. Transients can have different etiologies, the main ones being: viral, bacterial, medication. Among the chronic causes in childhood, congenital, cyclical and autoimmune neutropenia can be highlighted.

Investigating the cause of neutropenia and understanding its severity are extremely important not only for the hematologist, but also for the general pediatrician. Identifying the etiology can be difficult, but it is crucial for the therapeutic approach and assessment of the prognosis. Especially in childhood, hospitalizations and sometimes unnecessary antibiotic therapy are common.

GOAL

Primary objective:

1- Review the literature in the form of a narrative review on neutropenia.

2- Propose to pediatricians the application of a flowchart for investigating neutropenia in the pediatric age group and when to refer to a specialist.

Secondary objectives:

1- Describe the main causes of neutropenia in childhood;

2- Describe the basic investigation of neutropenia for pediatricians and hematologists.

METHODOLOGY

Study conducted in the form of a narrative review, with a non-systematic bibliographic review, using the terms described in the keywords of this work for the research. The search was carried out in the MEDLINE/PUBMED database of review articles, in Portuguese and English, from 2015 to 2020. The selection of articles was carried out by a single reviewer and the selected materials were read in full, categorized and analyzed critically. The exclusion criteria were articles with content referring to neutropenia in adults.

A brief review of the most frequent causes on the topic will be described and a flowchart for investigating neutropenia in childhood aimed at the pediatric public will be created.

ETHICAL ISSUES

The project was submitted to Plataforma Brasil no. 41712821.6.0000.5264, in line with current legislation, in compliance with resolution no. 466, of December 12, 2012.

LITERATURE REVIEW:

Neutrophils are leukocyte blood cells originating in the bone marrow and components of the immune system. Together with eosinophils and basophils, they form the class of granulocytes. Considering cellular morphology, the term neutrophil encompasses rods, segmented metamyelocytes and myelocytes. However, in clinical practice, neutropenia is considered based on the sum of absolute rod and segment values.

Neutropenia in the pediatric age group is a topic widely addressed in hematology and pediatrics due to its marked frequency in clinical practice and diversity of causes and clinical manifestations. It can be classified according to its etiology (hereditary or acquired causes), temporality and intensity. Neutropenia is considered in terms of duration as acute or transient and chronic (duration of less than 6 weeks and more than 6 weeks, respectively)[5].Conditions lasting more than 7 days are associated with a worse prognosis. The intensity of reduction in the number of neutrophils classifies neutropenia as severe (<500 neutrophils/μL), moderate (<1000 neutrophils/μL) and mild (<1500 neutrophils/μL). It is worth mentioning that children under one year of age have the limit for defining neutropenia set at <1000 neutrophils/μL.

INFECTIOUS NEUTOPENIA:

Viral infections are the main cause of neutropenia in the pediatric age group, although bacteria, fungi and medication use are also involved in the causes of acute acquired neutropenia, with a generally benign course and resolution within 6 weeks. In general, they are mild to moderate and the course tends to be benign, with spontaneous resolution within six weeks.

In viral cases, they coincide with the period of viremia and usually appear in the first 24 to 48 hours of illness. In most cases, they do not require therapeutic intervention. More severe and long-lasting neutropenias can occur with specific viral infections such as Cytomegalovirus, HIV, Epstein-Barr and Parvovirus B19. As they are the main cause of neutropenia in childhood, it is essential that the pediatrician knows how to identify it and is aware of the benignity of the condition.

MEDICATION-CAUSED NEUTOPENIA:

Neutropenias caused by drugs can range from mild neutropenia to agranulocytosis. The mechanisms of neutropenia are diverse, from direct spinal cord aggression, immune mechanisms and idiosyncratic reaction. Idiosyncratic reactions are more common in men and older patients. The main drugs involved in drug neutropenia are anticonvulsants, antibiotics, antipsychotics and medications used in rheumatological pathologies. Chemotherapy drugs are a class of medications that frequently cause neutropenia and sometimes require intervention with the introduction of empirical antibiotic therapy. Neutropenia secondary to chemotherapy varies depending on each drug. Drug-induced conditions generally resolve within 6 weeks after stopping medication use. However, myelosuppressive drug reactions can last from days to months or years, while immune-mediated reactions typically last 6 to 8 days. During recovery, reactional ("rebound") leukocytosis may occur, accompanied by a left shift in peripheral blood.

CHRONIC NEUTROPENIA:

In children, chronic neutropenias can be caused by different etiologies with variable prognoses. They can be divided into congenital and acquired. Severe congenital neutropenia, cyclic neutropenia, benign congenital neutropenia, refractory childhood cytopenia and autoimmune neutropenia will be addressed.

Severe congenital neutropenia constitutes a heterogeneous group of rare hereditary hematological diseases, characterized by a defect in the maturation of neutrophils and generally presenting with neutrophils below 200 cells per µL. The incidence is approximately two cases per million in the world population.

Of great historical relevance, Kostmann syndrome was described for the first time by Rolf Kostmann in individuals from a Swedish family who died at a very early age.6 These first cases followed an autosomal recessive hereditary pattern. Subsequently, other cases of severe congenital neutropenia with different hereditary patterns were described. Before the advent of genetic studies, this entire class of neutropenias was considered to be Kostmann's and as different genetic alterations were discovered, it was realized that it was not a single pathology, but rather a heterogeneous group of diseases. The prognosis of these patients is grim, with severe and recurrent infections from the first months of life. In approximately 50% of cases, autosomal dominant mutations occur in the ELANE gene, responsible for encoding neutrophil elastase. This enzyme is a protease capable of digesting elastin, present in several microorganisms, leading to their death. In 10 to 20% of cases, autosomal recessive mutations occur in the HAX1 gene, which contributes to the activation of the granulocyte colony-stimulating factor signaling pathway[7]. However, to date 24 genes have been described in this heterogeneous group of diseases [8].

Congenital neutropenias may predispose to myelodysplastic syndrome and acute myeloid leukemia. The gold standard for diagnosis is evidence of genetic abnormality, although genetic tests are not easily accessible.9 Diagnosis can be made based on a combination of the clinical picture, the intensity of neutropenia in peripheral blood and bone marrow assessment. It is possible to demonstrate eosinophilia and monocytosis in peripheral blood, in addition to neutropenia. Although uncommon, an association with anemia and thrombocytopenia has been described in the literature. Bone marrow examination characteristically shows a myeloid "maturation arrest" at the myelocyte stage of development, with normal to increased numbers of promyelocytes, but few more mature forms. Promyelocytes may show dysplastic morphology, including large size, atypical nuclei, and vacuolated cytoplasm. Marrow eosinophilia and monocytosis are common and do not change during treatment. Cellularity is generally normal or slightly decreased. Megakaryocytes are normal in number and morphology.

The hypothesis of congenital neutropenia in neutropenic babies with severe and recurrent infections should always be considered. Due to the severity of the condition, it is important to use granulocyte colony-stimulating factor prophylactically to improve the quality of life of these patients. Before the advent of this therapy in the 1980s, the majority of these patients died within the first two years of life. More than 95% of patients respond to treatment with granulocytic colony-stimulating factor, with an increase in neutrophil counts to more than 1000 per µL, with a documented reduction in the number of episodes and severity of infections [10].Long-term therapy, however, it can occur with a mutation in the CFS3R receptor gene and lead to the occurrence of myelodysplasia and genetic changes, especially monosomy 7. As a consequence, progression to acute myeloid leukemia is possible. During the course of the disease, it is recommended that annual

bone marrow assessments be carried out, due to the risk of progression to myelodysplastic syndrome and acute myeloid leukemia. The only curative treatment is hematopoietic stem cell transplantation, which must be performed early if the patient presents cytogenetic changes or does not respond to high doses of granulocytic colony-stimulating factor.

Benign chronic neutropenia in childhood is generally idiopathic, and patients are asymptomatic, with a mild to moderate degree of neutropenia, without serious infections. In general, it is just a finding on a blood count performed for another reason and should be a diagnosis of exclusion. It has a good prognosis, although spontaneous resolution does not occur. Within this subtype of neutropenia, there is ethnic benign neutropenia, associated with individuals of African, Arab or Jewish descent[11].Treatment is not necessary for these patients as the chance of infections is the same as in the pediatric population in general. In addition to neutropenia, they may also present an absence of Duffy erythrocyte antigen, which corroborates the diagnosis of ethnic neutropenia. The mechanism for neutropenia and leukopenia in patients with negative Duffy antigen/null Duffy antigen receptor for chemokines (DARC) was not clear until recently. Currently, studies have demonstrated that DARC-null hematopoietic cells will differentiate into DARC-null myeloid progenitors and subsequently into DARC-null mature activated neutrophils. The latter in the circulation, together with the normal expression of DARC in endothelial cells, will lead to its exit to the spleen, causing relative neutropenia. In addition to neutropenia, Duff-negative patients also have protection against malaria, as DARC is a receptor for plasmodium vivax and plasmodium knowlesi[12].

Cyclic neutropenia is a hematological disorder in which the absolute count of neutrophils in peripheral blood fluctuates, varying in cycles of approximately 21 days, reaching up to 6 weeks. Severe neutropenia occurs, lasting at least 3 to 5 days with values below 200 cells/µL. The incidence is 0.5 to 1 case per million inhabitants. The majority of these patients have mutations in the ELANE gene, responsible for the production of neutrophil elastase, and the genetic diagnosis can be confirmed by sequencing the ELANE gene. These patients do not usually suffer from high morbidity, having a low risk of progression to myelodysplastic syndrome and acute myeloid leukemia. Bone marrow aspirates show highly variable morphology depending on the phase of the cycle. Myeloid series may show hypoplasia with "maturation arrest" at the myelocyte stage during the declining phase of the neutrophil swing, but normal or hyperplastic myelopoiesis may be evidenced during neutrophil recovery.

The diagnosis of cyclic neutropenia is made with serial blood counts to detect cycles. Most authors suggest 2 to 3 weekly blood counts for 6 weeks to 8 weeks.2 When the use of granulocyte colony-stimulating factor is required, low doses are sufficient to increase the absolute neutrophil count in peripheral blood to >1,000 neutrophils/µL. Despite this, when the neutrophil count is less than 500 neutrophils/µL for more than seven days, oral ulcers, skin abscesses and upper respiratory tract infections are common. Treatment with granulocyte colony-stimulating factor does not nullify the cycle, but increases the absolute neutrophil count and shortens the period of neutropenia, in addition to preventing serious infections and should be prescribed for patients with <500 neutrophils/µL. In cyclic neutropenia, the use of granulocytic colony-stimulating factor does not predispose to the development of myelodysplastic syndrome and acute myeloid leukemia. Many patients who were symptomatic in childhood show clinical improvement as they age.

The pathophysiology of cyclic neutropenia is still uncertain, but there are two main theories: ELANE mutations would cause an unfolded protein response in the endoplasmic reticulum, leading to the activation of its proteins and consequently to endoplasmic reticulum stress. This stress would cause a temporary inhibition of the proliferation/differentiation of hematopoietic cells and neutrophil progenitor cells in the bone marrow.13 The second theory, proposed by TIDWELL et al., suggests that the ELANE mutation would result in the production of full-length proteins and levels reduced amino-terminal truncated isoforms. This mutated protein would be associated with the unfolded protein of the endoplasmic reticulum and consequently, with its stress leading to neutropenia.

Cyclic neutropenia cycling occurs because at the lower end of neutropenia, high levels of misfolded protein and reactive oxygen species lead to damage of progenitor cells, but already differentiated precursor cells can still generate enough neutrophils to increase the neutrophil count. Interestingly, why cyclic neutropenia is considered a milder disease than congenital neutropenia despite both being caused by the same mutation is based on the hypothesis that the inhibition of proliferation and differentiation of hematopoietic and progenitor cells induced by unfolded proteins and reactive species of oxygen in cyclic neutropenia is not complete when compared to congenital neutropenia. Some cells escape damage and respond to granulocytic colony-stimulating factor more effectively[13].

NEUTOPENIA FROM AUTOIMMUNE CAUSE:

Among the chronic acquired causes, the most common is primary autoimmune neutropenia in childhood, with an incidence of 1 in every 100,000 children per year. There is production of antibodies against neutrophil antigens. Autoimmunity can occur as an isolated process, as a manifestation of other autoimmune diseases, or as a secondary complication of infection, drugs, or malignancy. There is peripheral destruction of neutrophils linked to antibodies, but these can also directly interfere with myelopoiesis. The peak incidence occurs in infants and young children, with an average age of diagnosis between 8 and 11 months. Serious infections are uncommon in childhood

autoimmune neutropenia, generally restricted to mild skin or respiratory infections, and the degree of neutropenia can vary from mild to agranulocytosis. In general, they have an excellent prognosis and have a self-limited course, with no treatment required. Remission occurs in 6 to 54 months. Clinical characteristics and absolute neutrophil counts were described in a large cohort of 157 patients. The median neutrophil count was 0.45x 10^9/L and the age at diagnosis was 1.06 years, with a median time to resolution of 2[14]. years. Serious infections occurred in only 9.6% of cases and recovery from neutropenia occurred in approximately 89.9% of patients within 5 years of diagnosis[14].

Associated monocytosis can be seen in the peripheral blood and in the bone marrow, the cellularity is normal to increased, with myeloid hyperplasia. The measurement of antineutrophil antibodies is not necessary for the diagnosis, as part of the healthy population may only have them as a finding and without repercussions. Furthermore, it is possible that patients with autoimmune neutropenia do not present antibodies in laboratory tests. Most tests have high rates of false negative and false positive results. Glucocorticoid stimulation can be performed to clarify the diagnosis. This test demonstrates the mobilization of the reserve pool of neutrophils in the bone marrow. Neutrophils in peripheral blood are measured and then the child ingests a single dose of 1 to 2 mg/kg of predsnisone. After 4 to 6 hours of medication, absolute neutrophils in peripheral blood are measured again. In autoimmune neutropenia, an increase of 2-fold or more in the initial absolute neutrophil count in peripheral blood is expected. It is important to emphasize that children with atypical autoimmune neutropenia, including late onset, slow recovery or involvement of a lineage other than neutrophils, should be investigated thoroughly. These deserve immunological evaluation, with immunoglobulin dosage and evaluation of double negative T lymphocytes, thinking mainly about the diagnosis of autoimmune lymphoproliferative syndrome (ALPS).14

OTHER CAUSES OF NEUTROPENIA:

Refractory childhood cytopenia is also an important diagnosis to be considered during the diagnostic elucidation of a neutropenia condition. It is a rare group of clonal disorders of hematopoietic stem cells characterized by varying degrees of cytopenias, ineffective hematopoiesis and dysplasias, with a risk of leukemic transformation. It is the main form of presentation of childhood myelodysplastic syndromes and constitutes half of the cases. Bone marrow evaluation with biopsy is necessary, showing <5% blasts in the bone marrow.

In nutritional deficiency of vitamin B12, folic acid, copper or even severe protein malnutrition can lead to ineffective granulopoiesis and neutropenia. The onset is slow, often with suggestive clinical signs, and it is common to present with pancytopenia. Correction of the underlying cause leads to resolution of neutropenia.

NEUTROPENIA INVESTIGATION:

To begin the investigation, the first step is to repeat the blood count to confirm neutropenia. With confirmation, it is important to assess its intensity, whether there are other associated changes and cytopenias and in which series they occur. Repeated blood counts often show an improvement, meaning that referral to a hematologist is not necessary initially.

Children and adolescents with moderate to severe neutropenia or more than one cytopenia should be referred directly to a hematologist for investigation. Patients with mild neutropenia can be initially investigated by the general pediatrician (figure 1).

Initially, a detailed anamnesis and physical examination must be carried out, with the purpose of guiding the diagnostic investigation.

Anamnesis:

– Family members with neutropenia or hematological diseases?

– Did the child already present neutropenia in previous exams?

– History of serious infections or repeat infections? Hospitalization due to infection?

– Use of medications that lead to neutropenia?

– Previous diagnosis of any pathology that leads to neutropenia?

– Recent viral infection?

– Isolated neutropenia?

- Fever?

– Jewish, Arab or Afro-descendant?

Physical exam:

– Weight and height

– Bleeding

– Perianal fissures and abscesses

– Hepatomegaly

– Splenomegaly

– Mucositis/gingivitis

– Dental infections

– Respiratory tract infections

– Changes in the skin, nails, skeleton compatible with syndromic diagnoses

After the physical examination and anamnesis, it is necessary to continue with the laboratory investigation. This will be guided by the main clinical suspicions:

Infection →Serologies for toxoplasmosis, HIV, HTLV, cytomegalovirus, hepatitis, herpes virus, Epstein Barr and parvovirus B19;

Immunodeficiencies → Immunoglobulins

Hypovitaminosis → Folic acid and vitamin B12

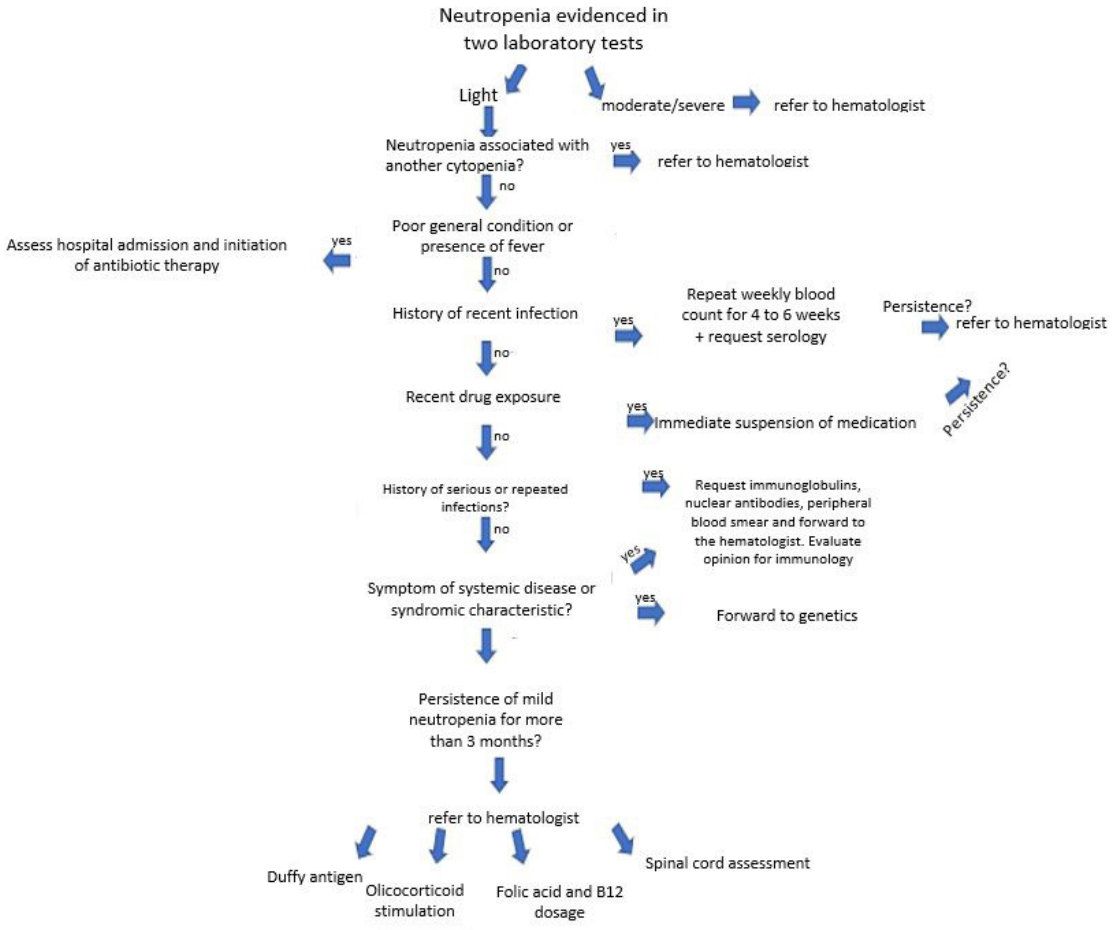


Figure 1. Neutropenia flowchart.

Hematological → Peripheral blood slide and serial blood counts

Rheumatological diseases → Antinuclear antibodies

Malformations/syndromic characteristics → Geneticist assessment

Flow chart guiding investigation and follow-up in patients with neutropenia in pediatric outpatients

CONCLUSION

The following work discussed neutropenia, its causes, clinical presentations and management. The outcome of this work was the development of a neutropenia investigation flowchart for pediatricians. It is extremely important for pediatricians to have in-depth knowledge about neutropenia due to the wide variety of causes and wide possibility of clinical manifestations. Furthermore, with this work we thought about optimizing resources and time, thus avoiding unnecessary referrals and delays in the specialist's investigation in more serious cases.

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