ITALIAN PRIMARY IMMUNODEFICIENCIES STRATEGIC SCIENTIFIC COMMITTEE

CHRONIC GRANULOMATOUS DISEASE

Recommendations for diagnosis and treatment

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AIM

The recommendations for the diagnosis and treatment of Chronic Granulomatous Disease (CGD) have been devised to optimize the approach to the diagnosis and treatment of “orphan diseases” like primary immunodeficiencies.

Establishing nationwide diagnostic and therapeutic recommendations and analysing patient outcome will allow ongoing adjustments and updates designed to offer all patients high uniform standards of care.

The aim of these recommendations is to:

- Establish standard diagnostic criteria;
- Define updated therapeutic recommendations to be applied nationwide for all CGD patients;
- Record the natural history and any complications of the disease as well as any side effects of treatment;
- Adjust treatment protocols on the basis of the outcome of any controlled experimental trials and results obtained by other research groups.

The first part of these diagnostic and therapeutic recommendations presents the clinical and pathogenetic state-of-the-art of CGD. The second part outlines the diagnostic and therapeutic recommendations; *basic indications are in italics*. The third part offer suggestions for the management of infections and complications in CGD patients. The fourth and last part deals with disease prevention from a genetic standpoint. The last two sections are not part of the diagnostic and therapeutic protocol as such, but updated information on the clinical, diagnostic and therapeutic aspects of CGD.

1. INTRODUCTION

1.1 What is Chronic Granulomatous Disease?

Chronic Granulomatous Disease (CGD) was described for the first time by both B.H. Landing and R.A. Good in 1957. CGD comprises a rare group of genetically determined changes affecting the immune system characterized by the inability of the body's phagocytic cells (neutrophil and monocyte granulocytes) to kill certain phagocytosed microorganisms. This phagocytic cell defect is caused by mutations in the gene coding for the NADPH oxidase enzyme essential for the microbicide activity of phagocytic cells.

The disease affects an average of one in every 250,000 live births. Affected children are subject to frequent severe bacterial and fungal infections with the granulomatous hallmark of inflammatory lesions in histological specimens from which the name CGD derives.

1.2 Biochemistry and genetics of CGD

Under normal conditions, exposure of phagocytes to opsonized germs results in prompt metabolic activation, namely the hexose monophosphatase shunt accompanying an up to 100-fold increase in oxygen and glucose consumption known as the "respiratory burst".

A key role in the respiratory burst is played by the nicotinamide-adenine-dinucleotide-phosphate oxidase (NADPH oxidase) system. This enzyme is a membrane flavoprotein transferring electrons from NADPH oxidase to molecular oxygen (O_2) with the formation of the superoxide ion (O_2^-), which is then transformed into hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl) within the phagosome by superoxide dismutase and lysosomial myeloperoxidase. The killing of phagocytosed microorganisms is linked to the production of these reactive oxygen products which damage the bacterial membrane.

The NADPH oxidase complex is composed of four subunits: two molecules, p22 *phox* (alpha subunit) and gp9l *phox* (beta subunit) forming the complex known as cytochrome *b558,* constitutively present on the cell membrane and on that of specific granules and secretory vesicles of the neutrophil granulocyte. This complex contains two haeme groups and two phagosome groups required to transport electrons from cytoplasmic NADPH to the O_2 contained in the phagosome. Another two proteins, weighing 47 and 67 kDa respectively, are present exclusively in the cytoplasm. A third protein, p40 phox, is found in the cytosol and seems to be involved in stabilizing the p47/p67 phox complex in resting phagocytes. After cell activation induced by a series of stimuli (microorganisms or opsonized bacterial peptides, complement C5a fraction, etc.) the secretory vesicles merge with the phagocyte plasma membrane thereby leading to the passage of cytochrome b 558 on the phagocytic cell membrane. At the same time, the phosphorylated cytosolic proteins p47 and p67 phox shift onto the plasma membrane where they interact with the b558 complex completing the assembly of the NADPH enzyme complex able to carry out its full oxidase activity.

Other low molecular weight GTP-binding proteins belonging to the *rac* family are involved in the translocation process*:* in particular *rac 1*, which binds to the p47, p67 and p40 phox cytosol protein complex. Another low molecular weight protein *rap* 1 A, associated with cytochrome b 558 and localized on the granule and secretory vesicle membrane, is involved in oxidase regulation.

In CGD one of the proteins belonging to the NADPH oxidase system is missing, reduced or functionally impaired. This means that phagocytosis occurs normally but the phagocyte is unable to process oxygen to produce the toxic metabolites needed for killing. As a result, the phagocytosed microorganisms survive within the cells and antibodies and most antibiotics have difficulty reaching them. CGD may be caused by a defect in any of the four protein subunits and this accounts for the genotypic heterogeneity of the disease (Table 1).

In 60% of cases CGD is caused by a mutation in the gene coding for the gp91 *phox* subunit located on the short arm of chromosome X (Xp21.1). Autosomal recessive variants are caused by mutations in the gene coding for the p22 phox subunit mapping to the long arm of chromosome 16 (16q24). Around 5% of cases depend on the genes for p47 phox or p67 phox respectively mapping to the long arm of chromosome 7 (7q11.23) and the short arm of chromosome 1 (1q25) and representing 25% and 5% of all cases of CGD. The mutations involving p40 phox are not known.
In addition, there are variants (+) and (–) for each of the protein subunits gp91 and p22 phox caused by mutations which leave the protein intact but abolish its enzymatic activity (+) or attenuate protein expression with residual functional activity (-).

Table 1: Classification of Chronic Granulomatous Disease

Impaired protein	Chromosome	Gene locus	Inheritance pattern	Subtype[a] (%)	NBT (% positive cells)	O_2^- Production (%)	Cyt. b (%)	Frequency of cases (%)
Gp91 phox	Xp21.1	CYBB	XR	$X91^0$	0	0	0	55-60
				$X91^-$	80-100 (weak)	3-30	3-30	5
				$X91^+$	0	0	100	1-3
P22phox	16p24	CYBA	AR	$A22^0$	0	0	0	~ 5
				$A22^+$	0	0	100	~ 1
P47phox	7q11.23	NCF1	AR	$A47^0$	0	0	100	25
P67phox	1q25	NCF2	AR	$A67^0$	0	0	100	5

(Modified from J.T Curnutte, Immunodef. Rev 1992; 3: 149)

[a] indicates protein expression is normal (+), reduced (-) or absent (0).

1.3 Molecular biology of CGD

1.3.1 X-linked CGD

With the exception of gene conversion, all of the more than 60 mutations in CGD have been identified in the CYBB gene. Deletions and insertions in this gene are present in 35% of XCGD patients, whereas single nucleotide substitutions have been disclosed in the remaining 65%. Nucleotide substitutions include mutations in the splicing site (16%), missense mutations (23%) causing a single aminoacid substitution which may lead to the formation of an inactive gp91 protein, and nonsense mutations resulting in premature termination of protein synthesis (27%). When gene deletions are large, genes adjacent to the CYBB gene may also be affected so that patients will have other clinical disorders like Duchenne's muscular dystrophy, retinitis pigmentosa and McLeod's syndrome: haemolytic anaemia with acanthocytosis due to deletion of the gene coding for a 37 Kda erythrocyte protein required for the expression of Kell antigens.

1.3.2 Autosomal recessive CGD

Fewer mutations have been identified in the autosomal recessive form of CGD. They include insertions, deletions, missense and splicing site mutations. The heterogeneity of the molecular defect of the A67 form is greater than that of A47 CGD which is almost always caused by a GT (guanine-thymidine) deletion in exon 2 due to recombination events between the p47 phox gene and one or more closely linked pseudogenes.

1.3.3 Genotype-phenotype correlation

Patients with X-linked CGD tend to have a more severe clinical disease course than those with the autosomal recessive form due to persistent residual NADPH oxidase activity in the neutrophils of $A47^0$ and $A67^0$ patients. This suggests that the b558 membrane complex plays a more critical role than cytosolic proteins in the oxidative process. XCGD patients with $X91^0$ and $X91^+$ phenotype should be distinguished from those with the $X91^-$ phenotype. Patients with the $X91^-$ phenotype have 10-30% residual oxidase activity and therefore tend to have a better clinical course, but this is not always the case because oxygen independent auxiliary antimicrobial systems probably enter into play. Generally speaking, insertions, nonsense and splicing site mutations lead to an $X91^0$ phenotype, whereas missense mutations are responsible for all three phenotypes.

In addition, a third of the X-linked forms of CGD are caused by *de novo* gene mutations in which the results of biochemical tests and molecular gene analysis are normal even in presumed disease carriers. Instead, obligate CGD carriers occasionally present clinical manifestations similar to those of hemizygote patients. This is usually the result of extreme lyonization when carrier females have fewer than 10% normal phagocytes and always present missense mutations.

1.4 What are the symptoms of CGD?

Two thirds of patients with CGD develop the signs of disease within the first two years of life with a wide range of clinical manifestations. The onset of X-linked CGD is usually earlier than that of autosomal recessive CGD which may even present in adulthood. Any organ may be affected, but the usual sites of infections are the lungs, lymph nodes, and skin (Tables 2 and 3).

Table 2: Common sites of infection in Chronic Granulomatous Disease

Very common (> 60%)	**Common (20-60%)**	**Sporadic (< 20%)**
Lungs	Middle ear	Paranasal sinuses
Lymph nodes	Bones	Hearing apparatus
Skin	Perirectal region	Central nervous system and meninges
	Systemic (septicaemia)	Pericardium

Table 3

CGD : clinical manifestations	**% cases**
Purulent dermatitis	60-70
Middle ear otitis	15-20
Conjunctivitis	10-20
Sinusitis	<10
Ulcerative stomatitis	5-25
Pneumonia	70-80
Bowel infections	5-15
Abscesses/ perirectal fistulae	15-30
Urinary tract infections	5-15
Lymphadenopathy	98
Lymphadenitis	60-80
Hepatosplenomegaly	50-90
Liver/perihepatic abscesses	30-40
Sepsis	10-20
Osteomyelitis	20-30
Kidney/perirenal abscesses	<10
Meningitis	<5
Brain abscesses	<5
Pericarditis	<5
Chorioretinitis	<10
Gengivitis	50
Pulmonary fibrosis	<10
Oesophagitis	<10
Gastric antrum stenosis	<10
Ileocolic granulomatosis	<10
Glomerulonephritis	<10
Hydronephrosis	10-25
Granulomatous cystitis	<10
Anaemia	Common
Hypergammaglobulinaemia	60-90
Weight Deficit	70
Failure to thrive	50
Delayed wound healing	Common
Discoid lupus erythematosus	Rare
McLeod’s syndrome	Extremely rare

The hallmarks of CGD are recurrent infections, unusual etiologic agents and the granulomatous evolution of inflammatory lesions. These granulomata, made up of giant cells and macrophages full of lipids, destroy the parenchyma and result in frequent stenosis of the gastrointestinal or urinary tracts and require surgical removal.

The dissemination of infection is facilitated by the fact that microoorganisms phagocytosed but not killed by leucocytes in the site of primary infection can be transported to distant sites to then involve the kidneys, muscles, pericardium, CNS and other organs.

Although many of the clinical manifestations of CGD are aspecific, infections caused by Aspergillus, recurrent pyodermitis, granulomatous liver abscess, atypical forms of tuberculosis and osteomyelitis of the metatarsal bones resistant to treatment may provide a valuable clue to the diagnosis (Table 4).

Patients may also present associated illnesses like lymphadenopathy, hepatosplenomegaly, malabsorption, hydronephrosis, failure to thrive and microcytic anaemia caused by chronic disease which usually resolves spontaneously at the end of the first decade of life.

The commonest microbial species include: catalase positive germs which breakdown the H_2O_2 they produce, *Staphylococcus aureus, Escherichia Coli, Salmonella, Klebsiella,* various strains of *Pseudomonas,* saprophytic bacteria like *Serratia marcescens, Staphylococcus epidermidis, Enterobacter, Burkholderia Cepacia,* and fungi like *Aspergillus* and *Candida* (Table 5).

Table 4: Valuable clues to the diagnosis of Chronic Granulomatous Disease.

Aspergillus infection at any age
Serratia infection at any age
Osteomyelitis
Lymphadenitis caused by Staphylococcus
Liver abscess
Granulomatous colitis
Obstruction of the airways and/or digestive and/or urinary tracts by granulomatous inflammation

Table 5: Spectrum of infectious agents in CGD

MICROORGANISMS	% isolates
Gram + bacteria	**~ 40**
Staphylococcus aureus	30-50
Staphylococcus epidermidis	5
Streptococcus	4
Nocardia	2
Actinomyces	<1
Gram - bacteria	**~ 40**
Escherichia Coli	5-10
Klebsiella	5-10
Salmonella	5-10
Serratia marcescens	5-10
Enterobacter	3
Proteus	3
Burkholderia cepacia	5-10
Chromobacterium violaceum	<1
Francisella philomiragia	<1
Fungi	**~ 20**
Aspergillus	10-20
Scedosporium	?
Candida albicans	3
Torulopsis	<1
Other	
BCG	<1
Mycobacterium fortuitum	<1
Pneumocystis carinii	<1

CGD carriers are usually asymptomatic with some major exceptions: around half of the X-linked carriers have recurrent stomatitis and gingivitis, while a quarter develop discoid lupus erythematosis on the skin of the face, shoulders, arms and back, probably linked to antibodies against microbial antigen fractions. These lesions do not usually progress towards a systemic form of CGD.

1.5 Complications

Complications are caused by the mechanical obstruction ensuing from the typically granulomatous evolutions of inflammatory lesions mainly affecting the gastrointestinal and urinary apparatus and resulting in pyloric stenosis, chronic bowel inflammation, hydronephrosis, urethral stenosis and granulomatous cystitis.

1.6 Diagnostic criteria for CGD (established by ESID and PAGID, 1999)

Certain diagnosis**:**
Male or female with abnormal NBT or unchanged "respiratory burst" in activated neutrophils (less than 5% of control) with one of the following:

1) Mutation in gp91, p22, p47, p67 phox
2) Absent mRNA for one of the above genes by Northern Blot analysis.
3) Maternal cousins, uncles or nephews with an abnormal NBT or respiratory burst

Probable Diagnosis**:**
Male or female with abnormal NBT or unchanged "respiratory burst" in activated neutrophils (less than 5% of control) with one of the following:

1) Liver, perirectal or lung abscess, adenitis or osteomyelitis due to Staphylococcus, Serratia marcescens, Candida or Aspergillus.
2) Diffuse granulomata I respiratory, gastrointestinal or urinary tracts
3) Failure to thrive and hepatosplenomegaly or lymphadenopathy.

Differential diagnosis:

1) Leucocyte adhesion deficit (LAD)
2) Sarcoidosis
3) Hyper-IgE

2. DIAGNOSTIC PROTOCOL

2.1 Diagnosis

When Chronic Granulomatous Disease is suspected diagnosis is made by establishing impaired granulocyte activity using one of the following tests:

- ***Nitro-blue tetrazolium test (NBT)*** *is a semiquantitative test. Under normal conditions, NBT is yellow and soluble in the presence of superoxide and is reduced to formazan, a dark blue insoluble reagent precipitated in phagocytic cells previously activated with phorbol myristate acetate (PMA), with particles opsonized with zymosan (OPZ) or N-formyl-methionyl-leucyl-phenylalanine (fMLP). In a CGD patient the superoxide is not formed and therefore NBT is not reduced to formazan.*

- ***Flow cytometric analysis by dihydrorodamine 123 (DHR)*** *is a quantitative test based on the use of DHR as a fluorescent marker of oxidase activity. In many laboratories this test has replaced NBT as it is highly sensitive and can be performed on whole blood up to 24 h after sampling.*

- ***CYTOCHROME "C"*** *is a quantitative test based on the reduction of C ferricytochrome by the superoxidase produced by PMA-stimulated neutrophils and monocytes. The drop in C ferricytochrome is measured by spectrophotometry at 550 nm.*

- ***Chemiluminescence*** *is a quantitative test based on the ability of stimulated phagocytes to interact with oxidizable substrate. On return to the baseline energy state the phagocytes emit light which is measured by a liquid phase beta scintillator.*

Which test is chosen will depend on the experience of the centre dealing with CGD patients which will already have developed a reliable diagnostic test.
Hospitals lacking a specialized laboratory for the diagnosis of CGD can refer to one of the Italian laboratories listed below for CGD testing of blood samples. These laboratories use flow cytometric analysis by dihydrorodamine 123 for CGD diagnosis.

2.2 Sending samples

After the meeting on 14th December 2000, the centres available to carry out dihydrorodamine 123 for CGD diagnosis were contacted and five are already using the test on a routine basis and are prepared to act as national reference centres. Blood samples from CGD patients, their mothers and normal subjects were sent at room temperature by a courier guaranteeing delivery within 24h to ascertain the reproducibility of results.
The results were reproducible and reliable for the diagnosis of CGD but not for the identification of carrier status. For this reason, blood samples can be sent to these centres below for diagnosis of CGD, whereas the diagnosis of carrier status must await the outcome of further standardization tests to be carried out in the near future.
Centres opting to undertake the identification of carrier status by this method are free to do so outside these recommendations.

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Telephone arrangements should be made for sending blood samples to one of the approved centres to carry out flow cytometric analysis by dihydrorodamine 123 for CGD diagnosis.

The following must be sent for this test:

- *A test tube containing 7 cc heparinized blood per patient. A test tube containing 7 cc heparinized blood from a normal subject should also be sent as an internal control.*

- *Samples also be accompanied by Form A duly compiled and sent the TRACO 10 service which guarantees delivery of samples by 10 a.m. on the following day. Please note that samples must be analyzed immediately to ensure reliable results.*

- *The doctor requesting the test will be notified of the results in writing (fax or e-mail) within 48 hours.*

2.3 Inclusion criteria

Diagnosis of CGD is made in male and female subjects with impaired granulocyte metabolism disclosed by one of the tests listed above.
*A registration form (**Form 1.03**) and a diagnosis form (**Form 22.01**) will be filled in for patients meeting these inclusion criteria. Annual follow-up forms (**Form 22.02**) will then be filled in and sent to the AIEOP Operation Office in Bologna.*

2.4 Genetic typing

No gene mutation analysis is planned for the first year.

3. TREATMENT RECOMMENDATIONS

Once a diagnosis of CGD has been established, the first mainstay of treatment is the prevention of infections by the following measures:
1) Drug management
2) Helping to prevent infections
3) Vaccinations

3.1 Drug management

Therapy is aimed at preventing bacterial and fungal infections with the following drugs:

Co-trimoxazole: 6-8 mg/Kg/die trimethoprim per os once or twice daily up to a maximum of 160 mg trimethoprim a day. In the case of sulphonamide allergy dicloxacillin can be given at a dose of 25-50 mg/Kg/die per os, in 4 divided doses. Co-trimoxazole is contraindicated in patients with a G6PD deficiency.

Itraconazole: 10 mg/Kg die per os once daily (up to a maximum dose of 200 mg/die)

Interferon γ: 50 μg /m² in patients with a body surface area of > 0.5 m²; 1.5 mcg/kg/dose in patients with a body surface area of <0.5 m². The drug is administered by subcutaneous injection three times a week (e.g. Monday, Wednesday, Friday).

- *Side effects: fever, headache, chills, myalgia, fatigue, nausea, vomiting and rashes.*
- *These effects are usually well-controlled by premedication with paracetamol (10-15 mg/kg/dose per os, 30-60 minutes before drug administration) or nimesulide at a dose of 2 mg/Kg up to a maximum of 100 mg. Nimesulide is not recommended for children under 12 years.*
  In some patients interferon γ can give rise to autoantibodies, rheumatoid factor, development of LES, leucopenia and thrombocytopenia.
- *Contraindications to the use of interferon γ: autoimmune disease, depressive syndromes.*

3.2 How patients can help prevent infections

The following lifestyle recommendations are very important for the prevention of infections:

▪Thorough personal hygiene; use mild soap; brush teeth twice daily with hydrogen peroxide and bicarbonate toothpaste; use mouthwash to reduce the likelihood of gingivitis.
▪Avoid alcohol and smoking (tobacco contains Aspergillus).
▪Prevent constipation.
▪Avoid granaries, caves and other dusty or damp areas (Aspergillus).
▪To avoid inhaling large amounts of fungi do not work in environments with mould, hay, wood chippings, compost or other rotten or fungi infested grass or wood.
▪Antibiotics should be taken before and after any dental treatment.
▪Any cut or graze should be washed thoroughly with soap and water then disinfected and rinsed with hydrogen peroxide; patients are urged to consult their physicians promptly if any rash or irritation around the lesion site, presence of pus or fever.
▪Walking barefoot is not recommended.
▪Do not use wood chip playing fields but those with a smooth or gravel surface.
▪Wear an air filter mask for gardening.
▪Avoid planting houseplants as mould often grows in the earth.
▪Add a teaspoon of bleach to vases of fresh flowers to reduced the formation of mould and algae.
▪Avoid living in old buildings or newly built or refurbished buildings before they have been thoroughly cleaned.
▪Do not lift or move carpets or tiles; thoroughly clean rooms before use.
▪Pets are tolerated but avoid using sawdust for litter; make sure pets are vaccinated regularly; keep water bowl and litter clean.
▪Empty vaporizers daily and wash with bleach to avoid mould.
▪Patients are urged to consult their physicians promptly in case of fever especially when accompanied by cough.

3.3 Vaccinations

Chronic granulomatous disease is not a contraindication to the administration of the regular scheduled vaccinations. CGD patients should also have annual anti-influenza, anti-pneumococcal and anti-meningococcal vaccinations.

3.4 Tests at diagnosis and during follow-up:

Every 6 months:

- Full blood count
- Transaminase levels
- Azotaemia, creatininaemia
- ESR. PCR
- FAN
- Anti-aspergillus antibodies (with titre)

Every year:
To monitor any organ specific damage:
X-ray
Ultrasound scan
CT and/or MR scan
Spirometric tests

Every 2 years:
High resolution lung CT scan

3.5. Bone marrow transplantation

Conventional treatment has the following limitations:
- poor lifetime compliance with prophylaxis against infections;
- difficulty in preventing the inflammatory sequelae of infections;
- impaired quality of life due to frequent hospital admissions and permanent organ damage.

Around 30% of patients die from infectious complications.
Bone marrow transplant is currently the only possible cure for CGD and should be entertained especially for patients with X-linked CGD at the time of diagnosis whenever an HLA-identical family donor is available.

4. TREATMENT OF INFECTIONS

•Each infectious episode is potentially dangerous so every effort must be made to isolate the microorganism responsible, focusing on Aspergillus.
•Every episode of fever must be treated promptly by an aggressive use of drugs able to cross the phagocyte cell membrane and accumulate within the phagocytic cells (see Table 6).
•Initial empirical therapy should include at least two antibiotics against Gram+ and Gram- bacteria (see Table 7).
•Treatment should be continued for weeks or months even when there is a significant improvement in the inflammatory index and the patient's clinical conditions to eradicate the infection completely.

Table 6: Relation between intra (I) and extra (E)-cellular concentration of antibiotics in neutrophil PMN

ANTIBIOTIC	I/E in PMN
Penicillin G	0.4
Gentamicin	1
Isionazid	1.5
Phosphomycin	2
Cloramphenicol	2.6
Trimethoprim	4
Clindamycin	11
Erythromicin	13.3
Rifampicin	14
Azithromycin	40
Teicoplanin	60

(modified from: Il Bambino Immunodepresso, A.Ugazio et al.)

Drugs against Gram+ bacteria (e.g.. Staphylococcus aureus):
Teicoplanin: 6 mg/kg by intravenous injection every 12 hours for the first 2 days (attack dose), continue with the same dose once daily. Side effects: mild hearing loss, appearance of Factor VIII antibodies has been reported.
Clarythromicin: 15 mg/kg/die by intravenous injection twice daily. Side effects: gastrointestinal disorders and hepatic impairment.
Rifampicin : 20 mg/kg/die in a single intravenous injection. Do not exceed the maximum dose of 600 mg/die. Monitor liver function for possible hepatic impairment.
Drugs against Gram- bacteria
Phosphomycin: 200 mg/kg/die by intravenous injection 3-4 times daily.
Ciprofloxacin: 15 mg/kg/die by intravenous injection twice daily. This drug is a quinolone and may damage the tendons and weight-bearing joints in children. Treatment should be discontinued in case of pain and/or oedema in the Achilles' tendon (possible rupture) or persistent severe diarrhoea (possible onset of pseudomembranous colitis).

Table 7: Treatment of infections

Treatment	Indications	Duration	Drug	Dose
Antibiotics	Gram+ infections	Months	Rifampicin Clarithromycin	20 mg/kg/die 15 mg/kg/die
	Gram– infections	Months	Ciprofloxacin Phosphomycin	15 mg/kg/die 200 mg/kg/die
	Fungal infections	At least 6 months	Liposomial amphotericin B	1-3 mg/kg/die
Transfusion of granulocytes	Resistant infections	Until resolution	Leucocytes stimulated with G-CSF+ dexamethasone	1 Unit / 10 Kg
Anti-inflammatory treatment	Obstructing granulomata	7-10 days→tapered	Prednisone	0.5-1 mg/kg/die

4.1 Tests to be done at every infectious episode

1. Inflammatory indices: blood cytochrometric test, ESR, PCR, serum immunoglobulins. Neutrophil leucocytosis, raised ESR, PCR and hypergammaglobulinaemia are also markers for monitoring infection and response to treatment.
2. Culture tests: blood, urine, faeces, culture from infected sites, culture of exudates or transudates, sputum or broncheoalveolar lavage.

N.B. Culture tests must also be accompanied by the request for an antibiogram.

3.
4. Diagnostic imaging: X-ray, ultrasound, CT, MR scans,.

In case of lymphadenitis and/or liver abscess an echo-guided needle aspirate should be performed. Biopsy may sometimes be necessary for accurate identification of the etiologic agent.

4.2 Fungal infections

Aspergillus infection is the most serious infectious event and the prime cause of death in CGD patients. The microorganisms most commonly involved are *Aspergillus Fumigatus* and *Aspergillus Flavus*, but other species may also give rise to Aspergillosis.
The organs most frequently affected are lungs, bones, muscles and brain. Diagnosis is based on:

1. Clinical and radiological evidence of infection.
2. Isolation and identification of the Aspergillus on culture specimens (expectorate, broncheoalveolar lavage); blood culture is seldom positive even in the course of disseminated Aspergillosis.
3. Biopsy of the affected organ is often required for diagnosis.
4. Search for anti-Aspergillus IgM and IgG antibodies by immunofluorescence, immunodiffusion or ELISA. Other techniques have now been developed to search for the antigen by immunoblotting, ELISA, radioimmunologic tests and PCR, but are only available in highly specialised laboratories.

If a fungal infection is identified or strongly suspected, treatment includes:

Amphotericin B
This drug is used for systemic fungal infections and is active against *Candida* and *Aspergillus* binding to ergosterol, a protein in the fungal cell wall, causing its rupture and hence cell lysis.
Preparation of the infusion: the vial containing 50 mg Amphotericin B must be dissolved in 10 ml sterile distilled water, shaking until a clear solution is obtained; the solution must be further dissolved in 490 ml dextrose at 5% with pH > 4.2 to obtain an Amphotericin B concentration equal to 0.1 mg/ml. The infusion solution must be prepared until rigorously sterile conditions.
Dose:
Day 1: test dose of 0.25 mg
Day 2: 0.25 mg/Kg/die
Day 3: 0.5 mg/Kg/die
Day 4: 0.75 mg/Kg/die
Day 5 onwards: 1 mg/Kg/die.

The solution should be administered slowly by intravenous infusion lasting from 2 to 6 hours and the solution kept out of the light during infusion.

Treatment must be prolonged for months and can be administered on alternate days after 4-6 weeks; shorter treatment may give rise to relapse.

Amphotericin B does not cross the blood brain barrier making intrathecal administration necessary if the fungal infection involves the central nervous system: the Amphotericin B solution is prepared as above (at a concentration of 0.1 mg/ml) diluted 1:10 in 10% glucosate solution. At this concentration (0.01 mg/ml) the drug can be administered by intraspinal infusion twice weekly as follows:
Day 1: 0.025 mg
Day 2: 0.05 mg
Day 3: 0.075 mg
Day 4: 0.1 mg
If necessary continue with:
Day 5:0.25 mg
Day 6: 0.5 mg (maximum dose)
Interactions: Amphotericin B synergy increases cellular uptake of flucytosine so that the doses of Amphotericin B can be reduced thereby attenuating its toxicity.
Corticosteroids when given with Amphotericin B carry an increased risk of hypokalaemia. In addition, acute pulmonary reactions have been reported when Amphotericin B was administered during or just after leucocyte transfusions. For this reason it is recommended that these infusions be given separately monitoring lung function.
Side effects:
-early: general reactions (febrile reactions, nausea, vomiting, muscle and joint pain, epigastric pain), local reactions (thrombophlebitis at the injection site).
-late: varying degrees of renal toxicity with possible renal tubular acidosis and hypokalaemia, convulsions, arrythmias, hypo and hypertension, blood dyscrasias, abnormal liver function.
Treatment of adverse reactions:
Early side-effects can be minimized by

1) reducing the infusion speed
2) administering small doses of heparin to minimize thrombophlebitis
3) administering salicylates (20 mg/Kg) or hydrocortisone (10 mg/Kg) before Amphotericin B infusion.

Amphotericin B induced hypokalaemia can be prevented by good hydration and oral or parenteral administration of potassium salts 2 mEq/Kg/die.
Tests to be done during treatment: renal function indices, serum electrolytes, urine test and full blood count.
The drug's high toxicity sometimes prevents its administration. In these cases lipid formulations of Amphotericin B are recommended as they are significantly less toxic.

Amphotericin B lipid formulations (Ambisome, Amphocil 50: vial 50 mg)
With respect to Amphotericin B, the uptake of lipid formulations by liver and spleen is greater than that in the kidneys and lungs. Lipid formulations are also less toxic and have better tissue penetration thanks to ingestion by macrophages.
Dose: 1-3 mg/kg/die single intravenous infusion for at least 3 months after which treatment can be discontinued on alternate days followed by Itraconazole for life.
Preparation:

1. add 12 ml sterile water to the vial to obtain and injection solution containing 4mg/ml Amphotericin.
2. use the 5 micron filter supplied to dilute the total infusion amount with 5% dextrose to obtain a dilution of 0.5 mg/ml Amphotericin.
3. Infuse intravenously over 30-60 minutes.

The drug is usually well tolerated and does not give rise to major side effects. Diuresis and renal function should be monitored during treatment.

Voriconazole: (UK-109,496) (Pfizer Central Research, Sandwich, England)
This drug can be given as an alternative if response to Amphotericin and its lipid formulations is poor. Voriconazole is a new broad spectrum triazolic molecule active against fungal infections caused by *Aspergillus*, *Candida Krusei* and *Candida glabrata* and has been successfully used as an adjunct to treat invasive Aspergellosis in patients with AIDS and CGD. However, its efficacy and long-term safety have yet to be assessed in controlled clinical trials.

4.3 Treatment of infectious complications

4.3.1 <u>Lymphadenitis due to Staphylococcus aureus</u>

- Incision/ Drainage
- Rifampicin and Teicoplanin at the doses indicated above.
- If treatment fails: exeresis

4.3.2 Liver abscess due to Staphylococcus aureus

- Echo-guided needle aspiration;
- Rifampicin and Teicoplanin for months;
- If treatment fails: Resection of the abscess wall;
  Line the abscess cavity with omentum;
  Transfusion of granulocytes.

4.3.3 Pneumonia due to Burkholderia Cepacia

- High doses of Co-trimoxazole: 20-100 (TMP/SMZ) mg/kg/die intravenous infusion in 4 divided doses;
- Transfusion of granulocytes.

4.3.4 Granuloma of the urinary tract

- Prednisone 0.5-1 mg/kg/die for ten days tapering off gradually;
- If treatment fails internal drainage (Pigtail catheter)

4.4 Complementary treatments

4.4.1 Corticosteroids

Corticosteroid treatment should be avoided as it induces secondary immunosuppression. This applies to both topical steroids and inhalants.
The use of prednisone in CGD is justified in some situations as it has proven efficacy in shrinking granulomatous lesions in the urinary tract and attenuating the symptoms of chronic inflammatory bowel disease correlated to immune deficiency.
Low doses of prednisone are sufficient for this purpose: 0.5-1 mg/kg/die per os in two doses for 7-10 days and subsequently withdrawn gradually to avoid relapse.

4.4.2 Transfusion of granulocyte concentrates

Granulocyte transfusions can be given for minor infections which fail to respond to drug management. They can also be administered directly into the abscess cavity after drainage. Granulocyte concentrates are collected from volunteer donors after informed consent and pretreated with subcutaneous G.CSF 5 mcg/kg + Dexamethazone 8 mg per os. 12 hours before leucopheresis to raise the granulocyte count.
Dose : 1 Unit/ Kg.

It has already been mentioned that patients with X-linked CGD must receive transfusions of Kell negative blood products as deletion of the CYBB gene could also affect the Xk locus controlling the expression of kell antigens on the erythrocyte membrane. These patients (with the McLeod phentoype) could therefore deteriorate following repeated transfusions with K+ blood products and risk severe adverse reactions.

4.4.3 G-CSF

Patients with CGDgp 91- presenting residual NADPH oxidase activity can be given the growth factor for granulocytes during acute infection to enhance the respiratory burst of phagocytes.
Dose: 5 mcg/kg/die by subcutaneous injection. Treatment should be suspended in case of neutrophil leucocytosis (G.B.>50 x 10^9/L) and/or thrombocytopenia (PST< 100 x 10^9/L).
Transient abnormalities have been reported including haematuria, proteinuria, raised liver enzymes and anaemia.

4.4.4 Gene therapy

The outcome of a phase I clinical trial on ex vivo gene transfection using retroviral vectors of CD34+ stem cells from patients with CGD gp47phox^0 was recently published. Preliminary findings show that only a small number of positive oxidase neutrophils (1:5000) can be obtained for a short period of time (2-6 months).

This treatment could be used in the future during acute infections in CGD patients in whom even a limited production of genetically correct neutrophils could enhance the immune response.

5. PREVENTION

5.1 How to identify disease carrier status

Functional tests

Carriers of X-linked CGD can be identified by searching for a mosaic pattern of positive and negative oxidase neutrophils using the NBT test, dihydrorodamine cytometric analysis or a search for the gp91 *phox* protein. However, given the random inactivation process of chromosome X, carriers may present an almost normal pattern using these tests.
It is very difficult to identify carriers of autosomal recessive CGD as NADPH oxidase activity and expression of p47, p67 and p22 phox are normal.
Direct mutation analysis is required if p67 and p22 phox expression is normal. When the mutation is known, carrier status can be established by PCR amplification of the DNA genome and subsequent sequence analysis.

5.2 Prenatal diagnosis

Prenatal diagnosis of CGD requires certain diagnosis established in the family as all invasive prenatal diagnostic techniques carry a risk of pregnancy termination. Although this risk is low, it is only justified when there is clear evidence that the family is actually affected by the disease. Before proceeding to prenatal diagnosis, the couple must be offered genetic counselling to give them a detailed picture of disease characteristics and currently available treatments.

In the past prenatal diagnosis was based on the search for CGD biochemical changes in phagocytes obtained from cord blood by funiculocentesis at the 20th week of gestation. However, this procedure has two drawbacks: it yields information late in pregnancy with obvious psychological implications in case of termination, and there is a technical difficulty linked to the low concentration of neutrophil granulocytes in cord blood at that time of gestation.

Nowadays cloning of genes coding for the various subunits of NADPH oxidase will identify the mutation and hence allow prenatal diagnosis by DNA analysis of the chorionic villi at the 15th-16th weeks of gestation. Prenatal diagnosis is possible only when the gene mutation has already been identified in another affected family member. This is one of the reasons gene typing is not recommended for all cases identified. When the mutation is not known, analysis of DNA polymorphisms can be carried out.

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Form A date_______________

Patient's Surname______________________ Name ______________________

Date of birth I_I_I_I_I_I_I
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