Chronic granulomatous disease – primary phagocytic immunodeficiency

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Abstract
Chronic granulomatous disease (CGD) is a rare hereditary primary immunodeficiency, in which defective production of microbicidal oxidants by phagocytes (neutrophils, eosinophils, monocytes, and macrophages) leads to severe recurrent infections. This article reviews pathogenesis, diagnostic criteria, clinical signs, and treatment of CGD.

Key words: chronic granulomatous disease.


Introduction
Chronic granulomatous disease (CGD) is an inherited disorder affecting the phagocytic cells and characterized by the absence of a respiratory burst that results in recurrent, sometimes life-threatening, bacterial and fungal infections. Although it is a rare primary immunodeficiency, it creates serious clinical problems connected with long – term therapy and in most cases with bad prognosis and high mortality. The cause of CGD is well documented. An early diagnosis and a prophylactic treatment are very important. The pathogenesis, diagnostic criteria, clinical signs, and treatment for CGD have been reviewed in this study.

Etiology
Chronic granulomatous disease was first described as “fatal granulomatosis of childhood” in 1957 in boys who died in early childhood with signs of severe infections, hepatosplenomegaly, lymphadenopathy, hypergammaglobulinemia and infiltration of granulomas in many organs [1]. In 1967 the underlying cellular mechanism that causes CGD was described by Baehner and Nathan [2]. They found that the intact leukocytes of two children with CGD don’t increase oxygen consumption due to diminished oxidase for reduced nicotinamide adenine dinucleotide and fail to reduce nitroblue tetrazolium during phagocytosis.

Acute or chronic infection is associated with an increased neutrophil’s influx into the tissue where the neutrophils play important role in nonspecific immune responses and host defenses. Oxidative products, such as hydrogen peroxide, are generated by the phagocytes in the respiratory burst process in order to kill ingested microbes. CGD is a dysfunction of the production of microbicidal oxidants by phagocytes (neutrophils, eosinophils, monocytes, and macrophages) [3]. Phagocytic cells with defects in respiratory burst activity usually have normal adhesion, chemotaxis and phagocytic uptake but abnormal intracellular microbicidal activity. The persistence of microorganisms within the phagosomal vacuoles are the stimulus to the prolonged and a recurrent inflammatory state and responds slowly to antimicrobial agents. Phagocytes require a cellular enzyme – nicotinamide adenine dinucleotide phosphate (reduced form: NADPH) oxidase (termed: “phagocyte NADPH-oxidase” – phox) and generated by itself reactive oxygen species (ROS) to destroy the ingested microbes [4]. NADPH oxidase system is a multiprotein enzyme including the membrane-associated proteins (gp91phox, p22phox), NADPH oxidase, flavocytochrome b558, cytosolic proteins (p47phox, p67phox, p40phox), and two small G proteins (Rac2 and Rap1A/B).

CGD is a very heterogeneous disorder. CGD is a result of molecular defects (absence, malfunctioning, low expression) in one of the four essential subunits of phox (gp91phox, p22phox, p47phox, p67phox) leading to decreased or complete absence of phagocytes oxidative burst. There are two main types of CGD – X-linked and autosomal recessive [5-7], but over 410 possible defects in the phox enzyme
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complex that can lead to chronic granulomatous disease are known [8]. On the basis of molecular data it was noticed that most cases (about 70%) of CGD patients have a mutation of CYBB gene on the X chromosome which codes the glycoprotein gp91phox (one of the two subunits of the flavocytochrome b558 of the NADPH oxidase) called an X-linked or X-CGD [9]. In the most of cases flavocytochrome b558 is absent – X(9)0. In some rare cases, the mutated gp91phox is normally expressed but no NADPH oxidase can be detected X91(+) [10], and less than 10% of patients have a form with low levels of expression of flavocytochrome b558 – X(9)1(-) [11]. Several novel mutations in the CYBB gene are identified in about 10% patients [12, 13]. The autosomal recessive forms of CGD have mutations in the genes encoding components p47phox (chromosome 7), p22phox (chromosome 16), and p67phox (chromosome 1) [14]. Mutation in the p47phox gene (NCF-1) is present in about 30% of the patients with CGD and has a single deletion in more than 90% of patients [15]. About 10% of all cases of CGD have mutation in the p22phox (CYBA) [16] and p67phox (NCF-2) [17]. The mutation affecting p40phox in CGD patients has not yet been reported.

ROS have been implicated also as signaling molecules in various processes e.g. in activation of the transcription factor NF-xB. Bylund et al. describe that in CGD leukocytes display a hyperinflammatory phenotype with increased production of proinflammatory cytokines in response to stimulation with Toll-like receptor agonists. These data indicate that ROS were nonessential for activation of NF-xB [18].

Neutrophils have been demonstrated in numerous studies to play a role not only in cellular immunity, but also in the pathogenesis of host tissue damage. This unfavorable effect is mainly attributed to proteolytic enzymes and free oxide radicals released by neutrophils. Elastase is thought to be one of the most potent proteolytic enzymes present in azurophil granules of neutrophils. Free elastase plays an important function in degrading phagocyted substances. After being released from neutrophils elastase is very quickly inactivated by natural inhibitors in systemic fluid mainly by α1-proteinase inhibitor. Excessive amount of unbounded elastase may result in the enzyme becoming hazardous for surrounding tissues mainly the essential elements of the interstitium. It is known that free radicals inactivate neutrophil elastase and its inhibitors [19]. The studies indicate a hyperactivity of the CGD neutrophils, which may be one of several pathways leading to tissue injury. Tintinger et al. found that neutrophils from blood of CGD subjects show accelerated Ca2+ influx resulting in hyperactivation of the cells according to excessive release of elastase [20]. In CGD patients, when there is a lack of free radicals, the high intracellular concentration of free elastase may be favorable for killing ingested bacteria, especially Gram-negative. But on the other hand, overstimulation of neutrophils may lead to uncontrolled destruction of the host tissues, chronic inflammatory conditions and other complications.

The data indicate that myeloperoxidase (also the important enzyme for killing microbes) activity in CGD leukocytes is normal [21]. Interleukin-8 (IL-8), a pro-inflammatory cytokine produced by various cells, is a potent chemoattractant for neutrophils. Levels of IL-8 were significantly raised in various disorders. When neutrophils from healthy subjects were treated with catalase (H(2)O(2) scavenger) or diphenyleneiodonium chloride (NADPH oxidase inhibitor) exhibit IL-8 responses comparable to those of CGD neutrophils were found. But when cells were stimulated only with formyl-Met-Leu-Phe (fMLP) in CGD patients increased production of IL-8 and a sustained IL-8 mRNA response was observed [22]. These results indicate that elevated concentration of IL-8 in CGD may raise influx of neutrophils into inflammatory focus and contribute to creating a vicious circle and support the inflammation. Warris et al. describe that in blood of CGD patients after stimulation with Aspergillus fumigatus concentration of both proinflammatory cytokines [tumour necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6)] and anti-inflammatory cytokine [interleukin-10 (IL-10)] was elevated. Authors suggest that this dysregulation between pro- and anti-inflammatory cytokines contributes to the increased susceptibility to invasive aspergillosis [23]. Sanford et al. found that patients suffering from CGD had abnormal apoptosis and abnormal clearance of apoptotic cells, which may be a cause of prolonged infections and an increased risk of developing lupus [24].

Microscopic examination

The changes observed in microscopic examination of biopsy specimens from CGD patients present active suppurative inflammations, sometimes with granuloma formation as a result of chronic inflammation. Neutrophils are the predominant cells. The pigmented macrophages and histiocytes containing golden-brown granular deposits (lipofuscin) are more characteristic findings. Histochemical stains show that this material is composed of unsaturated fatty acids, phospholipids, and glycoproteins [25-27].

Demographic findings

Symptoms usually first appear during early childhood but sometimes not until adolescence. The initial diagnosis of CGD is at a median age of 5 years [28, 29]. CGD appear in males four times more often than in females. No racial or ethnic predilection is reported. The frequency of occurrence of all CGD forms is estimated at about 1 case in 220 000-500 000 live births (X-CGD – 1 : 250 000; p47phox: 1 : 500 000, p22phox: < 1 : 2 000 000, p67phox: < 1 : 2 000 000). Analysis of the data indicate that both morbidity and mortality rates are highest in X-linked form CGD. However, Mouy et al. have not found difference in survival rates between patients with X-linked and those
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Pathogenic microorganisms in CGD

Most infections in CGD are caused by catalase-positive microorganisms that produce catalase to protect themselves against the hydrogen peroxide generated by the macrophages and neutrophils that engulf them. Patients suffering from CGD are usually infected by Staphylococcus aureus, E. coli, Klebsiella species, Salmonella, Burkholderia cepacia (Pseudomonas cepacia), Serratia marcescens, Legionella, Chromobacterium violaceum, Mycobacterium species, Candida glabrata, Candida albicans, Aspergillus fumigatus, Aspergillus nidulans, Nocardia species [6, 29, 30]. In past few years significantly more infections are caused by fungi. Almost all episodes involved pulmonary infection. Aspergillus fumigatus is more common pathogen in CGD compared with Aspergillus nidulans, but Aspergillus nidulans is more virulent and is significantly more likely to result in death compared with Aspergillus fumigatus [6]. Dorman et al. described 28 episodes of Nocardia infection in 23 patients with CGD. Patients receiving prophylaxis with IFN-gamma and/or a sulfonamide were significantly less likely to have disseminated nocardiosis. Nocardia infections in patients with CGD are not usually fatal if treated properly [32].

Diagnosis

The diagnosis of CGD may be made based on the presence of the clinical history, characteristic syndrome, and laboratory features [33]. Specialized laboratories perform various tests of the function of phagocytes. Diagnosis of CGD according to European Society of Immunology (ESID) criteria [33] is present in Table 1.

Biochemical diagnosis of CGD

Bactericidal and fungicidal capacity of leukocytes may be evaluated by different biochemical superoxide release assays, which are used in the initial diagnosis of patients with CGD or X-linked carriers.

The sensitive diagnostic screening of oxidase function test for CGD is cytochemical nitroblue tetrazolium (NBT) reduction test (expressed as percent reducing neutrophils) [34]. It depends upon the direct reduction of nitroblue tetrazolium by ROS to form an insoluble formazan. A clear yellow dye, NBT is normally reduced by neutrophil metabolism, resulting in a color change from yellow to blue. Quantifying this color change estimates the degree of neutrophil metabolism. One of the versions is the NBT slide test [35] in which a drop of whole blood is placed on a microscope slide coated with an activating agent, such as lipopolysaccharide. It may be also improved by precoating coverslips with phorbol myristate acetate (the NBT-PMA slide test) [36]. In CGD patients absent reaction is observed or fewer than 5% of the cells are positive compared to about 100% positive cells in healthy subjects. However, NBT test is not specific enough to detect phagocyte dysfunctions in the oxidative metabolism. False-negative test occurs when formazan accumulates in cells with low levels of active NADPH oxidase. There are described cases with normal NBT slide test in X-linked CGD patients [37]. NBT slide test does not detect carriers of autosomal recessive mutations.

### Table 1. Diagnosis of CGD according to European Society of Immunology (ESID) criteria

<table>
<thead>
<tr>
<th>Probable diagnosis</th>
<th>Definitive diagnosis</th>
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<tr>
<td>Male or female patient with abnormal NBT or respiratory burst in activated neutrophils (less than 5% of control) who has one of the following:</td>
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<tr>
<td>1. Deep seated infection (liver, perirectal or lung abscess; adenitis; or osteomyelitis) due to Staphylococcus, Serratia marcescens, Candida or Aspergillus.</td>
<td>1. Mutation in gp91, p22, p47 or p67 phox.</td>
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<tr>
<td>2. Diffuse granulomatata in respiratory, gastrointestinal or urogenital tracts.</td>
<td>2. Absent mRNA for one of the above genes by northern blot analysis.</td>
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<tr>
<td>3. Failure to thrive and hepatosplenomegaly or lymphadenopathy.</td>
<td>3. Maternal cousins, uncles or nephews with an abnormal NBT or respiratory burst.</td>
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The manifestation of the microbial activity (respiratory burst activity) of the cell may be chemiluminescence (CL), the process of photon emission during activation of leukocytes [38]. It is used in the diagnosis of patients with CGD or X-linked carriers. Phagocytes from patients with CGD do not produce response or have extremely low luminoi/lucigenin – dependent chemiluminescence production after activation by opsonized zymosan, PMA or FMLP [39]. This method may be used to differentiate MPO deficiency and CGD. In neutrophils from MPO-deficiency it is increased, whereas in CGD the response is decreased.

The respiratory burst of neutrophils may be measured by flow cytometry with fluorescent probes such as 2′7′-dichlorofluorescein diacetate (DCF), 5,6-carboxy-2′f′-dichlorofluorescein diacetate.bis (acetoxymethyl) ester (C-DCF) or dihydrorhodamine 123.

Dihydorhodamine 123 (DHR) assay using flow cytometry is in a highly sensitive method and may help to differentiate between the X-linked and autosomal recessive forms. Phagocytic cells reduce DHR to the strongly fluorescent compound rhodamine [40, 41]. The DHR assay may be performed in PMA stimulated and non-stimulated neutrophils [42]. This test may be also positive (decreased DHR signal) in MPO-deficient patients when neutrophils are investigated, but eosinophils (functionally normal in these cases) show an enhancement of the DHR signal [43]. Vowells at al. demonstrate that DHR test is more sensitive than DCF or C-DCF in detection of ROS for evaluating patients and carriers [44].

Quantitative superoxide released by phagocytes may be assessed by a superoxide dismutase-inhibitable cytochrome c reduction assay [45]. CGD patients have less than 10% of the amount of superoxide released than healthy control.

Molecular diagnosis

To clarify the CGD genotype, additional tests should be made. Molecular methods including polymerase chain reaction (PCR), single-strand conformational polymorphism (SSCP), Southern, Northern, and Western blots, DNA fingerprinting, and nucleotide sequencing may be performed.

Gene expression may be assessed by reverse transcription – polymerase chain reaction (RT-PCR) analysis in EBV-transformed B lymphocytes from CGD patients [46]. Western blot analysis detects a specific phox protein by means of antibodies. Absent mRNA for one of the genes may be detected by Northern blot analysis, fragments of DNA by Southern methods. For a final diagnosis, a genetic diagnosis should be also established in maternal cousins, uncles, or nephews with an abnormal biochemical tests.

Prenatal diagnosis

Prenatal diagnosis can be considered in pregnant CGD carriers that may be used for antenatal diagnosis of affected boys, carrier females and may help to differentiate between X-linked and autosomal recessive forms of disease. Moreover, genetic correction of an affected fetus is now a goal within reach [9].

Tests which may be performed on fetal blood (obtained by umbilical venous puncture under fetoscopy at the 20th gestational week) or in amniocytes are the following: NBT, cytotoxicity test with PMA as an activator (PMA-stimulated NBT test) [47], luminol enhanced chemiluminescence with activation by serum opsonized zymosan or PMA [48], superoxide anion (0-2) production by measurement of the superoxide dismutase inhibitable reduction of cytochrome c with PMA as activator [49] and using a denaturing high-performance liquid chromatography (DHPLC) system [50]. Prenatal diagnosis of CGD may be performed with restriction fragment length polymorphism (RFLP) analysis using probes flanking the gene [51, 52].

Chorionic villus sampling performed to obtain enough DNA to identify affected fetuses is preferred for the diagnosis because of its applicability early in gestation and reduced risk of fetal loss.

Differential diagnosis

Other diagnoses to consider include: leukocyte adhesion deficiency (LAD), Bruton agammaglobulinemia, common variable immunodeficiency (CVID), complement deficiency, human immunodeficiency virus infection (HIV), hyperimmunoglobulinemia E – (Job syndrome), severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome, hyper-immunoglobulin M (IgM) syndrome, Wegener’s granulomatosis, sarcoidosis.

Signs

CGD is a serious and sometimes life-threatening disease. CGD is usually manifested as several to long term and recurrent bacterial and fungal infection and forming of granulomas [30, 29]. The retrospective data show that once signs of CGD occur in early childhood, but diagnosis is made late, sometimes, although rarely, in adulthood [53, 54].

X-linked CGD patients are diagnosed significantly earlier and have a more serious clinical course than patients with the autosomal recessive forms. Fungal infections occur in up to 20% of patients. Infections may be locally invasive or disseminated.

This disease is characterized mainly by infections of the respiratory tract [55], gastrointestinal tract [56], cutaneous disease (occurs in 60% to 70% of cases) [26], chronic lymphadenopathy [30, 55], but may reach any part of the body. Recurrent and often multiple abscesses of deep organs most often occur in hepar and spleen [30, 57], and around the anus [29]. Some disorders that rare occurred in CGD are: brain lesions [27], hydrocephalus and brain granulomas [58], meningitis [59], chorioretinal lesions [60].

A characteristic manifestation of CGD is the development of granulomas as the result of chronic inflammation.
Table 2. Diseases/disorders that occurs in CGD patients

<table>
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<tr>
<th>Disease</th>
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<tr>
<td>Fever of unknown origin (FUO)</td>
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<tr>
<td>Lymphadenitis/chronic lymphadenopathy</td>
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<tr>
<td>Superficial skin infections</td>
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<tr>
<td>Gum disease</td>
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<tr>
<td>Infection of upper and lower respiratory tract</td>
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<tr>
<td>Gastrointestinal infection</td>
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<tr>
<td>Urinary tract infection</td>
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<tr>
<td>Hepatosplenomegaly</td>
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<tr>
<td>Bone infections/osteomyelitis</td>
</tr>
<tr>
<td>Brain lesions</td>
</tr>
<tr>
<td>Abscesses of the skin, liver, kidney, spleen, lungs, bones, brain, around the anus</td>
</tr>
<tr>
<td>Bacteremia/fungemia/septicemia</td>
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<tr>
<td>Granulomas – tumor-like masses in the skin, lungs, lymph nodes, liver, bones, gastrointestinal tract, urinary tract</td>
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Sometimes the granuloma formation may cause obstruction of gastrointestinal tract or urogenital tract. Symptoms in this case include abdominal pain, dysphagia, nausea, vomiting, bloody diarrhea, dysuria or urinary retention, haematuria [56].

Some of the diseases/disorders that occur in CGD patients are presented in Table 2. Phagocyte morphology, phagocytic chemotaxis, cell-surface adhesion proteins, and phagocytosis in CGD patients are normal. Also, other cell-mediated and humoral immunity are usually correct, although some patients have hypergammaglobulinemia. Recurrent liver, lung or urinary tract infection may eventually cause abnormal function studies. Other blood abnormalities (as a sign of chronic infection or inflammation) includes increased number of white blood cells, elevated erythrocyte sedimentation rate and low number of red blood cells.

The quality of live may be deteriorated by the chronic illness and frequent hospitalizations. Data indicate that about 25% CGD patients have cognitive deficits (IQ of 70 or below) [61]. The CGD patients also may grow slowly.

Treatment

Treatment may involve pharmaceuticals and general care. Conventional therapy consists of prophylactic and intensive management that lead to limitations of morbidity and mortality of CGD patients. CGD is on the list of diseases to use the gene therapy and in utero stem cell transplantation.

Prophylaxis

Most patients before the diagnosis are treated only symptomatically. Several studies have shown that continuous (long-term) antimicrobials and antifungal prophylaxis is effective in preventing recurrent inflammation, reduced number of complications, and have influence on improve the prognosis of this disease [29].

The fundamental medicine applied in prophylaxis of CGD is trimethoprim-sulfamethoxazole (TMP-SMZ). The data show that long-term oral treatment with TMP-SMZ, decrease the incidence of nonfungal infections without increasing the incidence of fungal infections both with autosomal CGD and in X-linked CGD patient [62]. Daily oral prophylaxis with TMP-SMZ (adult dose based on: TMP – 5 mg/kg body weight per day, SMZ – 18-30 mg/kg body weight per day in 2 divided doses; pediatric dose – administered as in adults) is recommended. TMP-SMZ has a broad spectrum of microbicidal activity (by inhibiting synthesis of dihydrofolic acid which leads to the failure of bacterial growth) and a high selective concentration within phagocytes. Tsuji et al. describe that production of highly reactive nitric oxide (NO) by neutrophils from CGD patients treated long-term with TMP-SMZ was significantly increased. Results of data indicate that NO plays an important role in bactericidal activity in these patients [63].

In sulfa-allergic patients – dicloxacillin (20-25 mg/kg body weight per day) may be used for antibiotic prophylaxis. Fungal infections in CGD patients have been reported to account for approximately 20% of infections. Currently, among drugs for prophylaxis against fungal infections itraconazole is recommended (adult dose: per os – 200 mg; not to exceed 400 mg/day; pediatric dose (body weight < 50 kg): 5 mg/kg body weight/day; not to exceed 100 mg/day). A fungicidal effect is cause by alteration of RNA and DNA metabolism, the permeability of the fungal cell membrane and an intracellular accumulation of peroxide that is toxic to the fungal cell. A retrospective analysis indicated that itraconazole administered orally for the prophylaxis against aspergillosis has been shown to be more effective than treatment with ketoconazole [30, 64].

Another standard treatment for the prevention of infection in CGD approved by the Food and Drug Administration (FDA) is human recombinant interferon-gamma (rIFN-gamma) as “biologic response modifier” [65]. rIFN-gamma improves bactericidal activity in neutrophils and monocytes [66] e.g. stimulates nonoxidative bactericidal pathways. Similarly as TMP-SMZ, an increased production of NO from neutrophils was found in patients receiving interferon-gamma [67]. Other data suggest that IFN-gamma can reprogram the myeloid progenitor cells to express a partially corrected phenotype [68]. Condino-Neto and Newburger found that...
IFN-gamma partially corrects a nuclear processing defect due to the intronic mutation in the CYBB gene by augmentation of nuclear export of normal transcripts, and by improvement in the fidelity of splicing at the first intron. Incubation with IFN-gamma produced a 3-fold increase in CYBB total messenger RNA levels in the CGD patients cells, and decreased nuclear transcripts [69]. Treatment with rIFN may be associated with increases side effects as flu-like illness, fever and diarrhea. But data show that in most cases it is well-tolerated treatment, reduces the frequency of serious infections and is safe for a long-term (even a few years) prophylaxis therapy (50 mcg/m2/dose received subcutaneously 3 times per week) [70, 71].

Reconstitution of bactericidal activity of CGD granulocytes can be possible by using glucose-oxidase-containing liposomes [72].

**Therapy**

Early and aggressive treatment of infections with broad-spectrum systemic antibiotics and antifungal agents is very important. In cases when abscesses are occurring, sometimes surgical drainage can be necessary.

Antibiotic must be given in high doses, sometimes administered intravenously.

Conventional antymyotic agents (amphotericin B, itracanzole) [32] administered orally or intravenously, are not always successful in fungal disseminated infection and must be applied more aggressively as voriconazole [73, 74], caspofungin [75] or combination of caspofungin and liposomal amphoteric B [76]. In the past years in very serious cases a new antifungal drug – posoconasole was used [77].

Allogeneic granulocyte transfusion from granulocyte colony-stimulating factor and dexamethasone stimulated donors may be an effective treatment in CGD patients especially with invasive aspergillosis [78].

**Corticosteroid therapy**

Corticosteroids as immunosuppressive agents are considered to be contraindicated in acute infection, because they may mask or favor fungal dissemination. But in individual cases in CGD patients with refractory infections it may be successfully used with concomitant antibiotics [79]. Noninfectious granulomas, typical for CGD, may resolve spontaneously.

The literature describes cases of obstructive lesions caused by excessive inflammation, e.g. bronchopneumonia [80], in gastrointestinal tract, in cholecystitis [81] or genitourinary tract successfully treated with corticosteroids [82]. A typical treatment in cases of granuloma formation and obstruction signs of organs is prednisone 1-2 mg/kg body weight as an initial dose.

**Bone marrow transplantation**

The treatment of CGD with bone marrow transplantation is controversial and rarely performed because of the high risk of serious complications, which can even lead to death. Conventional bone marrow transplantation (BMT) with unmodified hemopoietic stem cells and myeloablative regime after preconditioning with busulphan and cyclophosphamide may be use when CGD patients have very serious infection and prophylactic therapy is non effective and only to patients with HLA-identical sibling or matched unrelated donors. This therapeutic method is recommended to be performed in infancy or early childhood when the risk of death from infection or graft versus host disease is minimal. The survival after treatment is especially good in patients without infection at the moment of transplantation [83]. A conventional and curative therapy used in CGD is also the allogeneic hematopoietic stem-cell transplantation (HSC) [84] especially when nonmyeloablative conditioning regimen consisting of cyclophosphamide, fludarabine, and antithymocyte globulin is applied, as described by Horwith et al. In this method the allograft was depleted of T cells to reduce the risk of severe graft-versus-host disease [85].

Another method that may be used in CGD is a non-myeloablative, T-cell depleted allogeneic peripheral blood stem cell (PBSC) transplantation [86].

Sometimes a combined aggressive treatment must be used. Ozsahin et al. describe a case of an 8-year-old patient with invasive multifocal *Aspergillus nidulans* infection and nonresponsive to treatment with amphotericin B and IFN-gamma who underwent HLA- genoidentical bone marrow transplantation, granulocyte-stimulating factor (G-CSF) and liposomal amphotericin B with success [87].

**Gene therapy**

Gene transfer into hematopoietic stem cells has been used to correct some immunodeficiencies. CGD is a disease in which the genetic defect may be removed by gene therapy. For this reason precisely defining the mutation in CGD is very important to provide an appropriate genetic therapy. The gene therapy studies are still in the experimental stage and include animal studies, *in vitro* studies of human bone marrow-derived cells, and a report of adoptive transfer of *ex vivo* modified cells into human patients [88, 89]. Data on a murine model of X-linked CGD showed that retroviral-mediated gen transfer may cause a long-term high level correction of neutrophil gp91phox expression and reconstitution of NADPH oxidase activity [90]. In Germany two X linked CGD adults patients successfully underwent the gene therapy and blood cell precursor stem cell transplantation to their bone marrow in 2006 [91].

**General care**

Some important preventing methods in CGD patients are: educating the patients about their condition, skin and fingernails hygiene, dental debridement, evading exposure on to mould. Avoidance of live vaccines is necessary because of probability of occuring the severe adverse
reaction. Regular follow-up visits are very important in patient suffering from CGD, as they help to prevent from serious complications [92].

Complications

Nearly 50 percent of CGD patients suffer from chronic (infectious and non-infectious) complications, though its cause sometimes is poorly understood [93]. Recurrent and chronic inflammation leads to permanent damage of tissues and the development of granulomas which may impair function of many organs. Some complications come into being in response to immunomodulatory and immunosuppressive treatment (e.g. prednisolone, interferon- gamma, BMT). Similarly to other primary immunodeficiency diseases, as common variable immunodeficiency (CVID) or selective IgA deficiency (SIgAD), in CGD patients some autoimmune disorders may occur [94].

Disseminated bacterial and fungal infections may be very serious complications. Complications from respiratory tract include pulmonary and mediastinal abscesses, bronchiectasis, bronchopulmonary aspergillosis, fibrosis, cor pulmonale [55]. Respiratory failure is common cause of death [29, 95]. Gastrointestinal complications include chronic gastrointestinal inflammation, gastric obstruction and Crohn-like disease [96]. In 2006 two young CGD patients with sarcoidosis was described [97]. Rheumatologic disorders include the following – discoid lupus erythematosus, systemic lupus erythematosus, Raynaud syndrome, nodular vasculitis, juvenile rheumatoid arthritis, immune-mediated thrombocytopenia [93, 98, 99]. Among other complications may occur obstruction of the urogenitary tract [93, 100], chorioretinitis [101], severe aphthous stomatitis, gingivitis [102], granulomatous cheilitis [103]. Patients with deletion of the CYBB gene can become sensitized to Kell antigens after red blood cell transfusion and can have hemolytic complications [104]. Vieira et al. described complication after BCG vaccination in 15-month old boy who developed lymphadenopathy and ulcer at the site of vaccination [105].

Prognosis

Patients suffering from CGD have a disturbed defense against microorganisms which cause infections often leading to death. Therefore, careful physical examination, early diagnosis and therapy including use long-term antimicrobial and antifungal prophylaxis, immunomodulative agents (rIFN-gamma), and aggressive treatment of acute infection may improve prognosis. The autosomal recessive forms of CGD have further onset of sign of disease, milder clinical signs and a better prognosis. Although most of X-linked CGD are associated with serious infections and high mortality, less than 10% of these patients have low but detectable levels of flavocytochrome b588, and have a relatively mild clinical course. The average patient survives to the age of 20 years. The most common cause of death is pneumonia and/or sepsis due to Aspergillus or Burkholderia cepacia [29].

Conclusions

CGD is the rare, hereditary, chronic disorder associated with abnormal oxidase function of phagocytes. Patients have recurrent bacterial and fungal infections that may affect any part of the body. Defective bactericidal activity in this disease may be detected by the biochemical and molecular assays. The most common form is X-linked CGD caused by mutations in the CYBB gene encoding gp91 phox protein. The general treatment of CGD patients that reduces the frequency and severity of serious infections associated with CGD is prophylaxis of inflammatory disorders, including antimicrobials (trimethoprim-sulfamethoxazole) and antifungal (itraconazole) agents as well as rIFN-gamma. The more aggressive treatments, such as bone marrow transplantation and gene therapy are possible especially in very serious cases. The prognosis for long-term survival of CGD patients with X-linked mutation is worse than those with autosomal recessive form. An early diagnosis and early therapeutic intervention improve the prognosis, quality of life and a survival of CGD patients.

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