Primary Immune Deficiency

AN OVERVIEW OF NEUTROPHIL DISORDERS AND THEIR DIAGNOSIS

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ABSTRACT

Neutrophil disorders are commonly encountered in clinical practice. However, these encompass a variety of different conditions with variable presentation, making neutrophil disorders a diagnostic challenge to most clinicians. These disorders can be better understood when approached by distinguishing between disorders affecting mainly neutrophil numbers or function and by identifying which normal neutrophil processes are affected. The choice of assays for investigation of neutrophil disorders depends on a variety of factors, for example family history, clinical and laboratory findings.[1–3] This article aims to provide an overview of the clinical presentation and recommended diagnostic tests for the most important neutrophil disorders.

INTRODUCTION

Neutrophil disorders may be asymptomatic or present as recurrent infections – often of the skin – deep organs and respiratory tract. These disorders are usually associated with suggestive signs, including delayed umbilical cord separation, periodontal disease, abscesses and oral ulcers. The infections associated with neutrophil disorders are due mainly to organisms that are destroyed by phagocytes and include certain sentinel organisms that should prompt investigation for underlying neutrophil defects, among others recurrent *Staphylococcus aureus* infections, *Mycobacterium tuberculosis, Salmonella spp, Burkholderia cepacia, Serratia marcesens, Listeria monocytogenes, Nocardia spp, Actinomyces spp* and fungi (especially *Aspergillus spp*). These patients have a normal resistance to viral infections.[1,2,4]

Essentially, neutrophil disorders can be divided in two subgroups: defects in neutrophil numbers and defects in neutrophil function. The first can be identified by the ubiquitously available full blood count (FBC). The second group can be identified by phenotypic or functional neutrophil assays and, more recently, by genetic testing.

1. DEFECTS IN NEUTROPHIL NUMBERS (NEUTROPAENIA)

Neutropaenia can be defined as a neutrophil count less than 1.5 × 10^9/L in adults and children and less than 2.5 × 10^9/L in neonates.[5] Patients with neutropaenia may be asymptomatic, but may also present with recurrent infections or severe and catastrophic sepsis. The main causes of neutropaenia are summarised in Table I:

TABLE I: CAUSES OF NEUTROPAENIA[6]

Congenital	Constitutional neutropaenia/ethnic neutropaenia Benign familial neutropaenia Cyclic neutropaenia Severe congenital neutropaenia (SCN) As part of an inherited bone-marrow failure syndrome (e.g. Shwachman-Diamond)
Acquired	Infection-associated Drug-induced Autoimmune Malignancy: • T-cell large granular lymphocytic leukaemia (T-LGL) • Myelodysplastic syndromes/neoplasm (MDS) • Acute leukaemia Dietary

Neutropaenia can be further classified as congenital or acquired. The most relevant causes of congenital and acquired neutropaenia respectively are addressed below:

1.1. Congenital neutropaenia

1.1.1. *Constitutional or ethnic neutropaenia* - This is characterised by mild, chronic neutropaenia, usually with an absolute neutrophil count (ANC) >1.0 × 10^9/L in a patient with no history of recurrent infections. Constitutional neutropaenias are more common in

patients of certain ethnic backgrounds, particularly those of Mediterranean and African descent. Neutropaenia in populations of African origin is linked to polymorphisms in the Duffy Antigen Receptor Complex (DARC) gene; however, the mechanism by which the Duffy-negative phenotype is linked to neutropaenia is unknown.[6] Once diagnosed no further workup is indicated.

1.1.2. *Benign familial neutropaenia* - This form of neutropaenia is phenotypically similar to constitutional neutropaenia but, although clearly hereditary, it is not linked to a particular ethnic group. The genetic basis is unknown.[6]

1.1.3. *Cyclic neutropaenia* - Patients with cyclic neutropaenia commonly have regular oscillations of peripheral blood neutrophil counts with periods of severe neutropaenia occurring every 21 days and lasting for 4–6 days. During these periods of profound neutropaenia, the patients are predisposed to developing painful mouth ulcers, fevers and bacterial infections. Children are particularly at risk of developing severe consequences of profound neutropaenia, including life-threatening complications of bacterial infections, for example gangrene, bacteraemia and septic shock. The diagnosis of cyclic neutropaenia can be established by serial differential white counts 2–3 times per week for a minimum of six weeks. It is imperative to observe at least two neutrophil nadirs.[7]

Cyclic neutropaenia is a rare, autosomal-dominant disorder arising from mutations in the gene for neutrophil elastase (ELANE or ELA-2), observed in 80 per cent of affected subjects. Genetic testing for ELANE mutations is available in South Africa. Patients with cyclic neutropaenia share mutations in the ELANE gene with patients with severe congenital neutropaenia (SCN); however, unlike patients with SCN, patients with cyclic neutropaenia do not have an increased risk of leukaemia or myelodysplasia.

1.1.4. *Severe congenital neutropaenia (SCN)* - SCN is characterised by ANCs consistently below 0.2 × 10^9/L with recurrent, severe infections, often developing in the first months of life. Patients with SCN may suffer from chronic gingivitis, oral ulcers, skin abscesses, recurrent pneumonia or septicaemia.

Bone marrow (BM) examination characteristically shows a myeloid 'maturation arrest' at the promyelocyte–myelocyte stage of development. The apparent maturation arrest helps to differentiate SCN from idiopathic and immune neutropaenia.[7]

SCN can either be inherited as an autosomal-dominant disorder or an autosomal-recessive disorder, also known as 'Kostmann's syndrome'. An ELANE gene mutation is seen in approximately 40–60% of patients with SCN. This mutation is associated with the most severe infectious complications. The cumulative incidence of leukaemia among patients with SCN has ranged from 10–20% and equally affected those with and without ELANE mutations after 15 years of treatment.[8]

1.2. Acquired neutropaenia

1.2.1. *Infection-related neutropaenia* - Post-infectious neutropaenia is most commonly seen in children after viral infections. Almost any viral infection can be implicated, although neutropaenia is most commonly seen after varicella-zoster, measles, rubella, influenza, hepatitis, Epstein-Barr virus or HIV infection. Most post-infectious neutropaenias are self-limiting, although a prolonged neutropaenia may develop after Epstein-Barr virus or HIV infection. Bacterial infections are a rarer cause of significant neutropaenia, with notable exceptions including Brucellosis, rickettsial- and mycobacterial infections.[6] In addition, any cause of severe sepsis can result in neutropaenia; this is most commonly seen in infants and the elderly. This type of neutropaenia is thought to result from exhaustion of BM granulocyte reserves.[9]

1.2.2. *Drug-induced neutropaenia* - Drug-induced neutropaenia can lead to a severe neutropaenia with an ANC <0.5 × 10^9/L. The incidence of this condition increases with age, is associated with a high rate of infectious complications and has a mortality rate ranging from 2.5–10%. The highest mortality rate is observed in older patients and in those with concomitant renal failure, bacteraemia or shock.

Almost all classes of drugs have been implicated. The most common drugs associated with severe neutropaenia are anti-thyroid medications, ticlopidine, clozapine, sulfasalazine, trimethoprim-sulfamethoxazole and dipyrone. The CD20 monoclonal antibody (rituximab) may also cause late-onset neutropaenia. A summary of drugs implicated in neutropaenia and the frequency of reactions is summarised in Table II.

The pathogenesis of drug-induced neutropaenia is heterogeneous and is not completely understood. In some cases, neutropaenia occurs after prolonged exposure to drugs, resulting in decreased myeloid production from a hypoplastic BM. Other cases occur after repeated but intermittent exposure to offending agents. This suggests an immune mechanism and in some cases anti-neutrophil antibodies are implicated.[6]

TABLE II: DRUGS ASSOCIATED WITH NEUTROPAENIA[6]

CLASS	DRUG	FREQUENCY REPORTED
Antibiotics	Vancomycin TMP-SMX Chloramphenicol Dapsone Cephalosporins Amoxicillin, ampicillin Macrolides	High High High High Intermediate Intermediate Low
Anti-inflammatory agents	Sulfasalazine Diclofenac Ibuprofen	High Intermediate Low
Antithyroid drugs	Methimazole Propylthioracil	High Intermediate
Psychotropic agents	Clozapine Phenothiazines (e.g. Chlorpromazine) Tricyclic agents (amitriptyline)	High Intermediate Low
Antiepileptics	Carbamazepine Phenytoin Valproate Ethosuximide	Low Intermediate Low Low
Cardiovascular drugs	Anti-arrhythmic agents, for example Procainamide Ticlopodine ACE-inhibitors Digoxin Propranolol	High High Intermediate Intermediate Low
Diuretics	Thiazides Furosemide Spironolactone	Low Low Low
Anti-malarials	Quinine Chloroquine	Intermediate Low
Biologics	Rituximab	Intermediate

1.2.3. *Autoimmune neutropaenia* - Autoimmune neutropaenia is caused by autoantibodies directed at specific neutrophil antigens. Detection of neutrophil antibodies can be routinely performed by flow-cytometry. Autoimmune neutropaenia can be grouped into autoimmune neutropaenias occurring in babies which can be alloimmune or primary, or autoimmune neutropaenias occurring in later childhood and adulthood that can be idiopathic or secondary.

1.2.3.1. Neonatal alloimmune neutropaenia - Neonatal alloimmune neutropaenia is due to the transplacental passage of IgG antibodies to neutrophil-specific antigens inherited from the father that can cause a significant neutropaenia. These babies can be asymptomatic, present with a delayed umbilical cord separation or with severe infections and even septicaemia. These antibodies usually disappear after 12–24 weeks. Occasionally, this is associated with the transfer of other alloimmune antibodies, for example SSA and SSB antibodies associated with neonatal lupus.[10]

1.2.3.2. Primary autoimmune neutropaenia of infancy - Primary autoimmune neutropaenia usually appears between 5 and 15 months, without other signs or symptoms of an underlying autoimmune disorder. This may be associated with other infections (e.g. hepatitis B), collagen vascular disease, primary abnormalities of B-, T- or natural-killer (NK) cells (e.g. autoimmune lymphoproliferative syndrome (ALPS)), immune thrombocytopenia (ITP) and autoimmune hemolytic anaemia. In most cases the cause cannot be identified. Neutropaenia can be moderate to severe and may be complicated by serious infections. The disease usually remits in approximately 95 per cent of cases, mostly at age five.[10]

1.2.3.3. Chronic idiopathic autoimmune neutropaenia - Chronic idiopathic autoimmune neutropaenia tends to occur in later childhood and adulthood with no apparent cause. There is a female preponderance and usually no significant infection history.[11]

1.2.3.4. Secondary autoimmune neutropaenia - Secondary autoimmune neutropaenia is seen primarily in adults and usually occurs within the context of systemic autoimmune disease. Autoimmune neutropaenia is frequently a benign disorder that manifests as mild neutropaenia.[6] However, some patients with Felty syndrome (the triad of rheumatoid arthritis (RA), splenomegaly and neutropaenia) have substantial morbidity from serious bacterial infections.

1.2.4. *Malignancy associated with lymphopenia*

1.2.4.1. Large granular lymphocyte leukaemia (LGL) - associated neutropaenia shares many features with Felty syndrome, including an association with RA, frequent splenomegaly, and a strong association with HLA-DR4.[6] LGL-associated neutropaenia is often severe.

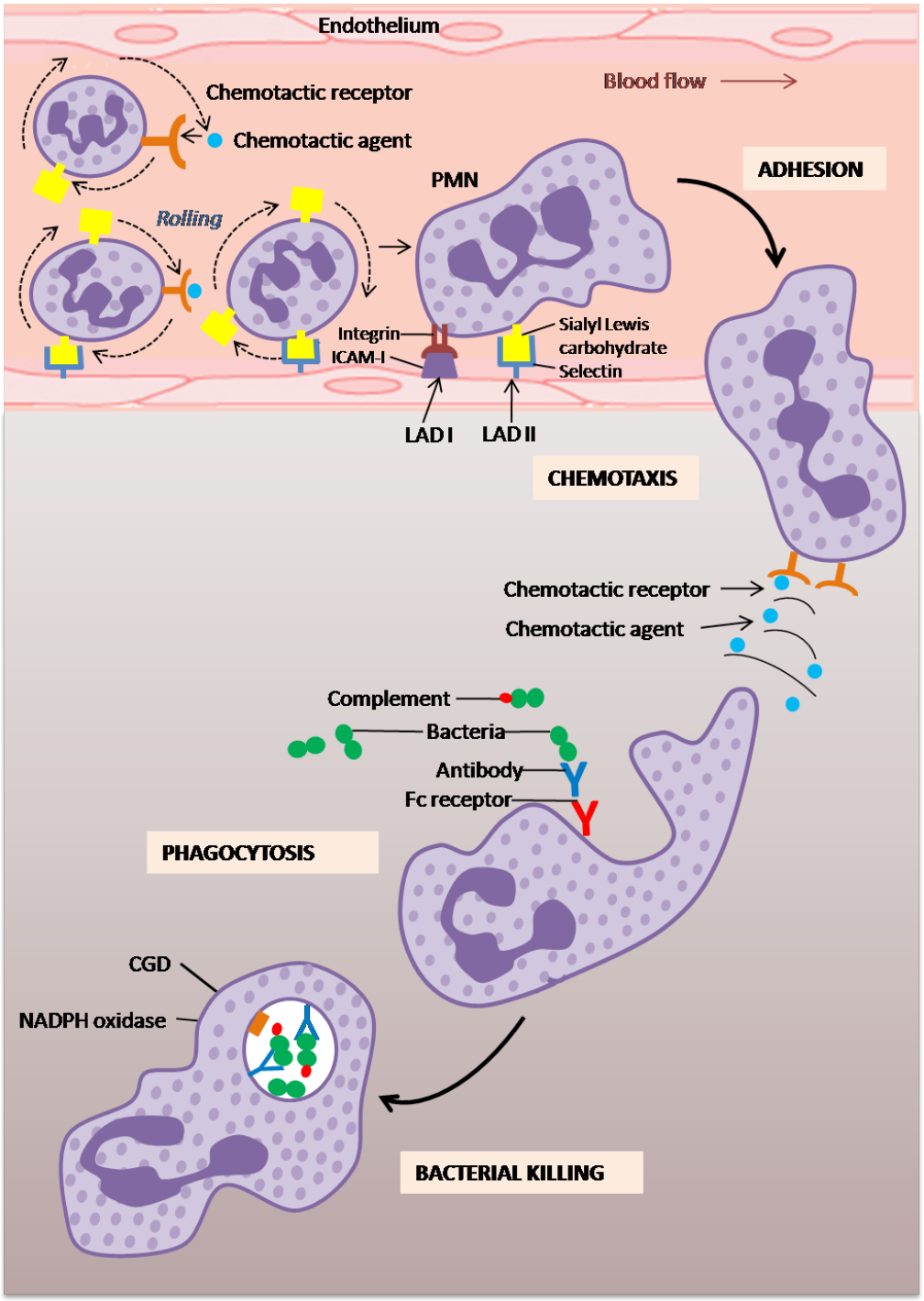
1.2.4.2. Myelodysplastic syndrome (MDS) - MDS is a clonal haematopoietic stem-cell disease characterised by cytopaenia(s), dysplasia in one or more myeloid lineages, ineffective haematopoiesis and increased risk of developing acute myeloid leukaemia.

Cytopaenias in MDS are defined as an ANC $<1.8 \times 10^9$/L, haemoglobin <10 g/dL and platelets $<100 \times 10^9$/L. Most patients with MDS present with anaemia and less commonly with an isolated neutropaenia or thrombocytopaenia. The disease occurs more commonly in older patients, with a median age of 70 years and a male predominance. The aetiology for MDS includes primary and secondary causes (e.g. inherited BM failure syndromes, post-exposure to alkylating agents,

ionising radiation and topoisomerase II inhibitors).

The diagnosis is usually confirmed with a BM examination and appropriate cytogenetic studies. The most common cytogenetic abnormalities observed in association with MDS are deletions of 7q, 5q or 20q and trisomy 8.[12]

1.2.5. *Dietary* - Patients with severe caloric malnutrition (e.g. patients with anorexia nervosa) can be leukopaenic, but this is usually mild. Patients with folate or vitamin B_{12} deficiency can be neutropaenic, but this rarely occurs without concomitant macrocytosis and pancytopenia. Copper deficiency – a less common cause of leukopaenia – has some clinical features that overlap with those of vitamin B_{12} deficiency and is most commonly found in patients who have undergone certain types of gastric bypass surgery.[6]



Adhesion defects
- Leukocyte adhesion defect (LAD) types I, II and III

Chemotactic defects
- LAD I
- Chediak-Higashi syndrome
- Hyper IgE/Job syndrome
- Actin polymerisation defect
- Localised aggressive periodontitis
- Acquired motility defects

Phagocytic defects
- LAD I
- Actin polymerisation defect

Intracellular or bacterial-killing defects
- Chronic granulomatous disease (CGD)
- Chediak-Higashi syndrome (CHS)
- Neutrophil- specific granule deficiency
- Myeloperoxidase deficiency (MPO)
- Neutrophil G6PD deficiency

Figure 1: Normal neutrophil functions and associated defects.[14]

2. NEUTROPHIL FUNCTIONAL DISORDERS

Causes of neutrophil dysfunction can be better understood as defects affecting one or more steps in normal neutrophil processes, including adhesion to vascular endothelium, chemotaxis of neutrophils to the site of infection, phagocytosis of pathogens and intracellular or bacterial killing. These processes, as well as the functional defects associated with them, are described in Figure 1. The most important of these neutrophil functional deficiencies are discussed in more detail below.

2.1. Chronic granulomatous disease (CGD) - CGD is the most significant inherited neutrophil functional defect, but is still a rare primary immunodeficiency. The estimated incidence is 1/250 000 described in Europe, with only a couple of cases reported on the South African Primary Immunodeficiency Disease (PID) registry, making it underdiagnosed.[15,16] CGD is caused by mutations in the gene components encoding the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex (gp91 phox, p22 phox, 47 phox, 67 phox and Rac). This results in reduced production of reactive oxygen species and decreased intra- and extracellular killing of micro-organisms. Ultimately, this leads to increased susceptibility to infections and reduced regulation of pro-inflammatory mediators, with subsequent systemic granulomatous manifestations and autoimmune disorders.[17,18]

Most patients present in infancy and the early toddler years, however, a growing number of patients are diagnosed only in later childhood or adulthood. A narrow yet profound spectrum of organisms and infections should raise suspicion of CGD, although in some cases the initial presentation can be non-specific and mimic other PIDs. CGD should therefore be included in the differential diagnosis of all patients with recurrent infections.[15,17]

The most prevalent clinical manifestation is recurrent and serious infections that can affect many organs. Patients with CGD may develop only a few clinical signs and symptoms, despite the presence of sinificant infection. Bacterial infections often present with fever and a leukocytosis.

One of the most important features of CGD is the relatively narrow spectrum of disease-specific infections.[13] In developing countries, recurrent *S aureus* infections*, M tuberculosis,* severe localised BCG infections and *Salmonella spp* are most frequently reported. *S marcesens, L monocytogenes, Nocardia spp, Actinomyces spp* and fungi (especially *Aspergillus spp*), among a few others, are well described in this disorder.[13,16,17]

Fungal infections may not present in the typical fashion, but may have a more 'indolent' picture with only moderately increased C-reactive protein (CRP) and white-cell count. However, fungal infections may elicit a severe localised inflammatory response. Assays for the detection of 1,3 Galactomannan and β-D glucan (Fungitell) are often not of much help, due to localised disease, but when elevated it is very relevant. The non-fumigatus *Aspergillus spp,* as well as some species of fungi other than *Aspergillus,* are difficult to diagnose, therefore a molecular diagnosis should be pursued.[17,19,20]

Patients with CGD may present with failure to thrive, abnormal wound healing, diarrhoea, infected dermatitis, hepatosplenomegaly, lymphadenopathy, septicaemia or fungaemia. Organ involvement includes the skin, liver, lungs, gastrointestinal and genito-urinary tract, ears, eyes, bones and joints. The lungs are the most affected organ in CGD, with pneumonia the commonest pulmonary infection. In contrast to neutropaenic patients, fungal pneumonias do not generally cause cavitation in CGD, whereas *Nocardia* infections do.[15] Common sites for abscess formation are perianal/perirectal regions and liver. *S aureus* liver abscesses are pathognomonic for CGD.[17] Gastrointestinal inflammatory manifestations of CGD include abdominal pain, diarrhoea, colitis, proctitis, strictures, fistulae and obstruction. Granulomatous or ulcerative colonic lesions may mimic Crohn's disease.[17]. Genitourinary manifestations of CGD are also common and include bladder granulomata and ureteral obstruction. CGD patients may have recurrent gingivitis, stomatitis, aphthous ulcers and gingival hypertrophy. Manifestations of autoimmunity or autoinflammation have been claimed to be more prevalent in CGD patients than in the normal population, with discoid lupus the most common manifestation.[15]

Incidental abnormalities that may be found on routine laboratory tests include hypergammaglobulinaemia, anaemia of chronic disease, elevated ESR and CRP and hypoalbuminaemia.

The diagnosis of CGD is usually made by direct measurement of neutrophil oxidative burst. Measurement of Dihydrorhodamine (DHR) on flow-cytometry is the method of choice, because of its availability, relative ease of use and its ability to detect carrier status.[21] Mothers of boys with X-linked CGD may be identified as carriers, which is important not only for future family planning or investigation of siblings, but also to help identify carriers who may be susceptible to recurrent infections, suffer from discoid skin lesions or other disease manifestations. Molecular diagnosis of CGD has become part of the definitive European Society for Immunodeficiencies (ESID) diagnostic criteria for CGD.[22] Mutations in the CYBB gene, encoding for gp 91 phox, are responsible for X-linked CGD. This is the most common form of the disease and presents with the most severe clinical phenotype. Autosomal reces-

TABLE III: SUMMARY OF NEUTROPHI DISORDERS ND RECOMMENDED DIAGNOSTIC TESTS[1]

DIAGNOSIS	INITIAL TESTING	GENETIC TESTING
DISORDERS OF NEUTROPHIL NUMBER		
Severe congenital neutropaenia (SCN)	• **FBC** and **BM evaluation**	• **ELANE** or **ELA-2** mutation in approximately 40–60% of cases
Cyclic neutropaenia (CN)	• Serial differential **WBC count**, 2–3 times per week for six consecutive weeks.	• **ELANE** or **ELA-2** mutation in approximately 80% of cases
Nutritional	• **Serum vit B12** and **folate** levels • **Serum copper** levels	
Myelodysplastic syndrome (MDS)	• **FBC** and **BM evaluation**	• **Conventional cytogenetics/ karyotyping**: trisomy 8, del 5q, del 7q, del 20q
Shwachman-Diamond syndrome (SDS)	• **FBC** • Exocrine pancreatic function (e.g. reduced levels of **faecal elastase**) • Detection of fatty pancreas by **ultrasound, CT-scan** or **MRI** • **BM evaluation**	• SBDS mutation
DISORDERS OF NEUTROPHIL FUNCTION		
Chronic granulamatous disease (CGD)	• Flow-cytometry for **oxidative burst** using DHR (dihydrohodamine)	• **CYBB, NCF1, CYBA, NCF2, NCF4**
Chediak-Higashi syndrome (CHS)	• **FBC** and **BM evaluation**	• **LYST**mutation in approximately 80%
Leukocyte adhesion deficiency-I (LAD-I)	• **FBC**, flow-cytometry for deficiency of **CD11** a, **b, c/CD18**	• **ITGB2** mutation in over 90%
Leukocyte adhesion deficiency-II (LAD-II)	• **FBC**, flow-cytometry for deficiency of CD15a/SLeX	• GDP-fucose transporter
Leukocyte adhesion deficiency- III (LAD-III)	• **FBC**, Integrins (**CD 11**a,**b,c/ CD18**) are present but defective	• Cal DAG – GEF, kindlin – 3 (FERMT3)
Neutrophil specific granule deficiency	• **FBC**	• C/EBP- epsilon
DISORDERS OF NEUTROPHILS ASSOCIATED WITH OTHER PID'S		
Common variable immune deficiency (CVID)	• **IgA, M, G, specific antibodies to protein and polysaccharide antigens, lymphocyte subsets, memory B-cells**	• **ICOS**, **BAFF**, **CTLA4, TACI**
Wiskott-Aldrich syndrome (WAS)	• **IgA, M, G**, Small platelet volume on **FBC**	• **WAS**
X-linked Hyperimmuno-globulin E (IgE) syndrome (Job syndrome)	• **FBC, IgE levels, Th-17 cell** count	• **STAT3**
X-linked agammaglobulinaemia (XLA)	• **IgA-, M-, G-, B-cell numbers** • **BTK** on flow-cytometry	• **BTK**
Hyper IgM (HIGM)	• **IgA, M, G, lymphocyte subsets, CD40L** on flow-cytometry	• **CD40L**

FBC: Full blood count with exam of smear; BM: bone marrow; ANC: absolute neutrophil count; DHR: dihydrohodamine

sive CGD (AR-CGD) is caused mainly by defects in the other genes of the NADPH oxidase enzyme complex. Sequencing of gene components encoding the NADPH oxidase enzyme complex is recommended to determine the exact nature of the genetic defect.[15,17,23,24] Sequencing of the CYBB gene as well as the other genes of the NADPH complex, is available in South Africa.

Genetic testing could be considered as first-line test in patients who have a known or characteristic family history of CGD and/or characteristic clinical or laboratory findings. This particularly holds true in settings where specimens may not reach the laboratory within 24 hours – the cut-off time for functional testing. DNA is very stable; therefore time constraints do not affect genetic-testing results. Genetic confirmation should also be sought if the clinical picture is characteristic, but functional testing appears not to be diagnostic.[16,25,26] Please see Table III for a summary of diagnostic testing for CGD.

2.2. Leukocyte adhesion deficiency (LAD) - LAD is a group of rare disorders that is caused by defective adhesion of neutrophils to the endothelial wall of blood vessels. The defective adhesion prevents neutrophils from migrating from the circulation into the tissues and to the site of infection. This gives us a clue to the main features of these disorders, which often include a neutrophilia and a lack of inflammatory changes and pus formation at the site of infection. However, there is a variable phenotypic presentation of these disorders and there are at least three known subtypes, LAD type 1, 2 and 3, caused by three different genetic mutations.[1,2,4,27] Delayed umbilical cord separation (>10 days) is a significant feature of LAD. Other prominent features

are skin infections, perianal ulcers and fistulae and periodontitis, often resulting in tooth decay. Patients often present with recurrent infections of the oral and genital mucosa, skin, gastro-intestinal and respiratory tracts. Infecting pathogens may include gram-negative enteric bacteria, *S aureus*, *Candida spp* and *Aspergillus spp*.[4] There is usually a lack of inflammatory changes at the site of infection and an absence of pus formation. Patients may develop scarred nodules at immunisation sites. Infections are most prominent in LAD type 1 and 3, whereas LAD type 2 is accompanied by developmental abnormalities and LAD type 3 by a bleeding disorder.[27]

The diagnosis of LAD should be considered in patients with suggestive clinical features and a persistent neutrophilia. The diagnosis of LAD 1 depends on demonstrating reduced or absent adhesion molecules (CD11a,b,c/CD18) on stimulated neutrophils by flow-cytometry. Genetic testing should be performed to confirm the diagnosis of LAD type 1 or make the diagnosis of LAD type 2 or 3.[1,27] Please see Table III for a summary of diagnostic testing for LAD. Flow-cytometry for adhesion molecules as well as genetic testing for ITGB2 mutations are available in South Africa.

2.3. Neutrophil-specific granule deficiency - Neutrophil-specific granule deficiency is an abnormality of internal neutrophil structure which arises during myelopoeisis. Neutrophils have abnormal, bilobed nuclei and there is a deficiency of secondary granules (lactoferrin) and other enzymes (alkaline phosphatase). This leads to defective bacterial killing.[1,2] Patients may present with recurrent skin and sinopulmonary infections, commonly with organisms such as *S aureus, S epidermidis* and enteric bacteria.[4]

The diagnosis can usually be made by careful examination of the blood film. Genetic testing can confirm a mutation affecting a transcription factor in myelopoiesis (C/EBPe gene).[1] See Table III for a summary of diagnostic testing for neutrophil-specific granule deficiency.

2.4. Myeloperoxidase deficiency - Myeloperoxidase (MPO) deficiency is the most common inherited disorder of neutrophils. MPO is an enzyme in primary granules and catalyses the formation of hypochlorous acid, which plays a role in intracellular killing of micro-organisms. However, MPO deficiency is not generally associated with symptomatic disease. An important exception is diabetic patients with MPO deficiency, who are susceptible to disseminated candidiasis.[1,2,4]

Testing for this condition is not generally required, but MPO deficiency can be diagnosed upon careful examination of a blood film and staining for MPO.[1] Genetic testing is not routinely performed. Please see Table III for a summary of diagnostic testing for MPO deficiency.

2.5. Chediak-Higashi syndrome (CHS) - CHS is a primarily granule-fusion defect that affects not only neutrophils, but also other leukocytes, nerve endings and melanocytes. Benign and aggressive presentations of CHS may occur. Characteristic features are partial oculo-cutaneous albinism (premature silver streaks in the hair, depigmentation of the iris and the skin) and central nervous system (CNS) abnormalities. CNS abnormalities may include both central and peripheral abnormalities, for example mental retardation, nystagmus, fits, parkinsonian features, cranial nerve palsies and neuropathy. Patients may suffer from recurrent pyogenic infections and severe periodontal disease. Causative organisms are often *S Aureus* and β-haemolytic streptococci.[4] Hepato-splenomegaly occurs frequently and patients may experience easy bruising due to platelet dysfunction.[1,2,4]

The diagnosis of CHS can usually be made on the peripheral blood smear, where giant granules are visibly present in leukocytes and platelets. Patients usually have a mild–moderate neutropaenia, defective neutrophil chemotaxis and NK-cell dysfunction.[2] The genetic basis of this autosomal recessive disorder can be attributed to a mutation in the LYST gene, which can be tested in South Africa.[1] Please see Table III for a summary of diagnostic testing for CHS.

LABORATORY TESTING FOR SUSPECTED NEUTROPHIL DISORDERS

As primary neutrophil deficiencies account for <10% of PID, it is usually appropriate to first eliminate other more common disorders (e.g. antibody deficiencies) in patients presenting with recurrent infections. However, if there is a family history suggestive of neutrophil dysfunction or clinical suspicion of neutrophil dysfunction based on the type, site of infection or organisms involved, patients should always be screened for neutrophil disorders.[1–4,16]

The first step in evaluating any patient for a neutrophil disorder is usually a FBC and a differential white-cell count. This is affordable and widely available. The choice of assays for further investigation depends on a variety of factors, for example a positive family history with a known defect, clinical findings which may include suggestive infections or syndromic findings, or other laboratory findings.[1–3]

Patients with a clinical history which may be suggestive of CGD should be screened by flow-cytometry (or by other available methods) for abnormalities in neutrophil oxidative burst.[1,2,4,16] CGD screening should be considered early on

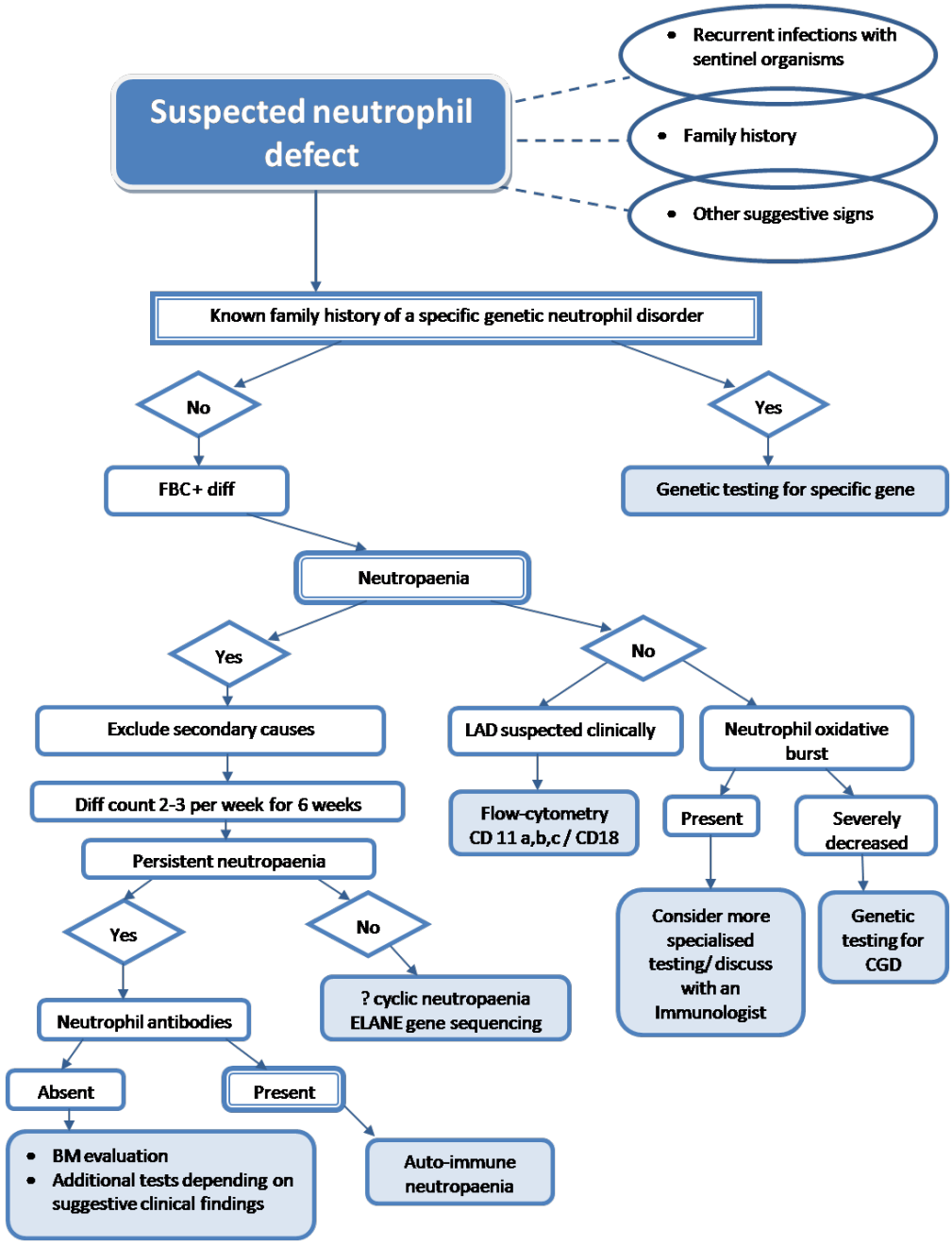


Figure 2: Approach to suspected neutrophil defects

in the work-up of a patient with a suspected neutrophil disorder, as CGD is the most significant neutrophil functional disorder and is relatively easy to diagnose. CGD can present at any age with variable clinical presentations. Carriers may also be affected clinically.

Patients who have a known family history of a specific genetic disorder or characteristic clinical or laboratory findings may be considered for first-line genetic testing.[1] A range of tests are available in South Africa to assist with the diagnosis of neutrophil deficiencies, for example neutrophil oxidative burst, neutrophil phagocytosis, neutrophil chemotaxis, expression of CD11b,c/CD18, anti-neutrophil antibodies, BM investigations with specialised haematology stains and genetic tests.

A simplified approach to a patient suspected of having a neutrophil disorder can be illustrated as a flow-diagram (see Figure 2).

Please refer to Table III as a guide on more specific testing to assist in the diagnosis of specific neutrophil disorders which may be suspected on history or initial test results. Please note that all tests in bold are available in South Africa at various laboratories and testing centres.

CONCLUSION

Neutrophil disorders occur commonly, however, primary or congenital neutrophil disorders account for <10% of PID. The most common cause of neutrophil disorders encountered in general practise is usually secondary, mostly due to infection, autoimmunity or drug-induced.

However, as primary neutrophil disorders may be life-threatening, they should be included in the differential diagnosis of any child or adult with recurrent fungal or bacterial infections, particularly when organisms such as *S aureus*, *Salmonella spp*, *Mycobacteria spp*, *B cepacia*, *S marcesens*, *L monocytogenes, Nocardia spp, Actinomyces spp* and *Aspergillus spp* are involved.[1,2,4,16] Since the clinical manifestations of infection are often blunted as a result of impaired inflammation, clinicians should have a low index of suspicion for screening patients for neutrophil dysfunction. Early identification and intervention in patients with severe primary neutrophil disorders may be life-saving.[1,4]

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REFERENCES

1. Coastes TD. Laboratory evaluation of neutrophil disorders. In: UpToDate 2017 Apr. Newburger P editor; Available at http://www.uptodate.com/contents/laboratory-evaluation-of-neutrophil-disorders.
2. Spickett GP. Primary Immunodeficiency. In: Spickett GP, editor. Oxford Handbook of Clinical Immunology and Allergy. 3rd ed. Oxford: Oxford Medical publications 2013;62–69.
3. Uzel G, Holland SM. Phagocyte deficiencies. In: Rich RR, Fleisher TA, Shearer WT Schroeder Jr HW et al. editors. Clinical Immunology principles and practice. 4th ed. Elsevier: Elsevier Saunders 2013;270–283.
4. Lekstrom-Himes JA, Gallin JI. Immunodeficiency diseases caused by defects in Phagocytes. N Engl J Med 2000;343(23):1703–1711.
5. Foucar K. Neutropenia. Diagnostic pathology blood and bone marrow. 2012;(1):4–12.
6. Gibson C, Berliner N. How we evaluate and treat neutropenia in adults. Blood 2014;124:1251–1258.
7. Boxer LA. How to approach neutropenia. Hematology Am Soc Hematol Educ Program 2012;2012:174–181.
8. Rosenberg PS, Alter BP, Link DC, Stein S et al. Neutrophil elastase mutations and risk of leukaemia in severe congenital neutropenia. Br J Haematol 2008;140(2):210–213.
9. Christensen RD, Rothstein G. Exhaustion of mature marrow neutrophils in neonates with sepsis. J Pediatr 1980;96(2):316–318.
10. Farruggia P. Immune neutropenias of infancy and childhood. World J Pediatr 2016;12(2):142–148.
11. Greenberg PL, Mara B, Steed S, Boxer L. The chronic idiopathic neutropenia syndrome: correlation of clinical features with in vitro parameters of granulocytopoiesis. Blood 1980;55(6):915.
12. Brunning RD, Orazi A, Germing U, Le Beau MM et al. Myelodysplastic syndromes/neoplasms, overview. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES et al, editors. WHO Classification of tumours of Haematopoietic and Lymphoid tissues. 4th ed. Lyon: International Agency for Research on cancer; 2007. P88–93.
13. Marciano BE, Spalding C, Fitzgerald A, Mann D et al. Common severe infections in chronic granulomatous disease. Clin Infect Dis 2015;60(8):1176–1183.
14. Coates TD. Laboratory evaluation of neutrophil disorders. In: UpToDate 2017 April. Newburger P, editor; Available at http://www.uptodate.com/contents/laboratory-evaluation-of-neutrophil-disorders.
15. Van den Berg M, Van Koppen E, Ahlin A, Belohradsky BH et al. Chronic granulomatous disease: The European experience. PLoS ONE 2009;4(4):1–10.
16. Frigati LJ, Esser M. Chronic granulomatous disease and the challenges of diagnosis and management in South Africa. Curr Allergy Clin Immunol J 2016;29(2):127–129.
17. Holland M. Chronic Granulomatous disease. Hematol Oncol Clin North Am 2013;27(1):89–99.
18. Rieber N, Hector A, Kuijpers T, Roos D et al. Current concepts of hyperinflammation in chronic granulomatous disease. Clinical and Development Immunology 2012:1–6.
19. Dotis J, Pana ZD, Roilides E. Non-Aspergillus fungal infections in chronic granulomatous disease. Mycoses 2013;56:449–462.
20. Akarcan SE, Karaca N, Aksu G, Bozkaya H et al. Necrotizing liver granuloma/abscess and constrictive aspergillosis pericarditis with central nervous system involvement: Different remarkable phenotypes in different chronic granulomatous disease genotypes. Case reports in Immunology 2017:1–9.
21. Jirapongsananuruk O, Malech HL, Kuhns DB, Niemela JE et al. Diagnostic paradigm for evaluation of male patients with chronic granulomatous disease, based on the dihydrorhodamine 123 essay. J Allergy Clin Immunol 2003;111(2):374–379.
22. CGD diagnostic criteria. Available at https://esid.org/Working-Parties/Clinical/Resources/Diagnostic-criteria-for-PID2#Q4 (accessed 18 May 2017).
23. Kuhns DB, Alvord GW, Heller T, Feld JJ et al. Residual NADPH Oxidase and survival in chronic granulomatous disease. N Engl J Med 2010;363:2600–2610.
24. Wu J, Wang W, Zhang Y, Chen T. Clinical features and genetic analysis of 48 patients with chronic granulomatous disease in a single centrr study from Shanghai, China (2005–2015): New studies and a literature review. J Immunol Res 2017:1–17.
25. Jones LBKR, McGrogan P, Flood TJ, Gennery AR et al. Special Article: Chronic granulamatous disease in the United Kingdom and Ireland: A comprehensive national patient-based registry. Clin Exp Immunol 2008;152:211–218.
26. Köker MY, Camcioglu Y, Van Leeuwen K, Kilic SS et al. Clinical functional, and genetic characterization of Chronic Granulomatous disease in 89 Turkish patients. J Allergy Clin Immunol 2013;132(5):1156–1163.
27. Etzioni A. Leukocyte-adhesion deficiency. In: UpToDate 2017 April. Notarangelo LD, editor. Available at http://www.uptodate.com/contents/leukocyte-adhesion-deficiency.