

REVIEW ARTICLE

Chronic granulomatous disease: Clinical, molecular, and therapeutic aspects

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Abstract

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency caused by defects in the genes encoding any of the NADPH oxidase components responsible for the respiratory burst of phagocytic leukocytes. CGD is a genetically heterogeneous disease with an X-linked recessive (XR-CGD) form caused by mutations in the *CYBB* gene encoding the gp91^{phox} protein, and an autosomal recessive (AR-CGD) form caused by mutations in the *CYBA*, *NCF1*, *NCF2*, or *NCF4* genes encoding p22^{phox}, p47^{phox}, p67^{phox}, and p40^{phox}, respectively. Patients suffering from this disease are susceptible to severe life-threatening bacterial and fungal infections and excessive inflammation characterized by granuloma formation in any organ, for instance, the gastrointestinal and genitourinary tract. An early diagnosis of and the prompt treatment for these conditions are crucial for an optimal outcome of affected patients. To prevent infections, CGD patients should receive lifelong antibiotics and antifungal prophylaxis. These two measures, as well as newer more effective antimicrobials, have significantly modified the natural history of CGD, resulting in a remarkable change in overall survival, which is now around 90%, reaching well into adulthood. At present, hematopoietic stem cell transplantation (HSCT) is the only definitive treatment that can cure CGD and reverse organ dysfunction. Timing, donor selection, and conditioning regimens remain the key points of this therapy. In recent years, gene therapy (GT) for XR-CGD has been proposed as an alternative to HSCT for CGD patients without a matched donor. After the failure of the first trials performed with retroviral vectors, some groups have proposed the use of regulated SIN-lentiviral vectors targeting gp91^{phox} expression in myeloid cells to increase the safety and efficacy of the GT protocols.

NADPH oxidase: function and disease

NADPH oxidase is a multicomponent enzyme complex containing both cytosolic and membrane-bound proteins that work together to produce reactive oxygen species (ROS) in activated phagocytes (neutrophils, monocytes, and macrophages) (1).

The catalytic core of phagocytic NADPH oxidase consists of the flavo-cytochrome *b*₅₅₈, a transmembrane protein composed of two subunits: the catalytic glycosylated gp91^{phox} and non-glycosylated p22^{phox}. In the cell membrane, gp91^{phox} is stabilized by p22^{phox}, which is also involved in the attachment of cytosolic components of the NADPH oxidase: p47^{phox},

p67^{phox}, p40^{phox}, and GTPase-Rac. Without bacterial and/or fungal infection, phagocytes are unstimulated and the NADPH remains inactive; however, interaction with pathogens and their engulfment in the phagosome activate the complex. During the phagocytic NADPH oxidase activation (Fig. 1), phosphorylation of p47^{phox} leads to conformational changes allowing interaction with p22^{phox}. The resultant membrane translocation of p47^{phox} with the activation of GTPase-Rac assembles the other cytoplasmic subunits p40^{phox} and p67^{phox} to form the active oxidase complex. During this process, extracellular superoxide anions (O₂⁻) and hydrogen peroxide (H₂O₂) are produced in the phagolysosome (respiratory burst). ROS and proteins from granules mediate together the killing of

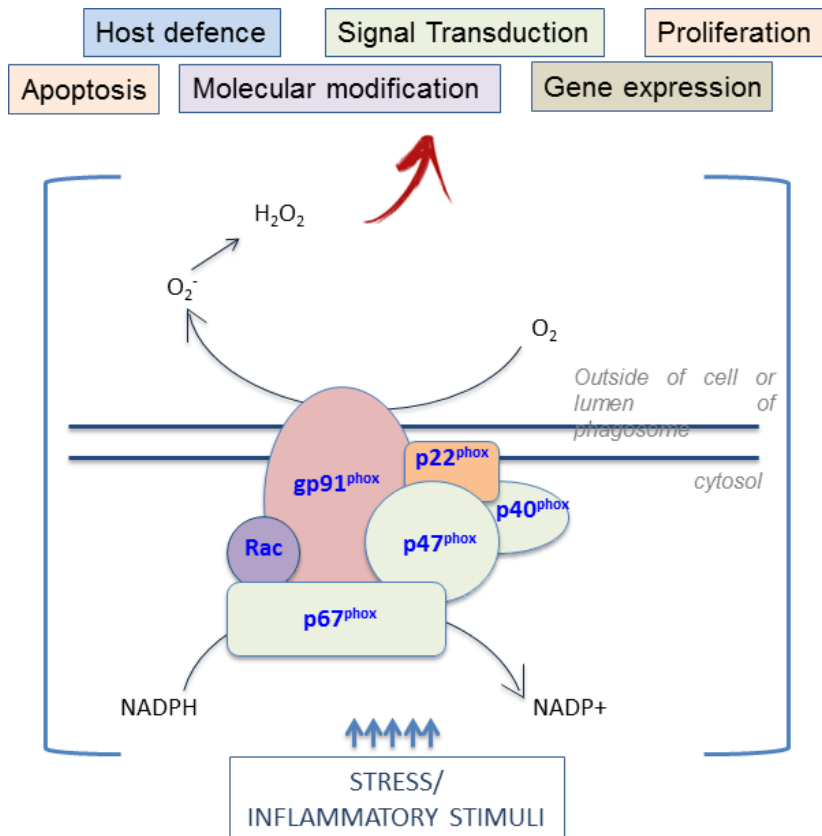


Figure 1 Scheme of NADPH oxidase system. Without stimuli, the oxidase complex remains inactive: gp91^{phox} and p22^{phox} cell membrane-bound forming the cytochrome *b₅₅₈* and p67^{phox}, p47^{phox}, and p40^{phox} in the cytosol. With inflammatory stimuli, the NADPH oxidase is activated and the phosphorylation of the cytosolic p47^{phox} subunit leads to the assembly of all cytosolic components (p67^{phox}, p40^{phox}) to p22^{phox}/gp91^{phox}. This is accompanied by the binding of Rac1/2. The active enzymatic complex transports electrons from the cytosol to the outside of the cell or the lumen of phagosome where oxygen (O_2) is converted into superoxide anions (O_2^-) and then into hydrogen peroxide (H_2O_2). Reactive oxygen species (ROS) is involved in killing of microorganisms.

the microorganisms (2). The quantification of O_2^- production can be obtained directly using the cytochrome *c* reduction assay or indirectly using the DHR test.

Professional phagocytic cells depend on functional NADPH oxidase for normal clearance of microbes. NADPH oxidase-derived ROS play two main biologic roles: They exhibit microbicidal effects when produced in large amounts in phagosomes within neutrophils and macrophages. However, they can also exert a role of second messenger by acting on a redox-sensitive cellular signaling system and thereby influencing many cellular pathways (3). Differently from its crucial role as a mediator of innate host defense, the contribution of the phagocyte oxidase to the exuberant inflammatory responses associated with chronic granulomatous disease (CGD) is poorly defined. Underlying mechanisms of hyperinflammation may include defective neutrophil apoptosis (4), skewed nuclear factor- κ B signaling (5), upregulation of tumor necrosis factor α , interleukin (IL)-17, IL-6, and granulocyte colony-stimulating factor (6), prolonged IL-8 messenger RNA activation (7), impaired activation of Nrf2 (8), defective tryptophan catabolism in mice (9), but not humans with CGD (10), decreased autophagy, and increased inflammasome activation (11).

Neutrophil extracellular traps (NETs) are a mix of externalized chromatin (DNA and histones) and cytosolic/granular proteins and may represent an important mechanism to control bacterial and fungal infection. Despite the fact that no direct

link has been described between ROS produced by NADPH oxidase and NET formation, it was extensively reported that the increased susceptibility to infection in CGD patient was linked with the impaired NETs formation (12, 13).

Although CGD patients provide the most striking evidence of the important role of phagocytic NADPH oxidase in the killing of bacteria by granulocytes, Bustamante et al. (14) recently described germline mutations in the gp91^{phox}-encoding gene (*CYBB*) conferring the phenotype of X-linked recessive mendelian susceptibility to mycobacterial disease type 2 (XR-MSMD) syndrome, but not XR-CGD. Unlike CGD, which is characterized by impaired respiratory burst in all types of phagocytes, XR-MSMD selectively affects the monocyte-derived macrophages and B cells, but not monocytes or granulocytes. This cell-specific impairment in the assembly of NADPH oxidase suggests that there may be some relation between the gp91^{phox} protein and MSMD, indicating that the macrophage respiratory burst is involved in protection against mycobacterial infections. The low levels of proinflammatory cytokines, particularly interferon gamma ($INF-\gamma$), probably induce a defect in the IL-12/ $INF-\gamma$ axis, which has a crucial role in protective immunity to mycobacteria (15).

Molecular genetic aspects of CGD

Chronic granulomatous disease is caused by defects in the genes encoding any of the NADPH oxidase components. The

X-linked recessive CGD (XR-CGD) is caused by mutations in the *CYBB* gene (ChrXp21.1) encoding the gp91^{phox} protein, while the CGD autosomal recessive forms (AR-CGD) are due to mutations in *CYBA* (Chr16q24), *NCF1* (Chr7q11.23), *NCF2* (Chr1q25), or *NCF4* (Chr22q13.1) encoding p22^{phox}, p47^{phox}, p67^{phox}, and p40^{phox}, respectively.

As stated in United States (16) and European (17) cohorts, the prevalence of CGD is between 1/200,000–250,000 live births, although the real incidence could be higher as estimations depend also on clinical expertise. Furthermore, the geographic context and social/cultural background, which influence the frequency of consanguineous marriages, change the balance between XR-CGD and AR-CGD and determine the relative distribution of the two inheritance forms in different countries. Reports from Europe (17–20), the United States (16), and Japan (21) showed that XR-CGD is the most common form of the disease (60% of cases) compared to AR-CGD (40%). About 30% of AR-CGD is due to p47^{phox} deficiency, and about 10% of cases are due to p22^{phox} and p67^{phox} deficiency (each about 5%). In contrast, both Iranian (22) and Turkish cohorts (23) reported AR as the predominant form of CGD due to the high frequency of consanguineous marriages. Generally, patients are characterized by mutations that may include small intragenic deletions/insertions, missense, non-sense, and splice-site mutations (24, 25) easily detected by sequencing. So far, large deletions and duplications are considered uncommon genetic mutation events in CGD patients, probably due to the difficulty to detect multiple exon deletions/insertions with conventional methods (sequencing, PCR-SSCP, Southern blot). Recently, array comparative genomic hybridization (array CGH) and multiplex ligation-dependent probe amplification were proposed for the detection of copy number variations (26, 27) in CGD patients also. Stasia et al. (27) reported two interesting cases of male XR-CGD patients, one with a 5.7-kb duplication spanning exons 6 and 8 of *CYBB* gene and the other with a deletion of the same region. Both mutations are probably caused by a single event of non-homologous meiotic or mitotic crossing-over between two high-similarity short-tandem-repeat (GT) repeats of introns 5 and 8.

When XR-CGD is caused by a deletion spanning the whole *CYBB* gene, the deletion can also involve adjacent genes resulting in the 'contiguous X-chromosome gene deletion syndrome' with manifestations of the McLeod syndrome (mutation in *XK* gene) (28), or Duchenne muscular dystrophy (mutation in *DMD*) (21), or X-linked retinitis pigmentosa (mutation in *RPGR*) (29). These clinical entities increase the severity of the disease and further complicate its management.

The most frequent XR-CGD mutations are missense and act on the level of gp91^{phox} expression in phagocytic cells leading to different variants: X91⁰ (protein and oxidase activity absent), X91⁻ (low levels of mutated protein and residual oxidase activity), and X91⁺ (mutated protein normally expressed but oxidase activity absent). AR-CGD mutations are associated with residual activity of NADPH oxidase in A47⁰ (protein absent), A22^{0/+} (mutated protein absent or normally expressed), and A67^{-/0} (low level or absent mutated protein).

Concerning genotype–phenotype correlation, XR-CGD patients show a more severe course of the disease with earlier presentation and diagnosis, more severe infections, and earlier death than AR-CGD patients (16). However, it is important to keep in mind that AR-CGD patients with early and extremely severe expression of the disease have also been described (17, 23). Recently, it has been reported that ROS produced by neutrophils are more predictive of survival than specific NADPH oxidase genes mutation. Indeed, a modest residual production of ROS is sufficient to confer a significantly less severe illness and a greater likelihood of long-term survival (23, 30).

Carriers and genetic counseling

Chronic granulomatous disease is characterized by mutations in a single gene following a predictable mendelian pattern of inheritance: X-linked recessive (XR-CGD) or autosomal recessive (AR-CGD). Accordingly, XR-CGD carriers are easy to identify because they have two populations of neutrophils/leukocytes, specifically positive and negative for gp91^{phox} expression and function (31, 32). Because of skewed non-random X-inactivation, some women may develop clinical evidence of CGD (33). Furthermore, skewing in myeloid cells increases during a lifetime, which means that clinical traits of the disease may develop or disappear at different ages (34).

Chronic granulomatous disease carrier detection is important not only with regard to genetic counseling for mothers and sisters of affected males and informing them about the risk of transmission, but also with regard to the treatment for the clinical manifestations. In recent years, CGD prenatal diagnosis has been performed on the fetus by sequencing; alternatively, FACS analysis offers a rapid and sensitive option when the facilities for the genetic diagnosis are not available (35, 36). Prenatal screening indicates the presence/absence of the mutation and allows parents to decide whether to carry the pregnancy to term. Moreover, CGD parents might consider having an additional child to serve as a hematopoietic stem cell (HSC) donor for their affected sibling. Recently, preimplantation genetic diagnosis combined with HLA typing has been used to identify the mutation and to select *in vitro* at the early embryonic stage the unaffected embryos (37). Despite the success obtained in XR-CGD patients treated with PDG/HLA typing and the fact that no errors in HLA typing have been reported thus far, the possibility of misdiagnosis due to technical/human errors either in HLA typing or in PDG cannot be excluded (38). At present, prenatal diagnosis is still the first option to rule out a CGD-affected embryo.

Survival rate: from the past to the present

Overall survival in CGD has changed remarkably over the last two decades and is now around 90%, reaching well into adulthood (23).

This change is due to a better recognition associated with an overall improvement of awareness concerning this disease.

There is substantial evidence that the severity of clinical manifestations of the disease reflects the degree of residual NADPH oxidase activity. Implementation of up-to-date laboratory methods, development of more effective antimicrobial agents (39), and HSC transplantation (HSCT) (40) constitute a significant step forward in the management of this disease. Infections are still a major cause of morbidity and mortality. Although antifungal therapies and prophylaxis have contributed to the improvement in survival during the last decade, the probability of contracting an invasive infection remains high (41, 42). In particular, fungal infections due to *Aspergillus* spp. remain the most common cause of mortality in CGD patients (43). Following the introduction in the 1980s of azole antifungals for the prophylaxis and treatment of fungal infections, the mortality rate decreased significantly. Before the introduction of oral antifungals, Winkelstein and colleagues (16) reported an XR-CGD mortality of *c.* 5% per year, compared with 2% per year for the AR variants. Van den Berg and colleagues (17) found 23% mortality in XR-CGD and 15% mortality in AR-CGD over almost 50 years of European survey. Mortality was higher in gp91^{phox}-deficient patients, mostly in those with lower superoxide production, which appears to still be one of the steady determinants of mortality (30).

Considering the age range at death, an overall best survival over the past 40 years has been obtained in CGD patients: In the 1970s, most deaths occurred in patients aged under 10, and in the 1980s, the mortality rate was about 50% at 10 years of age (44). Nowadays, as shown in recent studies, most of the patients survive well into adulthood (17, 18). A retrospective study on 268 CGD patients shows how age at death has increased substantially over time: In this population before 1991, the median age at death was 15.53 years; by 2001, it was 20.17 years; and by 2012, it was 28.12 years (43). Thus, the prognosis for patients with CGD appears to be much better than envisioned when the disorder was first described as a fatal granulomatosis of childhood.

Clinical manifestations

Infections

Bacterial and/or fungal infections are usually the first manifestations of CGD at the time of diagnosis. Most of the patients are infants and children aged <5 years; however, delayed diagnosis in adolescence and adulthood can occur depending on the degree of residual NADPH oxidase activity (18, 23, 45). In over 90% of patients, the respiratory burst defect is severe, resulting in poor or no production of ROS. The disease course in these patients usually begins early in life with severe or life-threatening bacterial or fungal infections (46). Although the frequency of infections in CGD patients has decreased due to the routine administration of antibacterial and antifungal prophylaxis, bacterial or fungal infections still occur at a frequency of 0.3/year (18). CGD patients are susceptible to catalase-positive microorganisms although the paradigm of catalase-dependent virulence in CGD is incomplete, because only a subset of catalase-positive pathogens is encountered in CGD (47). The most common site of infection is the lung, followed by lymph nodes, skin, liver, and gastrointestinal tract. The most frequent pathogens are *Aspergillus*, *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia*, *Nocardia*, and *Salmonella* spp. (16, 17, 43) (Fig. 2). Mycobacterial infections due to *BCG* and *Mycobacterium tuberculosis* have been reported in China, Iran, and Latin America. Patients, however, develop severe localized, not disseminated, BCG infection and pulmonary, not miliary, tuberculosis (48).

The recognition of fungal infections in CGD patients is very important because they are still a major determinant of morbidity. The most common fungal infection is invasive aspergillosis due to *Aspergillus fumigatus* followed by *Aspergillus nidulans* (43). Contrary to these filamentous molds, dimorphic mold infections such as histoplasmosis and blastomycosis do not occur in CGD, nor does cryptococcosis. Mucormycosis occurs in CGD but only in the setting of a

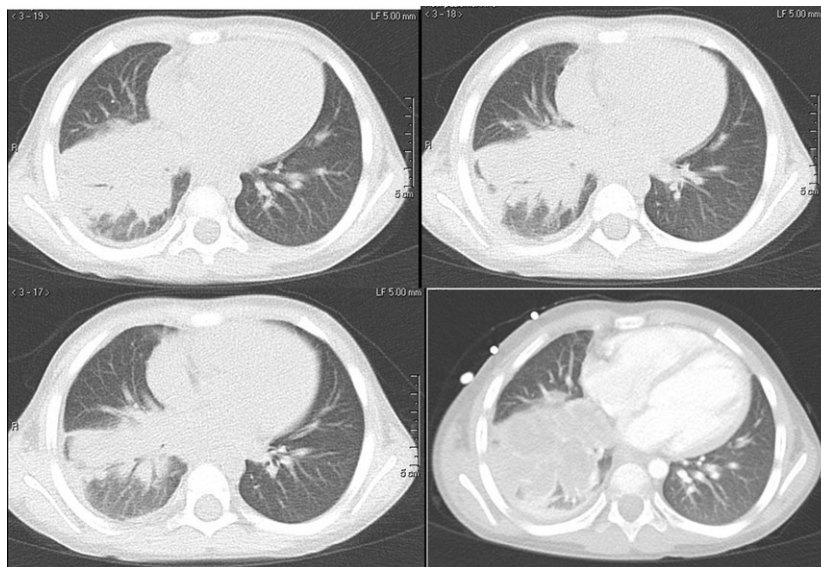


Figure 2 Chest TC findings of *Nocardia* infection in a 4-year-old CGD patient before the initiation of antimicrobial therapy.

significant immunosuppression. Much pneumonitis should always be considered in all cases of unexplained pneumonitis, especially with acute onset and hypoxia (49). In the last decades, some unusual pathogens (*Aspergillus tanneri*, *Phellinus tropicalis*, and *Granulibacter bethesdensis*) that are virtually pathognomonic for CGD have been reported (50–52), reinforcing the importance of vigorously microbial diagnostics with full assistance by a specialist microbiologic laboratory. To date, no evidence of correlation between patients' age and the onset and severity of bacterial or fungal infections has been reported. The susceptibility to infections of CGD patients arises from the neonatal period into advanced adulthood, and the outcome depends on prompt recognition of and therapy for the underlying infection (53).

Inflammatory complications

In addition to recurrent infections, inflammatory manifestations have become an increasingly relevant issue in CGD due to the increase in life expectancy of these patients. An abnormally exuberant inflammatory response leads to granuloma formation, which if it occurs in hollow viscera, may result in major complications, for instance, obstruction of the affected area. CGD granulomas are typically non-caseating and can be found in multiple organs, including the brain, lungs, liver, spleen, and gastrointestinal tract (54).

Magnani et al. (55) recently reported that gastrointestinal tract manifestations were the most frequent inflammatory complications in CGD patients, in terms of both the number of affected patients and the proportion of all episodes. The rate of GI involvement is much higher in the XR form than in the AR form as shown in a large survey of CGD patients followed at the National Institutes of Health (54). Winkelstein et al. (16) report GI involvement in 34% of CGD patients, and in the CGD *European experience*, 48% of the cohort suffered from one or more gastrointestinal tract symptoms (17). For most of these granulomas, no pathogen has been identified, and the patients respond rapidly to steroids, suggesting that the inciting event is not an invasive infectious one. The underlying

mechanism of the inflammatory dysregulation is still unknown (54) (Table 1).

Gastrointestinal tract symptoms in CGD patients are generally non-specific, such as (bloody) diarrhea, abdominal pain, nausea, vomiting, weight loss, and failure to thrive (56). Any portion of the GI tract may be involved, although the colon seems to be the site most affected, with perirectal disease especially common (54, 57). Sometimes GI involvement, and in particular inflammatory bowel disease (IBD), may be the first presentation of an undiagnosed CGD, which suggests the importance of considering CGD in the differential diagnosis of IBD particularly, but not exclusively, when it occurs early in life (57) (Fig. 3).

In addition to bowel involvement, liver involvement is frequent and important. Liver abnormalities include nodular



Figure 3 Inflammatory bowel disease (IBD)-like colitis in a CGD patient. Macroscopic appearance: colonic mucosa is hyperemic, edematous, congested with micro- and macroulcerations; spontaneous bleeding.

Table 1 Inflammatory and autoimmune manifestations in chronic granulomatous disease patients: comparison of published data

	Total number of Pts	Pts with gastrointestinal episodes	Pts with genitourinary episodes	Pts with autoimmune episodes	Pts with ocular episodes	Pts with inflammatory manifestations (%)
USA	368	124	37	41	8	57
Italy	60	4	1	4	1	17
UK	94	58	7	–	17	87
EU	429	208	98	26	8	79
Turkey	89	43	–	6	1	56
France	98	60	20	7	6	94

Autoimmune manifestations: lupus syndromes, idiopathic thrombocytopenic purpura, myasthenia, gravis, erythema nodosum, reactive arthritis, dermatomyositis, vasculitis, autoimmune hepatitis, celiac disease, sacroiliitis, rheumatoid arthritis, pericardic effusions.

Ocular manifestations: chorioretinitis, uveitis, ocular granuloma, keratitis.

Gastrointestinal tract manifestations: perianal abscess/fissure, diarrhea, stomatitis, aphthae, gingivitis/carries, colitis, pyloric outlet obstruction, esophageal granuloma/obstruction, appendicitis, pancreatitis.

Genitourinary tract manifestations: hydronephrosis, inflammatory cystitis, granulomatous orchitis, urinary tract obstruction.

regenerative hyperplasia, non-cirrhotic portal hypertension, hepatosplenomegaly, and liver abscesses (58). Liver abscesses are a common symptom of the disease, and their treatment remains a big challenge for medical teams. In the recent record of 268 CGD patients analyzed by Marciano et al. (43), liver abscesses occurred in 32% of the cohort, 47% of whom had recurrent liver infections. *Staphylococcus aureus* still represents the predominant organism isolated. Signs and symptoms are variable, the main ones being persistent fever and abdominal tenderness. Liver abscesses are often multiple, dense, and difficult to drain, and therefore in many cases, surgery is necessary (Fig. 4). Despite the low mortality rate of liver abscesses, the morbidity is still high with a high rate of recurrence. Therefore, avoiding surgery when possible seems prudent and the recent demonstration of the effectiveness of concomitant steroids and antibiotics in the complete resolution of liver abscesses offers an alternative treatment (59). It has also been reported that a subset of patients with CGD develop progressive non-cirrhotic portal hypertension, probably as a consequence of damage to the hepatic microvasculature caused by repeated liver abscesses. The authors suggest that portal hypertension contributes greatly to mortality and that a progressive decline in platelet count may be a useful non-invasive means of measuring the progression of portal hypertension (58). Genitourinary tract manifestations are also often reported in CGD (60, 61) and include bladder granulomata, ureteral obstruction, and urinary tract infection, typically in

those with gp91phox and p22phox (62). Eosinophilic cystitis, an uncommon inflammatory disorder of the bladder, has recently been reported in children with CGD (63).

Macrophage activation syndrome (MAS) represents a potential life-threatening inflammatory complication recently reported in CGD patients (64). Most of these patients developed the macrophage activation beyond the first year of life, and some in adulthood. In most of the younger CGD patients reported (<4 years), macrophage activation developed at or before the diagnosis of CGD. In the majority of cases, the macrophage activation was associated with severe infectious events and in one child suffering from CGD-associated colitis with no clinical signs of bacterial infections (64, 65). Treatment includes a wide range of MAS-directed treatment regimens, including corticosteroids alone or in combination with IVIG or cyclosporine, and generally, contrary to the familial form, MAS in CGD patients showed a good clinical outcome (64, 66).

Therapeutic options in CGD

Prophylaxis and treatment for infections

Management of CGD is predominantly with lifelong prophylactic antibiotics and antifungals (67).

Prophylactic therapy is based on daily doses of trimethoprim/sulfamethoxazole and itraconazole, which reduce the



Figure 4 Contrast-enhanced CT images of liver abscess in a 14-year-old CGD patient. Computed tomography (CT) shows the presence of a hypodense and round-shaped lesion (c. 10 mm diameter) within the VII liver segment, with fluid content and peripheral enhancement after contrast injection, compatible with liver abscess.

frequency of major bacterial and fungal infections, respectively (Table 2). Regarding the prophylactic use of INF- γ , this is still a matter of debate. More than one study demonstrates the capacity of INF- γ to reduce the number and severity of infections in CGD (68). However, a retrospective Italian study does not provide evidence justifying long-term prophylaxis with INF- γ in CGD patients (18).

Although antifungal therapy and more generalized prophylaxis are likely to have contributed to the improvement in survival over the last decade, patients experience at least one severe infection every 3–4 years, whether bacterial or fungal (69). Management of infections in the setting of CGD is rather complex. Serious infections, particularly those caused by fungi, may be asymptomatic or minimally symptomatic at presentation. Fever and leukocytosis may be absent, and elevated inflammatory markers (ESR and CRP) may be the only abnormal laboratory test. Serum galactomannan appears to be insensitive in CGD patients; this may be due in part to mold-active antifungal therapy decreasing the sensitivity of the serum galactomannan assay (70) as many CGD patients are on antifungal prophylaxis and in part related to the absence of fungal angioinvasion in CGD. Each infectious episode should be considered potentially dangerous, so every effort must be made to identify the microorganism responsible, focusing on *Aspergillus*. A definitive microbiologic diagnosis using molecular methods and susceptibility testing of difficult-to-grow organisms is essential for tailored antimicrobial therapy; if

non-invasive testing is non-diagnostic, an invasive procedure should be considered. Frequent imaging evaluation with plain radiographs, ultrasound scan, computed tomography (CT), or magnetic resonance imaging (MRI), depending upon the suspected organ involved, is critical to making early diagnoses and to follow the evolution of infections until resolution and/or stabilization of the lesions. Whole-body PET/CT scans can be useful to localize occult infections for biopsy and to exclude dissemination (71).

Treatment for acute infections must be aggressive: 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 (Table 2). Initial empirical therapy should include at least two antibiotics against Gram-positive and Gram-negative bacteria. In case of failure to respond within 48 h, empirical changes in antibiotic coverage may be needed before definitive pathogen identification, including the administration of an antifungal drug, if not administered from the very beginning. Treatment should be continued for weeks or months even when there is a significant improvement in the inflammatory index and the patients' clinical condition to eradicate the infection completely.

If a fungal invasive infection is identified or strongly suspected, intravenous voriconazole is recommended as initial treatment. Voriconazole serum concentrations present great variability, and drug monitoring is recommended to

Table 2 Treatment aspects in chronic granulomatous disease (CGD)

Prophylaxis of infections	<i>Trimethoprim/sulfamethoxazole (co-trimoxazole)</i>: 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 sulfonamide 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 <i>Itraconazole</i>: children 10 mg/kg die per os twice up to a maximum dose of 200 mg/die. Adult: 10 mg/kg die per os twice up to a maximum dose of 400 mg/die
Immunizations	The only routine immunization that adults and children with CGD should not have is BCG as it has been associated with BCG infection. An annual flu vaccine is recommended (over 6 months of age), because of the possible secondary bacterial complications of influenza
Management of infection	Empirical treatment • Initial treatment is with empirical broad-spectrum antibiotic treatment, including coverage of Gram-positive as well as Gram-negative organisms until pathogen identification • First-line agents: meropenem 60–100 mg/kg/die plus Ciprofloxacin: 20 mg/kg/die • Fungal infection voriconazole 16 mg/kg/die Liver abscess due to <i>Staphylococcus aureus</i> • Echo-guided needle aspiration • Linezolid 10 mg/kg/die or rifampicin 20 mg/kg/die and teicoplanin 10 mg/kg/die • If treatment fails: methylprednisolone 1 mg/kg/die, then slow taper Liver abscess due to <i>Aspergillus</i> spp. • Echo-guided needle aspiration • Voriconazole 16 mg/kg/die
Treatment for chronic granulomatous colitis	Mesalazine 50 mg/kg/die Prednisone 1 mg/kg/die, then slow taper Azathioprine 2.5 mg/kg/die Short course of metronidazole and ciprofloxacin

document bioavailability and efficient blood levels (72). Severe photosensitivity leading to squamous cell carcinoma and melanoma has been reported with long-term voriconazole treatment. Therefore, voriconazole should be used carefully for durations >6–9 months, particularly among patients with risk factors for skin cancer. In patients requiring prolonged voriconazole, diligent skin examinations, avoidance of excess sunlight, and liberal use of UV protectants are advisable (73).

When infections are refractory to voriconazole or when there is intolerance, intravenous liposomal amphotericin B and caspofungin have been shown effective. Posaconazole, an orally well-tolerated broad-spectrum triazole antifungal agent, has proven efficacy as prevention of and salvage therapy for invasive fungal infection (74). Unlike voriconazole, whose absorption is affected by food as well as potential interactions with concomitantly administered therapies, the new posaconazole tablets and capsule formulations have demonstrated improved bioavailability and are minimally affected by food (75).

When the fungal cause is uncertain, combined antifungal therapy can be considered. The most frequent combinations are voriconazole and caspofungin combination or caspofungin and liposomal amphotericin B. In addition to systemic antifungal treatment, surgical debridement or excision of consolidated infection is advised when possible. In case of multidrug refractoriness, life-threatening infections (e.g., aspergillosis), hematopoietic stem cell transplantation (HSCT) with reduced-intensity conditioning represents a valid curative option (76). Alternatively, a combination of sequential GT, leading to a transitory beneficial effect on pre-existing infections, and rescue HSCT has been proposed (77).

Corticosteroids as part of CGD treatment have previously been controversial and have been used principally for the treatment of inflammatory complications. There are now several reports for the successful use of steroids during acute infections when coadministered with appropriate antimicrobials. Mulch pneumonitis, invasive pulmonary *Nocardia* infection, and liver abscess appeared to benefit from steroids therapy when administered along with adequate antimicrobials (78, 79).

Hematopoietic stem cell transplantation

Since the beginning of the 21st century, there have been many reports on HSCT in patients with CGD and the encouraging results obtained with regard to survival rate, engraftment, and graft-versus-host disease (GVHD). In particular, it is notable that HSCT can cure CGD and reverse organ dysfunction (80). Three key points have been investigated during this period in CGD patients undergoing BMT: when to transplant (age and indications), which kind of donor to select, and which conditioning regimen to use.

There is continuing controversy about indications and optimum timing of HSCT in CGD. Patients with absent NADPH oxidase activity and poor prognosis have been recommended for early HSCT. Present HSCT indication

criteria in children are as follows: one or more life-threatening infections, non-compliance with antimicrobial prophylaxis, or steroid-dependent autoinflammation. Indication criteria in adolescents and young adults are more difficult to apply because organ dysfunction is frequent and transplant-related mortality after HSCT has been high (76). Myeloablative HSCT has been widely applied in CGD patients during the last decade with reports of excellent survival (81). However, besides the risks of myeloablative conditioning, survivors of myeloablative transplants are prone to long-term problems such as infertility (82). Ideally, reduced-intensity conditioning regimens would permit safe transplantation in high-risk patients while preventing long-term sequelae of high-dose chemotherapy (e.g., infertility). Unfortunately, previous efforts to apply reduced-intensity conditioning regimens in CGD were complicated by high rates of graft failure and GVHD (83, 84). In 2014, Güngör et al. (76) published a large prospective multicenter study, which included 56 patients, aged 0–40 years (median age: 13 years), using a reduced conditioning protocol. Importantly, 42 of the 56 were considered high-risk patients who are generally not deemed to be candidates for myeloablative HSCT. The results are impressive with a 2-year overall survival of 96% and event-free survival of 91%. The cumulative incidence of severe acute GVHD grades III–IV was only 4% and chronic GVHD was 7%. Stable myeloid donor chimerism was found in 93% of surviving patients. Furthermore, two adult patients were subsequently able to have children, showing that fertility might be preserved, at least in men.

When HSCT was first used in CGD patients, matched sibling bone marrow was the preferred source of stem cells. However, the probability of finding a matched sibling donor (MSD) is low (81). Nowadays, as confirmed in previous reports, outcomes with perfectly HLA-matched unrelated donors (MUD) are almost comparable with those using HLA-MSDs (40). Unfortunately, searches for a matched bone marrow or PBSC donor in the family or in unrelated adult donor registries could be unsuccessful. Thus, umbilical cord blood could be an option for these patients but available results concerning CGD patients are limited. At present, most authors concur that all patients with XR-CGD or AR-CGD with no residual oxidase activity should be offered HSCT as early as possible, with either an MSD or MUD.

Gene therapy for CGD

Gene therapy for hematopoietic cells (GT-HSC) represents an attractive alternative to HSCT as therapy for CGD patients without a matched donor. The observation that XR-CGD carriers with $\geq 10\%$ of normal neutrophils were healthy suggested that a minor functional correction of neutrophils would be sufficient to restore a normal phenotype in GT-HSC-treated patients.

Nevertheless, GT-CGD is difficult to perform because corrected HSC do not have a selective growth advantage compared to deficient cells and, at the same time, a large number of cells need to be corrected to ensure a good

restoration of neutrophil activity. Thus, myeloablative conditioning is necessary to ensure an efficient engraftment of progenitor cells (85).

Although recently Flynn et al. (86) proposed the site-specific clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 system to encourage repair of the endogenous gene by enhancing the levels of homologous recombination, to date GT using autologous BMT of HSC modified with retroviral vectors to express wild-type copy of mutated gene was favored for XR-CGD patients.

The first CGD clinical trial performed in the USA using γ -retroviral vector failed due to the lack of conditioning (87). Subsequently, clinical phase I/II GT trials for X-CGD (Frankfurt, Zurich, London, NIH and Seoul) were carried out using non-myeloablative conditioning and γ -retroviral gp91^{phox} vector. Initially, treated patients showed a restoration of the gp91^{phox} expression and functionality. However, the cell engraftment progressively decreased probably due to gene methylation events. In addition, myelodysplastic syndrome (MDS) with monosomy 7 and insertional mutagenesis with the transactivation of protooncogene MDS-EV11 and PRDM16 occurred in three patients (88–90). These results suggested that the ectopic expression of the gp91^{phox} transgene, as well as the highly activated bone marrow environment caused by CGD patients' chronic infections, promoted the loss of gene-corrected cell engraftment (91). To increase the safety and efficacy of the XR-CGD GT protocols, different groups proposed the use of the self-inactivating lentiviral vectors (SIN-LVs) with an internal promoter (92, 93). SIN-LVs integrate efficiently in HSC, allowing robust transgene expression and a safer profile of integration compared to γ -retroviral vectors. Santilli et al. (93) showed a restoration of NADPH oxidase activity in preclinical studies using a transcriptionally regulated LV where the gp91^{phox} protein was controlled by a synthetic chimeric myeloid promoter obtained by the fusion of cathepsin G and c-Fes minimal 5'-flanking regions and containing PU.1 and c/EBP α . A clinical trial with this vector is currently recruiting patients (92).

Recently, microRNAs were proposed as an alternative to post-transcriptionally regulating the therapeutic gene expression in GT protocols. Interesting results were obtained by Chiriaco et al. (94) with a novel XR-CGD GT approach based on the use of dual-regulated LV in which transcriptional (myeloid-specific promoter) and post-transcriptional (miR-126-restricted) regulations were combined to avoid the ectopic expression of the gp91^{phox} transgene. This regulated LV was shown to induce high levels of myeloid-specific transgene expression while entirely sparing the CD34⁺ HSC compartment, thus avoiding HSC toxicity. This dual-target LV construct represents a promising candidate for further clinical development.

Conclusion

Life expectancy of CGD patients has increased considerably thanks to progress in diagnosis and management. However, the morbidity of recurrent infections remains a major issue, and inflammatory manifestations have become an increasingly relevant issue in CGD patients. Autoimmune manifestations are also frequently found in patients and in carriers. The residual NADPH oxidase activity, measured by the dihydrorhodamine test, is an important parameter showing the severity of clinical manifestations and is predictive of survival (23).

The advent of highly active antifungal therapy has led to a significant reduction in mortality; however, the emergence of triazole-resistant *Aspergillus* and some new emerging pathogens remain difficult to treat and are important contributors to mortality. Treatment for inflammatory complications is often long term and difficult, and despite the immunocompromised condition of patients with CGD, low-dose steroids and/or other potent non-steroidal anti-inflammation agents are often beneficial or necessary in these conditions.

Although a conservative treatment has improved including infection and inflammatory management, complications and their consequence can accumulate over time. Children who have undergone HSCT are healthier than their counterparts conservatively managed, with few infections.

Allogeneic transplantation has also improved dramatically over the last decade because of improved conditioning regimens and GVHD prophylaxis, high-resolution sequence-based matching, and improved post-transplantation management. At present, most authors concur that all patients with XR-CGD or AR-CGD with no residual oxidase activity should be offered HSCT as early as possible, with either a MSD or MUD.

In addition to phagocytes, NOX 2 oxidase is expressed in a broad spectrum of cells and generation of moderate amounts of ROS has been described by several non-phagocytes leukocytes, including B lymphocytes and dendritic cells (95), suggesting that defects in these cells may have effects on the disease. In that context, we have recently reported that patients with CGD present a defective B-cell compartment in terms of frequencies of memory B cells, response to *in vitro* stimulation, and maintenance of long-term antigen-specific memory B cells (96). Furthermore, beyond its role in immune defense, it is now recognized that phagocytic NADPH oxidase is expressed in a wide variety of cells unrelated to the immune system, including endothelial cells, vascular smooth muscle cells, platelets, fibroblasts, and kidney, and that it might contribute to other important biologic processes.

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