CHRONIC GRANULOMATOUS DISEASE AND THE CHALLENGES OF DIAGNOSIS AND MANAGEMENT IN SOUTH AFRICA

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SUMMARY
Chronic granulomatous disease (CGD) is a rare genetic primary immunodeficiency disorder (PID) of phagocytes which manifests with severe bacterial and fungal infections but also granuloma formation that may be mistaken for Tuberculosis. CGD is caused by gene mutations or deletions of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (phox) complex which result in defective phagocytic respiratory burst function required for killing microbes. Patients with X-linked CGD and delay in diagnosis have a worse prognosis, as this is usually fatal in the first decade of life without prophylactic antibiotics and antifungals. Haematopoietic stem-cell transplantation and future gene-replacement therapy hold the only hope for a cure.

On the South African PID Registry, ten cases have been reported, of whom two have died prematurely. This article summarises the history, clinical presentation and laboratory investigations of two brothers with CGD, illustrating also the importance of family history and compliance.

INTRODUCTION
The reported incidence of CGD in Europe is 1/250 000,1 and in the United Kingdom and Ireland approximately 1/120,000.2 Seven cases of CGD have been described in the South African literature since 1983, and four patients could be genetically confirmed to date.3,4 Despite an increasing awareness of genetic susceptibility to infections and the realisation that PID is more common than previously thought,5,6 there is still a delay in diagnosis globally and especially in Africa. Even with the use of prophylactic antimicrobials and antifungals survival past the age of 30 years was until recently estimated to be approximately 50%, with an annual mortality rate of 2–5% and a worse outcome for those with a complete absence of phagocytic respiratory burst.7

CGD is due to the defective killing of phagocytosed microbes. It is caused by an inherited defect in the NADPH-oxidase enzyme complex present in neutrophils, eosinophils, monocytes and macrophages.

NADPH oxidase is required for the ‘respiratory burst’. This enzyme decreases molecular oxygen to superoxide, which subsequently reacts to form reactive oxygen species (ROS).

The NADPH oxidase enzyme complex comprises two membrane-spanning subunits, gp91phox and p22phox, as well as three cytosolic components, p47phox, p67phox and p40phox.8 Approximately 66% of all CGD cases are from mutations in the X-linked gp91phox gene (CYBB) and 30% are from the autosomal recessive forms of CGD, with defects in the gene coding for p47phox (NCF-1). The remaining 5% of the cases are due to mutations in CYBA, NCF-2 or NCF-4 that encode for p22phox, p67phox and p40phox, respectively.9,10

The impairment in the function of the complex results in the defective intracellular and extracellular killing of especially catalase-positive bacteria, for example Staphylococcus aureus, and of fungi (aspergillus species) and actinomyces species. Chronic inflammation is a feature of CGD, especially in the second decade of life, because NADPH oxidase deficiency also impairs the regulation of pro-inflammatory cytokine signals.

CASE REPORT
The index patient GD who is now 16 years old first presented in 2005 (at five years of age) with a liver abscess which cultured Staphylococcus aureus. He was also treated for Salmonella septicaemia and Tuberculosis (TB) in 2008 and had further episodes of liver abscesses (Staphylococcus aureus cultured on pus from an abscess)
in 2008 and 2009. In 2010, he presented again with a liver abscess. Chronic granulomatous disease was suspected and his neutrophil burst test was reduced but not absent, indicating possible autosomal recessive inheritance. Antimicrobial and antifungal prophylaxis was instituted but with variable patient compliance. GD then presented again in 2012 with a two-week history of fatigue, fever and significant weight loss. There was a TB contact in the home but investigations for TB were negative. An ultrasound of the abdomen revealed multiple liver abscesses; again, Staphylococcus aureus was cultured. He was treated with appropriate antibiotics and a prolonged course of corticosteroids, two pigtail drainage procedures, and surgery for near-fatal gut perforation followed by prolonged hospitalisation.

His younger brother BD was seen for CGD screening in the clinic in 2010 after GD was diagnosed with CGD. He also had an abnormal neutrophil burst response similar to his older brother’s with a reduced but not absent burst response. In both brothers the burst responses improved markedly in times of good health. The mother’s neutrophil burst response was normal; however, it showed two populations of neutrophils, indicating an X-linked, more severe mode of inheritance. At the time of screening BD was almost four years old and already had a prior history of acute gastroenteritis requiring hospital admission at seven months of age and tuberculous meningitis with multiple tuberculomas on CT scan in 2008. After completion of his TB treatment, he had a focal seizure which became generalised. In 2010, he had also suffered from measles complicated by bronchopneumonia and he had an abscess of the left thigh which was incised and drained. After his abnormal neutrophil burst findings he was started on antimicrobial prophylaxis with subsequent variable compliance.

However, in 2014 BD then presented with a liver abscess and Staphylococcus aureus septicemia complicated by bilateral pleural and pericardial effusions. As he was previously identified with a neutrophil function defect due to his brother’s history, and hence with a high suspicion of liver abscess formation, his treatment was prompt and recovery uneventful.

DISCUSSION
With a lack of awareness for the clinical manifestations and without access to specialised laboratories, including facilities for molecular diagnosis, the recognition and correct management of CGD can be very delayed and result in unnecessary morbidity and early death. Both brothers described in this article were diagnosed and treated for TB. As there is such a high prevalence of TB in the Western Cape, this did not raise suspicion for PID initially. Furthermore, CGD patients are known to be more susceptible to disease manifestation of TB as part of their immune defect. However, our patients also presented with unusual infections such as Salmonella (patient GD) and multiple and severe infections: measles, tuberculous meningitis and a deep-tissue abscess of the thigh (patient BD). They did however not present with infections due to Burkholderia cepacia, Serratia marcesens or Listeria, Aspergillus or Nocardia species which are other all indicator organisms associated with CGD.

The diagnosis of any PID can be suspected by using the ‘SPUR’ warning signs for PID: Severe, Persistent, Unusual or Recurrent infections or autoimmune/auto-inflammatory symptoms and the clinical phenotype. An example is the
The specific diagnosis of CGD should be pursued with the ‘gold standard’ nitroblue tetrazolium assay, which is available only in specialised laboratories. A flow cytometric-based assay also suggests the diagnosis of CGD. This assay, referred to as a ‘neutrophil burst test’, is based on the reduction of dihydrorhodamine-123 (DHR) by phorbol myristate acetate (PMA) stimulated phagocytic cells. It is particularly useful as it can demonstrate two populations of cells in carriers. Patients who are systemically ill from other causes may have a transient reduction in burst activity that will normalise as they improve.

Both BD and GD had neutrophil burst responses which were partially reduced but never normalised completely after recovery from their acute illnesses.

As the neutrophil burst tests with near-normal E coli stimulant response were atypical for CGD presenting with typical hallmark infections, genetic testing was requested at the Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University for confirmation of the diagnosis.

Both brothers were found to have a mutation in the CYBB gene. This protein change has previously been shown to be pathogenic for CGD.

Molecular testing finally confirmed the diagnosis of X-linked CGD and enabled the clinicians to offer the family genetic counselling and instructions on appropriate management. A third sibling (male) with failure to thrive and mild recurrent infections has subsequently tested negative for the CYBB mutation.

## TREATMENT MODALITIES

Previously reported mortality from CGD was high, but with antibiotic and antifungal prophylaxis as well as haematopoietic stem-cell transplantation (HSCT) outcomes have improved and reported mortality is currently less than 5%.

Both patients were started on prophylactic co-trimoxazole and isoniazid as well as itraconazole for GD. Patient GD also required a prolonged course of prednisone in 2012. This has been shown to be effective in controlling multiple liver abscesses that are not amenable to surgery. The use of interferon-gamma for CGD remains controversial and its cost is a major issue in South Africa. Both boys are now confirmed with X-linked CGD and would benefit from HSCT, but this is not a routine option in South Africa at this time. A study in the United Kingdom showed 90% survival at 15 years with and without HSCT; however, children who did not undergo HSCT had more frequent infections, hospital admissions and surgery, and had poorer growth. Currently, both boys are being looked after by their grandmother and are taking prophylaxis and attending clinic. At their last visit they were both healthy, gaining weight and attending school regularly.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES