Journal of the Pediatric Infectious Diseases Society

SUPPLEMENT ARTICLE

Considerations in the Diagnosis of Chronic Granulomatous Disease

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Chronic granulomatous disease (CGD) is a rare primary immunodeficiency that is caused by defects in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. The disease presents in most patients initially with infection, especially of the lymph nodes, lung, liver, bone, and skin. Patients with CGD are susceptible to a narrow spectrum of pathogens, and *Staphylococcus aureus*, *Burkholderia cepacia* complex, *Serratia marcescens*, *Nocardia* species, and *Aspergillus* species are the most common organisms implicated in North America. Granuloma formation, most frequently in the gastrointestinal and genitourinary systems, is a common complication of CGD and can be seen even before diagnosis. An increased incidence of autoimmune disease has also been described in patients with CGD and X-linked female carriers. In patients who present with signs and symptoms consistent with CGD, a flow cytometric dihydrorhodamine neutrophil respiratory burst assay is a quick and cost-effective way to evaluate NADPH oxidase function. The purpose of this review is to highlight considerations for and challenges in the diagnosis of CGD.

Keywords. chronic granulomatous disease; dihydrorhodamine assay; neutrophil oxidative burst; nicotinamide adenine dinucleotide phosphate oxidative complex.

Chronic granulomatous disease (CGD) is a rare primary immunodeficiency, first described in 1959, that is caused by defects in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. It was characterized originally by chronic suppurative lymphadenitis, hepatosplenomegaly, pulmonary infiltrates, and an eczematoid dermatitis and appropriately named fatal granulomatous disease of childhood, because all of the first 4 children diagnosed with the disease died before they were 6 years of age [1]. Although initially thought to have only an X-linked mode of inheritance, its discovery in females in 1968 led to the recognition of autosomal recessive (AR) forms [2]. CGD is thought to affect between 1 in 200 000 and 1 in 250 000 of live births without ethnic preference, although incidence rates can be higher in cultures in which consanguinity is more common [3–6]. Since its original description, advancement in microbial prophylaxis and stem cell transplantation (SCT) have improved survival well into adulthood [7–9].

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Journal of the Pediatric Infectious Diseases Society 2018;7(S1):S6–11

DOI: 10.1093/jpids/piy007

CLINICAL PRESENTATIONS

The diagnosis of CGD is usually established early in life, and the majority of patients are diagnosed with the disease before they are 5 years old [3, 5, 6, 10]. Although the disease can present in adulthood, most such cases are AR forms in which residual production of superoxide can be seen. Most patients present initially with infection, especially of the lymph nodes, lung, liver, bone, and skin [3, 10, 11]. Lymphadenitis has been reported as a common initial presentation of CGD, but pneumonia is diagnosed in 70% to 80% of patients, making it the most common infection overall [12]. Other pulmonary infections, including empyema, have also been described [13–15]. Abscesses are another common initial presentation of CGD and can occur at any site. Children who present with fever and abscess, especially of the liver, perirectal, or perianal areas, should be evaluated for the disease [3, 10, 16]. Other infections in young children that should lead to consideration of this diagnosis include osteomyelitis, genitourinary infections, and sepsis, particularly when they involve CGD-specific pathogens [3, 10, 12, 17].

Granuloma formation is a common complication of CGD and can be seen even before diagnosis. Granulomatous inflammation can affect various organ systems, but the gastrointestinal and genitourinary systems are involved most frequently [16, 17]. Approximately half of the patients with CGD have inflammatory bowel disease that might be indistinguishable

from Crohn disease [18]. Granulomata can form throughout the gastrointestinal tract, often starting in the pylorus, but later involving the rest of the gastrointestinal tract, including the colon and rectum in most patients [16, 18, 19]. Patients can present with abdominal pain, diarrhea, strictures, and fistulae. Gastrointestinal inflammation also can contribute to growth retardation, which is commonly seen in patients with the disease [20]. Genitourinary manifestations of CGD include bladder granulomata, ureteral or bladder outlet obstruction, and, in rare cases, eosinophilic cystitis [17, 21]. It is important to consider CGD for any patient who presents with granulomatous inflammation.

Aside from infections and granulomas, an increased incidence of autoimmune disease has been described in patients with CGD as well as in X-linked female carriers of the disease [22–25]. Ocular manifestations in patients with CGD include keratitis and uveitis. These patients also can develop chorioretinal lesions and granulomata with pigment clumping from previous infections [26]. In addition, juvenile idiopathic arthritis, myasthenia gravis, and immunoglobulin A nephropathy have been described in patients with CGD [22]. Interestingly, autoimmune disorders seem to be even more prevalent in X-linked female carriers. Skin manifestations such as photosensitivity, discoid/malar rash, and Raynaud phenomenon are the most common [24, 25].

COMMON PATHOGENS

The NADPH oxidase complex is responsible for generating the reactive oxygen species within phagocytes that are important for the killing of bacteria and fungi, both directly and through the activation of intraphagosomal proteases [27, 28]. Because of the defect in superoxide production, patients with CGD have an increased susceptibility to a remarkably narrow spectrum of pathogens. The most common infections in patients who live in North America are caused by *Staphylococcus aureus*, *Burkholderia cepacia* complex, *Serratia marcescens*, *Nocardia* species, and *Aspergillus* species [12]. Outside of the United States, infections caused by bacillus Calmette-Guérin, *Salmonella* spp, and *Mycobacterium tuberculosis* are seen also [4–6, 10]. Infectious organisms are typically catalase producing; however, catalase alone does not seem to be a significant virulence factor in animal models [29, 30]. The necessity for catalase production was further disproved in the report from a recent study of 10 patients with CGD who developed chronic infection with *Actinomyces*, a fastidious catalase-negative genus of pleomorphic rods [31]. A few uncommon bacterial infections are considered pathognomonic for CGD, including *Granulibacter bethesdensis*, *Chromobacterium violaceum*, and *Francisella philomiragia*. Infection with any one of these organisms can present as sepsis, and *G bethesdensis* was reported also to cause necrotizing lymphadenitis and meningitis [32–34].

Fungal infection is the leading cause of death in patients with CGD, and *Aspergillus* species are the most common cause of invasive fungal infection [35]. *Aspergillus nidulans* is a species found almost exclusively in patients with CGD and can cause severe and refractory disease [36]. Other fungal species that are diagnostic for CGD include *Paecilomyces lilacinus* and *Paecilomyces variotti* [37]. These pathogens can cause pneumonia and osteomyelitis and are rarely seen in patients with any other immunodeficiency. Another unique presentation of fungal infection in patients with CGD is mulch pneumonitis, which can be seen in adults and children after exposure to decayed organic matter such as fallen leaves, hay, or mulch [38]. Such patients typically present with acute-onset fever and dyspnea, similar to a hypersensitivity pneumonitis, and bilateral pulmonary infiltrates can be found with chest radiography. Bronchial biopsy is diagnostic for mulch pneumonitis yielding necrotizing granulomata and filamentous fungi, most commonly *Aspergillus* species.

DIAGNOSTIC TESTING

In patients with a compatible clinical history, a diagnosis of CGD can be made by functionally assessing the NADPH complex in stimulated neutrophils. The nitroblue tetrazolium (NBT) test has been used historically to measure superoxide generation. In this test, neutrophils are stimulated with phorbol myristate acetate (PMA) in the presence of NBT dye [39]. After stimulation, the yellow-colored dye is reduced by the NADPH oxidase complex in normal neutrophils to formazan, which is a dark-blue or purple precipitate that is retained within the cell. The cells then are visually analyzed by microscopy for this color change. The majority of normal neutrophils will be blue or dark in color, but neutrophils that lack a functional NADPH complex fail to change color. The NBT test sometimes can diagnose X-linked female carriers, because they should have a mixed population of positive and negative cells as a result of the random X-chromosome inactivation known as lyonization. However, the manual readout of the NBT test is semiquantitative, and often, carriers of X-linked mutations are not well identified. Carriers of an AR CGD mutation also are not well distinguished by the manual NBT test, because cells need only a small amount of functional NADPH oxidase to reduce the NBT. Although the NBT assay requires little blood to perform, the blood ideally should be fresh (not shipped). In addition, its semiquantitative nature, test variability that depends on technician experience, and additional problems with false-positive or false-negative readings among X-linked CGD carriers with very skewed lyonization have made the test difficult to maintain [40].

Since the late 1990s, the NBT test has been largely superseded by the flow cytometric dihydrorhodamine (DHR) neutrophil respiratory burst assay as the gold standard for diagnosing CGD [41]. In comparison to the NBT test, the DHR

assay is easier to perform, more reliable, more quantitative, and more sensitive. Similar to the NBT test, neutrophils are stimulated with PMA, but they then are incubated with DHR 123, which is oxidized by hydrogen peroxide (produced in the presence of a normal NADPH oxidase–myeloperoxidase [MPO] system in neutrophils) to rhodamine 123, which emits fluorescence. Then, the fluorescence of each cell is assessed by flow cytometry as a surrogate measure of NADPH oxidase activity. The mean fluorescence intensity of rhodamine 123 has been shown to quantitatively correlate with reactive oxygen intermediate production and subsequent survival in patients with CGD [42]. The DHR assay can usually differentiate between X-linked CGD, AR CGD, and X-linked carrier status (in females) (Figure 1) [43]. It also can assess the degree of lyonization in X-linked carriers, which has been shown to correlate with infection risk in females who have a normal neutrophil population of less than 20% [25].

Some important considerations regarding DHR test performance include neutrophil viability and nonspecific neutrophil activation, which can confound the analysis. The test is stable to most shipping artifacts, but to minimize error, cells should be analyzed within 24 to 48 hours from the time of the blood draw, and a control specimen of blood from an unrelated donor ideally would be shipped simultaneously with the patient sample to be tested if using a distant reference laboratory. Temperature fluctuations during shipping should also be taken into account, because they can affect cell viability [44]. Caution should be taken also to not confuse MPO deficiency with CGD. Because the oxidation of DHR also requires some MPO activity, MPO deficiency can appear with various levels of superoxide production on flow cytometry with either completely absent oxidation, similar to that seen with X-linked CGD, or with a positive but reduced oxidative shift [45]. Thus, abnormal DHR neutrophil respiratory burst assay results should be confirmed with gene mutation analysis for patients being evaluated for CGD.

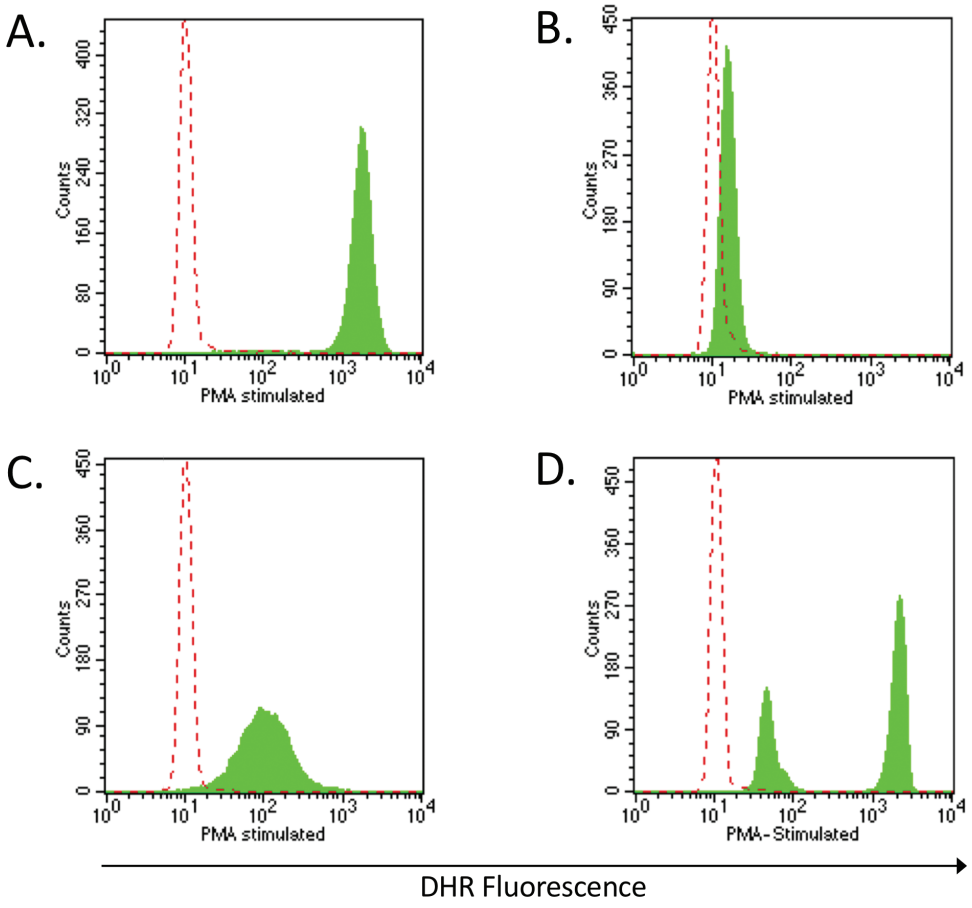

Figure 1. Assessment of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase function by flow cytometry using the dihydrorhodamine (DHR) assay. (A) Normal neutrophil respiratory burst showing a complete shift in fluorescence after stimulation. (B) X-linked chronic granulomatous disease (CGD) (gp91phox) histogram showing no significant change in neutrophil fluorescence after stimulation. (C) Autosomal recessive CGD (p47phox) histogram showing a proportion of unchanged stimulated neutrophils overlapping with the background fluorescence and some neutrophils with significantly reduced fluorescence. (D) X-linked female carrier status histogram with a pattern of 2 peaks reflecting the 2 populations of abnormal and normal neutrophils that result from X-chromosome inactivation. The dashed line indicates the background fluorescence of unstimulated neutrophils, and the green histogram indicates neutrophil fluorescence after phorbol myristate acetate stimulation. Adapted with permission from reference 43.

CGD is a genetically heterogeneous disease that results from mutations in any of the 5 subunits of the NADPH oxidase complex (see Rider N et al, this supplement; see also references 42, 46–62). Gene sequencing can be performed on DNA from peripheral blood leukocytes by using either single-gene or multigene panels [40]. A positive family history for X-linked CGD or previously identified mutation in a family member can help direct initial testing for the gene of interest. However, the high frequency of new mutations and the presence of AR mutations in some males might necessitate a multigene panel or more comprehensive genomic testing. It should be noted that because NCF1 is flanked by pseudogenes, care should be taken when selecting the appropriate primers, and complementary DNA should be verified with genomic sequencing [40]. If genetic testing is not readily available or cannot be performed, immunoblotting or flow cytometry analysis with specific antibodies for the individual NADPH components can be used to identify the deficient protein in a majority of cases [48]. Prenatal testing can be done for known female carriers by performing polymerase chain reaction testing for the implicated gene using amniocentesis or chorionic villus sampling. As an alternative, measurement of NADPH oxidase activity, whether by NBT or DHR, can be performed in peripheral blood neutrophils taken from the fetus but only after 16 to 18 weeks of gestation [40].

DIFFERENTIAL DIAGNOSIS OF CGD

The differential diagnosis of CGD involves primarily disorders associated with recurrent and/or unusual infection, particularly those caused by pathogens commonly associated with CGD or disorders associated with granuloma formation or hyperinflammation. When the entire clinical picture is taken into consideration, however, there are certain clues (described below) that can help differentiate these entities.

MPO Deficiency

MPO deficiency is the most common inherited disorder of phagocytes; complete deficiency occurs in approximately 1 in 4000 people [63]. Because MPO catalyzes the oxidation of hydrogen peroxide, patients with complete MPO deficiency typically have an abnormal DHR assay result. However, unlike CGD, MPO deficiency is rarely associated with clinical symptoms, with the exception of patients who have concurrent diabetes mellitus, in whom disseminated candidiasis and other fungal infections can occur. Also, the NBT test (and, perhaps more importantly, gene sequencing) is normal in patients with MPO deficiency [45, 64].

Hyperimmunoglobulin E Syndrome

Patients with both autosomal dominant STAT3-deficient hyperimmunoglobulin E syndrome (AD-HIES) and AR DOCK8-deficient hyperimmunoglobulin E syndrome (AR-HIES) commonly develop pulmonary *Staphylococcus* and *Aspergillus* infections [65, 66]. It should be noted that *Aspergillus* infections occur in the setting of specific lung lesions (eg, pneumatoceles or bronchiectasis), which are not common in patients with CGD [67]. In addition, patients with AD-HIES have characteristic facies and skeletal abnormalities, and both AD-HIES and AR-HIES result in markedly elevated immunoglobulin E levels that are not present in patients with CGD [65].

IRAK4/MyD88 Deficiency

Patients with interleukin 1 receptor–associated kinase 4 (IRAK4) deficiency and MyD88 deficiency develop recurrent severe infections (cellulitis, arthritis, meningitis, osteomyelitis, organ abscesses, and sepsis) caused by *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Unlike with CGD, patients do not develop fungal infections, and overall susceptibility to infection spontaneously improves with age. Another clue to the possibility of IRAK4 or MyD88 deficiency is the lack of significant fever and an unexpectedly low C-reactive protein (CRP) level in the setting of a serious bacterial infection. Although patients with CGD might not develop fever, sometimes even in the setting of severe infection, they tend to have an elevated CRP level and erythrocyte sedimentation rate [68].

Humoral Immunodeficiencies

Patients with common variable immunodeficiency and patients with agammaglobulinemia, such as Bruton tyrosine kinase (Btk) deficiency, can develop various types of inflammatory bowel disease [69, 70]. However, humoral immunodeficiencies predispose patients primarily to infections with an encapsulated organism and are relatively easy to diagnose with an assessment of immunoglobulin quantification and function. In contract, patients with CGD tend to have hypergammaglobulinemia, possibly as a result of chronic inflammation.

Inflammatory Bowel Disease

Similar to patients with CGD colitis, patients with Crohn disease can have intestinal inflammation that leads to bowel obstruction, fistulae, and strictures. However, Crohn disease is not commonly associated with severe infections (before iatrogenic immunosuppression). Although Crohn disease can involve any part of the gastrointestinal tract and might result in extraintestinal manifestations, CGD colitis is more often in the rectal and perirectal areas and is not associated with extraintestinal manifestations [71]. Bowel biopsy specimens from patients with CGD can have characteristic histopathologic pigment-laden macrophages with either brown granular cytoplasm or pink eosinophilic crystalline cytoplasmic inclusions, which are not typical of Crohn disease; however, these characteristics are not always seen in patients with CGD [16, 70–72]. Very-early-onset inflammatory bowel disease (before a few years of age, particularly in males) should raise the clinician question whether CGD colitis is the cause.

Cystic Fibrosis

Patients with cystic fibrosis (CF) develop recurrent respiratory infections and can develop pulmonary infections with *B cepacia* complex. However, in patients with CF, infections typically are limited to the lungs and usually occur in the setting of significant bronchiectasis, which is not common with CGD. Patients with CF also tend to develop a persistent infection with the same strain of *B cepacia* complex, whereas patients with CGD are prone to recurrent infections with different strains [73, 74]. Newborn screening programs should help reduce confusion of CGD with CF in the future.

Sarcoidosis

Sarcoidosis is a systemic immune disorder characterized by the formation of non–necrotizing epithelioid granulomas. Gastrointestinal and respiratory symptoms and hilar adenopathy are common in patients with sarcoidosis and in those with CGD. The angiotensin-converting enzyme (ACE) level is elevated in ~55% of children with sarcoidosis, but it typically is not elevated in patients with CGD. Also, perirectal abscesses, ulcers, and fistulae occur in patients with CGD, unlike in patients with sarcoidosis [75].

SUMMARY

CGD is a rare but serious primary immunodeficiency that results in recurrent bacterial and fungal infections and in hyperinflammation that results from a deficiency in the phagocyte NADPH oxidase complex. In patients who present with signs and symptoms consistent with CGD, the DHR assay is a quick and cost-effective way to evaluate NADPH oxidase activity. Once a diagnosis is made, DHR flow cytometry and genetic sequencing results can help predict the severity of the disease and identify patients who might benefit from hematopoietic SCT. Genetic and prenatal testing can be useful for screening for other female family member carriers.

Notes

Supplement sponsorship. This article appears as part of the supplement "Chronic Granulomatous Disease," sponsored by Horizon Pharma USA, Inc.

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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