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How we diagnose neutropenia in the adult and elderly patient

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Neutropenias (NP) comprise a wide spectrum of disorders with varying clinical significance. At one end of the spectrum are the severely neutropenic patients, where the NP, being either acute (such as the unexpected, idiosyncratic drug-induced agranulocytosis or the chemotherapy-induced agranulocytosis) or chronic (e.g. the severe congenital NP), represent serious conditions that, if not appropriately treated, lead to considerable mortality, primarily due to bacterial and fungal infections. Experience from chemotherapy-induced NP over the last 50 years has been valuable in order to develop strategies for severely neutropenic patients. At the opposite end of the spectrum are patients with mild NP, often detected during evaluation for other conditions. These subjects are rarely prone to infections, yet they might suffer from other disorders where NP might be a key part of another underlying disease. The HIV-associated NP is an example in this category. The levels of NP and the risk for infections are given in Table 1.

Against this background, most NP patients are in need of evaluation in order to determine the causes, risks and prognosis. Since non-chemotherapy-induced NP is a relatively rare condition, many hematologists may need support in approaching the NP patient workup. The Scientific Working Group on Granulocyte and Monocyte Disorders of the EHA has promoted science and education in this area since 2004. The approach to chemotherapy-induced NP is discussed elsewhere.[1]

Reviews on the diagnosis and treatment of the neutropenic child have been published recently.[2,3] However, the spectrum of diseases causing NP is different in children compared to adults, mainly because congenital disorders predominate in pediatric clinical experience, whereas other hematologic disorders, autoimmune and chronic viral diseases, and drug-induced agranulocytosis constitute the majority of cases in adults.

This review will focus on how we diagnose and treat acute and chronic NP in the adult patient, particularly in the elderly, through discussing one case.

The case

A 68-year old Swedish female, with a history of hypertension and lower back pain since approximately ten years, presented with a sore throat, fever (40°C) and chills since four days. She was clearly ill; blood pressure was 100/70 mm Hg. Physical examina-

Table 1. Stratification of neutropenia by severity and clinical context.

Neutropenia stratification	ANC (x10^9/L)	Clinical context	Risk of infection
Mild	1.0-1.5	General good health	Usually none
		Associated disease, debilitated, malnourished.	Minimal to severe*
Moderate	0.5-1.0	General good health	Usually minimal
		Associated disease, debilitated, malnourished.	Moderate to severe*
Severe	< 0.5	*All clinical settings*	Moderate to severe

**Often because of co-existent acquired immunodeficiency.*

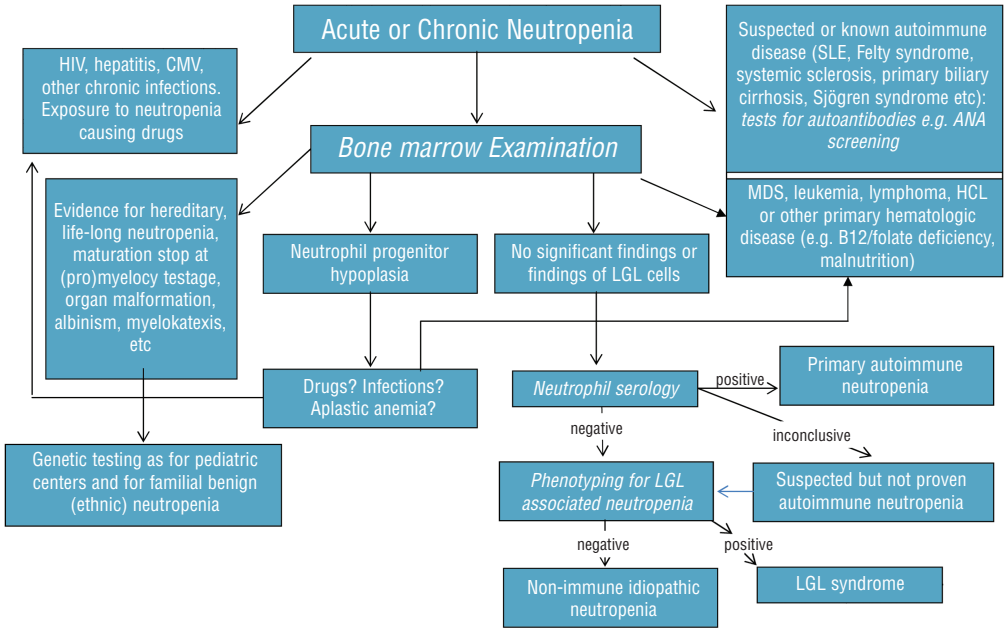

Figure 1. Algorithm for the investigation of a case of neutropenia in an adult patient. CMV: cytomegalovirus; ANC: absolute neutrophil count; SLE: systemic lupus erythematosus; ANA: antinuclear antibodies; MDS: myelodysplastic syndrome; LGL: large granular lymphocytes.

tion of skin, lungs, and abdomen was normal but she had severe tonsillitis. C-reactive protein (CRP) was 226 mg/L, blood leukocyte count 1.2x10⁹/L, hemoglobin 120 g/L and platelet count 140x10⁹/L. Absolute neutrophil count (ANC) was 0.2x10⁹/L.

How to approach the diagnosis

Our suggested workup for a patient with NP of any grade is shown in Figure 1. The first specific action (apart from standard treatments for severe infections) is discontinuation of all drugs not necessary for maintenance of vital functions. The reason for this is that drug-induced agranulocytosis may be fatal with continued exposure to a drug and that any acute severe NP in the adult/elderly should be regarded as drug-induced until proven otherwise. This general approach might also be appropriate in mild/moderate NP, although there is less urgency for action.

Discussion with the patient usually reveals intake of drugs that might cause NP. Any drug can cause mild-to-severe NP but some are incriminated more than others, e.g. trimethoprim sulphametoxazole (usually mild NP), anti-thyroids (sometimes causing agranulocytosis), etc.[4-6] Thus, antipsychotic drugs (such as clozapine) and an iron-chelating drug (deferiprone) are often used in young patients, whereas anti-thyroids are used in the young and middle-aged. Antibiotic-induced NPs can be found at any age. Elderly patients are often exposed to combinations of drugs, complicating identification of the drug causing the NP.

Likewise, patient history will disclose known or latent autoimmune disease. Apart from Felty syndrome, where NP can be severe and associated with infections, most autoimmune diseases display mild to moderate NP. Infection proneness is usually attributed to malfunctioning of other host defense systems (e.g. TNF inhibitors enhancing risk for tuberculosis). Findings such as the detection of antinuclear antibodies and an increase in polyclonal gammaglobulins support the diagnosis of an autoimmune disorder.

Similarly, chronic or acute viral infections (e.g. hepatitis, HIV, cytomegalovirus (CMV) or influenza, measles) might be associated with mild/moderate NP. Parvovirus-associated anemia and NP are typical childhood disorders rarely seen in adulthood.

Our patient showed no signs of autoimmune or chronic viral disorders. She denied intake of drugs other than a thiazide for hypertension and paracetamol for the back pain. Her ANC had been normal two years previously. There was no family history of NP.

Presentation of the mild-moderate familial benign (ethnic) NP, frequent in African populations, and the severe chronic NP (due to mutations in *ELANE*, *HAX1* and other genes) is very unusual after adolescence. Moreover, in severe chronic NP, infection load is normally evident from the first month of life. Thus, most of the evidence would point to other explanations for the patient's NP.

At this time point (Figure 1), a bone marrow examination (BME) is needed in order to exclude hematologic malignancies, aplastic anemia (the diagnosis of which requires demonstration of reduced total hematopoietic cellularity on bone marrow trephine biopsy), the so-called gelatinous metamorphosis that accompanies severe malnutrition, solid cancer metastatic disease to the bone marrow (BM), etc., and as a step in the decision-making process for specific treatment of severe NP (see below). This is in contrast to recommendations for pediatric NP where hematologic malignancies as cause of NP are rare and BME is not recommended as part of the initial evaluation.

However, the value of BME to discriminate the different types of NP remains rather limited. A paucity of myeloid progenitors and mature neutrophils in the BM is usually taken as a sign of poor myeloid proliferation, whereas normal numbers of progenitors and mature neutrophils is taken as evidence of destruction of the peripheral blood/tissues,

such as in autoimmune NP. This distinction, never scientifically validated, seems to be questionable based on the recent reports that senescent neutrophils return to the BM for final destruction.[7] Maturation stop at the promyelocyte stage is a widely accepted diagnostic criterion for severe chronic NP due to *ELANE*, *HAX1* and some other mutations.[8] However, it is also seen in drug-induced NP, such as the late onset NP that is observed months after treatment with rituximab.[9]

Notwithstanding, paucity of BM myeloid progenitors in a drug-induced NP patient is considered to be a poor prognostic sign, and together with older age, low blood pressure, renal insufficiency and other findings, is a recommendation for granulocyte-colony stimulating factor (G-CSF) and other intensive care treatments.[6]

The BME of our patient showed a low number of myeloid progenitors and few mature neutrophils, but no typical maturation arrest. According to the algorithm in Figure 1, she was next evaluated for autoimmune NP by analysis of anti-neutrophil antibodies with validated technology.[10] She was negative for specific autoantibodies as well as factors causing neutrophil aggregation. Next, the peripheral blood and BM were reanalyzed for presence of large granular lymphocytes (LGL), but these were negative.

The diagnosis of autoimmune NP is still a challenge. Direct antibody testing (detection of antibodies attached to the surface of the neutrophils) has a very low specificity that remarkably raises the rate of false-positive results. In the past, indirect testing (detection in the serum of antibodies to neutrophil antigens) when focused on search of antibodies to defined epitopes on neutrophil antigens has been hampered by technical problems. These autoantibodies, when detected with validated techniques,[10] are usually associated with primary autoimmune NP, a classical childhood disease sometimes found in young adults but very rare in the elderly patient. Other indirect tests, such as neutrophil agglutination and immunofluorescence, do not detect autoantibodies directed to defined epitopes, but might still reliably indicate autoimmune NP since the low sensitivity (i.e. a high number of false-negative results) of this method is in part overcome by repeated assessments.[11] When combined with other signs of autoimmunity, positive agglutination and immunofluorescence tests probably point to secondary autoimmune NP. As stated above, this topic area needs further clarification.

Apart from antibody-mediated neutrophil destruction, the workup has to consider cell-mediated reactions. Like immune thrombocytopenic purpura, where T-lymphocyte-mediated platelet destruction accounts for thrombocytopenia in a minority of the patients, large granular lymphocyte (LGL)-associated NP is a model for understanding what had previously been undefined NP. The LGL disorders span from polyclonal (reactive) to monoclonal (leukemic) conditions[12] and are mostly seen in the adult/elderly patient, often with other autoimmune disorders. The mechanism by which LGL cause NP is still poorly understood.

The algorithm recommends evaluation for idiopathic NP at this stage.

This term is used to define any unexplained reduction in the ANC below the lower reference values. Its frequency has been estimated in 34% among patients with incidental NP and is clinically characterized by occurrence in middle-aged women, a stable condition, and the usually benign and uncomplicated course with rare evolution to acute myeloid leukemia or myelodysplastic syndromes (MDS).[13] Although the etiology of the disease remains unknown, activated oligoclonal/monoclonal T lymphocytes with myelosuppressive properties were shown to have a pathogenic role.[14]

The diagnosis of the disorder is based mainly on exclusion criteria, namely the absence of clinical and laboratory evidence of any underlying disease, the absence of history of exposure to irradiation, and a normal BM karyotype.[15]

It is important to distinguish idiopathic NP of adults from MDS presenting with isolated NP. In MDS, a thorough examination of peripheral blood and BM smear will typically reveal distinct dysplastic features such as agranular or hypogranular or Pseudo-Hüet neutrophils in association with left-shifted myeloid series showing defective granule formation and/or reticulated nuclei with prominent Golgi apparatus of promyelocytes. In addition, the BM karyotype may reveal typical cytogenetic aberrations. The term "idiopathic cytopenia of undetermined significance" (ICUS) has been proposed by MDS study groups for patients presenting only with mild cytopenia and subtle dysplasia who do not fulfill the minimal diagnostic criteria for MDS but who may transform to MDS.[16] In our experience, an easily performed 4-color flow-cytometric analysis of the BM myeloid cells, based on the European LeukemiaNet recommendations, may become the most sensitive diagnostic tool for the identification of the pure idiopathic NP from pre-MDS (ICUS) and MDS cases presenting with NP only.[17] Variables to analyze are the proportion of the immature myeloid progenitor cells, the side scatter properties of the maturing neutrophils (as an indication of cell granularity), the expression of maturation markers (namely CD13/CD11b/CD16), the relationship between CD15 and CD10, and the presence of lineage infidelity markers such as CD56.

Overall, idiopathic NP is a distinct disease entity with T-cell mediated pathophysiology and a benign clinical course that should be distinguished from cases of pre-MDS and MDS.

So, what was the final diagnosis? At the end of the day, she recalled that she had bought a painkiller over the counter when on vacation in Southern Europe. Showing a box containing metamizole, she confirmed she had ingested a dozen of the tablets 1-2 weeks before admission to the hospital, believing that an over-the-counter painkiller could not be associated with serious side-effects. Metamizole has been withdrawn from the Swedish and other markets (but not all) because of the risk for agranulocytosis! The final diagnosis was, hence, a drug-induced NP.

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Age and aging in blood disorders: multiple myeloma

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Do new standards of care incorporating immunomodulatory agents and proteasome inhibitors benefit all elderly patients with multiple myeloma?

Multiple myeloma (MM) accounts for 1% of all types of cancer and for 2% of all cancer deaths. These numbers are approximately 13% for all hematologic malignancies and 20% for hematologic malignancy-related deaths.[1,2] MM is a disease of the elderly reflected by a median age at diagnosis of approximately 70 years, with 35-40% of patients being older than 75 years.[3]

The introduction of the immunomodulatory agents (IMiDs; thalidomide, lenalidomide and pomalidomide) and the proteasome inhibitors (PIs; bortezomib and carfilzomib) has not only greatly improved the prognosis of younger patients with MM, also in elderly MM patients aged 65 years or over. The addition of bortezomib or thalidomide to melphalan and prednisone (MP) improved overall survival (OS) by 13.3 and 6.6 months, respectively.[4,5] Although the addition of lenalidomide to melphalan and prednisone did not improve OS, progression-free survival (PFS) significantly improved by 18 months, provided that maintenance therapy was given.[6] However, when considering the outcome as described in population-based registries, reflecting real-life situations, the elderly patients appear to benefit less. Recently, in the Italian and Dutch population-based registry (PBR), the overall survival of very old patients (≥75 years of age) was found to be similar over time, without any improvement in OS after the introduction of novel agents in 2006[2] (SG Verelst, personal communication, 2014). This lack of impact does not seem to be explained by a biologically different, more aggressive disease in the elderly. Although differences in cytogenetic abnormalities have been observed between younger and older patients,[7] there is currently no evidence for a higher incidence of biologically high-risk disease in the elderly. Moreover, the French Intergroupe Francophone du Myélome (IFM) showed that the incidence of t(4;14) was even significantly less in patients aged over 75 years (8.3%) and aged 66-74 years (10.9%) *versus* those aged 65 years or under (14.3%). The incidence of del17p was similar (6.1% in patients aged over 75 years, 5.9% in patients aged 66-74 years and 6% in patients aged 65 years or under). Data on del1p and ampl1q were not available.[8] Finally, no increase according to age was found in the percentage of prognostic adverse hypermethylation of the tumor modulating genes *GPX3*, *RBP1*, *SPARC*, and *TGFBI*.[9]