A comparison of clinical outcome, quality of life, emotional well being and cognitive function in those with Chronic Granulomatous Disease managed conservatively and curatively

Volume 1 of 1

Theresa Cole

A9918903

Institute of Cellular Medicine
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Abstract

Background

Chronic Granulomatous Disease (CGD) is a primary immunodeficiency, characterised by serious infections and inflammation. It can be managed conservatively, with prophylactic antimicrobials, or curatively with haematopoietic stem cell transplant (HSCT). In the UK and Ireland there are cohorts of children managed both conservatively and curatively. Previous research has shown patients with CGD have low intelligence and increased emotional difficulties. Chronic diseases are known to result in poor quality of life.

This study aimed to evaluate: clinical outcome; quality of life; emotional well being and cognitive function in children managed conservatively and curatively.

Methods

Children were identified from specialists centres and advertising through special interest groups. Clinical data were collected from medical records. Children and parents completed questionnaires measuring quality of life, emotional and behavioural difficulties and self-esteem. Children underwent brief IQ tests. Results were compared to published norms for healthy children. Non-HSCT and post-HSCT groups were compared.

Results

78 children were identified. 59 (80%) living children were recruited. Clinical information was available for 62 children (four deceased). 30 (48%) children had undergone HSCT. Children with CGD had 0.71 episodes of infection/admission/surgery per CGD life year (95%CI 0.69-0.75 events per year). Post-HSCT children had 0.15 events per transplant year (95%CI 0.09-0.21 events per year). Post-HSCT survival was 90%.

Parents and children reported quality of life significantly below normal for in the non-HSCT group. Post-HSCT scores were not significantly different from

healthy norms. Parents of non-HSCT children reported increased emotional difficulties compared to healthy children. IQ was normal in both groups.

Conclusions

Children with CGD have more serious infections, episodes of surgery and admissions compared to post-HSCT children. They also have poorer quality of life and are at risk of emotional difficulties. Post-HSCT children have normal quality of life. Cognitive function is normal in both non-HSCT and post-HSCT children.

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Chapter 1 Introduction

Chronic Granulomatous Disease (CGD) is a rare inherited primary immunodeficiency, characterised by recurrent bacterial and fungal infections. Mutations in the genes encoding subunits of the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase result in failure of the oxidative burst required by phagocytes to eradicate organisms. CGD is associated with abnormal inflammatory responses leading to colitis, chorioretinitis and granulomas in such places as the liver, lung, skin and lymphoid tissue. Despite management of CGD improving over recent years, patients continue to have reduced life expectancy[1]. Outcomes following haematopoietic stem cell transplantation (HSCT) have shown great promise in patients with CGD with a marked reduction in hospital attendance, compared with pre-transplant attendance rates, despite ceasing all antimicrobial prophylaxis[2]. CGD has also been treated with gene therapy, albeit with limited success to date[3].

Chronic diseases are well recognised to impact on quality of life. Now that survival rates following HSCT for primary immunodeficiency have improved, there is a greater focus on wider measures of health and quality of life post-HSCT. There have also been concerns about cognitive outcome and behavioural difficulties in some children undergoing HSCT for primary immunodeficiencies[4].There are few published data on the psychological impact of CGD or curative treatment for it. However, one study of patients with CGD referred for psychological assessment indentified lower than expected cognitive function amongst this selected sample[5]. Emotional difficulties have also been demonstrated in children with CGD[6].

There continues to be discussion in the international community about which patients with CGD should be treated with HSCT. There are no data that show whether conservative management or HSCT have an effect in terms of quality of life and emotional well being. This thesis describes studies that review clinical outcome in children with CGD treated conservatively or curatively, and addresses the issues of cognitive function, emotional wellbeing and quality of life.

1.1 History of CGD

In 1957, Bridges et al published information on four boys with hypergammaglobulinaemia, recurrent infections and granulomatous lesions which were distinct from other granulomatous conditions[7]. At a similar time, Landing and Sharkey also published a description of two males with recurrent infections and infiltration of visceral organs with lipid histiocytes containing yellow-brown pigment[8]. The condition was described as fatal granulomatous disease of childhood as all of the initial cases died in early childhood. Over the next few years, however, the use of antibiotics and surgical drainage of abscesses improved survival in to the second decade of life[9]. With improved survival, the word "fatal" was dropped and it was renamed Chronic Granulomatous Disease of Childhood in 1967[10].

Nine years after the original descriptions of CGD, the immune defect started to be understood in more detail. In 1966 Holmes et al documented the failure of phagocytes to destroy ingested *Staphylococcus aureus* in three affected boys[11]. A year later the same group identified the failure of the oxidative burst as an important factor in defective intracellular killing by polymorphonuclear leukocytes in CGD patients[12]. Hydrogen peroxide production was identified as a key component of the process of destroying ingested micro-organisms and its role in CGD was highlighted when it was demonstrated that organisms that produce their own H_2O_2 could be destroyed by leukocytes from patients with CGD[13]. In 1968 Baehner and Karnovsky proposed two possible enzyme defects, either affecting nicotinamide-adenine dinucleotide (NADH) oxidase or nicotinamide-adenine dinucleotide phosphate(NADPH) oxidase[14]. In fact, they concluded NADH oxidase was more likely to be the primary enzyme involved in the oxidative burst. It wasn't until 1975 that NADPH oxidase was recognised as the defective enzyme in CGD[15].

1.2   NADPH oxidase

NADPH oxidase is an enzyme involved in oxidation of NADPH, releasing electrons and protons that move across a membrane to combine with oxygen, producing superoxide which is rapidly transformed to hydrogen peroxide by superoxide dismutase. Superoxide and hydrogen peroxide are known as primary reactive oxygen species (ROS). During the 1980s-1990s our understanding of the structure and function of NADPH oxidase increased dramatically. The first component to be described was a b-type cytochrome which was found to be absent in 19 men with CGD[16]. It was found to reside in the membrane of specific granules in unstimulated neutrophils but to move to the plasma membrane when cells were stimulated[17, 18]. It was proposed to form an electron transport chain to generate O2- required to form highly reactive oxygen species. In 1987 Segal identified the two components of the b-type cytochrome (cytochrome b-$_{558}$) [19], later known as the gp91phox (also known as NOX2[20]) and p22phox subunits. In the same year Umei et al identified the component that would come to be known as p67phox[21] and Curnette et al demonstrated the importance of the cytosolic factor, later to be known as p47phox, for a functional oxidative burst[22]. It was not until 1993 that the final major component, p40phox was identified by Wientjes et al[23].

It is now clear that there are six components to the dormant NADPH oxidase, five phox components plus the GTP-binding proteins Rac1/2. The gp91phox and p22phox are found in their dormant state in the membrane of specific granules within the phagocyte[24]. When activated, the cytochrome b-558 is moved to the plasma membrane which forms the phagosome[25](Figure 1). p47phox, p67phox and p40phox are found in the phagocyte cytosol. In vitro studies have demonstrated that activation of NADPH oxidase begins with a change in the phosphorylation of p47phox[26, 27]. This process alters the p47phox/p67phox/p40phox complex in the cytosol and results in it translocating to the membrane where it is then associated with the cytochrome b-$_{558}$ complex. Once in the membrane p47phox stabilises the interaction between p67phox and cytochrome b-$_{558}$[28] which is required to generate the superoxide[29]. Rac1

interacts with p67phox[30] and it has recently been demonstrated that preventing binding of Rac1 with p67phox prevents superoxide formation in a dose dependent manner[31]. Rac2 has been found to be associated with the cytosolic components of NADPH oxidase, migrating to the membrane with p47phox and p67phox in healthy cells but not p47phox deficient cells[32]. Inhibition of Rac2, similar to Rac1, inhibits superoxide production in a concentration dependent manner[33]. Rap1A is located in the granule membrane in association with cytochrome $_{b-558}$ and is moved to the plasma membrane alongside the other components of NADPH, it appears to play a regulatory role and may be involved in deactivation as well as activation of NADPH oxidase[25].

Figure 1:NADPH Oxidase activation, demonstrating the membrane and cytosolic components of the NADPH complex. On activation cytosolic components move to the membrane, resulting in oxidation of NADPH and release of hydrogen ions which combine with superoxide to produce reactive oxygen species. From: Assari T. Chronic Granulomatous Disease; fundamental stages in our understanding of CGD[9]

Defects in gp91phox, p22phox, p47phox and p67phox are well recognised to lead to CGD. Until recently, p40phox deficiency was not considered as a cause of a CGD phenotype. In a cell free assay, p40phox is not required for NADPH activity[25]. It has been shown that p40phox is a binding partner for p67phox and is reduced in cases of p67phox deficient CGD[23]. More recently it has been shown that p40phox is important in upregulation of NADPH oxidase activity through binding to phosphatidylinositol 3-phosphate (PtdIns(3)P) in membranes, so targeting the p67phox to the correct area necessary for activation of ROS formation[34]. Mutations in p40phox prevent binding of PtdIns(3)P. In p40phox-/- mice this results in failure of neutrophils to kill *Staphylococcus aureus* as seen in other causes of CGD[35]. In 2009 the first case of human p40phox mutation causing granulomatous colitis and impaired NADPH oxidase activity was described[36].

Rac2 deficiency has been described in humans but this does not lead to a typical CGD phenotype. A case report of an infant describes multiple, rapidly progressing soft tissue infections with poor wound healing. Defects in neutrophil chemotaxis, ingestion, degranulation and ROS formation were demonstrated and a point mutation in the Rac2 gene identified[37, 38].

1.3   NADPH oxidase and microbial killing

Opsonisation of a microbe, with complement or antibody, allows recognition by surface receptors on the phagocyte which triggers the process of ingestion and the production of a phagosome(Figure 2). The phagosome is derived from invagination of the cell membrane and various granules (gelatinase, specific and azurophil). The resulting phagolysosome, which is responsible for microbial killing, has cytochrome $_{b-558}$ in the membrane. The hydrogen peroxide generated by the NADPH oxidase is used to generate hypochlorous acid when combined with chloride, catalysed by myeloperoxidase [39, 40]. H_2O_2 and hypochlorous acid have a direct role in attacking ingested micro-organisms, but also optimise the local environment for cationic microbicidal proteases. Influx of ROS in to vacuoles results in a negative charge within the vacuole, driving a

compensatory movement of positively charged potassium ions across the membrane in to the vacuole[40]. Proteases, cathepsin G and elastase, are then activated through the generation of the potassium rich environment. Recent work has suggested that these neutrophil granule proteases play an important role in direct microbial killing. Studies in which the influx of potassium into the vacuole was blocked, demonstrated complete lack of microbial killing despite normal production of ROS[41]. Mice made deficient in the proteases succumbed to staphylococcal and candida infections despite having normal superoxide production[40]. However, contradictory work showed mice deficient in neutrophil elastase and cathepsin G did not succumb to Aspergillus fumigatus or *Burkholderia cepacia* infection, whereas, the CGD model p47phox deficient mice did[42]. Given that *staphylococcus*, *Aspergillus* and *Burkholderia* are all important pathogens in CGD this perhaps reflects the complexity of human defences against infection.
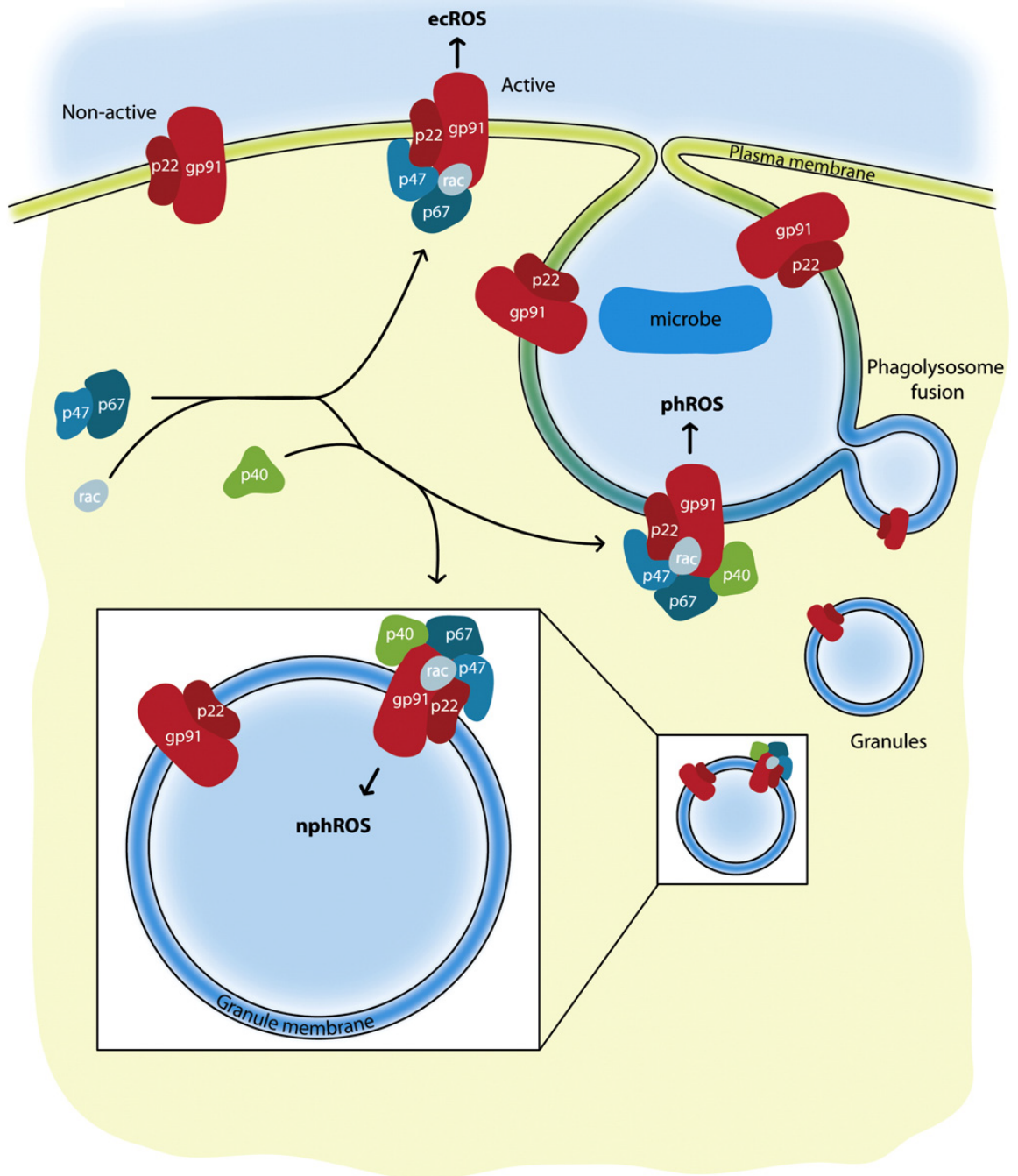
Figure 2:Extracellular & intracellular ROS formation. NADPH oxidase components gp91 and p22phox are present on granule and cellular membranes. Cytosolic components can move to these membranes for activation of NADPH oxidase and generation of ROS. From Intracellular generation of superoxide by the phagocyte NADPH oxidase: How, where, and what for? Bylund et al[43]

ecROS = extracellular reactive oxygen species
phROS = phagosome reactive oxygen species
nphROS = nonphagosomal intracellular reactive oxygen species

Another mechanism used by neutrophils to kill microbes has also recently been described, and shown to be defective in patients with CGD[44]. Neutrophil extracellular traps (NETs) are formed from cationic antimicrobial peptides and proteins mixed with chromatin. These are released from neutrophils to bind and kill bacteria[45] and fungi such as candida[46]. NETs kill micro-organisms by providing a high local concentration of antimicrobial peptides whilst, at the same time, minimising tissue damage by sequestering the granule enzymes. Neutrophils from patients with CGD fail to form NETs when activated with *Staphylococcus aureus* or phorbol 12-myristate 13-acetate (PMA). However, when stimulated with a hydrogen peroxide forming enzyme they do produce NETS similar to neutrophils from healthy people. Further studies have demonstrated that NET formation in neutrophils stimulated by PMA requires the presence of singlet oxygen. This is formed from hypochlorous acid and hydrogen peroxide[47] and does not occur in neutrophils from patients with CGD[48]. Bianchi et al demonstrated that NET formation could be restored by gene therapy in a patient with CGD, leading to removal of *A. nidulans* conidia and hyphae and cure of clinical pulmonary aspergillus infection[49]. This suggests the importance of NADPH oxidase driven ROS production in the process of NET formation and gives another mechanism by which people with CGD are prone to infection[44]. However, our understanding of NETS is rapidly evolving, and there have been contradictory studies which have shown NET formation to be NADPH oxidase independent[50]. These differences may reflect the different stimuli used and it is likely our understanding of this area will change and deepen over the next few years as more is published.

Recently, NADPH oxidase dependent ROS have also been recognised to be important in neutrophil chemotaxis. In a study screening for inhibition to neutrophil migration in-vitro, they identified that diphenyleneiodonium chloride (a flavoprotein inhibitor known to impair NADPH oxidase activity[51]) significantly impaired healthy neutrophil directionality on an EZ-TAXIScan chemotaxis device[52]. Further investigation using a mouse model demonstrated reduced chemotaxis of abnormal CGD neutrophils compared to wild type neutrophils in response to peritoneal inflammation. In-vitro testing of neutrophils from a single

patient with X-linked CGD demonstrated that they had a lack of directionality, more frequent formation of multiple pseudopodia, and slow migration toward the direction of higher chemoattractant[52]. Further work is required in a larger cohort of CGD patients to confirm whether this is a further mechanism causing the predisposition to infection seen in CGD.

Another role for NADPH oxidase may be in cell surface receptor expression. Hartl et al showed that neutrophils from CGD patients had reduced expression of Toll Like Receptor (TLR)5, TLR9, CD11b, CD18, CD35, and CXCR1 when compared to normal controls. Reduced TLR5 expression was associated with impaired neutrophil activation by bacterial flagella. Reduced CD11b and CD18 resulted in impaired phagocytosis of *Staphylococcus aureus* and reduced CXCR1 resulted in impaired chemotaxis. TLR5 and CD18 expression were inversely correlated with frequency of lymphadenitis and pneumonia respectively. Inhibition of NADPH oxidase in control phagocytes in vitro resulted in decreased TLR5 and TLR9 expression along with impaired function of TLR5[53].

1.4 Mechanism for CGD hyperinflammation

The mechanism of inflammatory complications of CGD remains poorly understood, however, our understanding is evolving all the time. Over recent years a number of hypotheses have been put forward implicating a number of different cells and pathways. Some of these have good evidence for involvement in CGD hyperinflammation and others less so. It is likely that the process is complex and many factors are involved. Patients with CGD certainly exhibit increased levels of proinflammatory cytokines [54, 55], but the reason for this and role of ROS remains unclear.

Inflammasomes, large cytoplasmic complexes that have a role in activating pro-inflammatory cytokines (interleukin -1β (IL-1β) and interleukin -8(IL-8)) in response to microbial products have been implicated in CGD hyperinflammation[56]. It was proposed that NADPH oxidase generated ROS were important in activation of the inflammasome[57]. However, this has more

recently been called in to question by findings from studies which show cells from CGD patients can exhibit high levels of caspase-1 and interleukin-1 despite having inactive NADPH oxidase. Recent work has demonstrated increased levels of caspase-1(required to activate IL-1β) and IL-1β itself in CGD monocytes although not macrophages. In particular, monocytes from patients with CGD colitis had high levels of caspase-1 and IL-1. Treatment of these patients with Anakinra (an IL-1 receptor antagonist) only produced minimal improvement in symptoms despite significant reduction in IL-1 production [55]. Another study has shown that cells from gp22phox deficient CGD patients are capable of secreting normal quantities of IL-1β in response to activators for stimulation of the inflammasome NLRP3. It is not in doubt that CGD patients can have high levels of caspase-1 or interleukin 1β, but it appears that this is not due to NADPH oxidase dependent ROS activation of the inflammasome [58].

Other recent work has demonstrated that NADPH oxidase is important in the activation of Nfr2, an anti-inflammatory transcription factor. In a p47phox deficient mouse model macrophages were shown not to have increased Nrf2 after stimulation with zymosan when compared to wild type[59].

Indoleamine 2,3-dioxygenase (IDO), a tryptophan catabolising enzyme, which is important in regulating immune responses and suppressing inflammation was widely believed to require ROS for enzymatic activity. A CGD mouse model demonstrated a block in the tryptophan metabolism pathway that resulted in defective regulatory T cell activity and acute lung inflammation in mice with Aspergillus infection so providing a good explanation for inflammation[60]. However, in vitro studies using monocytes, dendritic cells, and polymorphonuclear leukocytes from humans with CGD have failed to demonstrate reduction in metabolites therefore showing no block in the pathway[61, 62]. Recent work on the activation of IDO has helped explain this as it has been demonstrated that IDO is activated in-vitro by cytochrome b_5 rather than superoxide[63].

Another possible mechanism put forward is impaired peripheral regulatory T cell induction by macrophage derived ROS. Impaired regulatory T cell function has been demonstrated in rats and in-vitro studies with macrophages from patients with CGD[64]. However, defects in regulatory T cells are more commonly associated with the development of autoimmune disease which does not appear to be a major component of CGD.

A theory with good evidence to support it, is that of defective apoptosis. Clearance of activated and infected neutrophils occurs when macrophages recognise phosphatidylserine (PS), on the neutrophil surface[65] and this appears to be NADPH oxidase ROS dependent[66]. Macrophages also require functional NADPH oxidase in order to phagocytose the neutrophils and this has been shown to be defective in cells from X-linked CGD patients and in normal macrophages treated with an NADPH oxidase blocking agent[66]. Macrophages have to be "programmed" to clear apoptotic cells and this requires signalling through the peroxisome proliferator-activated receptor gamma (PPARγ). Using a mouse model of acute peritonitis it was demonstrated that CGD mice had decreased PPARγ signalling compared to wild type mice but that this could be restored with the administration of pioglitazone, a PPARγ agonist[67].

Because neutrophils from CGD patients are more resistant to apoptosis it results in accumulation of cells which then degrade and release their intracellular constituents, resulting in inflammation[68]. Recognition of apoptotic cells is also, in itself, anti-inflammatory as it leads to the release of anti-inflammatory mediators such as transforming growth factor beta (TGFβ), which suppress cytokine and chemokine release[69]. It was recognised back as early as 2003 that macrophages from CGD patients produce significantly less anti-inflammatory factors such as TGFβ[70].

1.5 Genetics

Initially CGD was thought to be purely an X-linked disease as the original cases were male. However, in 1968 Quie et al described two females with the same findings suggesting a second autosomal recessive pattern of inheritance[71].

With the advent of the ability to identify and map genes the inheritance of CGD has become clearer. The abnormal *CYBB* gene on the X chromosome (Xp21.1) was first cloned in 1986 by Royer-Pokora et al[72]. The gene for p47phox, later named *NCF1* located on chromosome 7q11.23, was identified and cloned by Volpp et al three years later[73] and the *NCF2* gene for p67phox on chromosome 1q25 was cloned in 1990[74] along with the *CYBA* gene located on chromosome 16q24 encoding for p22phox[75]. The gene for p40phox, *NCF4* located on chromosome 22q13.1 was the last to be identified in 1996[76].

Worldwide, the most common form of CGD is due to mutations in *CYBB*, accounting for approximately two thirds of cases[77]. No single mutation is responsible for all cases of X-linked CGD, single nucleotide substitutions are the most common defect but deletions and insertions are also found[78]. Most people with X-linked CGD have no gp91phox expression, however some have reduced or normal amounts of non-functional protein[79]. It has been shown that patients with nonsense, frameshift, splice or deletion mutations have no superoxide production or protein expression but certain missense mutations with little effect on protein expression have absent NADPH oxidase function[79]. A case of X-linked CGD in a 61 year old man has been described after he presented with an unusual skin rash. He was found to have normal gp91phox expression on Western blotting, but no superoxide production[80]. Somatic mosaicism has also been described in two patients with X-linked CGD, resulting in small populations of normal cells[81]. Interestingly, two cases of gp91phox deficient CGD patients have been described with no superoxide production whilst maintaining some degree of hydrogen peroxide production. These patients both had mutations that interfered with the interaction between gp91phox and p47phox. It was hypothesised that superoxide generation was bypassed by electron accumulation at FAD which then pass directly to oxygen to produce hydrogen peroxide[82].

Autosomal recessive CGD usually occurs as a result of defects in p47phox, p22phox and p67phox. The most common form, caused by mutations in the *NCF1* gene, is responsible for approximately 20-25% of cases of CGD overall,

whilst mutations in *CYBA* and *NCF2* are each responsible for approximately 5-6% of cases[83-85]. Only one patient has been described with a mutation in *NCF4*[85]. *CYBA* and *NCF2* mutations are heterogeneous, however, a single *NCF1* mutation is responsible for the majority of cases of p47phox deficient CGD (a dinucleotide deletion at the start of exon 2)[85]. The majority of autosomal recessive mutations result in absent protein expression. However, a few result in diminished, or occasionally normal protein expression[85]. Residual NADPH oxidase activity has been demonstrated in brothers with p67phox deficient CGD who were diagnosed at the ages of 58 and 53 due to their atypical history[86].

There are dramatic differences in the number of X-linked and autosomal recessive cases according to ethnic background. As can be seen from the European registry, the percentage of autosomal recessive cases varies between 11-83% according to ethnic background (Table 1).

Country/Region of origin	Total	CGD Type	
		X-linked (%)	Autosomal Recessive (%)
France	84	75 (89)	9 (11)
Germany	69	54 (78)	15 (22)
The Netherlands	62	43 (69)	19 (31)
Arab/North African	38	11 (29)	27 (71)
Poland	30	21 (70)	9 (30)
Spain	25	16 (64)	9 (36)
Sweden	24	12 (50)	12 (50)
Switzerland	24	19 (79)	5 (21)
Denmark	22	12 (55)	10 (45)
Israeli	16	9 (56)	7 (44)
Turkey	12	4 (33)	8 (67)
Former Yugoslavia	8	7 (88)	1 (12)
Belgium	6	5 (83)	1 (17)
East & South Asia	6	1 (17)	5 (83)
Italy	2	1	1
Austria	1	0	1
Total	**429**	**290 (67)**	**139 (33)**

Table 1: The cohort of CGD patients divided according to nationality/ethnic background and type of CGD from van den Berg JM, et al. [77]

It remains controversial as to whether there are any clinical differences according to genetic defect. Some epidemiological studies have shown a milder clinical course for those with p47phox deficient CGD when compared to X-linked forms, demonstrating fewer complications and longer life expectancy[77, 87]. However, this is more likely to be related to residual NADPH oxidase activity (and therefore microbial killing) rather than inheritance per se. In a large study of 287 patients from 244 kindreds, those with p47phox deficiency and gp91phox missense mutations had significantly better survival than other mutations in gp91phox[79]. It was demonstrated that these patients had greater residual NADPH oxidase activity when compared to those with nonsense, frameshift, splice, and deletion mutations in gp91phox. Even small amounts of residual ROS production (1% of normal) resulted in improved survival[79]. It is likely that the contradictory findings in epidemiological studies reflect the various different genetic mutations present in the local CGD population.

1.6   Diagnostic tests

CGD is diagnosed by demonstrating the failure of phagocytes to produce reactive oxygen species. The nitroblue tetrazolium test (NBT) is the most well known. With a normal respiratory burst, leukocytes reduce the nitroblue tetrazolium dye to insoluble formazan which is a deep blue colour. It is a measure of superoxide production which takes place inside phagocytes. The dye cannot leave the cells, making them easily visible through a microscope. The inability of leukocytes from children with CGD to reduce nitroblue tetrazolium was first identified in 1967[88] and since then it has remained a widely used investigation. It was also developed into a quantifiable test by lysing the cells in potassium hydroxide and dissolving the formazan allowing spectrophotometric measurement[89]. The NBT can be used to detect X-linked CGD carriers as they will demonstrate a mixture of normal and abnormal neutrophil populations. It must be noted, however, that diagnosis of CGD in

patients with residual ROS production may be missed on the NBT as will show faint blue staining[90].

Other methods to demonstrate NADPH oxidase activity, or lack of it, have included measuring oxygen consumption with an oxygen electrode[91], ferricytochrome c reduction and chemiluminescence assays[89]. However, these are rarely used now due to the introduction of flow cytometry methods with dyes sensitive to the production of reactive oxygen species. One of the most commonly used agents dihydrohodamine -1,2,3, (DHR) enters cells and is oxidised to rhodamine – 1,2,3 after stimulation of the cells with PMA. Histograms are produced for unstimulated and stimulated cells (Figure 3) and in CGD patients the expected peak of stimulated cells is not present (Figure 4). A broad histogram can be seen in patients with autosomal recessive forms of CGD and some with variant X-linked CGD. This test can also be used to assess for X-linked CGD carrier status (Figure 5). A peak of cells that do not respond following stimulation are identifiable. The peaks are quantifiable, giving a percentage of functioning respiratory burst. The limitation of this technique is that the oxidation reaction is dependent on peroxidise and therefore will be abnormal in anyone with myeloperoxidase deficiency. However, this is distinguishable from a truly absent response in CGD.
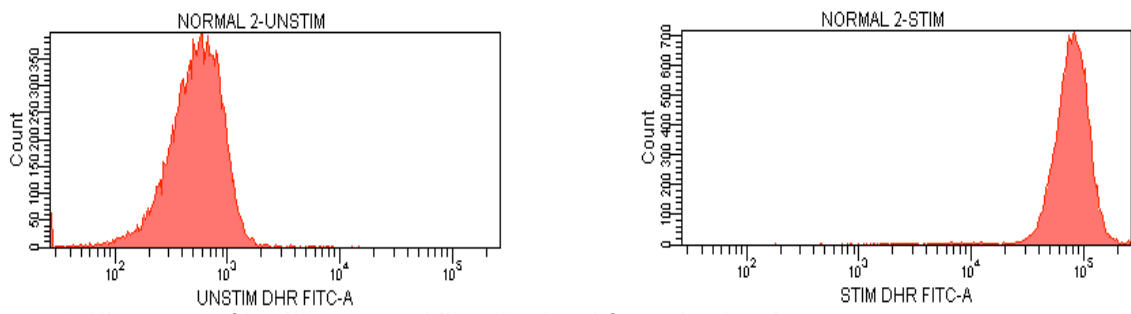
Figure 1: Histogram of healthy neutrophils, stimulated & unstimulated
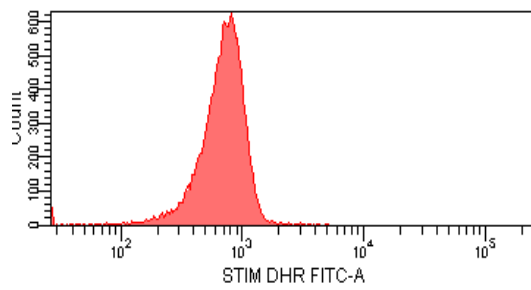
Figure 4: Histogram for stimulated neutrophils from CGD patient
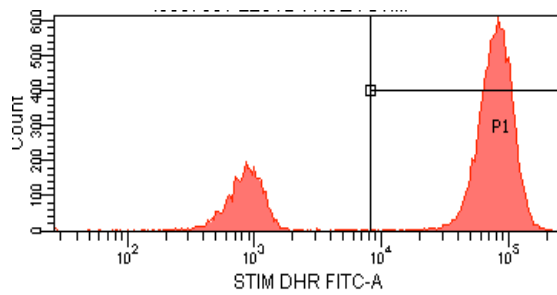
Figure 5: Histogram for female CGD carrier neutrophils

Once a diagnosis of CGD has been made by demonstrating failure of the oxidative burst, it is important to identify which component of NADPH is defective. Clinically it is important to know whether the condition is inherited in an X-linked or autosomal recessive pattern, to aid counselling of families. In many cases it is possible to identify absent NADPH oxidase components using Western blotting or flow cytometry[92, 93](Figure 6).
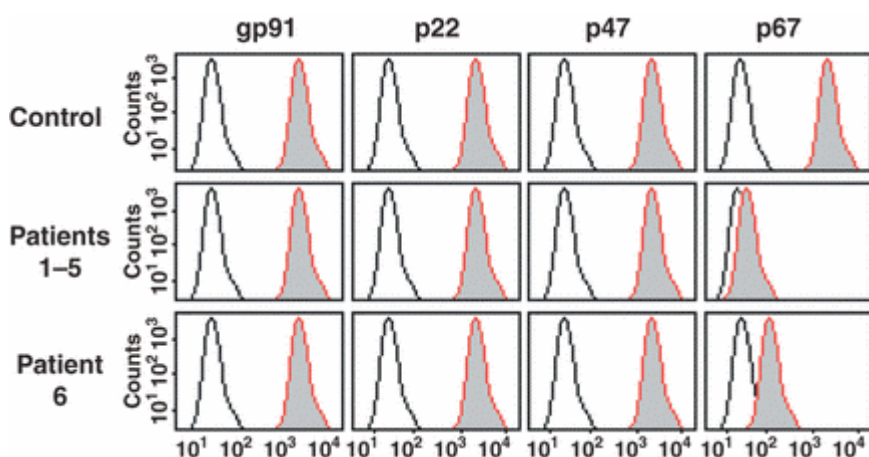
Figure 6: Flow cytometric analysis of neutrophils from patients and from a healthy (positive) control with specific antibodies against human NADPH-oxidase components. Koker et al. [93]

In those individuals that have a mutation resulting in normal protein expression, but absent or severely reduced enzymatic activity, it is possible to narrow the search slightly by using a cell free assay, to identify whether the defect is in the cytosolic or membrane bound compartment of NADPH oxidase[94]. It must be remembered, however, that gp91phox and p22phox are required to stabilise each other, so, if one is absent, the other will also be undetectable. In these cases it is possible to look for gp91phox expression in the neutrophils of female relatives.

Once the defective NADPH component has been identified mutation analysis can is performed. Gene sequencing for *CYBB*, *CYBA*, *NCF2* and *NCF4* can be performed from genomic DNA. Sequencing *NCF1* is more difficult because there are pseudogenes on either side. However, techniques have been developed to perform this analysis[95]. Demonstrating a genetic mutation is important for genetic counselling of families.

1.6.1 Carrier status

Random X chromosome inactivation early in fetal development of haematopoietic precursor cells in female carriers of CGD results in a mosaic pattern of wild type and mutated X chromosomes, producing a mixture of gp91phox positive and negative phagocytes. The overall degree of NADPH activity will be reduced, but can range from near normal to, in rare cases, severely depleted resulting in symptoms of CGD[96, 97]. It must be remembered however, that X-linked cases can occur as a result of a new mutation in the germ line and, therefore, mothers may not be identified as carriers[98]. For autosomal recessive CGD it is only possible to diagnose carriers of the mutation through genetic analysis.

1.6.2 Prenatal diagnosis

Prenatal diagnosis is possible for CGD in women known to be carriers of the disease. For many years it has been possible to measure NADPH oxidase activity in fetal neutrophils[99]. However, fetal blood sampling can only be performed from approximately 16-18 weeks gestation. More recently, genetic diagnosis has become possible from cells in amniotic fluid or chorionic villus sampling[100, 101]. This is important as it can allow families known to be at risk of having a child with CGD to make decisions about how they wish to progress with an affected pregnancy. This has been successfully used in Denmark to provide a healthy sibling for a child with CGD and provide umbilical cord blood for later HSCT for the affected child[102].

1.7 Clinical features

Because CGD is a rare disease, much of our early understanding of the clinical features come from case reports and small case series. However, data from a number of larger registries have been published in recent years, giving us a broader understanding of the clinical features of CGD at a population level. Three European series have been published; one from the UK and Ireland, consisting of 94 patients[1], one from Italy including 60 patients[103] and a

European group (excluding the UK and Italian data) consisting of 429 patients[77]. There are also small series from Israel (38 patients)[87] and Turkey (26 patients)[104]. The other very large series comes from the American registry which was compiled slightly earlier than the UK registry and results of which were published in 2000 for 368 patients[105].

The most common manifestations of CGD are bacterial and fungal infections, usually with catalase producing bacteria and fungi such as *Staphylococcus aureus*, Aspergillus species, enteric gram negative bacteria and *Burkholderia cepacia*[78]. Catalases are enzymes that convert hydrogen peroxide in to water and oxygen. Catalase negative organisms are thought to be less important in CGD because hydrogen peroxide is not broken down and can be used by the defective neutrophils in place of their own H_2O_2 production[106]. However, this theory has been opened up to debate. Catalase negative *Staphylococcus aureus* and *Aspergillus nidulans* have been shown to be as virulent as catalase positive organisms in mouse models[107, 108]. There have also been case series of infections with unexpected catalase negative organism in patients with CGD such as Actinomyces and Haemophilus species[109, 110]. Given that our understanding of the mechanisms by which NADPH oxidase is involved in micro-organism killing has evolved to encompass more than just the direct effects of ROS this is perhaps unsurprising. However, the virulence of catalase negative organisms in CGD remains yet to be fully explained.

CGD is usually diagnosed in early childhood, but the diagnosis can be delayed in to adulthood in some cases. The median age at diagnosis was 2.7 years in the UK [1]. Patients with X-linked disease are normally diagnosed at an earlier age than those with autosomal recessive disease. In the European series, mean age at diagnosis in X-linked disease was 4.9 years compared to 8.8 years for autosomal recessive disease[77]. Females are often older at diagnosis, in the UK series the mean age for females was 15.3 years, compared to 2.5 years for males[1]. This possibly reflects the fact that CGD is normally thought of as a male disease and can be over looked in girls. It may also reflect the less severe course seen in some series of autosomal recessive patients. There are

occasional reports of much older people being diagnosed with CGD, including people in their 60s[111]. The oldest man reported in the literature from America was aged 69 years at diagnosis[112]. He had x-linked CGD with non-functional protein expression and a small amount of residual NADPH activity. The diagnosis was made in him following an episode of *Burkholderia cepacia* sepsis. The family history came to light that his grandson had a presumptive diagnosis of CGD (after death from *Burkholderia cepacia* sepsis) and his daughter had been identified as a carrier.

1.7.1 Infection

In the UK and Ireland, the most common presentations of CGD are with suppurative adenitis and pneumonia[1]. Other common infections include liver abscesses, lung abscesses, osteomyelitis and septicaemia. These infections are severe and often result in hospitalisation for prolonged courses of treatment.

Worldwide, there appear to be a relatively small number of organisms responsible for the majority of infections, although case reports of more unusual organisms have become more frequent in recent years. In America the most common organisms are: *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens*, *Nocardia* and Aspergillus species[3]. In the UK *S. aureus* and Aspergillus species are the most commonly isolated organisms, with only a few cases of *Burkholderia*, *Nocardia* and *Serratia* infection[1]. European data demonstrate similar findings to that in the UK with a striking paucity of *Burkholderia* infections[77]. Localised skin disease as a results of Bacille Calmette-Guérin(BCG) vaccination occurred in 24 patients. However, it was not clear how many had received BCG as protocols vary across Europe [77]. A review of 38 patients in Israel, where autosomal recessive disease is more common due to high rates of consanguineous marriage, demonstrated a wider range of infectious agents. *S.aureus* and *Aspergillus* were again the most frequent, but there were also significant numbers of *Escherichia Coli*, *Salmonella*, *Candida albicans*, *Serratia marcescens*, *Pseudomonas* and *Burkholderia cepacia* infections[87].

Rare organisms reported to cause infection in CGD patients include the fungus *Geosmithia argillacea* (causing pulmonary infection in seven cases in America and two in France)[113, 114] and bacterial osteomyelitis caused by *Edwardsiella tarda* in a 17 year old male in Japan[115].

Aspergillus nidulans infection appears an important indicator for CGD. Many of the case reports of *A. nidulans* infection on Pubmed involve patients with CGD[116-121]. It also appears to be more virulent and difficult to treat than *Aspergillus fumigatus* in people with CGD[122]. A recent review of Aspergillus osteomyelitis in CGD demonstrated 55% mortality for *A.nidulans* infection compared to 13% for *A.fumigatus*[123]. The cause for this remains unclear as it has been shown that, compared to *Aspergillus fumigatus*, *Aspergillus nidulans* is not killed by ROS dependent means in vitro. Studies have demonstrated that swollen conidia of *Aspergillus nidulans* are resistant to hydrogen peroxide in vitro, furthermore, A.nidulans conidia and hyphae are poor stimulators of ROS[124]. Blocking NADPH oxidase in polymorphonuclear leukocytes (PMNs) also had no effect on A.nidulans hyphal damage. PMNs and peripheral blood mononuclear cells from X-linked CGD patients inhibited germination and damaged A.nidulans hyphae as effectively as controls [124]. These findings contrast with those for A.fumigatus, where hyphal damage is significantly reduced by blocking NADPH oxidase and in cells from X-linked CGD patients[124].

It may be that invasive fungal infection has become less prevalent in recent years. The French CGD registry, which collected data from 2005-2009 reported an incidence of 0.04 invasive fungal infections per patient year[125]. However, the study used the 2008 European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORT/MSG) definitions which were not available in the original study. It is not possible to determine incidence of fungal infection in the other registries as the data are not provided in a way which is comparable. The French group went on to look further at their proven mould infections. They identified 29 such infections in 24

patients, of whom only 54% were taking Itraconazole prophylaxis. In those that were taking prophylaxis, onset of fungal infection was later and *Aspergillus nidulans* and other opportunistic moulds were more common than *Aspergillus fumigatus*[126]. Prognosis improved over time, but was also better in those that received prophylaxis.

Tuberculosis may be more common in patients with CGD in endemic areas. A Chinese survey of 17 X-linked patients identified seven with tuberculosis, along with seven with abnormal scarring or abscess formation after BCG[127]. This was significantly higher than their background rate of childhood TB in the local population There is an increased risk of *Mycobacterium avium* infection as predicted using a mouse model which demonstrated increased risk of pulmonary infection (increased bacterial load) with *M.avium* and increased mortality compared to wild type mice inoculated with the same dose of *M.avium*[128]. There have been no published case reports of *mycobactium avium* in humans with CGD.

1.7.2 Inflammation

Inflammation is a significant component of the CGD phenotype. The typical inflammatory lesion is the granuloma (which gives CGD its name). Histological findings are of acute and/or chronic inflammation with fibrosis and non-caseating granulomata[129]. These have a predilection for hollow viscera such as the colon, stomach and bladder. Although the finding of granulomas is considered pathognomic for CGD they are not always present. In many organs and tissues the most common finding is non-specific inflammation[129]. Gastrointestinal manifestations can mimic inflammatory bowel disease, affecting any part of the gastrointestinal tract. In some instances patients have been diagnosed with Crohn's disease and treated as such before receiving a diagnosis of CGD[130] . A recent review of adult cases of CGD by Marks et al demonstrated histological features that would be consistent with a diagnosis of Crohn's disease[131]. Oesophageal, gastric outlet and small bowel obstruction can occur. Colitis was seen in 37% of patients in the UK series[1] but in only 9% of the European patients[77]. Impaired growth is common. In the UK series,

75% of those who had their height measured were below the population mean and 77% were below the mean for weight. Nasogastric nutritional support was required in 31% and a small number required total parental nutrition when acutely unwell[1]. Six patients in the series received growth hormone therapy for delayed growth. This information was not published in the European, Italian or American series so no comparisons can be drawn for different countries. Impaired growth may be related to poor nutrition as a result of bowel inflammation. However, in recent years it has been recognised that poor nutrition alone cannot explain impaired linear growth in Crohn's disease and proinflammatory cytokines have a significant role to play[132]. This has not been explored in CGD.

Urinary tract complications range from inflammatory cystitis to the development of urinary tract granulomata which can cause urinary frequency, as a result of reduction in bladder volume, through to ureteric opening or urethral obstruction in more severe cases. Ureteric opening obstruction can cause hydronephrosis with a resultant effect on renal function[133]. In the UK series, seven patients were found to have urinary tract granulomas[1].

Along with frequent pulmonary infections, chronic lung disease is also found in CGD. In the UK series, 77% of those that had chest CT scans had significant abnormalities including bronchiectasis, obliterative bronchiolitic and chronic fibrosis[1]. A study of histopathology specimens from CGD patients demonstrated a variety of changes including granulomatous inflammation in 40%, active neutrophilic inflammation in 40% and non-specific chronic inflammatory change with fibrosis in 11%[134]. However, both of these studies may over represent the changes as the patients underwent CT or lung biopsy for clinical indications.

Ocular complications can also occur with CGD, particularly chorio-retinal abnormalities. In a small UK series, Goldblatt et al identified nine children out of 38 who had Chorioretinal lesions on screening[135]. They described the typical lesion as a "punched out" lesion associated with pigment clumping lying along

major retinal vessels. The UK series by Jones et al also identified nine patients with chorio-retinal abnormalities, however, these may well be the same nine identified by Goldblatt et al. A series from Korea also frequently identified Chorioretinal lesions on screening, with six out of 17 patients affected[136]. This number compares to only 8 out of 368 in the American series reported as having chorioretinitis. However, this may reflect lack of screening at the time when data was collected. The significance of these findings has not been evaluated, Goldblatt et al describe the majority of lesions as "inactive". However, 2 patients had significant visual impairment as a result of extensive lesions. It is unclear how much of an impact this had on these individuals.

Skin rashes have also been described. these are typically Discoid Lupus Erythematosus(DLE) like but do not fulfil all the criteria for a diagnosis of DLE[137]. A case of acute neutrophilic dermatosis has also been described[138] as has dermatomyositis and leucocytoclastic vasculitis[139].

A number of autoimmune conditions have been described, including: antiphospholipid syndrome; IgA nephropathy; juvenile idiopathic arthritis; idiopathic thrombocytopaenic purpura and myasthenia gravis[140, 141]. However, these are case reports and small series and whether these are truly linked to CGD is yet to be established. Until our understanding of the inflammatory mechanisms in CGD improves it will be difficult to develop clear hypotheses about the connection between these conditions and CGD.

1.7.3 *Haemophagocytic Lymphohistiocytosis*

Haemophagocytic Lymphohistiocytosis(HLH) is characterised by fever, cytopaenias, splenomegaly, hypertriglyceridaemia and low fibrinogen levels. There are a small number of cases of patients with CGD presenting with evidence of HLH, most commonly in association with infection[142, 143]. HLH results from an exaggerated immune response with secretion of excessive cytokines such as IL1, IL6, IL18 and TNFalpha. This perhaps represents the severe end of the hyperinflammation seen in CGD.

1.7.4 Malignancy

There are reports of various malignancies in patients with CGD, including; lymphomas, leukaemia, retinoblastoma, rhabdomyosarcoma and glioblastoma[144-147]. In the 1980s it was suggested that hydrogen peroxide was required for neutrophil mediated tumor cell killing[148]. Therefore, it was hypothesized that CGD patients could be at increased risk of malignancy, however, this has not been reported in any of the large registries.

1.8 Treatment

1.8.1 Prophylaxis

As described above, the first documented cases of CGD were fatal. Recognition and management of infections significantly improved life expectancy in the early years of the disease. Antibiotic prophylaxis became widespread from the late 1970's reducing the number of non-fungal infections[149]. Sulphamethoxazole-trimethoprim(SMX-TMP) was identified as particularly useful for preventing the type of infections seen in CGD, a number of mechanisms were considered for this but it was established that, when compared with other antibiotics such as Penicillin, SMX-TMP had high levels of intra-cellular accumulation within leukocytes[150] . In 1983 Weening et al reported the safe and effective use of long term SMX-TMP in 9 patients, reducing the number of bacterial infections, reducing the need for surgical intervention and the number of days of hospitalisation per year[151]. In a description of the early European experience by Mouy et al in 1989, the introduction of prophylactic SMX-TMP significantly reduced the number of bacterial infections (from 2.06 to 0.43 per year) and the need for surgical intervention, but did not affect overall survival due to a large number of deaths being related to fungal infection[152]. They also tried ketoconazole prophylaxis, but this had no effect on the Aspergillus infections that CGD patients commonly suffered.

In the early late 1980s and early 1990s there was interest in the use of interferon gamma as a prophylactic agent due to its apparent ability to augment superoxide production[153]. A multicentre study showed a 70% reduction in the

number of infections in those receiving interferon gamma compared to the placebo group[154]. It therefore became common place to add interferon gamma in the United States. However, this was not adopted so widely in Europe, due to lower levels of serious infection compared to the American cohort it was trialled on[155], along with continued debate about its mechanism of action. It does not appear to up regulate NADPH oxidase activity[156] and there is a lack of evidence of effect when compared with Itraconazole prophylaxis. Long term follow up of 76 patients in America, from 1992 – 2001, suggested interferon gamma was well tolerated but serious infection rates continued at 0.3 per patient year for bacterial infection and 0.1 per patient year for fungal infection which were not dramatically different from rates in Europe where it was not being used[157]. An Italian retrospective study, as part of their registry, found no additional benefit when those receiving prophylactic interferon gamma were compared to those receiving standard antibiotic and antifungal prophylaxis[103]. Adverse effects from interferon gamma were also common, including fever, myalgia, headache and rash[103].Studies have failed to demonstrate improved neutrophil oxidative metabolism[158]. Only two patients in the UK registry received prophylactic interferon gamma[1]. In the European registry, 33% of patients received interferon gamma at some point, but this was not differentiated in to prophylactic and treatment groups[77]. More recently studies have suggested that only people with splice site mutations will benefit from interferon gamma therapy[159, 160].

Itraconazole prophylaxis was introduced later than interferon gamma, but has become common place in Europe and America. Adding antifungal prophylaxis, in the form of Itraconazole, showed a significant reduction in the number of fungal infections in a randomised double-blind placebo controlled trial by Gallin et al in 2003. In the UK cohort 93% received antifungal prophylaxis[1], mainly Itraconazole, while in the wider European experience only 53% had received anti-fungal prophylaxis[77].

1.8.2 Treatment of infections

Aggressive and early use of antibiotics and antifungals remains the mainstay of treatment of infections in CGD. These are empirically directed against the organisms known to be problematic in CGD, while awaiting culture confirmation. Adjunctive therapies have included interferon gamma, granulocyte infusions and granulocyte colony stimulating factor in some cases. These are most often used for severe infections not responding to conventional treatment[1, 77]. White cell infusions have, perhaps become less commonly used, with the development of better antifungals as there are associated risks, such as alloimmunisation to HLA class I antigens and the risk of Cytomegalovirus infection, which could complicate any later HSCT[161]. Surgery may be required for drainage of abscesses or removal of consolidated areas of infection that will not respond to antibiotics alone[133].

1.8.3 Treatment of inflammatory complications

Inflammatory components of CGD, for example bowel disease or obstruction of the GI or genitourinary tract, require immunosuppressive agents. The most commonly used agents are corticosteroids, however the benefits have to be weighed against the increased risk of infection. In particular, high dose steroid use has been associated with the development of mucormycosis in CGD patients[162]. The use of corticosteroids was first reported in 1987, for gastrointestinal and genitourinary granulomata causing obstruction[163]. Corticosteroids are also effective for granulomatous cystitis[164]. However, symptoms may recur on weaning and long term low doses may be required[133]. CGD colitis is often managed in a similar fashion to Crohn's disease, with 5–aminosalicylates (5ASA), steroids and, more recently, the Tumour Necrosis Factor alpha blocking agent Infliximab[3, 130, 165]. The review by Marks et al identified some response to 5-aminosalicylates, thalidomide and interferon gamma, with better response to azathioprine, infliximab and surgical resection. None of their patients responded well to steroids[131]. Infliximab has been useful in treating refractory colitis. However, there are concerns about increasing rates of infection in these patients, despite

continuing antimicrobial prophylaxis. Five American patients treated with infliximab, along with 6-Mercaptopurine, prednisolone and 5ASA compounds developed serious infections with Burkholderia, Staphylococcus, Candida and Aspergillus species[166]. A literature review of 17 published cases (11 autosomal recessive) where TNF alpha blocking agents (Infliximab, Etanercept or Adalimumab) were used demonstrated sustained improvement in only five cases but also raised concerns about increased serious infection risk as seven developed serious infection after use of TNF alpha blocking agents[167]. The interleukin-1 antagonist, Anakinra, has also been successfully used, due to fewer concerns about infectious complications[168].

Recent work in macrophages, from gp91phox deficient mice, has raised an interesting question about the use of interferon gamma for suppression of inflammation in CGD. It was demonstrated that priming with interferon gamma enhanced the uptake of apoptotic neutrophils by macrophages, which may contribute to a reduction in the inflammation seen in CGD[169].

1.8.4 HSCT

Stem cell transplant is now an attractive option for CGD as it can provide a complete cure. However, early attempts in the 1970s and early 1980s were essentially unsuccessful, with only one child out of five surviving with reasonable donor engraftment [170]. In America in the 1990s non-myeloablative T cell depleted HLA identical sibling transplants were attempted in 10 patients with 70% survival. Of these, two rejected their transplant and three of the four adults with donor engraftment developed acute Graft versus Host Disease (GvHD)[171]. From then there has been significant improvement. The European experience from 1985-2000 demonstrated 81% survival with complete cure using a more aggressive approach than the Americans, with myeloablative conditioning and unmodified haematopoietic allograft [172]. Over recent years there has been an improvement in survival following HSCT for many primary immunodeficiencies[173]. This results from a combination of factors including development of less toxic chemotherapy regimes, better recognition and management of infections and improved treatment of GvHD. Improvements in

transplant techniques have led to improved survival and disease cure with both matched sibling donors and unrelated donors for CGD[2, 172, 174]. Long term outcome has also been good, with a recent series of 20 patients from a single centre in the UK showing 90% survival with normal neutrophil function, remission of colitis and good catch up growth[2]. Concerns have persisted about the use of myeloablative conditioning in patients with significant infections or inflammatory complications. However, recent experience with a reduced intensity conditioning regime has resulted in a good outcome in older patients with a number of severe disease related complications[175], which may provide hope of a cure for those that were previously deemed too unwell for HSCT . HSCT has also recently been advocated for the treatment of people with severe bowel disease associated with their CGD, when conventional treatment fails or side effects are too severe[176].

There are potential long term complications of HSCT which have yet to be evaluated in children who have undergone transplants for immunodeficiency, including CGD. Late effects are well recognised in transplants for malignancy. These include, secondary malignancy, endocrine disorders, particularly thyroid dysfunction, and organ specific complications such as bronchiolitis obliterans and avascular necrosis of the femoral head[177]. Many of these complications are related to the use of total body irradiation and long term steroid use for chronic graft versus host disease which are not normally factors in transplant for children with CGD[178]. Gonadal failure and infertility are also a particular concern following HSCT. Again, infertility is associated with irradiation but also busulphan and cyclophosphamide conditioning regimes, which are used in CGD transplants. Sanders et al demonstrated that the partners of 24% male patients who had received cyclophosphamide but only 4% of those who had received busulphan and cyclophosphamide became pregnant. However, these were transplants for malignancy and aplastic anaemia in children over the age of 12 at transplant[179]. There has been no evaluation of fertility after HSCT in childhood for CGD as there is currently not long enough follow up.

In post-pubertal males undergoing HSCT there is the option for storage of sperm pre-transplant, which may give them the potential to father children later in life. This is obviously not an option for children who undergo transplant before puberty and is therefore an issue that families may wish to consider when thinking about the best time for their child to undergo HSCT. However, there is also the issue for men with X-linked CGD regarding potential transmission of their inherited disease, which is not present in those treated for malignancy. Males with X-lined CGD, including those who have undergone HSCT, can have healthy male children, but female children will be carriers, with the potential to have an affected child themselves. This may make the issue of whether to have children a very difficult decision for individuals.

1.8.5 Gene therapy

Similar to HSCT, gene therapy has been tried for CGD. In principle it appears a good option, as CGD is the result of a single gene defect, and incomplete correction will still eliminate symptoms. Unfortunately however, gene therapy has not seen particularly successful results to date. Gene corrected cells in CGD patients do not show a growth advantage over diseased cells as seen in patients with severe combined immunodeficiency treated with gene therapy. This results in very low numbers of the corrected granulocytes appearing and remaining in the peripheral blood long term[180]. Better results have been obtained if patients receive myeloablative conditioning but, as in HSCT, this has risks attached, such as herpes virus reactivation which could be fatal. There are also concerns about the potential development of leukaemia as has been seen in X-linked SCID patients treated with gene therapy[181]. This has not been found to date in CGD patients but clonal expansion of the modified cells has been found[180]. A report published in 2010 of two cases of CGD treated with gene therapy demonstrated vector inactivation of gene transduced cells, which then had no microbicidal activity. They also demonstrated clonal expansion and increased genetic instability, with myelodysplasia. One patient died of overwhelming sepsis 27 months after gene therapy and the other was treated with HSCT[182]. With the current concerns about gene therapy it is currently

mainly used as rescue therapy, to treat severe infections which are refractory to conventional management, with the expectation that it will not be curative but will prevent death from the immediate infection[183, 184].

There are a number of advances that may make gene therapy a more attractive option in the future. Recent work in mice has suggested it may be possible to develop lineage and stage restricted vectors, targeting only fully differentiated mature phagocytes, so preventing the risk of mutagenesis[185]. Another option appears to be the use of patient derived induced pluripotent stem cells (iPSCs) to generate functional neutrophils. The potential for this technique has been demonstrated to work in mice[186] and in human cells[187]. In theory it would be possible to use gene corrected iPSCs to create functional neutrophils that could be infused in to a patient, for example to treat refractory infection. This would have fewer risks than current strategies as the mature neutrophils would not have the ability to engraft or proliferate, so preventing the risk of myelodysplasia and malignancy.

1.9 Prognosis

CGD is no longer fatal in early childhood, but remains a life limiting condition. Data from the UK series demonstrated 88% survival at 10 years, but only 55% survival at 30 years[1]. The Italian series identified similar trends in survival, with 46% still alive 25 years after diagnosis[103]. Perhaps surprisingly, these figures do not appear to have improved significantly over recent years, despite presumed better recognition and management of the disease. Finn et al reviewed UK CGD patients from 1964-1989 and, similar to Jones et al, identified 50% mortality by the third decade[188]. Fungal infections have continued as an important cause of death, Aspergillus being particularly prominent. Aspergillus infection was the cause of death in 50% of the Italian cases and 15 out of 84 deaths in van den Berg et al's paper on the European experience [77, 103]. Survival was significantly reduced in the French cohort for those that had invasive fungal disease compared to those that did not[125]. Given that many patients now take anti-fungal prophylaxis it is difficult to say

why mortality remains so high. The causative organisms continue to be similar and should be sensitive to the azoles. There are few reports of azole resistance in CGD, one such report was for a patient treated over a two year period for aspergillus pulmonary infection with combination azole and echinocandin therapy, who developed resistant isolates on treatment[189]. None of the registries reported Itraconazole levels so it is difficult to tell whether patients were taking the prescribed prophylaxis. Other infectious causes of death have included Candida species, *Burkholderia cepacia* and Salmonella[77, 103]. Non-infectious causes of death described by van den Berg et al included: cardiac failure, chronic lung disease and malignancy[77]. Non-cirrhotic portal hypertension appears to have an important association with mortality in infectious episodes, with thrombocytopaenia, raised alkaline phosphatase levels and history of liver abscess all being independent predictors of mortality[190]. For those that live with CGD there is continued morbidity, with frequent infections requiring hospital treatment and long term growth failure.

Given the long term complications of the disease, one could argue that curative treatment should be advocated. However, there is not insignificant mortality associated with HSCT. Four out of 27 CGD patients that underwent HSCT died in Seger et al's paper on the European experience. These were all patients with pre-existing therapy-refractory fungal infection, which increases the risk of transplant[172]. Two out of 20 died in Soncini et al's paper, one during chemotherapy conditioning for HSCT as a result of overwhelming fungal infection and the other 73 days after transplant from haemorrhage, as a result of arterial erosion by a tracheostomy tube[2]. Currently, gene therapy remains experimental as there are still questions about its effectiveness and safety to answer before it can be used routinely. Therefore, families are left with a difficult decision, to risk the complications of HSCT or gene therapy, or live with the long term complications of CGD and expect the sufferer to have a reduced life expectancy.

1.10 Quality of life in Chronic Granulomatous Disease

As detailed above, patients with CGD have wide variety of infective and inflammatory complications. They can spend a large amount of time in hospital and have difficulties maintaining normal, everyday activities. From discussion in clinic appointments, there is anecdotal evidence of the impact this disease has on children and families. For example, those who give up their favourite sport as they feel embarrassed to get changed in front of their peers because they are short and not as physically mature. Or, those who can not go away on trips with school or friends because of the need to go to the toilet on a frequent basis. There are also restrictions placed on a child's activities in order to avoid infection, for example, the need to avoid playing outside in fallen leaves or wood chip due to the risk of fungal infection. There can also been an impact on schooling, with the need to avoid building work which can result in a child not being allowed in certain classrooms and potentially being isolated from their peers. There is also stress for the family caused by the knowledge that the child is living with a life limiting condition and maternal guilt about transmission of a genetically inherited disease.

Clinicians recognise from their interactions with these families that these issues are likely to have an impact on the patient's quality of life, their emotional wellbeing and their self esteem. The degree of impact the diagnosis of CGD has on quality of life, emotional well being and self esteem is likely to vary with a child's age and maturity, depending on their understanding of their condition and the degree of impact it has on their daily life. For example, it would seem likely that a teenager with a good understanding of their diagnosis and growth retardation due to colitis is likely to be more affected than a five year old, who has been relatively well, perhaps only in hospital with one infection and has little understanding of their condition. However, none of this has been formally been studied in CGD.

1.11 Quality of life in chronic disease

Quality of life has been defined by the World Health Organisation (WHO) as:

A individual's perception of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectation, standards and concerns[191].

An individual's state of health can impact on their quality of life.

Where the WHO defines health as:

A state of complete physical, mental and social well-being – not merely the absence of disease or infirmity[192]

Health related quality of life (HRQOL) is a concept that has evolved over recent decades to include aspects of quality of life connected with physical and mental health, including: health perceptions, functional status, social support and socioeconomic status[193]. Tools have been designed to measure HRQOL and it has become a recognised outcome in a wide range of research. HRQOL measures can be generic or disease specific. They are usually self-report questionnaires, as doctor's and patient's opinions do not correlate well, with clinicians rating a patients quality of life as poorer than the patient would themselves[194].

Chronic illnesses can result in poor quality of life in children and adults with a variety of conditions. For example, poor HRQOL has been documented in sickle cell disease, juvenile idiopathic arthritis and rare genetic conditions[195-199]. However, not all individuals with a disease or disability will rate themselves as having poor quality of life. Therefore, it is not possible to draw conclusions for patients with CGD without measuring it. A study of children with chronic kidney disease demonstrated higher quality of life scores for those with kidney disease compared to the normal population[200]. The authors concluded that the patients had successfully adapted to their diagnosis, rather than lowering their expectations of what they could achieve, indicating a true good quality of life.

Children often rate their quality of life better than their parents[201, 202]. This is thought to be because the children do not know anything different, whereas, parents are comparing their situation to other healthy children. Some research has been undertaken to evaluate whether it is possible to predict quality of life. In juvenile idiopathic arthritis the predictive factors have been demonstrated to be: functional ability, pain, subjective burden of medication and school absence[199]. However, disease severity does not necessarily correlate well with quality of life. A study in children with asthma showed quality of life was predicted by associated emotional and behavioural problems but not disease severity[203]. A study of children with JIA demonstrated impaired quality of life in children with minimal symptoms or even no symptoms[204].

Advances in medical treatments have resulted in improved survival for people with many chronic or life threatening conditions. As a result of this there has been an increased interest in the presumed improved quality of life provided by these treatments. HSCT has been demonstrated to have a positive impact on quality of life in children, although there is a drop immediately after the procedure there is a gradual increase from that point onwards[205, 206]. However, this work has mainly been done in paediatric haematology/oncology transplants where HSCT is rescue therapy for conditions with dramatically reduced life expectancy. There are no studies looking at quality of life in children undergoing HSCT for primary immunodeficiencies. There has been one study looking at quality of life in the primary immunodeficiency X-linked agammaglobulinaemia, which showed poorer quality of life compared to healthy controls, but not as bad as quality of life in children with rheumatological diagnoses. However, this group of patients do not undergo HSCT[207]. There has been a study of social functioning (an element of quality of life) in children treated with HSCT for primary immunodeficiency which included a small number of CGD patients (3% of the sample). This demonstrated impaired social functioning, with difficulties in peer relationships, fewer friends and being more likely to play alone when compared to healthy controls[208].

As has been discussed, it is not possible for clinicians to determine the quality of life of patients with CGD without measuring it. For it to be valid, it is necessary to undertake an assessment of patient/parent perspectives on quality of life not clinician ratings.

1.12 Emotional disorders in chronic disease

1.12.1 Psychological adjustment to chronic disease

Diagnosis of any chronic disease will impact upon a child and their family, as they are confronted with new challenges, for example, dealing with symptoms, limitations to their activities and the administration of treatments. Patients and families must find ways to cope with, and adjust to their new circumstances. The effects of diagnosing a chronic disease in a child are wide ranging, impacting on physical well being but also education, social relationships and emotional well being, along with family finances[209].

Studies in adults with chronic illness have shown adjustment is most successful when patients can maintain emotional balance, preserve healthy relationships and maintain a good functional status, for example continuing to work[210]. However, approximately 30% of adult patients have a prolonged or unsuccessful adjustment to their new situation[211]. There are many factors which influence adjustment. A study in children with type 1 diabetes mellitus showed characteristics such as age, sex, socioeconomic status, ethnicity and family environment can all have an impact on adjustment[212]. Abnormal psychological responses in this group include depression, anxiety, stress, behavioural disorders and eating disorders. Positive adjustment was associated with effective coping strategies, positive family functioning, good self management skills and social competence[212].

1.12.2 Emotional disorders in children with chronic disease

A meta-analysis of 87 studies of psychological adjustment to a diagnosis of a physical disorder, has shown children are at risk of psychological adjustment disorders such as depression, anxiety and stress[213]. A large epidemiological

study in Canada has shown children with chronic illness are at increased risk of a whole range of psychological disorders including: anxiety, depression, obsessive-compulsive disorder, conduct disorders and attention deficit hyperactivity disorder[214]. An even larger population study of nearly 12,000 children and adolescents, with a range of chronic conditions, in America also demonstrated an increased risk of behavioural problems, such as peer conflict and social withdrawal[215]. Social adjustment can also be affected. The Canadian study showed children with chronic conditions with associated disability (measured by limitations of normal function) had difficulties getting along with peers, were more isolated, had low participation in activities and low competence[214]. This was not true for those with chronic conditions without associated disability. It may be, however, that these findings are due to physical restrictions impacting on ability to be involved in activities with their peers.

Data from the original UK CGD registry demonstrated increased emotional difficulties, as reported by their parents, in children with CGD compared to the normal population when evaluated with the Strengths and Difficulties Questionnaire[6].

In recent years, it has been recognised that depression has a significant inflammatory component. Various markers of inflammation have been shown to be raised in people with depression, including: C-Reactive Protein, interleukin-1, interleukin-6 and tumour necrosis factor alpha[216-218]. Work in adults receiving haemodialysis has shown a correlation between IL6 levels and anxiety symptoms but not depression[219]. They also demonstrated inverse correlation between quality of life and levels of IL6, IL10 and TNF alpha.

Rates of depression have not been evaluated in people with CGD. It could be hypothesised that depression will be common, not just because of the implications of living with a life limiting disease, but also because CGD induces a hyperinflammatory state, which could itself induce depression.

1.13 Self esteem in patients with chronic disease

Self esteem is defined as:

An individual's self perception of his/her abilities, skills and overall qualities that guides and/or motivates specific cognitive processes and behaviours[220]

Harter argues that by the age of eight years, children have a view of their self-worth which is broader than their competencies in specific skills, for example, physical activities[221]. Physical appearance is an important predictor of global self worth[222]. Scholastic competence appears to become more important as children get older. A small study from Northern Ireland demonstrated that at age eight years social acceptability was second after physical appearance, but at eleven years old scholastic competence was second most important[222].

Self esteem can be affected by chronic illness. Low self esteem can result in depression, difficulty managing stress, poor social relationships and perceived worsening of symptoms, so having a significant impact on the quality of life of patients[220]. However, in one study of adolescents, those with chronic illnesses scored higher self esteem and lower stress than controls[223]. It has been argued that these children look to things other than physical health to provide value and meaning to their life. There has been no evaluation of self esteem in those with CGD.

1.14 Intelligence and Chronic Illness

Intelligence (IQ) can be difficult to define and continues to provoke much debate regarding its worth.

It has perhaps best been described in an editorial signed by 52 American academics as:

A general mental capability, that among other things, involves the ability to reason, plan, solve problems, think abstractly, comprehend complex ideas, learn quickly and learn from experience[224].

Many would agree that it is beneficial to consider (and measure) intelligence as it is associated with educational, occupational, economic and social outcomes[225]. It appears to be a predictor of long term survival in the general population. An association between childhood IQ and all cause mortality in adulthood has been described. A study from Scotland for children born in 1921 and in school in 1932 showed those with lower scores on the mental ability test were less likely to be alive in 1997[226]. However, a cohort from the North East of England identified a correlation between IQ at age 11 and mortality up to middle age (56 years) in men, but not in women[227]. The reasons for this association are complex and may be connected to those with higher IQs making healthier lifestyles choices and making better use of health care services.

Low IQ has been identified in a number of chronic illnesses, both with and without obvious neurological involvement, for example: multiple sclerosis[228], schizophrenia[229], chronic kidney disease[230] and inflammatory bowel disease[231]. This is important because it has the potential to impact on the health of patients, in terms of understanding their disease, making decisions about treatment options and complying with treatment (those with higher verbal IQ are more likely to comply with long term medication[232])

In recent years it has been demonstrated that inflammation is associated with cognitive decline and the development of dementia in the elderly[233, 234].

Increased levels of the non-specific inflammatory markers C-reactive protein (CRP) and the Erythrocyte sedimentation rate (ESR) have been shown to be associated with poorer cognitive function in adults[235, 236]. This association has even been demonstrated in adults as young as 18-20 years old, where ESR was inversely correlated with performance on an IQ test. However, this study did not identify causes for raised ESR and may have included those with acute infection, as well as on going inflammatory conditions[235]. Acute infection has been shown to alter cognitive function. A small study of 12 humans were administered a low dose of Escherichia coli endotoxin. They had TNF alpha and IL-6 levels measured at intervals after administration and were also asked to perform neuropsychological tests before and after administration of the endotoxin. This showed inverse correlation between circulating IL-6 levels and memory function, although not overall cognitive function[237].

There has also been a lot of interest in Interleukin 1 beta (IL1 beta), a pro-inflammatory cytokine which is expressed in the brain[238]. IL1 beta has been implicated in postoperative cognitive decline in the elderly[239]. In a mouse model of orthopaedic surgery (as a trigger of inflammation) memory impairment was demonstrated which was interleukin 1 dependent. IL-1 receptor knock-out mice, and mice treated with an IL-1 antagonist did not demonstrate the same memory impairment[239]. Another mouse model with hippocampal IL-1 beta over expression demonstrated impaired long term contextual and spatial memory when exposed to various fear conditioning experiments[240].

There is evidence that IQ can improve after certain solid organ transplants. In a study of children undergoing kidney transplant for end stage kidney disease, a group known to have neurocognitive deficits[241], IQ improved by a mean of 12 points after transplant compared to a group who had not received transplant[242]. This took the post-transplant group from the borderline to the low normal range. Although not evaluated, it could be hypothesised that this may help in patient compliance with their long term immunosuppression as well as having social and economic benefits.

1.14.1 Cognitive deficits in CGD

One small American study of 26 patients identified cognitive deficits in the X-linked CGD population, with 6 (23%) having an IQ less than 70, which is markedly below the normal range and will have an impact on educational and work performance[5]. The authors suggested the lower than expected IQ could be due to frequent illnesses and hospitalisations. However, this has not been seen in other chronic illnesses such as cystic fibrosis[243], a condition that would cause a similar pattern of frequent infections and hospital admissions. They, therefore, proposed other possible mechanisms related to the lack of superoxide production in CGD, including abnormal development of neurons and abnormal signalling within cerebral pathways[5]. Other proposed mechanisms include frequent brain infections or dysregulation of inflammatory processes[129]. However, the data from this study need to be interpreted with care as all of the patients had already been referred to neuropsychological services for educational or behavioural concerns and may not therefore reflect the true population of people with CGD.

There has also been some work in CGD mouse models (both gp91 and p47 phox deficient mice) which demonstrated impaired memory, thought to be due to lack of ROS as signalling molecules [244].NADPH oxidase is required for N-methyl-D-aspartate (NMDA) receptor dependant signalling through activation of extracellular signal-regulated kinase (ERK) in the hippocampus. In mice NMDA receptor dependent activation of ERK can be blocked by diphenylene iodonium, an NADPH oxidase inhibitor, and is also absent in p47phox deficient mice[245]. Blocking signalling impairs long term potentiation which is a mechanism associated with memory formation. In the mice this resulted in impaired spatial memory in those that were gp91phox deficient and impaired context dependent fear memory in those that were p47phox deficient[244]. The authors of this work suggested there may be mild cognitive defects in patients with CGD based on their work in mice. This has not been evaluated in detail.

Another area which has not been evaluated in CGD patients is the possible link between high levels of IL1 beta (which have been found in CGD[55]) and the cognitive decline and memory impairment as discussed above.

There has been one study looking at cognitive deficits and behavioural abnormalities in children undergoing HSCT for severe congenital immunodeficiencies, which included two patients with CGD[4]. This concluded that children undergoing HSCT for primary immunodeficiency were more likely to have cognitive deficits and behavioural abnormalities when compared to sibling controls. However, specific analysis was not carried out for the CGD patients as the numbers were so small. It is also important to recognise that this group included children with a diagnosis of adenosine deaminase deficient severe combined immunodeficiency, which has previously been shown to be associated with low IQ and increased behavioural difficulties and may have therefore influenced the sample results[246]. There have been no other published studies on cognitive factors in CGD patients undergoing HSCT.

1.15 Summary

Since the UK and Ireland CGD registry was compiled initially in 2000[1] there has been no further large scale study in this country looking at the prevalence, clinical features and mortality for CGD. Although rare it remains an important condition due to the high level of medical input required by each patient. There have been significant advances in management of the condition, including improved outcome from HSCT and further developments in gene therapy. This has led to improved survival and complete cure for some. It is not clear whether there has been an increase in survival for those treated conservatively. There is an increased awareness and interest in quality of life in primary immunodeficiencies, as other chronic conditions and the process of HSCT have both been shown to impact on life quality. There have also been questions raised about the cognitive function for people with CGD following the publication of the paper by Pao et al, as described in section 1.13.1[5].

Since issues of physical and mental health are interlinked it was appropriate to gather further clinical information on people with CGD in the UK and Ireland and look at their psychological wellbeing and cognitive function at the same time.

This information is vital for individuals with CGD, in terms of making the decision about whether conservative or curative management would be the best option for them. It is also important for the health services in order to better understand the current health needs of those with CGD and plan for appropriate service provision.

Chapter 2 Study Objectives & Hypotheses

The objectives of this study were to:

1. Survey the clinical course, treatment and mortality of children with CGD in the UK and Ireland, through an updated registry of CGD patients.
2. To compare rates of infection, admission and surgery in non-HSCT and post-HSCT children.
3. Evaluate cognitive function in children with CGD, comparing those that have and have not undergone curative treatment.
4. To document quality of life and emotional wellbeing in children with CGD and compare those who have and have not undergone HSCT.

The hypotheses for this study were:

1. Children with CGD have more infections, surgery and admissions to hospital than those who have undergone HSCT.
2. Children with CGD have impaired cognitive function, as demonstrated through lower than expected IQ test scores.
3. Children with CGD have poor quality of life, increased emotional and behavioural difficulties and poor self esteem compared to normal, healthy children.
4. Children who have undergone HSCT for CGD will have better quality of life, emotional wellbeing and self esteem compared to those who have not received HSCT.

Chapter 3 Methods

3.1 The UK and Ireland CGD registry

Disease registers, or registries, are a well recognised way of gathering clinical information for a variety of purposes. The terms are often used interchangeably. The definition according to the Dictionary of Public Health is:

Register, registry   A data file containing information about all the identified cases of a condition, such as cancer, ideally in a region (state, province, or nation) with a defined population. The information should include age, sex, other pertinent identifying data such as occupation and place of residence, and precise diagnosis…...Preferably a registry should provide statistically compiled data on all identified cases in a specified jurisdiction with a defined population, so that rates and prevalence can be calculated, but registries are useful even without a defined population because case fatality and survival rates can be calculated for the populations in specified diagnostic categories[247].

According to a report commissioned by the Department of Health Policy Research Programme for the White Paper Saving Lives: Our Healthier Nation, registers are databases that attempt to identify all cases of a disease in a defined population[248]. Registers are particularly useful for gathering data on rare diseases[249]. In a review of disease registers in England published in 2002 approximately 250 were identified, covering a variety of different conditions, such as cancers, diabetes, congenital anomalies and more specific diseases such as; Galactosaemia, Gaucher's and Fabry's diseases[248] .

In 2008 Jones et al published data from the UK and Ireland CGD registry, compiled in 2000-2001[1]. It attempted to identify all CGD patients in the UK and Ireland who were alive and diagnosed before 31st December 2001. Medical specialties that might care for CGD patients were determined and 1684 physicians were contacted in an attempt to identify all cases of CGD within the UK and Ireland. Those that reported having CGD patients were provided with information packs and consent forms for participants to enrol in the registry. 112

patients fulfilled inclusion criteria for the registry and 94 were enrolled. Detailed data were collected from medical records and death certificates obtained from the Office of National Statistics. This provided comprehensive information about the majority of patients with CGD in the UK and Ireland at that time. Due to the design, this study was successful in finding the majority of people with CGD rather than just those limited to large centres. The Italian and European registries only requested information from primary immunodeficiency network members and specialist diagnostic laboratories respectively, so potentially missed patients cared for in local hospitals only[77, 103]. The UK and Ireland Registry gathered very wide ranging clinical information, as the research team abstracted the data, rather than relying on local physicians to complete the forms. It was also able to collect information regarding people with CGD who died before referral to a specialist centre by collecting data from the Office of National Statistics regarding cause of death. Some additional updates were added to the Registry up to 2004. The registry is held within Newcastle University and is the basis for the current update.

3.2   Study population

This study aimed to recruit participants from across the entire UK and Republic of Ireland. Ninety-four patients were consented for the previous registry. It was not known exactly how many people were living with CGD in the UK and Ireland, as the previous registry was only able to provide a snapshot of prevalence up until 1999. Using the European Commission's Eurostat data which provides information on total number of live births in the UK for the decade 2000-2009 as 7,253,564 and the total number of births in the Republic of Ireland for 2000-2009 as 643,984[250] and Jones et al's birth prevalence of 7.5 cases per million it is possible to estimate the number of new cases between January 2000 and December 2009 as 59 (presuming the birth prevalence has remained the same). Jones et al also showed a mortality of 12% over 10 years. Therefore, it could be estimated that there would be approximately 150 people, both children and adults, living with CGD currently (from the 112 previously found and 59 estimated new cases).

3.3   Participant identification

Information from the original CGD registry was accessed and consultants that previously acknowledged that they were caring for patients with CGD identified. Those patients included in the original registry that would still be under 16 years of age at the commencement of this study (i.e. with birthdays after 1st June 1994) were identified.

The study was advertised by circulating information through a variety of special interest groups across the UK and Ireland, asking members to respond, identifying whether they had any CGD patients currently, or had previously had patients that had since died. These groups included: The British Paediatric Allergy, Immunology and Infection Group (BPAIIG), The Travellers (adult immunologists), The British Society for Paediatric Gastroenterology, Hepatology & Nutrition, The British Society for Gastroenterology, The British Society for Haematology, The British Paediatric Respiratory Society, The British Thoracic Society and The British Infection Association.

Two major centres for the care of paediatric patients with CGD have evolved since the registry was developed, one being Newcastle. Great Ormond Street Hospital is the other and has specific monthly CGD clinics for patients from the south of the UK.

Other centres with smaller cohorts of paediatric CGD patients were also identified and approached. These were identified from the original registry, from knowledge of teams at the two main centres and from the CGD Research Trust nurse specialist, based at Great Ormond Street. Centres were asked to confirm whether they had patients and whether they would agree to collaborate.

3.4   Case definition

Children, under the age of 16 years, with a confirmed diagnosis of CGD on NBT or flow cytometry, with or without protein expression abnormalities or identified genetic mutations, were eligible for the study.

3.5 Exclusion criteria

Individuals were excluded if they were not usually resident within the UK or the Republic of Ireland but had attended a UK centre for part of their treatment, for example HSCT. Children were excluded from the IQ test if they were under the age of 5 years as the WASI assessment is not appropriate for this age group. Those participants for whom English was not their first language were excluded from the standardised quality of life and psychology questionnaires if they were deemed not to be able to complete the questionnaires in English. They were included in the registry update (gathering of clinical information from their records) if they are able to provide consent.

3.6 Ethical approval

A favourable opinion was provided by the County Durham and Tees Valley 1 Regional Ethics Committee (09/H0905/73). Section 251 approval was obtained from the National Information Governance Board Ethics and Confidentiality Committee for access to records of patients in England and Wales that were deceased and therefore unable to consent for the study. Site specific research and development approval was obtained for each hospital with patients.

3.7 Recruitment and informed consent

Following appropriate ethical and research and development approval potential participants were provided with age appropriate information leaflets by the team of healthcare professionals looking after them or the study principle investigator (i.e. the author). This was either done in person at their routine clinic appointments or by post. Information leaflets were available for parents, children aged 6-10 years and 11-15 years.

Written consent was obtained from parents for living participants under the age of 16. The children were given the opportunity to complete written assent if they wished. The consent form gave parents the option to be included in all parts of the study or to choose not to complete the psychology questionnaires or the

WASI IQ test if they preferred. Those that were included in the original registry were re-consented for the new round of data collection as well as the psychology components of the study.

Each participant's General Practitioner (GP) was informed of their patient's involvement in the study.

Deceased patients were identified by clinicians. Their notes were accessed for addition of data to the registry following NIGB section 251 approval. Families of deceased patients were not approached as it was deemed likely to cause unnecessary distress to families to ask for permission to access their family member's records.

Consultants were asked to provide gender and age for those that did not consent to involvement in the study in order to calculate birth prevalence.

3.8   Evaluation of clinical data

3.8.1   Birth prevalence

Dates of birth/ages were used to calculate numbers of children with CGD born in the last 3 decades (1990s, 2000s, 2010 onwards). Data were available for live births in the UK and Ireland for decade 2000-2010.

Observed birth prevalence for 2000-2010 was calculated as:

Number of children born with CGD/number of live births UK + Ireland

3.8.2   Clinical outcome

For those patients that consented to involvement in the registry, medical records were accessed. Data were abstracted using a standard proforma to provide up to date clinical information on the problems commonly associated with CGD (Appendix 1 contains details of information collected on the proforma, although not the proforma itself as this was 44 pages long). Data included: growth, respiratory and gastrointestinal symptoms, infections, hospitalisations, medications, surgery and other complications or treatments received including

HSCT or gene therapy. Dates of specific events, hospitalisations, infections etc were documented where possible. If exact dates were unclear from medical records the closest possible estimate was made. For example, if an exact date of commencing a medication could not be determined the 1st of the month it was started in would be used. If the month was also unclear the date would be recorded as year only. Hospital admissions were classified according to the diagnosis given by the clinical care team at the time.

For patients who had been transplanted, data were collected regarding; pre-transplant health, conditioning for transplant, type of transplant, post transplant course and any complications. Further information was gathered for time since transplant including: growth, number of hospitalisations, infections, and medication.

In addition, for children diagnosed since the registry was developed, information was gathered in line with the original registry, covering gender, diagnostic tests and mode of inheritance. The proforma used for data collection was amended from the original registry proforma in order to be sure of collecting similar information.

For patients that had died, information from the medical records was collected regarding progress up until the point of death and cause of death, again using the standard proforma.

Data were entered in to the UK and Ireland CGD database held at Newcastle University. All data were checked manually for accuracy and to guard against duplication. Data from both the original registry and current update were used for analysis of clinical features.

Number of life years of CGD were calculated for all children managed conservatively (date of birth until either date of data abstraction or death) plus children managed with HSCT (date of birth until HSCT). Total number of transplant years was calculated (date of transplant until either date of data

abstraction or death). If children received more than one transplant the later date was taken for calculating number of transplant years.

Number of events were calculated for the commonly occurring infections; suppurative adenitis, pneumonia, perianal abscess, liver abscess, osteomyelitis, septicaemia, brain abscesses and splenic abscess. Median age at time of diagnosis of the infection was calculated. Numbers of X-linked and AR children diagnosed with these infections were recorded. Number of serious infections after transplant calculated. Number of admissions for other reasons and surgical procedures were calculated for pre and post transplant time.

3.9 Evaluation of Cognitive Function

3.9.1 History of IQ testing

Although ability testing for occupations can be traced back to 2200BC in China[251], modern assessment of intelligence really began to take off in the late 19th century. Francis Galton started to develop tests of sensory discrimination that he argued were related to intellect[252]. James McKeen Cattell took this further in 1890, describing 10 "mental tests" which included measuring the threshold for discerning difference in weights, judging time intervals and reaction times[253]. The correlation between these tests and intelligence were called in to question when Wissler compared college grades with test performance for Columbia University undergraduates in 1901 finding no correlation between the two[254]. Charles Spearman introduced the concept of "g", general intelligence, to explain attributes that underpinned a variety of different tasks which he tested using sensory experiments with sight, hearing and touch[255].

However, the first true intelligence test, not reliant on sensory tests, was developed by Alfred Binet and colleagues in Paris in the early 1900s. The French Minister of Public Instruction had commissioned a committee, including Binet, to develop a test that would differentiate normal children from those requiring special education[256]. These tests were refined over a number of years to include an evaluation of "mental age" compared to the normal population. In 1912 a German psychologist, William Stern, suggested the idea of the "mental quotient" which was gained by dividing the mental age by chronological age. The term Intelligence Quotient (IQ) was first used by American psychologist, Lewis Terman who multiplied the mental quotient by 100[251]. He adapted the French Binet-Simon scale and re-named it the Stanford-Binet scale. Terman was the first to set the average IQ at 100 with average variability of 15. Obviously there are problems with this for adults whose chronological age continues well beyond the continued development of

their intelligence. For this reason Terman assigned all adults the chronological age of 16 years.

In the 1930s Wechsler started to develop intelligence scales which have since been modified but remain some of the most common and well validated forms of intelligence assessment. Wechsler devised measures for both children (Wechsler Intelligence Scale for Children –WISC) and adults (Wechsler Adult Intelligence Scale –WAIS). One of the benefits of the Wechsler scales over the previous Stanford-Binet scale was the introduction of a performance scale (for example: assembling block patterns, pointing to missing details) which does not involve verbally answering questions, so overcoming difficulties with language or education. The Wechsler Scales are organised in to four dimensions: verbal comprehension, working memory, perceptual organisation and processing speed. Within each of these dimensions a number of aspects of cognitive function are tested including: logical and abstract thinking, short term memory and attention, perception, concentration and attention to detail, motor skills, visual motor dexterity and psychomotor speed. Rather than relying on mental and chronological age for scoring Wechsler used actual test score compared with expected test score for people of the same age and then multiplied by 100. The scales provide scores for total IQ, but also verbal and performance IQ.

Brief intelligence tests have been developed from the longer more in-depth tests, for example the Wechsler Abbreviated Scale of Intelligence (WASI) which is a standardised short form of the WISC and WAIS. Brief intelligence tests were designed mainly as screening tools, when a fast reliable measure of IQ is required, but when it is not practical or desirable to undertake a full assessment. They can be useful for testing a broad age range of individuals rather than being specific to children or adults. They can be administered quickly so are often more convenient than the longer, more in-depth tests. They can be useful when looking at the effect of cognitive function in association with emotional and behavioural problems[257]. However, they are not as accurate as the full scales and therefore do have some limitations. They are not recommended for making diagnoses of cognitive disorders in individuals without further assessment[258].

3.9.2 *Measurement of IQ in CGD patients*

Cognitive function was measured using the Wechsler Abbreviated Scale of Intelligence (WASI). Children aged 5 -15 years were eligible for completion of the WASI. Children were excluded if they were unable to complete the assessment in English or if they had a second diagnosis that would impact on their ability to perform the assessment. It was administered by the study PI after training from a clinical psychologist. It is composed of four sub-tests, two verbal and two performance tests and is a useful short screening tool, taking approximately 30 - 45 minutes to administer. Although not as comprehensive as the Wechsler Intelligence Scale for Children (WISC), it was more acceptable to patients and families as the WISC can take 1.5-2 hours to complete.

3.10 Evaluation of psychological health

Children and their parents were asked to complete questionnaires covering different aspects of psychological wellbeing. These were the Strengths and Difficulties Questionnaire and the Harter Self Perception Profile.

3.10.1 Strengths and Difficulties Questionnaire

The Strengths and Difficulties Questionnaire (SDQ) was designed to measure social, emotional and behavioural functioning in children. There are published norms for UK children[259]. The SDQ has been used in a wide variety of research settings. It is validated as a screening tool for screening for psychosocial problems in children with chronic illness. Amongst others, it has been used in children with asthma[260], cancer[261] and juvenile idiopathic arthritis[262] and to screen for psychological difficulties in paediatric outpatient clinics[263]. Parents of children over the age of 5 completed parent report questionnaires. Children aged 11 and over completed the self report questionnaire.

The questionnaire provided respondents with statements which they had to agree or disagree with. Questions are divided in to five sections regarding emotional difficulties, conduct disorders, hyperactivity, peer difficulties and prosocial behaviour. Each question can score 0, 1 or 2 points according to how much the respondent agrees with the statement. Scores are generated for each of the sections. A total score is calculated by summing scores from all sections apart from the prosocial score.

3.10.2 Harter Self Perception Profile for Children

Harter first developed the perceived competence scale for children in 1979 to measure children's perceived competence in three domains; cognitive, social and athletic competence and from this to gain an understanding of global self-esteem. This has since been refined to include further domains (physical appearance and behavioural conduct), so providing a broader, more differentiated measure of self-concept compared to single self concept

scores[264]. The title was also changed to reflect the assessment of a child's self adequacy as well as competence. In 1988 Harter developed the Self-Perception Profile for Adolescents, with three additional domains which are of relevance to adolescents but not children; job competence, close friendship and romantic appeal.

The Harter Self Perception Profile for Children (SPPC) is a self report questionnaire that measures self esteem in children over the age of 8. The child self report form was used for 8-12 year olds and the adolescent version for those over 12. The questionnaire provides a number of pairs of statements. The respondent must decide which of the pair is most applicable and then decides whether they strongly agree or partially agree with it. The child questionnaire has 36 statements and the adolescent questionnaire has 45 statements, to incorporate the additional sections. Each answer has a score of 1 to 4. Mean scores for each section were calculated for each patient for scholastic competence, social acceptance, athletic competence, physical appearance and behavioural conduct.

3.11 Evaluation of quality of life

The Pediatric Quality of Life Inventory 4.0 (PedsQL) is a health related quality of life instrument designed for children aged 3 – 18 years[265]. It is designed with child self report and parent proxy report scales. There are Generic Core Scales that have been validated for use in healthy children and those with medical disorders[266]. It is used widely in medical research and has been translated into many languages for use across the world. There are published data for healthy children in the UK[267]. There are also disease specific scales, for asthma, diabetes, cancer and cardiac conditions[265] but none have been developed that are appropriate for children with primary immunodeficiency or CGD. Therefore, only the Generic Core Scales were used.

Parents of children aged 3-15 years were provided with parental questionnaires. Children aged 5 years and over were provided with age appropriate questionnaires. It has been shown that children from 5 years of age can reliably report their quality of life using the PedsQL generic core scales[268].

The questionnaire is divided into four sections: physical, emotional, social and school functioning. Each section contains a number of statements about how the respondent feels and what they may have difficulties with. Respondents must rate how frequently they experience the difficulty, from never to always, which generates a score between 0 and 4. The raw score values of 0 – 4 are assigned a scale value between 0 and 100[265]. For each respondent, scores were calculated for each of the four domains. This was done by summing each individual question scale score and dividing by the number of questions in the section. Domain scores were then summed to provide an overall total score and also a psychosocial score (emotional, social and school functioning). Maximum score for each domain is 100 and the higher the score the better the quality of life.

3.12 Statistical analysis

Statistical analysis was performed using IBM SPSS v.17.0. For the clinical data, comparisons were made for non-HSCT and post-HSCT serious infections, surgical procedures and other admissions. Comparisons of categorical data, for example numbers with colitis in the non-HSCT and post-HSCT groups, were compared using the Chi-squared test and, when expected numbers were too small, the Fishers exact test. Non-normally distributed continuous data were analysed using the Mann-Whitney U test. Normally distributed data were analysed using the independent sample t-test, paired t-test and one sample t-tests as appropriate.

95% Confidence intervals (CI) were calculated for prevalence rates and rates of infections/admission, using the formula:

Upper limit = (1/n)(d + 1.96 x√(d(1-(d/n))))

Lower limit = (1/n)(d - 1.96 x√(d(1-(d/n))))

Where d= number of events and n=denominator of rate (life years/transplant years)

z-scores were calculated for weight for age (up to age 10 years), height for age and BMI for age using WHO growth standards with WHO Anthro software version 3.2.2[269], for children aged 0-5 years, and WHO Anthro plus software for children aged over 5 years[270]. Comparisons were made for those who had not undergone HSCT with those that were at least 2 years post-HSCT, as growth has previously been shown to significantly improve in post-HSCT children 2 years after transplant[2].

z-scores were calculated for spirometry results (FEV1, FVC and FEV1/FVC ratio) using Growing Lungs software available from the lungfunction.org website which provides z-scores for children from age 3 upwards[271], according to recently published data[272]. Mean z-scores were calculated for FEV1, FVC and FEV1/FVC ratio.

Survival was plotted on a Kaplan-Meier curve.

PedsQL mean scores were calculated. Mean scores for the non-transplant and post-transplant groups were compared with healthy norms using the one sample t-test. Mean scores for the non-transplant and post-transplant groups were compared using the independent sample t-test. Mean scores for non-HSCT children were compared to mean scores for UK children with inflammatory bowel disease and cancer[267], as markers of severe and life threatening disease, using the one sample t-test. Values for cancer and IBD were taken from the paper by Upton et al[267].

SDQ scores were calculated for each patient for each section plus a total score. Mean scores were calculated for non-transplant and post-transplant groups. Comparisons were made with UK population norms for the non-transplanted and the post-transplant groups using the one sample t-test[259]. Comparisons were also made between the non-transplant and post-transplant groups using the independent sample t-test.

Global self-esteem score was calculated along with subscales for: scholastic competence, social acceptance, athletic competence, physical appearance and behavioural conduct. There are no published norms for Harter results for healthy children in the UK. When designed, the questionnaire was validated on multiple groups of American children and Harter provides these norms in the questionnaire manual. However, there is no one single mean value for healthy children. Therefore, comparisons were made with American norms used in work published by Radcliffe et al[273] using the one-sample t-test. Comparisons were made between those that had and had not undergone HSCT using the independent sample t-test.

WASI full scale, verbal and performance scores were calculated for each patient. Mean scores for non-transplant and post-transplant groups were calculated for each scale. The value of 100 ± 15 was used as normal. Comparisons were made for non-transplant and post-transplant groups with the normal population score of 100 using the one sample t-test. Comparisons were

made between the mean scores for the non-transplant and post-transplant group using the independent sample t-test. The proportion of children with IQ below 85 was compared with the expected numbers for the normal population (15%) using the exact binomial test.

A p value of <0.05 was used for significance in all tests.

Chapter 4 Clinical outcome in conservatively and curatively managed CGD

4.1 Identification and recruitment

Interrogation of the existing UK and Ireland CGD Registry identified 30 children with a date of birth after 1st June 1994, who were eligible for inclusion in the new round of data collection. The last of these were added to the registry in September 2004.

Advertising the study through special interest groups resulted in 20 responses from hospitals around the UK. Five consultants identified a total of 16 children with CGD.

Discussion with the Paediatric Immunology team in Newcastle identified 30 potential participants, 9 of whom were included in the original registry. Discussion with the CGD Nurse Specialist at Great Ormond Street identified a further 37 children, of whom 12 were in the original registry.

A total of 78 potential participants were identified(Figure 7). Five children from the original registry were excluded for reaching their 16th birthday before they could be approached to participate. 73 living children were therefore eligible for inclusion, of whom 59(80%) were recruited in to the study. Five children with CGD who died before the commencement of the study were also identified.
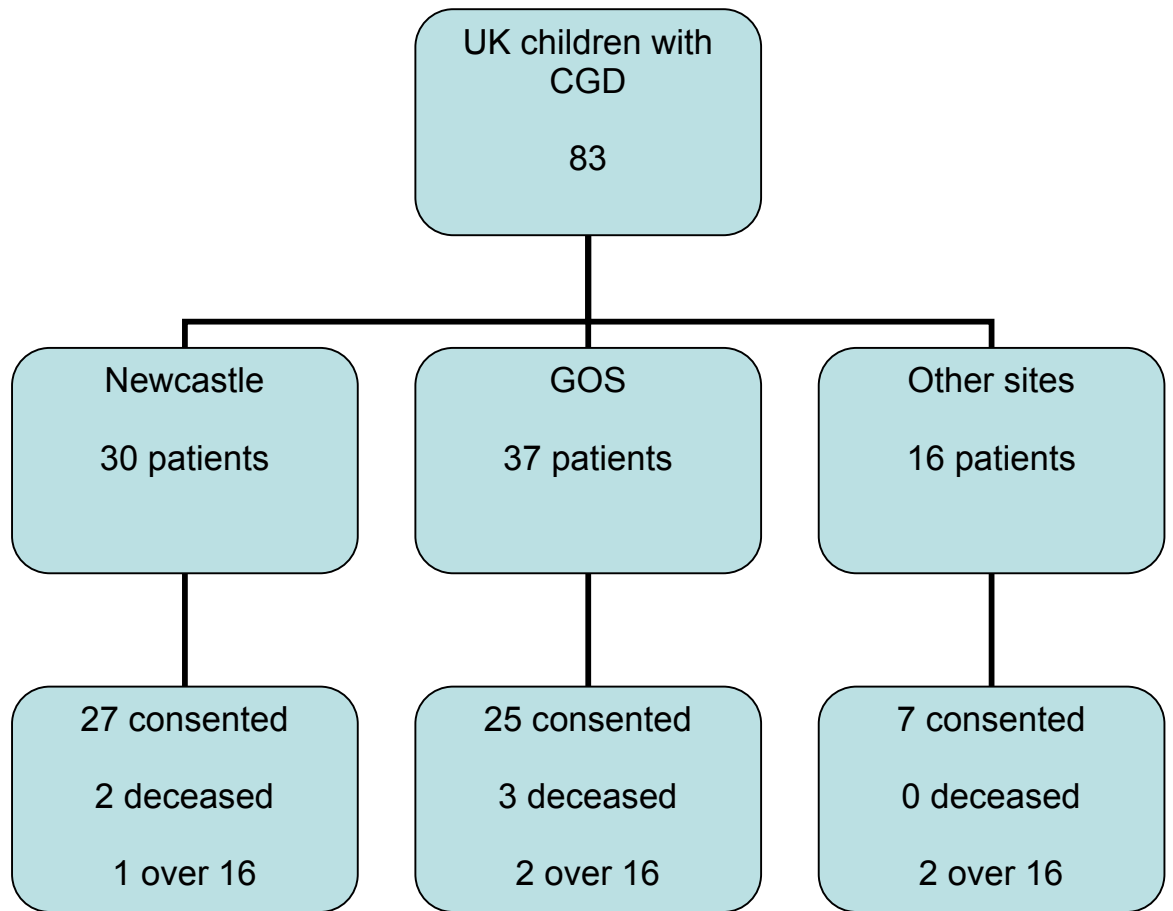
Figure 2: Patient identification and recruitment

4.2 Birth prevalence

30 children were born before 2000. 44 were born between 1st January 2000 - 31st December 2009. 3 were born after 1st January 2010. Date of birth was not available for one deceased patient.

Observed birth prevalence = 5.6/million live births for 2000-2010 (95% CI 4.05 – 7.48).

4.3 Demographics

Clinical information was abstracted for 62 children, including 4 who were deceased prior to the commencement of the study. No notes could be found for one deceased patient. 30 children were post-HSCT. Median age at time of data collection for the non-HSCT group was 8.95 years (range 0.51 – 16.41 years). Median age of the post-HSCT group at the time of data collection was 10.57 years (range 3.90 – 15.22 years). This was not significantly different (p=0.360).

57 (92%) children were male. 53 (85%) were X-linked. Of those with autosomal recessive (AR) inheritance three had identified p47phox deficiency, one had p40phox deficiency and five had no clear identification of the NADPH oxidase component involved.

Date of diagnosis was available for 57 children. 48 (86%) children were diagnosed before the age of 5 years. 55 (96%) were diagnosed by the age of 10.

Median age at diagnosis for X-linked patients was 1.81 years (range: at birth – 11.88 years). Median age for AR patients was 4.20 years (range: 0.31 – 14.82 years). There was a significant difference between median age at diagnosis according to mode of inheritance (p=0.017). For those with AR inheritance, the median age for diagnosis for males was 5.21 years, compared to 4.06 years for

girls (p=0.451). Genetic mutation was documented in the notes of 24 (39%) patients. Other than for siblings, the mutations were different in each patient.

There were a total of 470 life years for CGD (286 life years for non-transplanted patients and 184 pre-transplant years for transplanted patients).

For those that did not consent to join the study; 12 (80%) were male. Median age at time of data analysis was 12.5 years (range 0.9-15 years). This was not significantly different from those that did consent (p=0.287).

4.4   Curative treatment
4.4.1   HSCT

At the time of data collection 30 (48%) children had under gone a total of 34 HSCT procedures. 30 were first transplants, four were second transplants. 28 (93%) were male and 27 (90%) X-linked inheritance. Median age at first transplant was 5.25 years (range 0.7 – 15.3 years). Median age at second transplant was 8.2 years (range 4.2 – 7.3 years). Time between first and second transplant ranged from 0.42 – 6.6 years. Median post-transplant follow up for living patients was 3.84 years (range 0.2-9.9 years). Total transplant years were 124.

21 transplants were conditioned with busulphan and cyclophosphamide, 8 with treosulphan and fludarabine, 3 with busulphan and fludarabine and 1 with treosulphan with cyclophosphamide.

11 transplants were complicated by acute skin Graft versus Host disease (GvHD), two also had gut GvHD. 11 had virus reactivation. Two developed veno-occlusive disease. Two developed disseminated fungal disease (of which 1 died). Three transplants were followed by chronic GvHD (2 liver and 1 skin).

At the time of data collection 12 (40%) children were still receiving some prophylactic medication (Cotrimoxazole, Aciclovir, antifungal agents, immunoglobulin replacement or penicillin). For those that were over one year from transplant, 9/23 (39%) were still receiving some form of prophylaxis.

4.4.2 Gene therapy

Two children underwent rescue gene therapy (without expectation of cure) for fungal infection not responding to conventional management. One died, the other did not have long term correction of NADPH oxidase activity. He recovered from his fungal infection after changing to the newer antifungal drug, Posaconazole.

4.5 Survival

At commencement of the study five deceased children with CGD were identified. Two were post-transplant deaths and three were in non-transplanted patients. Information was unavailable for one deceased non-HSCT patient. Two further children died after recruitment to the study, one after HSCT and the other whilst awaiting a second round of gene therapy.

Causes of death were varied: respiratory failure secondary to mulch pneumonitis, *Burkholderia cepacia* sepsis and cerebro-vascular event following central venous catheter insertion in the non-HSCT children, with fungal infection, multi-organ failure following conditioning and severe influenza in the post-HSCT children.

Median age at death in the non-HSCT group was 6.84 years (range 0.92 – 16.41). Median age at death in the post-HSCT group was 9.18 years (range 8.55 – 12.58). There was no statistically significant difference in age at death for the two groups (p=0.513). All deceased children were male. 4 were x-linked inheritance.

Kaplan Meier plots were generated for the non-HSCT group

Figure 8), which demonstrated 90% survival at 15 years of age, and for the post-HSCT group

Figure 9) which showed 90% survival 9 years after transplant (longest follow up). All 3 children in the post-HSCT group who died received their transplant at over the age of 5 years. However, survival in those that were transplant younger than age 5 was not statistically significantly better when compared to those that were transplanted later (p=0.228)
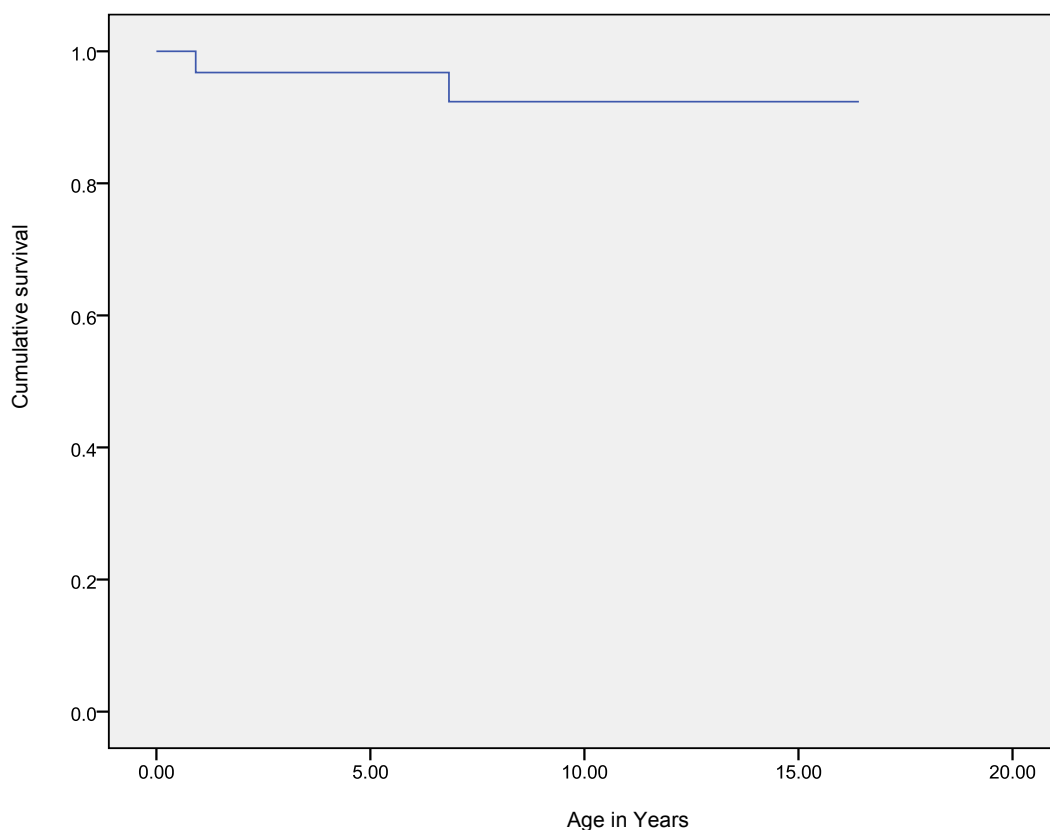
Figure 8: Survival in non-HSCT group
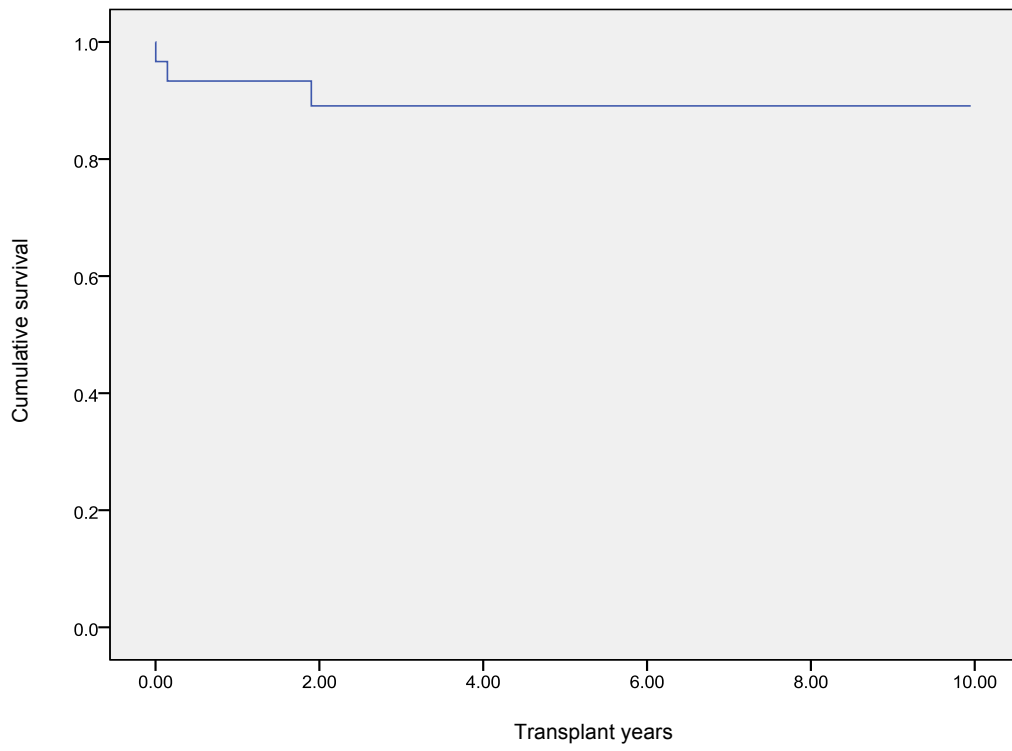
Figure 9: Survival post-HSCT

4.6 Infectious complications of CGD

There were a total of 138 episodes of serious infection (0.29 per CGD life year, 95%CI 0.24 – 0.33 infections per life year)(Table 2). Suppurative adenitis was the most common infection, with 34 children having at least 1 episode. Median number of episodes was one (range 1 – 4). Pneumonia was the second most common serious infection, with 27 children having at least one episode. Median number of episodes per patient was one (range 1 – 4 episodes). 12 children had at least one perianal abscess (median one perianal abscess, range 1 – 8 episodes). Nine children had a single episode of liver abscess and one had two separate episodes. Four children had a single episode of osteomyelitis, one child had two episodes (one in the foot and one in the skull). Three children each had two episodes of septicaemia and one child had a single episode. There were no significant differences between number of children with at least one episode of each of the significant infections according to genetic inheritance.

	No of patients			Total no. episodes		P value XL vs AR
	Total N=62 (%)	XL N=53 (%)	AR N=9 (%)		Median age (years)(range)	
Suppurative adenitis	34 (55)	31 (58)	3(33)	49	2.20 (0.04-11.6)	0.161
Pneumonia/empyema	27 (44)	24 (45)	6 (66)	37	4.50 (0.25-13.4)	0.432
Perianal abscess	12 (20)	12 (23)	0	24	1.63 (0.3-12.6)	0.185
Liver abscess	10 (16)	10 (19)	0	11	2.38 (0.25-4.2)	0.332
Osteomyelitis	5 (8)	3 (6)	2 (22)	6	5.82 (0.5-10.3)	0.149
Septicaemia	4 (6)	4 (7)	0	7	2.67 (0.5-4.1)	0.525
Brain abscess	2 (3)	2 (4)	0	2	7.13 (3.5-7.18)	0.343
Splenic abscess	2 (3)	2 (4)	0	2	2.66 (1.4-4.0)	0.729

Table 2: Infectious complications in children with CGD & those prior to HSCT

4.6.1 Causes of infection

Identifiable causes of serious infection were found in a proportion of cases

Figure 10). Of the suppurative adenitis episodes, causative organisms were found in 15 (31%) episodes. Eleven were *S.aureus* (one also had *S.epidermidis*). There was one each of *Burkholderia cepacia*, Haemophilus *influenzae, histoplasma* and *Nocardia*.

For the pneumonias, organisms were identified in 19 episodes (51%). Fifteen were presumed or proven fungal infection. Seven were presumed. Five were *A.fumigatus*, two *A.nidulans* and one *Scopulariopsis*. Three were bacterial and one was viral (*respiratory syncytial virus*). One was multiple organisms(*Nocardia, aspergillus flavus, aspergillus terreus, mycobacterium tuberculosis and Escherichia coli*).

Three liver abscesses had identified organisms, two *S. aureus* and one coagulase negative staphylococcus. Three episodes of osteomyelitis had identified organisms, two aspergillus species and one *S. aureus*. All but one episode of septicaemia had bacteria identified: three were *Acinetobacter*, one *S.aureus*, one *streptococcus mitis* and one histoplasmosis. Both non-HSCT brain abscesses were presumed fungal.
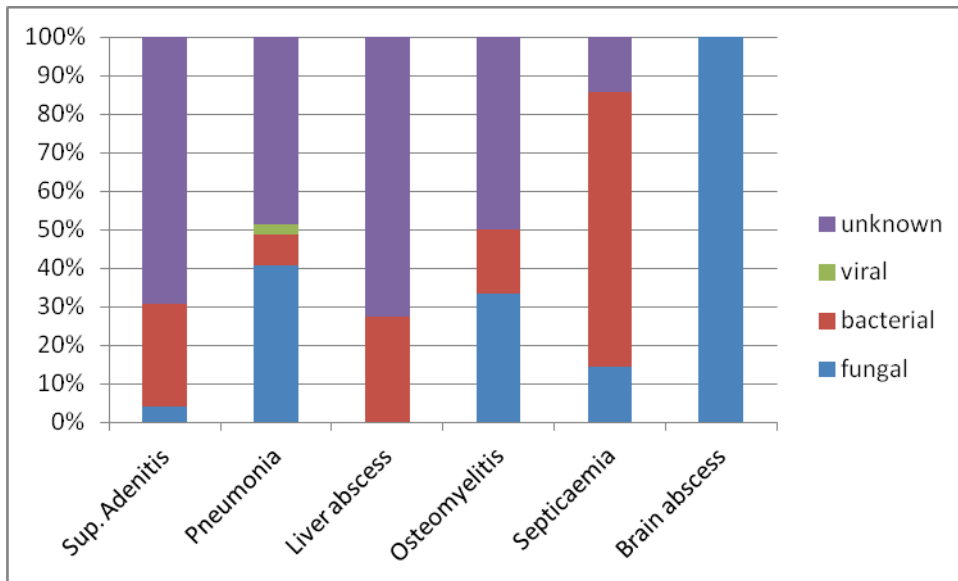
Figure 10: Causes of serious infection

4.6.2 Post-HSCT serious infections

One child had two episodes of suppurative adenitis after HSCT, 2.04 and 3.17 years after transplant. Three children had pneumonia diagnosed after transplant, however, 2 were during their transplant admission and 1 a week after discharge following HSCT. One child was diagnosed with a fungal brain abscess during his transplant admission following his second HSCT. No child had a perianal abscess, liver abscess, osteomyelitis, septicaemia or brain abscess after discharge following their HSCT.

4.7 Inflammatory complications

Of the 25 (40%) children diagnosed with CGD colitis, 24 were x-linked cases. Median age was 3.99 years at diagnosis (range 0.28 -12.24 years). Six received an alternative gastroenterology diagnosis before they were diagnosed with CGD (Crohn's disease or cows milk protein allergy).

23 children underwent a total of 36 colonoscopies, of which 10 were documented to have granulomata on histology. Four children diagnosed with colitis had no documented colonoscopies. Four children never diagnosed with colitis underwent colonoscopy for diarrhoea, poor weight gain and an abnormal white cell scan. One child underwent two colonoscopies after HSCT for evaluation of GvHD.

Six children had 11 episodes of admission for gastrointestinal symptoms related to colitis; including exacerbation of known colitis (3), bloody diarrhoea (3), poor weight gain (3), melaena (1) and 1 planned admission for establishing nasogastric feeds.

18 (75%) children with colitis had documentation of having received 5-aminosalicylic acid compounds. Eight (33%) also received steroids (prednisolone).No child was maintained on steroids without a 5-ASA compound. Five (21%) children received azathioprine, all of whom had 5-ASA compounds and steroids. One child received infliximab for treatment of colitis prior to HSCT.

Three children had pyloric outlet obstruction. However, one of these was asymptomatic and diagnosis was only made on endoscopy. The two symptomatic children received treatment with prednisolone. Five children had bladder involvement, four of whom were treated with prednisolone.

No child was described to have HLH. However, three children received immunomodulation for hyperinflammatory states. One received methylprednisolone alone, one methylprednisolone and infliximab and one methylprednisolone, infliximab, anakinra and pioglitazone.

15 (24%) children were documented to have had at least one ophthalmological examination. Four children had more than one examination. No non-HSCT child had chorio-retinal abnormalities. One child had a post-HSCT examination which showed chorioretinitis.

32 children had a total of 98 chest CTs, 90 (92%) of which were reported as abnormal. One was reported as basal changes indicative of fibrosis. One child was reported to have small airway disease on two CTs. The other episodes were reported as infective (consolidation, cavities or nodules) or were resolving infection after an acute episode. Six children had normal CT scans, two children having 2 normal CTs each.

Eight pre-transplant/non-transplant children had at least one spirometry result documented (median number of tests was one, range 1-3). Median age at first spirometry was 7.79 years (range 5.42 – 11.42 years). Mean FEV1 z-score was -0.98 (range -2.97 to 0.96). Mean FVC z-score was -1.00 (range -2.83 to 0.68). Mean FEV1/FVC score was 0.03 (range -0.69 to 1.18). Four children had a second measure at median age 8.66 years (range 8.17 – 12.83 years). Mean FEV1 z-score was -0.88 (range -2.33 to 0.38). Mean FVC z-score was -0.83 (range -2.31 to 0.37). Mean FEV1/FVC z-score was -0.24 (range -0.37 to -0.06).

 Four children had post-HSCT measures, only one of whom had a pre-transplant measure as well as post transplant measure. Two had two measures of spirometry. Median age at first measurement was 9.71 years (range 5.67 – 11.67 years). Mean FEV1 z-score was -0.95 (range -3.83 to 1.53). Mean FVC z-score was -0.80 (range -3.25 to 1.36). Mean FEV1/FVC z-score was -0.46 (range -1.44 to 0.26).

No child had return of colitis or bladder involvement after HSCT.

4.8   Nutrition

Recent height and weight measures were available for 30 non-HSCT children and 15 children with greater than 2 years post-HSCT follow up. Median age for the non-HSCT group was 9.0 years (range 0.83 – 15.25). Median age for the post-HSCT group was 10.1 years (range 4.17 – 13.8 years). Median time post-HSCT was 4.42 years (range 2.25 – 9.00 years). Six (20%) children in the non-HSCT group had low height for age (z-score less than -2). No child in the post-HSCT group had low height for age. Five children had low BMI for age in the non-HSCT group. No post-HSCT child had low BMI for age. There was a statistically significant difference in z-scores for height for age and BMI for age(Table 3).

	Non-HSCT N=29	Post-HSCT N=16	P value of difference
Mean Height for age **(SD)** **range**	-1.15 (1.15) -3.42, 1.85	-0.29 (0.75) -1.62, 1.11	0.012
Mean BMI for age **(SD)** **range**	-0.37 (1.55) -3.26, 2.50	0.58 (0.88) -0.70, 2.25	0.012

Table 3: z scores for height and BMI for age in non-HSCT and post-HSCT children

Weight for age z-scores were calculated for 18 non-HSCT and 7 post-HSCT children under the age of 10 years. Mean score for non-HSCT children was -0.82 (range -3.53 to 0.90). Mean score for the post-HSCT children was -0.24 (range -1.45 to 1.44). Four children had low weight for age scores (z-score less than -2) in the non-HSCT group. No post-HSCT child had low weight for age. There was no significant difference in weight for age between non-HSCT and post-HSCT children (p=0.141).

Pre and post-transplant weights and heights were available for 14 children who had undergone HSCT with at least 1 year post-transplant follow up (Figure 11). Median time post-HSCT was 4.3 years (range 1.3 – 9.0 years). Seven children did not have z-scores for weight for age calculated as they were over 10 at most recent measurement. Therefore, weight for age comparisons pre and post transplant were not performed. Three (21%) children had height for age z-scores below -2, deemed low height for age pre-HSCT. One child continued to have low height for age post-HSCT. No child had low BMI for age, pre or post HSCT. There was a significant improvement in height for age but no significant difference in BMI for age when comparing pre and post transplant(Table 4).

	Pre-HSCT	Post-HSCT	P value of difference
Mean Height for age (SD)	-1.41 (1.03)	-0.54 (1.07)	0.011
Mean BMI for age (SD)	0.20 (0.77)	0.49 (1.04)	0.417

Table 4: Pre and post-HSCT z scores for height and BMI
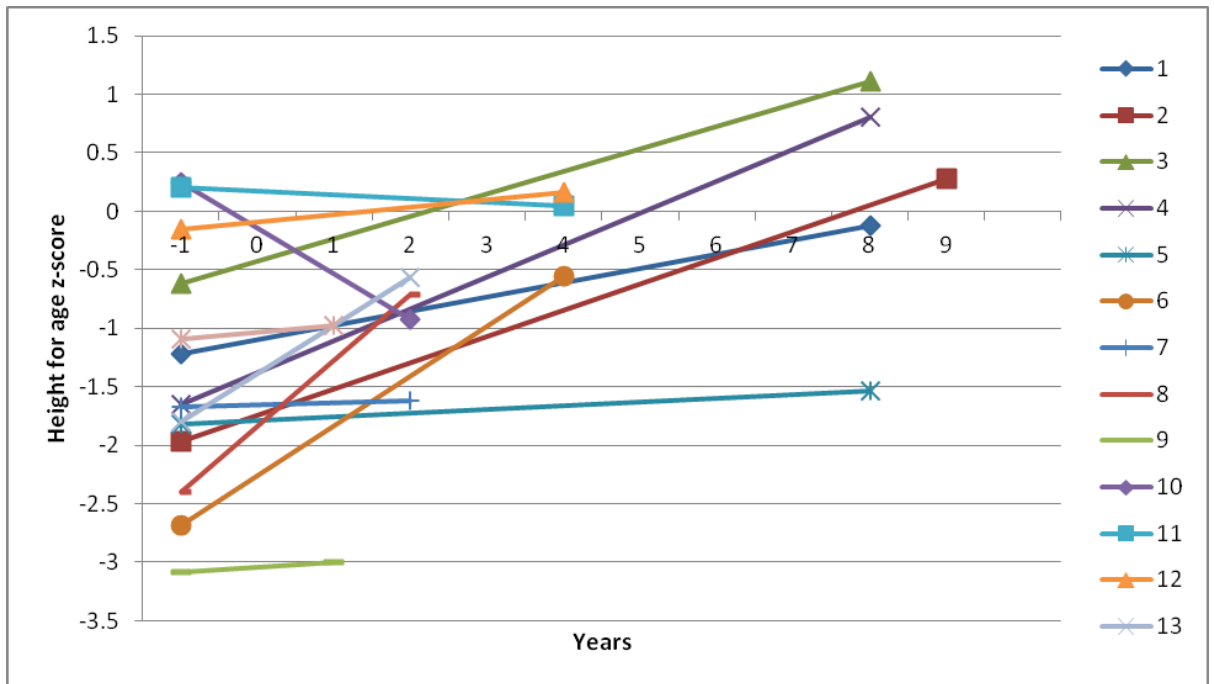
Figure 11: Height for age z-score pre and post HSCT in 14 patients

Four (6%) children received parenteral nutrition due to poor nutritional status. Two (3%) received nasogastric feeds. Three (5%) children received growth hormone therapy for poor growth and delayed puberty, all were being conservatively managed at the time, although one did go on to receive HSCT.

4.9   Other admissions

There were a total of 52 admissions for other reasons in 30 children (0.11 admissions per CGD life year, 95%CI 0.08-0.14 admissions per year). Median number of admissions was two (range 1 – 4 admissions). Median age at admission was 3.5 years (range birth – 12.1 years). 14 episodes were upper or lower respiratory tract disorders not treated as pneumonia (tonsillitis, pharyngitis, croup, bronchiolitis, cough, otitis media, exacerbation of asthma). Eight children had nine episodes of skin or soft tissue infection requiring admission and antibiotics. One child had preseptal orbital cellulitis. Two had severe *Varicella zoster* infection requiring intravenous Aciclovir. Three admissions were for seizures. Five admissions were with acute diarrhoea (not colitis). Two were for CVL infection. One child had three admissions related to an expanding mediastinal mass. One admission each for: superior vena cava obstruction, Kawasaki's, Bell's palsy, ascites of unknown cause and mesenteric adenitis. Six admissions were for non-specific viral illness.

Two neonates were admitted to the neonatal unit at birth for observation due to antenatal diagnosis of pericardial effusion.

4.9.1 Post-HSCT admissions

There were a total of 15 admissions in 10 children after discharge following their HSCT (0.12 admissions per transplant year, 95%CI 0.06 – 0.18 admissions per year). Median age at admission was 6.4 years (range 1.4 – 12.4 years). Median time post-HSCT at admission was 0.75 years (range 0.08 – 4.83 years). Ten (67%) admissions were within the first year after HSCT.

Within a year of transplant the admissions were: three with fever, two with viral reactivation (*Cytomegalovirus* and *Varicella zoster*), two with diarrhoea, one with difficult GvHD, one seizure and one urinary tract infection.

The later admissions were for: two upper respiratory tract infections, headache and confusion, a seizure and new onset type 1 insulin dependent diabetes mellitus.

4.10 Surgery

37 children underwent a total of 97 surgical procedures in relation to their diagnosis of CGD. Median number of surgical procedures per child was 1 (range 1-12). Median age at surgery was 3.2 years (range 0.28 – 13.6 years). This results in a rate of 0.21 surgical procedures per CGD life year (95% CI 0.17 – 0.24 events per year).

29 episodes were incision and drainage (I&D) or aspiration for suppurative adenitis. 23 were I&D or aspiration for other collections (soft tissue abscesses, thymic abscess, liver abscess, osteomyelitis). Seven episodes were for management of perianal abscesses (I&D or laying open of fistula). Nine were laparotomies (for liver haemorrhage, partial hepatectomy, partial pancreatectomy, splenectomy, splanchnic haematoma, gastric perforation, ascites, jejunocolic fistula and removal of packing following previous abdominal surgery). Two were cystoscopies for CGD bladder involvement. Eight were biopsies of lymph nodes or soft tissue masses. Two children had lymph node excision and one had fine needle aspiration. Two had bone marrow aspiration and trephine. One child had lobectomy for fungal pneumonia. One child required pulmonary artery stenting and a further procedure to dilate the stent. One child had a diagnostic nasal space washout related to maxillary osteomyelitis. One child had examination under anaesthesia and rectal biopsy for constipation and later required anal dilatation. One child needed two episodes of debridement for poor wound healing following laparotomy. One child required a transjugular portosystemic shunt. Three children required central venous access during treatment of infection. One child had gastroenterostomy insertion for poor nutritional intake which was later removed.

Two children had genitourinary procedures which were not directly connected with their diagnosis of CGD (hypospadias repair, orchidopexy).

All children that underwent HSCT had at least one central venous line for the procedure. One child required evacuation of intracranial haematoma following fungal brain abscess during his transplant admission.

Two children underwent surgical procedures after discharge following HSCT. One was a roux en y enterostomy for a bile duct stricture. The other was excision of a lower lip lesion.

4.11 Total admissions pre & post-HSCT

There were a total of 0.71 events per CGD life year (95%CI 0.69-0.75 events per year) and 0.15 events per transplant year (95%CI 0.09-0.21 events per year)(Table 5). Children with CGD were 4.7 times more likely to need admission to hospital for serious infection, inflammatory complications or surgery when compared to post-HSCT children.

	Pre-HSCT	Post-HSCT
Serious infection	138	0
Colitis	11	0
Colonoscopy	36	2
Other	52	15
Surgery	97	2
Total	334	19
Events per person-year	0.71	0.15

Table 5: Pre and post-HSCT admissions and events

4.12 Prophylaxis

All children with known CGD received antibiotic prophylaxis with Cotrimoxazole. One child died during his presenting acute illness and therefore never received prophylaxis.

54 (87%) had antifungal prophylaxis with Itraconazole. Six were treated for fungal infection at diagnosis and therefore remained on other antifungals rather than Itraconazole at the time of data collection. One child never received antifungal prophylaxis as he died during his presenting illness. Three children were switched to voriconazole prophylaxis due to difficulties with Itraconazole. No child received prophylactic interferon gamma. Although 13 did receive it as part of treatment for severe infection.

4.13 Summary

- 85% of children in the UK and Ireland have X-linked inherited CGD.

- Median age at diagnosis is significantly younger for X-linked compared to AR inherited cases.

- 48% of children with CGD had undergone HSCT at the time of data collection, with 90% survival.

- Children with CGD had 0.29 serious infections per CGD life year (suppurative adenitis and pneumonia were the most common).

- There were no serious infections after the peri-transplant period.

- 39% of children with CGD had colitis.

- Post-HSCT children demonstrated improved height for age compared to non-transplant children.

- Children with CGD had 0.71 episodes of admission or surgery per CGD year for all reasons, compared to 0.15 episodes in post-HSCT children.

Chapter 5 Quality of life, emotional wellbeing and cognitive function in CGD

5.1 Study of quality of life in CGD

5.1.1 Parent report scores

47 parents completed PedsQL questionnaires. Median age of children was 10 years (range 3-15 years). 44 were parents of male patients. 21 were post-HSCT. Median age for non-HSCT group was 9 year (range 3-15 years). Median age for post-HSCT group was 10 years (range 4-14 years). The post-HSCT group were median 3 years post transplant (range 1-9 years). Number of children with colitis in the two groups did not differ significantly, 10 in the non-HSCT group and 8 in the post-HSCT group respectively (p=0.807). 8 children in the non-HSCT group had suffered presumed or proven fungal infection (pneumonia, brain abscess or osteomyelitis) compared to only 2 in the post-HSCT group (p=0.150).

Scores for the non-HSCT group were significantly lower across all domains when compared to population norms(Table 6). Scores for the post-HSCT group were not significantly different from population norms. Scores for the post-HSCT group were significantly higher than those for the non-HSCT group in all domains.

	UK norms	No HSCT N=26	P value: norms vs no HSCT	Post HSCT N=21	P value: norms vs post-HSCT	P value: no HSCT vs post HSCT
Domain	**Mean (SD)**	**Mean (SD)**		**Mean (SD)**		
Total	84.6 (11.2)	65.31 (20.43)	<0.001	81.93 (18.74)	0.521	0.006
Psychosocial	82.2 (12.7)	66.74 (19.25)	<0.001	81.17 (16.80)	0.782	0.010
Physical	89.1 (12.3)	60.35 (25.74)	<0.001	85.27 (25.27)	0.495	0.002
Emotional	78.3 (15.5)	65.53 (20.08)	0.003	78.81 (18.57)	0.901	0.024
Social	86.8 (15.4)	71.88 (21.48)	0.002	88.33 (17.42)	0.691	0.007
School	81.5 (16.1)	55.88 (21.92)	<0.001	79.13 (22.99)	0.641	0.001

Table 6: Parent report PedsQL mean scores

Compared to UK children with cancer and inflammatory bowel disease, children with CGD scored significantly worse for physical, social and school domains(Figure 12).
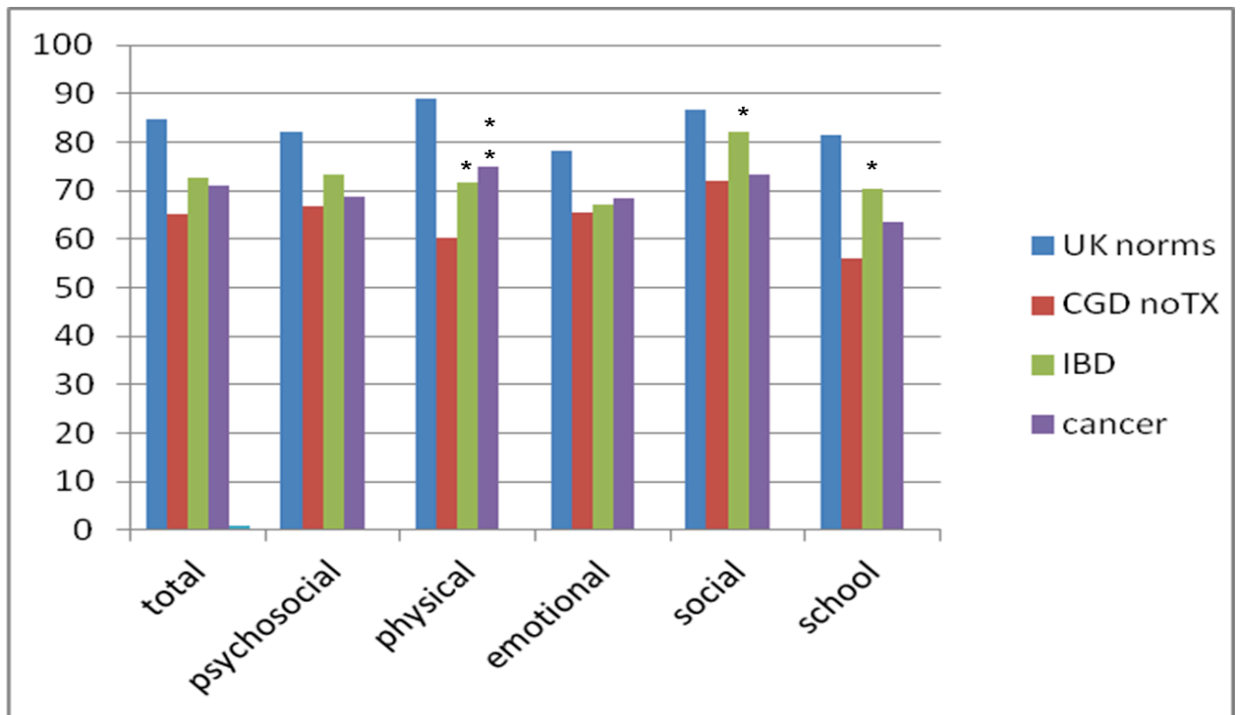
*p=<0.05, **p=<0.01

Figure 12: Parent report scores for non-transplanted CGD patients compared to other diseases

5.1.2 Self report scores

35 children (33 males) completed self-report PedsQL questionnaires. 18 were post-HSCT. Median age was 10 years (range 5-15 years). Median age for both groups (non-HSCT and post-HSCT) was 10 years. The post-HSCT group were median 4 years post transplant (range 1-9 years).

Non-HSCT children reported significantly lower scores than population norms in all except the emotional domain(Table 7). Post-HSCT children had scores not significantly different from normal in all domains. There were significant differences in scores for the individual domains (physical, emotional, social and school) but not the combined scores when comparing non-HSCT and post-HSCT groups.

	UK norms	No HSCT N=17	P value: norms vs no HSCT	Post HSCT N=17	P value: norms vs post-HSCT	P value: no HSCT vs post HSCT
Domain	Mean (SD)	Mean (SD)		Mean (SD)		
Total	83.9 (11.8)	75.39 (12.99)	0.016	81.79 (20.22)	0.664	0.276
Psychosocial	81.8 (13.2)	74.61 (13.27)	0.040	80.28 (19.10)	0.739	0.318
Physical	88.5 (11.6)	69.68 (17.69)	<0.001	89.93 (17.20)	0.729	0.002
Emotional	78.5 (17.9)	72.64 (14.37)	0.113	85.83 (16.20)	0.072	0.016
Social	87.7 (16.5)	73.53 (17.12)	0.004	89.71 (19.16)	0.672	0.014
School	78.9 (15.9)	64.12 (15.64)	0.001	79.12 (18.73)	0.962	0.016

Table 7: Self reported PedsQL mean scores

Children with CGD had scores similar to those reported by children with IBD and cancer in all except the social domain, where the CGD score was significantly lower than that for IBD(Figure 13).
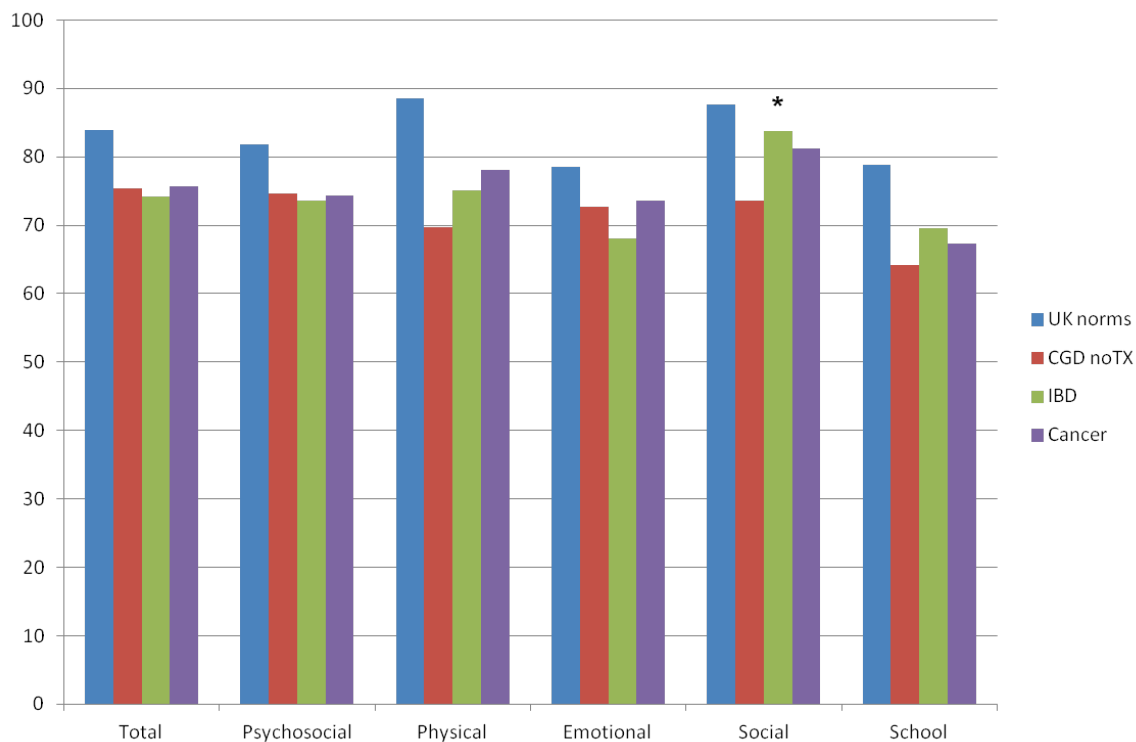
*p=<0.05

Figure 13: Self report scores for non-transplanted CGD patients compared to other diseases

5.2 Study of emotional and behavioural difficulties in CGD

5.2.1 Parent report scores

42 parents completed SDQs. 39 were parents of male patients. Median age of all children was 10 years (range 3-15 years). 19 (45%) were post-HSCT. Median age of non-HSCT group was 9 years. Median age of post-HSCT group was 10 years. The post-HSCT group were median 4 years post transplant (range 1 -9 years).

When compared to population norms for males the non-HSCT group had significantly higher total and emotional scores(Table 8). The post-HSCT group mean scores were not significantly different from population norms. The post-HSCT total score was significantly lower than the non-HSCT group. The post-HSCT score for hyperactivity was significantly lower than the score for the non-HSCT group.

	UK norms for males	No HSCT N=23	P value: normal vs no HSCT	Post HSCT N=19	P value: Normal vs post-HSCT	P value: no HSCT vs post HSCT
Domain	Mean (SD)	Mean (SD)		Mean (SD)		
Total	9.1 (6.0)	12.09 (6.19)	0.030	7.74 (5.70)	0.311	0.024
Emotional	1.8 (2.0)	2.83 (2.10)	0.029	1.74 (2.05)	0.895	0.099
Conduct	1.7 (1.8)	2.00 (1.41)	0.320	1.63 (1.86)	0.875	0.471
Hyperactivity	4.0 (2.7)	5.04 (2.44)	0.052	3.16 (2.28)	0.123	0.014
Peer	1.5 (1.7)	2.22 (1.95)	0.092	1.21 (1.40)	0.379	0.067
Prosocial	8.3 (1.6)	8.3 (1.40)	0.988	8.05 (1.58)	0.504	0.587

Table 8: Parent report SDQ scores for non-HSCT and post-HSCT groups

5.2.2 Self-report scores

19 children (all male) completed self-report SDQs. Median age was 13 years (range 10-15). 10(53%) were post-HSCT. Median age of the non-HSCT group was 14 years. Median age of the post-HSCT group was 13 years. The post-HSCT group were median 4.5 years post transplant (range 1-9 years).

There were no significant differences between mean scores for the non-HSCT group and population norms(Table 9). The mean conduct score was significantly lower in the post-HSCT group than the population norm. There were no significant differences between the non-HSCT and post-HSCT groups.

	UK norms for males	No HSCT N=9	P value: normal vs no HSCT	Post HSCT N=10	P value: normal vs post-HSCT	P value: no HSCT vs post HSCT
Domain	**Mean (SD)**	**Mean (SD)**		**Mean (SD)**		
Total	10.5 (5.1)	10.56(4.77)	0.973	7.70 (5.49)	0.139	0.244
Emotional	2.6 (1.9)	2.00 (2.18)	0.433	2.00 (1.83)	0.326	1.000
Conduct	2.4 (1.7)	2.00 (1.73)	0.508	1.30 (1.25)	0.021	0.323
Hyperactivity	3.9 (2.2)	4.33 (1.94)	0.521	2.50 (2.76)	0.143	0.116
Peer	1.6 (1.4)	2.22 (1.97)	0.375	1.90 (1.73)	0.597	0.710
Prosocial	7.5 (1.7)	7.11 (1.61)	0.491	7.50 (1.90)	0.936	1.000

Table 9: Self-reported mean SDQ scores

5.3 Study of self-esteem in CGD

25 children (24 males) completed the Harter questionnaires. Median age was 12 years (range 8-15 years). 13 children were post-HSCT. Median age for the non-HSCT group was 14, whereas median age for the post-HSCT group was 10 (this was not statistically significant p=0.085). The post-HSCT group were median of 4 years post-HSCT (range 1-9 years).

The non-HSCT group had a significantly lower score for athletic competence when compared to published norms(Table 10). The post-HSCT group had significantly better scores for global self-esteem along with conduct compared to norms.

	norms	Non-HSCT N=12	P value: norms vs non-HSCT	Post-HSCT N=13	P value: norms vs post-HSCT	P value: non-HSCT vs post-HSCT
Global	3.02 (0.67)	3.19 (0.52)	0.266	3.49 (0.68)	0.028	0.239
Athletic	3.02 (0.73)	2.15 (0.57)	<0.001	2.76 (0.91)	0.333	0.059
Conduct	2.80 (0.55)	3.09 (0.58)	0.108	3.38 (0.75)	0.017	0.294
Appearance	2.79 (0.71)	3.06 (0.61)	0.157	3.16 (0.61)	0.052	0.951
Scholastic	2.85 (0.67)	3.00 (0.60)	0.406	3.02 (0.98)	0.542	0.856
Social	2.94 (0.65)	2.96 (0.65)	0.901	3.29 (0.65)	0.087	0.232

Table 10: Mean Harter self-esteem scores for non-HSCT and post-HSCT groups

5.4 Study of cognitive function in CGD

23 children (all male, 22 X-linked) completed the WASI. Median age for all children was 10 years (range 7-15 years). One child was excluded due to a diagnosis of autistic spectrum disorder that would impact on ability to perform the test. One child, with intracranial fungal infection and sensorineural hearing impairment was unable to concentrate to complete the test and therefore did not receive a score. 10 children completed the WASI post-HSCT. Median age for both non-HSCT and post-HSCT groups was 10 years. The post-HSCT group was median 3.5 years post transplant (range 1-9 years).

There were no significant differences when comparing non-HSCT and post-HSCT groups with the population mean score 100 for verbal, performance or total IQ. There were no significant differences in mean score for verbal, performance or total IQ when comparing non-HSCT and post-HSCT groups (Table 11).

Four children (17%) had IQ <85 (2 post-HSCT). This was not significantly different from the expected proportion of children in a normal population (p=0.46). No children had IQ<70.

	Non-HSCT Mean (SD) N=13	Post-HSCT Mean (SD) N=10	P value: Non-HSCT vs post-HSCT
Verbal	102.08 (21.16)	96.50 (15.55)	0.492
Performance	100.00 (10.34)	103.30 (16.37)	0.560
Total	101.00 (16.69)	100.40 (17.84)	0.935

Table 11: Mean IQ scores for non-HSCT and post-HSCT groups

5.5 Summary

- Parents and children with CGD reported poor quality of life compared to healthy norms.

- Parents and children that had undergone HSCT for CGD reported quality of life that was normal.

- Parents of children with CGD reported increased risk of emotional difficulties when compared to the healthy population.

- Children that had undergone HSCT for CGD had higher global self-esteem than those managed conservatively.

- Children with CGD reported lower self-esteem in terms of their athletic competence.

- Children with CGD and those transplanted for it had normal IQ.

Chapter 6 Discussion

6.1 Discussion of clinical outcome studies

6.1.1 Identification and recruitment

This study aimed to survey the clinical outcome in the UK and Ireland paediatric CGD cohort. Due to the cooperation of the supra-regional centres, the CGD Nurse Specialist and wide advertising of the study it was possible to identify virtually all children with CGD. It is unlikely that any children known to have CGD were missed and not given the opportunity to join the study. It is, however, possible that further children are yet to be diagnosed, especially those born in recent years. It is also unlikely that any deceased children with a known diagnosis of CGD were missed. However, death certificates were not reviewed to identify any further children who may have had CGD without confirmation. This study managed to recruit 80% of the known children living with CGD. This is a large proportion of the UK and Ireland paediatric CGD cohort and is similar to the percentage of both adults and children, recruited by Jones et al when the UK and Ireland CGD registry was set up[1]. Those that did not consent to involvement were mainly male, as would be expected for CGD, and were spread across the paediatric age range. Median age was not significantly different from the children that did join the study. However, no other information was available about them to evaluate whether they differed significantly from those that did join the study, in terms of their clinical condition. Therefore, it is not possible to be certain that no bias has been introduced in to the sample. Because of the high level of recruitment, the results of this study should be considered representative of the UK and Ireland paediatric CGD population.

6.1.2 Birth prevalence

The observed birth prevalence for the 2000-2010 was 5.6/million. This is lower than that described by Jones at el for 1990-1999 (7.5/million), which was in turn lower than the prevalence for the decade 1980-1989 (8.5/million)[1]. However, the confidence intervals are wide, with the upper limit closer to Jones et al's

calculation. It is also likely that the calculated prevalence is an under estimate as some children, particularly those born later in the decade, may yet to be diagnosed. It is, therefore, most likely that the prevalence rate is relatively stable. There is no reason to expect a dramatic change. For example, pre-implantation diagnosis is not yet widely available to result in a reduction in numbers of births of children with CGD.

6.1.3 Demographics

As expected the majority of patients were male and X-linked inheritance. The proportion of X-linked cases (83%) is similar to that described by Jones et al previously for the UK[1]. Similar to previously reported in UK, Italian and European registries the median age at diagnosis was lower for X-linked cases compared to AR cases[1, 77, 103]. The median age for diagnosis in both X-linked and AR cases was lower in this study than the published registries but this is a result of it being a paediatric study, which does not include any late adult diagnoses. It is, therefore, not possible to determine whether we are getting better at recognising and diagnosing CGD in early life. It has been argued that the later diagnosis in AR inherited cases has previously been because clinicians do not think about CGD in girls. However, in this study the median age at diagnosis for AR girls was not different to AR boys. This suggests that in the UK clinicians do consider the diagnosis of CGD in a girl if she presents with typical features. Historically, there has been lots of discussion about whether AR cases are less severe and, therefore, present later. However, as has more recently been highlighted, genetic mutation and resultant residual NADPH oxidase activity are associated with disease severity[79]. This study, although attempting to document known genetic mutations, did not consider residual NADPH oxidase activity and cannot, therefore, contribute further to this area.

Interestingly, within the AR inheritance group this study includes one child with p40phox deficiency. This has only been described once before, in a patient with colitis[85]. Whereas, the child in this study presented with significant infections. This is a new description of presentation of p40phox deficiency.

6.1.4 Curative treatment

Approximately half of the children in the UK and Ireland had undergone HSCT at the time of data collection. Since data analysis was undertaken, further children have also undergone HSCT and more are awaiting it. This is a significant change from historical practice. This study includes a larger group of transplanted children than any published case series. Gene therapy for CGD remains experimental. This study has shown it has been used in the UK when no suitable donor for HSCT has been available. In one case in this study it appeared to have minimal benefit and in the other it did result in improvement in clinical condition.

For the post-transplant children, 60% were off all prophylactic medication by one year after transplant. However, it must be noted that the two main centres for primary immunodeficiency HSCT have different policies. One aims to stop all medication, whilst the other continues life-long pneumococcal prophylaxis. Therefore, with current policy it will not be possible to achieve 100% of children off all medication. This is, perhaps, an issue for discussion by the two centres to see if consensus can be reached. However, this is a positive point for families considering HSCT. Without HSCT it is absolutely necessary to remain on prophylaxis life long, whereas, following HSCT there is a good chance they will not need medication. This is particularly attractive for teenagers who are known for their poor compliance in other diseases such as diabetes, inflammatory bowel disease, and even in life threatening states such as after solid organ transplant[274-276].

6.1.5 Infectious complications

Overall, suppurative adenitis was the most common infection. Whereas, it was only second most common behind pneumonia in the Jones et al paper and the Italian registry[1, 103]. Comparison with the European Registry is slightly more complicated as they divide episodes by site of disease rather than infectious and non-infectious complications. Lymphadenitis appears the second most common infection behind pneumonia and lung abscess together[77].

The difference between published work and this study may reflect the fact that it is purely a review of paediatric cases. In Jones et al's paper median age for pneumonia was 10.2 (range 0.2 – 56.9 years), compared to median age 2 years (range 0.2 – 28.8 years) for suppurative adenitis, reflecting the fact that suppurative adenitis is a disease of earlier childhood and pneumonia occurs later[1].

Liver abscesses appeared to be less common than in the original UK registry, which reported 79 episodes in 27 (29%) patients[1]. The European registry also reported liver abscesses in 32% patients[77]. Whereas, the current study shows only 16% of cases had a liver abscess. This is similar to the frequency reported in the Italian Registry[103]. Again, this apparent reduced frequency may be due to only collecting data on children. According to Jones et al the median age for liver abscess was 14.3 years, with a maximum age of 38 years. Therefore some of the children presented here may go on to develop liver abscesses as they get older.

S.aureus was the most common bacteria causing infection, as would be expected. There were, however, some more unusual organisms identified, for example, *H.influenzae, Acinetobacter* and *Histoplasma*. The histoplasmosis occurred in a child from the Republic of Ireland. Although histoplasmosis is unusual in this geographic region, there are cases reported in Europe[277]. History of travel to an endemic area was not recorded in this child. It is reassuring to know the spectrum of bacteria causing infection in CGD has not changed significantly. We can be reassured that our empiric antibiotics are still likely to cover the majority of organisms.

As expected, fungal infections were common. Of those that were proven, *A. fumigatus* remained the most common pathogen. However, many were presumed rather than proven infection. This shows that it remains difficult to identify fungus. It may be that some of these presumed fungal pneumonias were, in fact, not infection at all but rather an inflammatory process as part of the CGD phenotype. However, it is unlikely any clinician would feel confident

withholding antifungal treatment from a child with a known predisposition to fungal infection in the face of clinical suspicion. Because the treating clinician's diagnosis was taken as the diagnosis for each episode, rather than applying hindsight to amend the diagnosis at time of data collection, it is not possible to determine how many were infectious or inflammatory episodes.

Serious infections were only seen in non-HSCT patients and HSCT patients prior to the time of transplant. The serious infections that occurred in the post-transplant group (all around the time of transplant) were likely to have been present pre-HSCT and unmasked by conditioning with chemotherapy.

Other, less serious infections were relatively common in children with CGD, particularly upper respiratory tract and skin or soft tissue infections. It is difficult to draw conclusions about the rate of admission for upper respiratory tract infection compared to other children, as it is difficult to establish what would be expected from a normal, immunocompetent population. There are also many variations from season to season and year to year for the population in terms of respiratory infections, these include the seasonal peaks for respiratory syncytial virus[278], and in recent years, pandemic influenza[279] and the current outbreak of pertussis[280]. It is likely that children with CGD present to hospital for evaluation more frequently as parents are told to seek medical help if concerned about fever or respiratory infection in order not to miss a significant and potentially life threatening infection.

Of the less serious infections in the post-HSCT, two thirds were within the first year after transplant and were transplant related. The causes for admission at greater than a year after transplant were varied and not specifically transplant related. This suggests that by a year after transplant the children are relatively well. However, it is not possible to determine whether the rate of admission is higher than the background rate for healthy children for all causes.

No children were seen with disseminated BCG. Although the UK policy is to only vaccinate children deemed to be at high risk, the Irish policy is to vaccinate all children at birth. Vaccination records were not recorded for the children in

this study so it is not possible to determine how many, if any had received BCG. One child did have mycobacterium tuberculosis identified on broncho-alveolar lavage, at initial presentation, as part of a mixed group of organisms causing pneumonia. However, this child had travelled to a high incidence country prior to presentation.

6.1.6 Inflammatory complications

40% children developed CGD colitis. This is similar to the findings of Jones et al, with 37% having colitis[1]. This is much higher than the frequency described in the Italian Registry 6/47 (13%)[103]. It is also much higher than the figures in the European Registry. However, this divides gastrointestinal manifestations in to colitis and diarrhoea separately without clarifying the difference and also counts gastroenteritis as distinct from these. Van den Berg et al report colitis in only 9% of patients and diarrhoea in 13%[77]. The reasons for these differences are unclear and may relate to definition and detail of data collection. It may be that in the UK we are actively asking our patients about and recording their gastrointestinal symptoms. This information was also actively sought during the data abstraction process. Given that the European registries relied on reports from labs and immunology specialists it may be they did not have information regarding gastrointestinal symptoms available to them.

 It seems the most commonly used agents for management of CGD colitis are 5-ASA compounds. Steroids were also required in one third of those with colitis, suggesting that 5-ASA compounds alone do not keep the colitis in remission, as has previously been suggested by Marks et al[131]. Azathioprine was also used in a fifth of children. Detailed data were not collected on response to treatment so it is not possible to draw further conclusions about the effectiveness of any particular therapy for CGD colitis. The need for multiple agents again suggests CGD colitis remains difficult to manage.

This study is the first to demonstrate the difference in height for age between non-HSCT and post-HSCT groups. The non-HSCT group has significantly lower height for age when compared to the post-HSCT group. However, the mean z-

score for the non-HSCT group is still within 2 standard deviations of expected and therefore not counted as low for age. It also demonstrates improvement in height for age z-scores pre and post transplant in a small group of patients. Soncini et al showed good catch up growth 2 years after HSCT[2]. This study further adds to this, showing that there is significant improvement in height after 1 year of follow up post-HSCT when compared to pre-transplant heights. It would have been interesting to further evaluate weight for age comparisons in a larger group of children as one would expect improvements post-HSCT with resolution of colitis symptoms. Only small numbers were available in this study with documented weights, so results of any such comparison would be very limited in their usefulness. The lack of recorded weights was surprising, and not explainable, given that this is normal practice in most paediatric clinics.

This study did not evaluate pulmonary inflammatory complications in detail. Although many children had chest CT these were mainly for infection, with only two children having any evidence of chronic lung disease. Only a small number of children had spirometry results, which was surprising, given previously identified concerns about chronic lung disease. Mean z-scores for FEV1, FVC and FEV1/FVC ratio were not outside the normal range but on such small numbers it is very difficult to draw any conclusions. With such small numbers, evaluating change over time or pre/post-HSCT has little meaning. It is likely that adults are more at risk of chronic lung disease and the paediatric population is the wrong cohort to evaluate.

6.1.7 Prophylaxis

It is reassuring that all children received appropriate prophylaxis with Cotrimoxazole. This is better than reported by Jones et al where not all patients were receiving Cotrimoxazole which is now standard practice for CGD[1]. In the original registry 93% patients were receiving Itraconazole. It is slightly difficult to compare with the current study as Jones et al have not documented whether the reason some patients were not on prophylaxis was because they were being treated for fungal infection, as was the case in the current study. It is reassuring that all children diagnosed with CGD, without evidence of fungal

infection, were receiving antifungal prophylaxis. A small number were on voriconazole prophylaxis due to difficulties with Itraconazole. Voriconazole is often used as first line antifungal treatment in CGD, therefore, there would be concerns about potential resistance if widely used prophylactically. However, it is not unreasonable to use in those cases where Itraconazole cannot be tolerated as it has a good spectrum of activity against fungi of concern in CGD, namely aspergillus species. No child in this study received prophylactic interferon gamma. Whereas, a recent American series by Martinez et al regarding HSCT in CGD states that all children were on interferon gamma prior to transplant.[174] This demonstrates the continued division between Europe and America on this issue.

6.1.8 Survival

Three children in both the non-HSCT and post-HSCT groups died. 90% of the non-transplanted children were still alive at age 15 years. This is slightly better than survival reported by Jones et al, where survival for males at age 15 years was approximately 80% and survival for females was closer to 75%[1]. Pneumonia and septicaemia were common causes of death in the original registry. These were also the causes of two out of the three deaths in the non-HSCT group in the current data. One death was a child who had not previously been diagnosed with CGD so was not taking prophylaxis. One was an unexpected event following surgery, so, although he would not have been having surgery if he did not have CGD, it was not a death directly due to infection. This suggests we may have become better at managing children with recognised CGD so that they are now rarely dying in childhood. It is not possible to draw any other conclusions about long term survival as data collection stopped at age 16 in this study. Further analysis of adult deaths would be important to assess how much the survival curve has changed and whether long term survival has really significantly improved.

When analysed by age the post-HSCT group have 80% cumulative survival at age 15 years. However, this is a misleading view of transplant survival and reflects the age at which the children were transplanted, along with the age at

which the deaths occurred. A far better way to understand transplant survival is to look at years of survival after transplant.

Long-term post-HSCT survival is good, deaths all occurred relatively early after transplant (the latest at 2 years). All children that died received their transplant later in childhood but the difference in survival was not statistically significant when compared to those that received their transplant before the age of five. This is something that has historically been identified in HSCT for Wiskott-Aldrich Syndrome[281] but was not evident in more recent work[282]. It is reassuring that older children in this study appear to do well after transplant, however, the lack of significance may reflect the small numbers involved. The deaths all occurred within two years of transplant and survival has remained constant since then. It is the same survival rate as demonstrated by Soncini et al, which is one of the largest groups of CGD transplants described in recent years. However, this study does include some children who will have also been included in that paper[2]. Survival in this study is better than the post-HSCT survival quoted for Seger et al's historical European series of 27 cases (85% survival)[172] and in the European registry which was only 80%[77]. However, no details are provided about the cases in the study by van den Berg et al. The data were collected from 2000-2003 so they may have been early CGD transplants. Better survival in the current study may reflect overall improvement in HSCT survival for primary immunodeficiency. Gennery et al showed that survival following HSCT for primary immunodeficiency improved after 1995 compared to previously[283]. The recent case series by Martinez et al demonstrated 100% survival which provides hope for the future[174]. The American children were slightly younger than the children in this study, with a median age of 3.8 years. They had a similar infection history, with bacterial and fungal infections and two had colitis. They were all conditioned with a Busulphan based regime and had good neutrophil engraftment, although four did have grade I GvHD. Although, it is unlikely we will ever achieve 100% survival across all CGD transplants, in all circumstances, it does provide reassurance that HSCT can be a safe and effective procedure in children with CGD.

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6.1.9 Strengths of the study

CGD is unusual in terms of primary immunodeficiencies as there are two distinct cohorts of similar ages, pre and post-HSCT. Many other primary immunodeficiencies are either treated early in life (e.g. severe combined immunodeficiency) or are managed with prophylaxis (e.g. antibody deficiencies). This is the first study to compare the outcome of these two different groups in CGD. Due to the cooperation of specialists across the UK and Ireland we can be confident we identified all the known paediatric patients. This is different from other studies which have relied on tertiary specialists to report cases. Although there would be an expectation that all children with such a rare condition are managed at centres of excellence a number of cases scattered around the UK, without input from the specialist centres, were identified. We had good levels of recruitment, particularly from face to face recruitment. The data collection was also very detailed and because it was done by a single researcher it was recorded consistently. By not relying on local clinicians to provide information it was possible to obtain detail that may otherwise not have been completed. Therefore, the findings of this study should be representative of the UK cohort of children with CGD, or post-HSCT for it.

6.1.10 Limitations of the study

One of the key limitations to the study is the small numbers involved. However, this is a result of the rarity of CGD and is the largest such study that can take place in the UK. The other key limitation is that it was a retrospective study. Ideally a prospective study would be performed looking at both clinical and quality of life aspects over time in the non-transplant and post-transplant groups. However, due to rarity of the disease and the fact that many are now going on to have earlier HSCT, it would not be possible to generate two groups of children to compare over time within the UK.

There were also some other specific difficulties. Recruitment was very good for face to face contact but less successful for those approached by post. The postal approach was only used when it was not possible to set up face to face

meetings at clinic appointments, due to time constraints, clashes of clinics at different sites or families not attending for booked appointments. Clinical information was available for 62 of the children. Notes could not be found on one deceased child. Some of the records that were available were limited, for example, in some cases only the second volume could be found. Some records have been transferred to electronic format and the quality of these is variable. It is therefore possible that some information was missed and rates of infection etc are an under estimate of true values. At commencement of the study it was planned to review medical records at all centres a patient was seen at. However, this was found to be impractical due to time constraints and difficulty obtaining access to records. Therefore, only records from major centres significantly involved in the care of the patient were reviewed. Accuracy of data, therefore, relies on clinicians at these centres recording any events that may have happened elsewhere. For example, some minor admissions may be missed if patients were seen at their local hospital. Despite this, the data are probably more accurate than in those studies that relied on specialist centre clinicians to complete a proforma. With busy schedules they would have limited time to complete the information and may not have had opportunity to review old notes.

There were particular issues for data collection from patients originating from the Republic of Ireland. The original intention was to travel to Ireland to collect data. However, due to the ethics process required and the set up of the health service with individual private hospitals, this was not possible. Irish patients were therefore recruited on their visits to the UK and data were only gathered from their UK medical records. These records did included copies of letters from their clinic appointments in Ireland which were attended by the visiting consultant immunologist from the UK. All other correspondence from Ireland was also reviewed to check for details of admission, infection surgery etc. However, it is possible some details were missed for the small number of patients from Ireland as their local records could not be directly accessed.

Due to the way data were collected and entered in to the database by one individual (the author) there was the possibility of omissions or errors in data entry. Data were manually checked when entered to prevent duplication and new data were checked against that already held within the registry for existing patients but it was possible some errors could have been introduced. This could have been addressed by arranging for an independent person to audit a percentage of the entries to check for accuracy. However, this was an individual research project and there was no one available to do this. If there are inaccuracies it is likely these take the form of omissions of detail regarding admissions, infections etc. Therefore, again the rates may be an underestimate of the true infection, admission and surgery rates.

This study did not endeavour to address the issue of genetics and disease severity. Although genetic mutations were documented in some cases, they were not available for all and details of residual NADPH oxidase activity were not recorded therefore no conclusions can be drawn about this. The CGD registry also does not contain detail about ethnic origin, which would be an important factor to address. This may have implications for inheritance, genetic mutations and residual NADPH oxidase activity.

This study is not able to make any conclusions about long term morbidity and survival in patients with CGD as it only includes paediatric data. To really evaluate what has happened over the last decade it would be important to look at adult survival as well.

6.2 Discussion of quality of life, emotional wellbeing and cognitive function studies

6.2.1 Quality of life

This study demonstrates that children with CGD have poor quality of life when compared to the normal population. This may seem obvious, but as previously discussed clinician and patient perspectives on quality of life do not correlate well and it has never before been measured in CGD[194]. It is therefore important to measure quality of life directly from the patient and family perspective. This is the first study to do so in CGD. According to the parents, quality of life in children with CGD appears to be similar to those with inflammatory bowel disease and cancer. In fact, it is rated as worse for specific areas of quality of life (physical, social and school) when compared to IBD. Parents also reported more impact on physical quality of life for CGD when compared to cancer. This demonstrates how significant an impact CGD has on a child.

The children reported significantly poorer quality of life compared to healthy children in most domains. Interestingly, however, they did not report worse emotional quality of life compared to healthy children, whereas their parents did. It is well know that children report better quality of life for themselves than their parents often do. This may be because they do not know any different, whereas, their parents will be comparing to what they expect from a "normal" child. It may be that these children feel they have adjusted to their diagnosis.

Most interesting is the fact that post-HSCT children have quality of life that is normal when compared to healthy children. This is the first study to demonstrate this difference between non-HSCT and post-HSCT children with primary immunodeficiency and has been demonstrated in both parents and the children themselves. Parental scores were also significantly better for post-HSCT children compared to non-HSCT children across all domains.

When child self report non-HSCT and post-HSCT groups were compared there were no statistically significant differences in total and psychosocial score. This

lack of significance may be related to the small sample size and may also be because the non-HSCT group started from a higher point (although still significantly below normal). This would, again, be explained by children reporting better quality of life than parents.

This study cannot determine causality for improved quality of life post-HSCT as it is a cross-sectional survey carried out at one time point. It also does not evaluate whether the clinical features of the two groups could result in differences. However, it would be difficult to determine which features of the disease may impact on perception of quality of life. As already discussed clinician perception of quality of life differs from the patient perspective and therefore making decisions about what is "severe" disease and what is "mild" may have no bearing on what the patient feels. In other conditions, for example, asthma and juvenile idiopathic arthritis, there is not always correlation between disease severity and quality of life[203, 204]

To assess whether the differences are due to treatment with HSCT it would be necessary to carry out a longitudinal study, evaluating quality of life in the same children over time, pre and post transplant. However, due to the rarity of this disease, and, because some patients are managed conservatively, and some curatively, it would take many years to gather a significant amount of data. The sample size for this study was small but still represents a large proportion of the children with CGD in the UK and Ireland. It would be difficult to undertake a larger study of quality of life in CGD without performing an international study. This would have limitations because of the need to complete questionnaires in different languages, having different normal values for different populations and different protocols for when to undertake HSCT. The only country that could perhaps undertake a study with a larger population would be the United States.

6.2.2 Emotional and behavioural difficulties

This study demonstrates that parents report children with CGD are at increased risk of emotional difficulties when compared to the normal population. This has been described previously in a cohort of children with CGD in the UK[6]. This

study did not include the same children as previously tested as they are now older than 16. This would suggest that it is likely to be a true finding as it has been replicated. Parents of the post-HSCT group did not demonstrate the same increased risk of emotional difficulties, instead, having scores similar to normal.

Parents of non-HSCT children also reported a higher total score, presumably at least in part, due to the increased emotional score. This represents an overall increased risk of emotional and behavioural difficulties. The post-HSCT group had a significantly lower total score compared to the non-HSCT group showing that they are at less risk of emotional and behavioural difficulties.

The post-HSCT group also had a significantly lower score for hyperactivity compared to the non-HSCT group. Although the non-HSCT group hyperactivity score was not significantly abnormal there was a trend towards significance ($p=0.052$), which when compared to the much lower score for the post-HSCT group resulted in a significant difference. The lack of significance for the non-HSCT group compared to normal may be a reflection of the small sample size. Samples were compared to population norms for boys given the very high percentage of boys in the group so this is not a reflection of hyperactivity being more common in males than females.

There were no significant differences from normal or when comparing non-HSCT and post-HSCT groups for the child self report data. However, the numbers involved in this were very small and therefore it would be difficult to draw any conclusions.

The SDQ is not designed to diagnose emotional difficulties, for example, anxiety or depression, but it is a useful screen to identify risk. It is important for clinicians to recognise that children with CGD may have increased risk of emotional and perhaps behavioural difficulties. It is not always easy to identify these problems in a routine clinic appointment but by raising awareness of these issues for both clinicians and families it may be easier to talk about in clinic. Early recognition will allow for formal diagnosis of any emotional

problems and then for interventions to be put in place which may improve the situation.

6.2.3 Self-esteem

There were no differences in global self-esteem for non-HSCT and post-HSCT children when compared with each other and compared to published norms. Non-HSCT children did have significantly lower scores compared to norms for athletic competence. Whereas, the post-HSCT group had normal scores for athletic competence. This suggests that those with CGD do feel their disease impacts on the athletic abilities, compared to their peers. Interestingly, both groups had higher scores for conduct compared to the norms and this was statistically significant for the post-HSCT group. This is difficult to explain. It may be that children with a chronic illness such as CGD have less ability to get involved in activities that they feel would be seen as bad behaviour. They may have less physical and emotional energy for such activities.

These results are limited by the fact that only small numbers of children were eligible (needing to be 8 years old or over) and completed the questionnaire. The questionnaire is not "user friendly", being 3 pages long and involving a complex style of questioning. A number of questionnaires had to be excluded due to incorrect completion. Despite explanation some children struggled with the concept of either/or questions and, rather than choosing one side of the questionnaire to answer they ticked both sides. There is also a lack of UK norms for comparison. The questionnaires were validated in American schools and a number of different sample results were generated for children of different ages. The only published data for comparison encountered the same difficulty so generated combined scores from these sample groups in the questionnaires manuals[273]. These are the results that were used as norms, however, they may not reflect current UK norms. In order to improve the quality of conclusions it would be important to have up to date UK data from a control group to compare to. Given these limitations, it is very hard to draw any useful conclusions about self-esteem in CGD and those transplanted for it.

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6.2.4 Cognitive function

This study demonstrates that children with CGD and those transplanted for it have normal IQ. This differs from the study by Pao et al which showed a higher than expected proportion of patients with significantly low IQ[5]. It also differs from the findings by Titman et al which showed lower than expected IQ in children treated with HSCT for a range of primary immunodeficiencies[4]. The differences from Pao et al can perhaps be explained because that group was selected because of recognised psychological difficulties. Whereas, the children tested in this study were taken from across the UK cohort. This makes the results of the current study more generalisable. The differences from Titman et al's work may be due to the fact that that study included children with primary immunodeficiencies that also have neurological involvement.

The number of children involved in this study is small, although not markedly different from the numbers in Pao et al. It does represent a reasonable proportion of the UK paediatric CGD population. Some families were reluctant to participate in IQ testing because of the extra time commitment when they were keen to get home after clinic appointments. Some families were also put off by the word "test". It is difficult to tell if those who did not participate were different in any way that may have biased results.

Given the normal results it is impossible to draw any conclusions about the impact of inflammation on IQ in CGD. It may be that prolonged exposure to inflammation is required to demonstrate any reduction in IQ. Most studies looking at inflammation and cognitive function are in adults rather than children. The question may be better addressed in adults with CGD. It may also be that the test used was too limited to pick up any subtle cognitive difficulties. The work in CGD mice has focussed very much on specific areas of memory[244, 245] that the WASI is not designed to test in detail. It may be that a more detailed assessment would identify subtle differences. The trade off for this study was convenience over detailed assessment. Given that a number of families did not participate in the WASI due to the time involved it is likely even fewer would have stayed for a more detailed evaluation.

It is reassuring, for families, however, that a screening test has not identified any major difficulties that will impact on education.

6.3 Areas for further research

Over the years a number of CGD registries have been established. These are important to document clinical features of the disease in different populations. It will be important to analyse data for adult patients with CGD that was collected alongside the data in this study to evaluate morbidity and mortality and consider whether it has changed since the findings of the original UK and Ireland registry were published. Adult data are also necessary to make comparisons with the published results from other registries. The European Society for Immunodeficiencies (ESID) does provide an online database for a wide range of immunodeficiencies including CGD[284]. However, at the moment this is unable to record the wide variety of detail which is a key strength of the UK and Ireland Registry. Work has been undertaken to collect more detail and hopefully this will be available soon. This will then run as a continually updated registry and reduce the need for manual updates of the UK and Ireland registry.

This study has provided no additional knowledge regarding residual NADPH oxidase activity and clinical outcome, which is an important area for further work. A small number of patients obviously do well into late adulthood, as seen from case reports. This is significant because, it may be that a subgroup of patients does not require HSCT. As more evidence points to the benefits of HSCT it will be important to identify whether this is an unnecessary risky procedure in a few select patients. It would be interesting to see if it is possible to predict clinical course from NADPH oxidase levels measured in childhood. This is only going to be possible while we are not routinely transplanting all children early. It may be that international collaboration is needed with countries that are not as rapid in their move to transplant.

This study did not identify any cognitive difficulties on gross screening. However, it may be that subtle deficits could be identified on more detailed examination. Given that mouse models highlight issues with memory this would

be an area for further work, specifically using measures to evaluate memory rather than IQ. It would also be interesting to evaluate cognitive function in adults with CGD as it may be that older patients do suffer from more difficulties due to longer exposure to inflammation and the risk of more infections. If any cognitive difficulties are identified it would be useful to look at any correlation with levels of inflammatory markers. As IL1β has been implicated in cognitive decline it would be interesting to measure this in CGD patients and correlate it with cognitive function. It would be interesting to also look at this in the post-HSCT group. Given that our understanding of inflammation in CGD remains incomplete we cannot be sure at this stage that we ameliorate all complications from this with HSCT.

Further examination of emotional difficulties in a larger cohort of CGD patients would be important. This study was only able to identify significant differences in parent reports. The lack of significant difference in child self-reports may be due to the small numbers involved. It would be important to evaluate self-reported emotional and behavioural difficulties in a larger sample of children. Again, this would probably need international collaboration and may be complicated by different normal values for different populations.

The SDQ is, like the WASI, a screening tool so it cannot provide detail about what type of emotional difficulties a patient is experiencing. Further evaluation would be useful in those identified to high scores on the SDQ to elucidate the type of difficulties experienced. This is important in order to put in place the correct form of support.

As discussed above, a longitudinal study of quality of life in the same patients pre and post transplant would be really useful to examine whether the better post-HSCT quality of life really was a result of the procedure and to examine reasons for this. However, this would be difficult to carry out due to the rarity of CGD. If that is not possible, a study of quality of life in a larger sample of non-HSCT and post-HSCT patients would be beneficial, however, complicated by international variation.

The questionnaires used in this study were validated for children. However, there are also adults living with CGD and some who have undergone HSCT for it (either as a child or an adult). It is important to evaluate emotional well being and quality of life in these patients as well. If children are at risk of emotional difficulties and poor quality of life, then it is quite possible adults are as well. It would be important to recognise this, again, in order to provide appropriate support.

Another area that this study has not addressed is the cost of treatment. There is significant interest in cost effectiveness of treatment options within the health service. CGD is likely to be an expensive condition to manage, for example, long courses of intravenous antifungal treatment have significant costs in terms of drugs and nursing time to administer them (in hospital or at home). Prolonged hospital admissions or frequent hospital visits also potentially result in time off work for parents. Although HSCT is thought of as an expensive procedure, it may be that by providing a cure and reducing the need for admission or surgery after HSCT it is less expensive in the long term. Further detailed information about length of hospital admissions and medication given, along with time off work for parents would be needed to estimate life-time cost of managing CGD before comparisons could be made with HSCT.

There are wider implications for the findings of this study, in terms of other primary immunodeficiencies and other non-malignant conditions treated with HSCT. As HSCT survival is improving it is, perhaps, time to evaluate the emotional wellbeing and quality of life of these children, particularly in those where best treatment remains open to debate. It is not true the HSCT results in improved quality of life in all conditions. In those treated for leukaemia, the post-HSCT group had worse quality of life than the non-transplanted group[285]. Presumably, this is because they had more intensive treatment, with more complications and side effects. Providing further information in these areas is important for families when considering their options. Although a demonstration of better quality of life, for example, does not necessarily mean we should be

recommending HSCT for all it may help provide more information for families to consider when faced with incredibly difficult decisions.

Chapter 7 Conclusions

These studies set out to evaluate the clinical outcome, cognitive function, emotional wellbeing and quality of life for children with CGD, treated conservatively or curatively. It hypothesised that children with CGD would have more morbidity when compared to those who have undergone HSCT. This has been confirmed. It has shown that children who have undergone transplant for CGD have fewer infections and admissions to hospital than children that are managed conservatively. Post-HSCT survival is good 90%. Post-HSCT children also have better height for age compared to non-transplanted children and demonstrate improved height for age following HSCT.

These studies have also shown that children with CGD are at increased risk of emotional and possibly behavioural problems when compared to healthy norms. These differences are not present in the post-HSCT group. There was little difference in self-esteem between the two groups. There was marked difference in perceived quality of life between non-transplanted and post-transplant children. This supports the hypothesis set out in chapter two. What was not expected was the post-transplant group rating their quality of life as similar to healthy norms. In contrast to the stated hypothesis and previously published work, children with CGD and those transplanted for it have normal IQ.

These studies suggest children that have undergone HSCT for CGD fare better clinically and emotionally. This information is important for clinicians and families that care for children with CGD and also has wider implications for other children with primary immunodeficiencies. Although this does not provide an answer to the question "should we transplant all patients with CGD" it contributes important information to the on-going debate.

References

1.   Jones, L.B.K.R., et al., *Special Article: Chronic granulomatous disease in the United Kingdom and Ireland: a comprehensive national patient based registry.* Clinical and Experimental Immunology, 2008. **152**(2): p. 211-18.

2.   Soncini, E., et al., *Unrelated donor and HLA-identical sibling haematopoietic stem cell transplantation cure chronic granulomatous disease with good long-term outcome and growth.* British Journal of Haematology, 2009. **145**: p. 73-83.

3.   Holland, S.M., *Chronic Granulomatous Disease.* Clinical reviews in allergy and immunology, 2010. **38**: p. 3-10.

4.   Titman, P., et al., *Cognitive and behavioral abnormalities in children after hematopoietic stem cell transplantation for severe congenital immunodeficiencies.* Blood, 2008. **112**(9): p. 3907-13.

5.   Pao, M., et al., *Cognitive function in patients with chronic granulomatous disease: a preliminary report.* Psychosomatics, 2004. **45**(3): p. 230-34.

6.   Cole, T.S., et al., *Emotional and behavioural difficulties in chronic granulomatous disease.* Arch Dis Child, 2012. **97**(1): p.87

7.   Berendes, H., R.A. Bridges, and R.A. Good, *A fatal granulomatosus of childhood:the clinical study of a new syndrome.* Minnesota Medicine, 1957. **40**(5): p. 309-12.

8.   Landing, B.H. and H.S. Shirkey, *A syndrome of recurren infection and infiltration of viscera by pigmented lipid histiocytes.* Pediatrics, 1957. **20**(3): p. 431-38.

9.   Assari, T., *Chronic Granulomatous Disease; fundamental stages in our understanding of CGD.* Medical Immunology, 2006. **5**(1): p. 4.

10.   Quie, P.G., et al., *In vitro bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood.* Journal of Clinical Investigation, 1967. **46**(4): p. 668-679.

11.   Holmes, B., et al., *Fatal granulomatous disease of childhood. An inborn abnormality of phagocyte function.* Lancet, 1966. **1**(7449): p. 1225-1228.

12.   Holmes, B., A.R. Page, and R.A. Good, *Studies of the metabolic activity of leukocytes from patienst with a genetic abnormality of phagocytic function.* Journal of Clinical Investigation, 1967. **46**(9): p. 1422-1432.

13.   Kaplan, E.L., T. Laxdal, and P.G. Quie, *Studies of polymorphonuclear leukocytes from patients with chronic granulomatous disease of childhood: bactericidal capacity for streptococci.* Pediatrics, 1968. **41**(3): p. 591-599.

14.   Baehner, R.L. and M.L. Karnovsky, *Deficiency of reduced nicotinamide-adenine dinucleotide oxidase in chronic granulomatous disease.* Science, 1968. **162**(3859): p. 1277-1279.

15.   Hohn, D.C. and R.I. Lehrer, *NADPH oxidase deficiency in X-linked chronic granulomatous disease.* Journal of Clinical Investigation, 1975. **55**(4): p. 707-713.

16.   Segal, A.W., et al., *Absence of cytochrome b-245 in chronic granulomatous disease. A multicenter European evaluation of its incidence and relevance.* New England Journal of Medicine, 1983. **308**(5): p. 245-251.

17.   Borregaard, N., et al., *Subcellular localisation of the b-cytochrome component of the human neutrophil microbicidal oxidase: translocation during activation.* Journal of cellular biology, 1983. **97**(1): p. 52-61.

18.   Calafat, J., et al., *Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b558 and the adhesion molecule CD11b/CD18.* Blood, 1993. **81**(11): p. 3122-3129.

19.   Segal, A.W., *Absence of both cytochrome b-245 subunits from neutrophils in X-linked chronic granulomatous disease.* Nature, 1987. **326**(6108): p. 88-91.

20.   Sumimoto, H., *Structure, regulation and evolution of Nox-family NADPH oxidases that produce reactive oxygen species.* FEBS J, 2008. **275**(13): p. 3249-77.

21.   Umei, T., K. Takeshige, and S. Minakami, *NADPH - binding component of the superoxide-generating oxidase in unstimulated neutrophils and the neutrophils from the patients with chronic granulomatous disese.* The Biochemical Journal, 1987. **243**(2): p. 467- 472.

22.   Curnutte, J.T., R. Kuver, and P.J. Scott, *Activation of neutrophil NADPH oxidase in a cell free system. Partial purification of components and characterization of the activation process.* The Journal of Biological Chemistry, 1987. **262**(12): p. 5563-5569.

23.   Wientjes, F.B., et al., *p40phox, a third cytosolic component of the activation complex of the NADPH oxidase to contain src homology 3 domains.* The Biochemical Journal, 1993. **296**(3): p. 557-561.

24.   Jesaitis, A.J., et al., *Ultrastructural localization of cytochrome b in the membranes of resting and phagocytosing human granulocytes.* J Clin Invest, 1990. **85**(3): p. 821-35.

25. Leusen, J.H.W., A.J. Verhoeven, and D. Roos, *Interactions between the components of the human NADPH oxidase: a review about the intrigues in the Phox family.* Frontiers in Bioscience, 1996. **1**(1): p. 72-90.

26. Segal, A.W., et al., *Stimulated neutrophils from patients with autosomal recessive chronic granulomatous disease fail to phophorylate a M-44000 protein.* Nature, 1985. **316**(6028): p. 547.

27. Rotrosen, D. and T.L. Leto, *Phosphorylation of neutrophil 47-kDa cytosolic oxidase factor. Translocation to membrane is associated with distinct phosphorylation events.* Journal of Biological Chemistry, 1990. **265**(32): p. 19910-5.

28. Cross, A.R. and J.T. Curnette, *The cytosolic activating factors p47phox and p67phox have distinct roles in the regulation of electron flow in NADPH oxidase.* Journal of Biological Chemistry, 1995. **270**(12): p. 6543-48.

29. Smith, R.M., et al., *The cytosolic subunit p67phox contains an NADPH binding site that participates in catalysis by the leukocyte NADPH oxidase.* Journal of Clinical Investigation, 1996. **98**(4): p. 977-83.

30. Diekmann, D., et al., *Interaction of Rac with p67phox and regulation of phagocytic NADPH oxidase activity.* Science, 1994. **265**(5171): p. 531-533.

31. Bosco, E.E., et al., *Rational design of small molecule inhibitors targeting the Rac GTPase-p67(phox) signaling axis in inflammation.* Chem Biol, 2012. **19**(2): p. 228-42.

32. el Benna, J., J.M. Ruedi, and B.M. Babior, *Cytosolic guanine nucleotide-binding protein Rac2 operates in vivo as a component of the neutrophil respiratory burst oxidase. Transfer of Rac2 and the cytosolic oxidase components p47phox and p67phox to the submembranous actin*

cytoskeleton during oxidase activation. Journal of Biological Chemistry, 1994. **269**(9): p. 6729-6734.

33. Knaus, U.G., et al., *Regulation of phagocyte oxygen radical production by the GTP-binding protein Rac 2.* Science, 1991. **254**(5037): p. 1512-1515.

34. Ellson, C.D., et al., *PtdIns(3)P regulates the neutrophil oxidase complex by binding to the PX domain of p40phox* Nature Cell Biology, 2001. **3**(7): p. 679-82.

35. Ellson, C.D., et al., *Neutrophils from p40phox-/- mice exhibit severe defects in NADPH oxidase regulation and oxidant-dependent bacterial killing.* The Journal of Experimental Medicine, 2008. **203**(8): p. 1927-37.

36. Matute, J.D., et al., *A new genetic subgroup of chronic granulomatous disease with autosomal recessive mutations in p40phox and selective defects in neutrophil NADPH oxidase activity.* Blood, 2009. **114**: p. 3309-15.

37. Kurkchubasche, A.G., et al., *Clinical features of a human Rac2 mutation: a complex neutrophil dysfunction disease.* J Pediatr, 2001. **139**(1): p. 141-7.

38. Ambruso, D.R., et al., *Human neutrophil immunodeficiency syndrome is associated with an inhibitory Rac2 mutation.* Proc Natl Acad Sci U S A, 2000. **97**(9): p. 4654-9.

39. Clifford, D.P. and J.E. Repine, *Hydrogen peroxide mediated killing of bacteria.* Molecular and Cellular Biochemistry, 1982. **49**(3): p. 143-9.

40. Reeves, E.P., et al., *Killing activity of neutrophils is mediated through activation of proteases by K+ flux.* Nature, 2002. **416**(6878): p. 291-297.

41. Cross, A.R. and A.W. Segal, *The NADPH oxidase of professional phagocytes--prototype of the NOX electron transport chain systems.* Biochim Biophys Acta, 2004. **1657**(1): p. 1-22.

42. Vethanayagam, R.R., et al., *Role of NADPH oxidase versus neutrophil proteases in antimicrobial host defense.* PLos one, 2011. **6**(12): p. e28149.

43. Bylund, J., et al., *Intracellular generation of superoxide by the phagocyte NADPH oxidase: How, where, and what for?* Free Radical Biology and Medicine, 2010. **49**(12): p. 1834-1845.

44. Fuchs, T.A., et al., *Novel cell death program leads to neutrophil extracellular traps.* The Journal of Cell Biology, 2007. **176**(2): p. 231-241.

45. Brinkmann, V., et al., *Neutrophil extracellular traps kill bacteria.* Science, 2004. **303**(5663): p. 1532-5.

46. Urban, C.F., et al., *Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms.* Cell Microbiol, 2006. **8**(4): p. 668-76.

47. Klebanoff, S.J., *Myeloperoxidase: friend and foe.* Journal of Leukocyte Biology, 2005. **77**(5): p. 598-625.

48. Nishinaka, Y., et al., *Singlet oxygen is essential for neutrophil extracellular trap formation.* Biochemical and Biophysical Research Communications, 2011. **413**(1): p. 75-79.

49. Bianchi, M., et al., *Restoration of NET formation by gene therapy in CGD controls aspergillosis.* Blood, 2009. **114**(13): p. 2619-22.

50. Pilsczek, F.H., et al., *A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to Staphylococcus aureus.* J Immunol, 2010. **185**(12): p. 7413-25.

51. Cross, A.R. and O.T. Jones, *The effect of the inhibitor diphenylene iodonium on the superoxide-generating system of neutrophils. Specific labelling of a component polypeptide of the oxidase.* Biochemical Journal, 1986. **237**: p. 111-6.

52. Hattori, H., et al., *Small-molecule screen identifies reactive oxygen species as key regulators of neutrophil chemotaxis.* Proceedings of the National Academy of The United States of America, 2010. **107**(8): p. 3546-51.

53. Hartl, D., et al., *Dysregulation of innate immune receptors on neutrophils in chronic granulomatous disease.* J Allergy Clin Immunol, 2008. **121**(2): p. 375-382 e9.

54. Bylund, J., et al., *Enhanced inflammatory responses of chronic granulomatous disease leukocytes involve ROS-independent activation of NF-kappa B.* Eur J Immunol, 2007. **37**(4): p. 1087-96.

55. Meissner, F., et al., *Inflammasome activation in NADPH oxidase defective mononuclear phagocytes from patients with chronic granulomatous disease.* Blood, 2010. **116**(9): p. 1570-3.

56. Martinon, F., A. Mayor, and J.r. Tschopp, *The Inflammasomes: Guardians of the Body.* Annual Review of Immunology, 2009. **27**(1): p. 229-265.

57. Dostert, C., et al., *Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica.* Science, 2008. **320**(5876): p. 674-7.

58. van Bruggen, R., et al., *Human NLRP3 inflammasome activation is Nox1-4 independent.* Blood, 2010. **115**(26): p. 5398-400.

59. Segal, B.H., et al., *NADPH oxidase limits innate immune responses in the lungs in mice.* PLoS One, 2010. **5**(3): p. e9631.

60.   Romani, L., et al., *Defective tryptophan catabolism underlies inflammation in mouse chronic granulomatous disease.* Nature, 2008. **451**(7175): p. 211-5.

61.   De Ravin, S.S., et al., *Tryptophan/kynurenine metabolism in human leukocytes is independent of superoxide and is fully maintained in chronic granulomatous disease.* Blood, 2010. **116**(10): p. 1755-60.

62.   Jurgens, B., et al., *Intact indoleamine 2,3-dioxygenase activity in human chronic granulomatous disease.* Clin Immunol, 2010. **137**(1): p. 1-4.

63.   Maghzal, G.J., et al., *Cytochrome b5, Not Superoxide Anion Radical, Is a Major Reductant of Indoleamine 2,3-Dioxygenase in Human Cells.* Journal of Biological Chemistry, 2008. **283**(18): p. 12014-12025.

64.   Kraaij, M.D., et al., *Induction of regulatory T cells by macrophages is dependent on production of reactive oxygen species.* Proceedings of the National Academy of Sciences, 2010. **107**(41): p. 17686-17691.

65.   Fadeel, B., D. Xue, and V. Kagan, *Programmed cell clearance: molecular regulation of the elimination of apoptotic cell corpses and its role in the resolution of inflammation.* Biochem Biophys Res Commun, 2010. **396**(1): p. 7-10.

66.   Sanmun, D., et al., *Involvement of a functional NADPH oxidase in neutrophils and macrophages during programmed cell clearance: implications for chronic granulomatous disease.* Am J Physiol Cell Physiol, 2009. **297**(3): p. C621-31.

67.   Fernandez-Boyanapalli, R., et al., *PPAR{gamma} activation normalizes resolution of acute sterile inflammation in murine chronic granulomatous disease.* Blood, 2010. **116**(22): p. 4512-4522.

68. Fadok, V.A., et al., *Differential effects of apoptotic versus lysed cells on macrophage production of cytokines: role of proteases.* J Immunol, 2001. **166**(11): p. 6847-54.

69. Freire-de-Lima, C.G., et al., *Apoptotic cells, through transforming growth factor-beta, coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages.* J Biol Chem, 2006. **281**(50): p. 38376-84.

70. Brown, J.R., et al., *Diminished production of anti-inflammatory mediators during neutrophil apoptosis and macrophage phagocytosis in chronic granulomatous disease (CGD).* Journal of Leukocyte Biology, 2003. **73**(5): p. 591-599.

71. Quie, P.G., et al., *Defective polymorphonuclear-leukocyte function and chronic granulomatous disease in two female children.* N Eng J Med, 1968. **278**(18): p. 976-980.

72. Royer-Pokora, B., et al., *Cloning the gene for an inherited human disorder - chronic granulomatous disease - on the basis of its chromosomal location.* Nature, 1986. **332**: p. 32-38.

73. Volpp, B.D., et al., *Cloning of the cDNA and functional expression of the 47-kilodalton cytosolic component of the human neutrophil respiratory burst oxidase.* Proceedings of the National Academy of Sciences of the United States of America, 1989. **86**(18): p. 7195-7199.

74. Leto, T.L., et al., *Cloning of a 67-kD neutrophil oxidase factor with similarity to a noncatalytic region of p60csrc.* Science, 1990. **248**(4956): p. 727-730.

75. Dinauer, M.C., et al., *Human neutrophil cytochrome b light chain (p22phox). Gene structure, chromosomal location and mutations in cytochrome-negative autosomal recessive chronic granulomatous disease.* Journal of Clinical Investigation, 1990. **86**(5): p. 1729-37.

76.   Zhan, S., et al., *Genomic Structure, Chromosomal Localization, Start of Transcription, and Tissue Expression of the Human p40-phox, A New Component of the Nicotinamide Adenine Dinucleotide Phosphate-Oxidase Complex* Blood, 1996. **88**(7): p. 2714-21.

77.   van den Berg, J.M., et al., *Chronic granulomatous disease: The European experience.* PLos one, 2009. **4**(4): p. e5234.

78.   Meischl, C. and D. Roos, *The molecular basis of chronic granulomatous disease.* Springers seminars in immunopathology., 1998. **19**: p. 417-434.

79.   Kuhns, D.B., et al., *Residual NADPH Oxidase and Survival in Chronic Granulomatous Disease.* New England Journal of Medicine, 2010. **363**(27): p. 2600-2610.

80.   Isman-Nelkenbaum, G., et al., *Chronic granulomatous disease of childhood: an unusual cause of recurrent uncommon infections in a 61-year-old man.* Clinical and Experimental Dermatology, 2011. **36**(7): p. 759-762.

81.   Yamada, M., et al., *Somatic mosaicism in two unrelated patients with X-linked chronic granulomatous disease characterized by the presence of a small population of normal cells.* Gene, 2012. **497**(1): p. 110-115.

82.   Wolach, B., et al., *Two X-Linked Chronic Granulomatous Disease Patients with Unusual NADPH Oxidase Properties.* Journal of Clinical Immunology, 2011. **31**(4): p. 560-566.

83.   Clark, R.A., et al., *Genetic variants of chronic granulomatous disease: prevalence of deficiencies of two cytosolic components of the NADPH oxidase system.* New England Journal of Medicine, 1989. **321**(10): p. 647-52.

84.   Roos, D., et al., *Mutations in X-linked and autosomal recessive forms of chronic granulomatous disease.* Blood, 1996. **87**(5): p. 1663-81.

85.   Roos, D., et al., *Hematologically important mutations: The autosomal recessive forms of chronic granulomatous disease (second update).* Blood Cells, Molecules, and Diseases, 2010. **44**(4): p. 291-299.

86.   Roesler, J., et al., *P67-phox (NCF2) Lacking Exons 11 and 12 Is Functionally Active and Leads to an Extremely Late Diagnosis of Chronic Granulomatous Disease (CGD).* PLoS One, 2012. **7**(4): p. e34296.

87.   Wolach, B., et al., *Chronic granulomatous disease in Israel: Clinical, functional and molecular studies of 38 patients* Clinical Immunology, 2008. **129**: p. 103-114.

88.   Baehner, R.L. and D.G. Nathan, *Leukocyte oxidase: defective activity in chronic granulomatous disease.* Science, 1967. **155**(3764): p. 835-6.

89.   Elloumi, H.Z. and S.M. Holland, *Diagnostic assays for chronic granulomatous disease and other neutrophil disorders.* Methods in Molecular Biology, 2007. **412**: p. 505-23.

90.   Vowells, S.J., T.A. Fleisher, and H.L. Malech, *Testing for chronic granulomatous disease.* Lancet, 1996. **347**(9007): p. 1048-9.

91.   Weening, R.S., D. Roos, and J.A. Loos, *Oxygen consumption of phagocytozing cells in human leukocyte and granulocyte preparations: a comparative study.* The Journal of laboratory and clinical medicine, 1974. **83**: p. 570-7.

92.   Casimir, C., et al., *Identification of the defective NADPH - oxidase component in Chronic Granulomatous Disease: a study of 57 European families.* European Journal of Clinical Investigation, 1992. **22**(6): p. 403-6.

93.   Köker, M.Y., et al., *Four different NCF2 mutations in six families from Turkey and an overview of NCF2 gene mutations.* European Journal of Clinical Investigation, 2009. **39**(10): p. 942-51.

94.   Molshanski-Mor, S., et al., *Cell-free assays: the reductionist approach to the study of NADPH oxidase assembly, or "all you wanted to know about cell-free assays but did not dare to ask".* Methods in Molecular Biology, 2007. **412**: p. 385-428.

95.   Dekker, J., M. de Boer, and D. Roos, *Gene-scan method for the recognition of carriers and patients with p47(phox)-deficient autosomal recessive chronic granulomatous disease.* Exp Hematol, 2001. **29**(11): p. 1319-25.

96.   Chollet-Martin, S., et al., *Severe X-linked chronic granulomatous disease in two unrelated females.* Eur J Pediatr, 2007. **166**(2): p. 153-9.

97.   Lun, A., J. Roesler, and H. Renz, *Unusual Late Onset of X-linked Chronic Granulomatous Disease in an Adult Woman after Unsuspicious Childhood.* Clin Chem, 2002. **48**(5): p. 780-781.

98.   Patiño, P.J., et al., *Molecular analysis of Chronic Granulomatous Disease caused by defects in gp91phox.* Human Mutation, 1999. **13**(1): p. 29-37.

99.   Matthay, K.K., et al., *Prenatal diagnosis of chronic granulomatous disease.* American journal of Medical Genetics, 1984. **17**(4): p. 731-9.

100.   De Boer, M., et al., *Prenatal diagnosis in two families of autosomal p47-phox deficient chronic granulomatous disease due to a novel point mutation in NCF1.* Prenatal diagnosis, 2002. **22**: p. 235-40.

101.   De Boer, M., et al., *Prenatal diagnosis in a family with X-linked chronic granulomatous disease with the use of the polymerase chain reaction.* Prenatal diagnosis, 1992. **12**: p. 773-7.

102.   Degn, B., et al., *Preimplantation genetic diagnosis for HLA typing in a case of X-linked chronic granulomatous disease.* Acta Obstet Gynecol Scand, 2012. **91**(7): p. 876-8.

103. Martire, B., et al., *Clinical features, long term follow up and outcome of a large cohort of patients with Chronic Granulomatous Disease: An Italian multicentre study.* Clinical Immunology, 2008. **126**: p. 155-64.

104. Turul-Ozgur, T., et al., *Clinical features of chronic granulomatous disease: a series of 26 patients from a single center.* Turk J Pediatr, 2010. **52**(6): p. 576-81.

105. Winkelstein, J.A., et al., *Chronic Granulomatous Disease: Report on a National Registry of 368 Patients.* Medicine, 2000. **79**(3): p. 155-69.

106. Pitt, J. and H.P. Bernheimer, *Role of peroxide in phagocytic killing of pneumococci.* Infection and Immunity, 1974. **9**(1): p. 48-52.

107. Chang, Y.C., et al., *Virulence of catalase-deficient aspergillus nidulans in p47(phox)-/- mice. Implications for fungal pathogenicity and host defense in chronic granulomatous disease.* J Clin Invest, 1998. **101**(9): p. 1843-50.

108. Messina, C.G.M., et al., *Catalase negative Staphylococcus aureus retain virulence in mouse model of chronic granulomatous disease.* FEBS Letters, 2002. **518**(1-3): p. 107-110.

109. Kottilil, S., et al., *Infections with Haemophilus species in chronic granulomatous disease: insights into the interaction of bacterial catalase and H2O2 production.* Clinical Immunology, 2003. **106**(3): p. 226-230.

110. Reichenbach, J., et al., *Actinomyces in Chronic Granulomatous Disease: An Emerging and Unanticipated Pathogen.* Clinical Infectious Diseases, 2009. **49**(11): p. 1703-1710.

111. Zeeli, T., et al., *Chronic granulomatous disease in a sexagenarian.* Journal of the American Academy of Dermatology, 2011. **64**(2, Supplement 1): p. AB102-AB102.

112. Schapiro, B.L., et al., *Chronic Granulomatous Disease presenting in a 69-year old man.* New England Journal of Medicine, 1991. **325**(25): p. 1786-90.

113. De Ravin, S.S., et al., *Geosmithia argillacea: An Emerging Cause of Invasive Mycosis in Human Chronic Granulomatous Disease.* Clinical Infectious Diseases, 2011. **52**(6): p. e136-e143.

114. Machouart, M., et al., *Emergence of Disseminated Infections Due to Geosmithia argillacea in Patients with Chronic Granulomatous Disease Receiving Long-Term Azole Antifungal Prophylaxis.* J. Clin. Microbiol., 2011. **49**(4): p. 1681-1683.

115. Kawai, T., et al., *Osteomyelitis due to trimethoprim/sulfamethoxazole-resistant Edwardsiella tarda infection in a patient with X-linked chronic granulomatous disease.* Infection, 2011. **39**(2): p. 171-173.

116. Bukhari, E. and A. Alrabiaah, *First case of extensive spinal cord infection with Aspergillus nidulans in a child with chronic granulomatous disease.* J Infect Dev Ctries, 2009. **3**(4): p. 321-3.

117. Dellepiane, R.M., et al., *Invasive Aspergillus nidulans infection in a patient with chronic granulomatous disease.* Mycoses, 2008. **51**(5): p. 458-60.

118. Dotis, J., et al., *Femoral osteomyelitis due to Aspergillus nidulans in a patient with chronic granulomatous disease.* Infection, 2003. **31**(2): p. 121-4.

119. Dotis, J. and E. Roilides, *Osteomyelitis due to Aspergillus spp. in patients with chronic granulomatous disease: comparison of Aspergillus nidulans and Aspergillus fumigatus.* Int J Infect Dis, 2004. **8**(2): p. 103-10.

120. Kim, M., et al., *Aspergillus nidulans infection in a patient with chronic granulomatous disease.* J Korean Med Sci, 1997. **12**(3): p. 244-8.

121. Rosen-Wolff, A., et al., *Successful elimination of an invasive Aspergillus nidulans lung infection by voriconazole after failure of a combination of caspofungin and liposomal amphotericin B in a boy with chronic granulomatous disease.* Pediatr Infect Dis J, 2004. **23**(6): p. 584-6.

122. Segal, B.H., et al., *Aspergillus nidulans infection in chronic granulomatous disease.* Medicine (Baltimore), 1998. **77**(5): p. 345-54.

123. Dotis, J. and E. Roilides, *Osteomyelitis due to Aspergillus species in chronic granulomatous disease: an update of the literature.* Mycoses, 2011. **54**(6): p. e686-e696.

124. Henriet, S.S.V., et al., *Human Leukocytes Kill Aspergillus nidulans by Reactive Oxygen Species-Independent Mechanisms.* Infect. Immun., 2011. **79**(2): p. 767-773.

125. Beaute, J., et al., *Epidemiology and Outcome of Invasive Fungal Diseases in Patients With Chronic Granulomatous Disease: A Multicenter Study in France.* The Pediatric Infectious Disease Journal, 2010. **30**(1): p. 57-62

126. Blumental, S., et al., *Invasive Mold Infections in Chronic Granulomatous Disease: A 25-Year Retrospective Survey.* Clinical Infectious Diseases, 2011. **53**(12): p. e159-e169.

127. Lee, P.P., et al., *Susceptibility to mycobacterial infections in children with X-linked chronic granulomatous disease: a review of 17 patients living in a region endemic for tuberculosis.* Pediatr Infect Dis J, 2008. **27**(3): p. 224-30.

128. Fujita, M., et al., *Impaired host defence against Mycobacterium avium in mice with chronic granulomatous disease.* Clinical & Experimental Immunology, 2010. **160**(3): p. 457-60.

129. Schäppi, M.G., et al., *Hyperinflammation in chronic granulomatous disease and anti-inflammatory role of the phagocyte NADPH oxidase* Seminars in Immunopathology, 2008. **30**: p. 255-71.

130. Marciano, B.E., et al., *Gastrointestinal involvement in chronic granulomatous disease.* Pediatrics, 2004. **114**(2): p. 462-8.

131. Marks, D.J., et al., *Inflammatory bowel disease in CGD reproduces the clinicopathological features of Crohn's disease.* Am J Gastroenterol, 2009. **104**(1): p. 117-24.

132. Walters, T.D. and A.M. Griffiths, *Mechanisms of growth impairment in pediatric Crohn's disease.* Nat Rev Gastroenterol Hepatol, 2009. **6**(9): p. 513-23.

133. Seger, R., *Modern management of chronic granulomatous disease.* British Journal of Haematology, 2008. **140**: p. 255-66.

134. Levine, S., et al., *Histopathological features of chronic granulomatous disease (CGD) in childhood.* Histopathology, 2005. **47**(5): p. 508-16.

135. Goldblatt, D., et al., *Chorioretinal lesions in patients with chronic granulomatous disease.* Journal of Pediatrics, 1999. **134**: p. 780-3.

136. Kim, S.J., J.G. Kim, and Y.S. Yu, *Chorioretinal lesions in patients with chronic granulomatous disease.* Retina, 2003. **23**: p. 360-5.

137. Chowdhury, M.M.U., A.A. Anstey, and C.N.A. Matthews, *The dermatosis of chronic granulomatous disease.* Clinical and experimental Dermatology, 2000. **25**: p. 190-4.

138. Lyon, C.C. and C.E.M. Griffiths, *Chronic granulomatous disease and acute neutrophilic dermatosis.* Clinical and experimental Dermatology, 1999. **24**: p. 368-71.

139. Magnani, A., et al., *Rheumatologic manifestations in a large cohort of patients with Chronic Granulomatous Disease.* Clinical and Experimental Rheumatology, 2011. **29**(2): p. 450.

140. Rosenzweig, S.D., *Inflammatory manifestations in Chronic Granulomatous Disease.* Journal of Clinical Immunology, 2008. **28**(Supplement 1): p. S67-72.

141. De Ravin, S.S., et al., *Chronic granulomatous disease as a risk factor for autoimmune disease.* Journal of Allergy and Clinical Immunology, 2008. **122**: p. 1097-103.

142. Parekh, C., et al., *Hemophagocytic lymphohistiocytosis in children with chronic granulomatous disease.* Pediatric Blood & Cancer, 2011. **56**(3): p. 460-462.

143. van Montfrans, J.M., et al., *Fatal hemophagocytic lymphohistiocytosis in X-linked chronic granulomatous disease associated with a perforin gene variant.* Pediatr Blood Cancer, 2009. **52**(4): p. 527-9.

144. Aguilera, D.G., et al., *Glioblastoma multiforme in a patient with chronic granulomatous disease treated with subtotal resection, radiation, and thalidomide: case report of a long-term survivor.* J Pediatr Hematol Oncol, 2009. **31**(12): p. 965-9.

145. Lugo Reyes, S.O., et al., *Hodgkin lymphoma in 2 children with chronic granulomatous disease.* Journal of Allergy and Clinical Immunology, 2011. **127**(2): p. 543-544.e3.

146. Weel, E.A., W.K. Redekop, and R.S. Weening, *Increased risk of malignancy for patients with chronic granulomatous disease and its possible link to the pathogenesis of cancer.* Eur J Cancer, 1996. **32A**(4): p. 734-5.

147. Wolach, B., et al., *Acute lymphoblastic leukemia in a patient with chronic granulomatous disease and a novel mutation in CYBB: first report.* Am J Hematol, 2005. **80**(1): p. 50-4.

148. Clark, R.A. and S. Szot, *The myeloperoxidase-hydrogen peroxide-halide system as effector of neutrophil-mediated tumor cell cytotoxicity.* J Immunol, 1981. **126**(4): p. 1295-301.

149. Margolis, D.M., et al., *Trimethoprim-sulfamethoxazole prophylaxis in the management of chronic granulomatous disease.* The Journal of Infectious Diseases, 1990. **162**(3): p. 723-26.

150. Gmunder, F.K. and R.A. Seger, *Chronic granulomatous disease: Mode of action of Sulfamethoxazole/Trimethoprim.* Pediatric Research, 1981. **15**: p. 1533-7.

151. Weening, R.S., et al., *Continuous therapy with sulfamethoxazole-trimethoprim in patients with chronic granulomatous disease.* The Journal of Pediatrics, 1983. **103**(127-30).

152. Mouy, R., et al., *Incidence, severity and prevention of infections in chronic granulomatous disease.* The Journal of Pediatrics, 1989. **114**: p. 555-60.

153. Ezekowitz, R.A.B., S.H. Orkin, and P.E. Newburger, *Recombinant Interferon Gamma Augments Phagocyte Superoxide Production and X - Chronic Granulomatous Disease Gene Expression in X-Linked Variant Chronic Granulomatous Disease* Journal of Clinical Investigation, 1987. **80**(4): p. 1009-16.

154. International Chronic Granulomatous Disease Cooperative Study Group, *A controlled trial of interferon gamma to prevent infection in chronic granulomatous disease.* New England Journal of Medicine, 1991. **324**: p. 509-16.

155. Mouy, R., et al., *Interferon gamma for chronic granulomatous disease.* N Engl J Med, 1991. **325**(21): p. 1516-7.

156. Woodman, R.C., et al., *Prolonged recombinant interferon-gamma therapy in chronic granulomatous disease: evidence against enhanced neutrophil oxidase activity.* Blood, 1992. **79**(6): p. 1558-1562.

157. Marciano, B.E., et al., *Long-Term Interferon-gamma Therapy for Patients with Chronic Granulomatous Disease.* Clinical Infectious Diseases, 2004. **39**(5): p. 692-699.

158. Muhlebach, T.J., et al., *Treatment of patients with chronic granulomatous disease with recombinant human interferon-gamma does not improve neutrophil oxidative metabolism, cytochrome b558 content or levels of four anti-microbial proteins.* Clin Exp Immunol, 1992. **88**(2): p. 203-6.

159. Condino-Neto, A. and P.E. Newburger, *Interferon-gamma improves splicing efficiency of CYBB gene transcripts in an interferon-responsive variant of chronic granulomatous disease due to a splice site consensus region mutation.* Blood, 2000. **95**(11): p. 3548-3554.

160. Ishibashi, F., et al., *Improved superoxide-generating ability by interferon {gamma} due to splicing pattern change of transcripts in neutrophils from patients with a splice site mutation in CYBB gene.* Blood, 2001. **98**(2): p. 436-441.

161. Seger, R., *Chronic granulomatous disease: recent advances in pathophysiology and treatment.* Netherlands Journal of Medicine, 2010. **68**(11): p. 334-40.

162. Vinh, D.C., et al., *Mucormycosis in chronic granulomatous disease: Association with iatrogenic immunosuppression.* Journal of Allergy and Clinical Immunology, 2009. **123**(6): p. 1411-1413.

163. Chin, T.W., et al., *Corticosteroids in treatment of obstructive lesions in chronic granulomatous disease.* The Journal of Pediatrics, 1987. **111**: p. 349-52.

164. Collman, R.J. and J.D. Dickerman, *Corticosteroids in the management of cystitis secondary to chronic granulomatous disease.* Pediatrics, 1990. **85**: p. 219-21.

165. Schappi, M.G., et al., *Colitis in chronic granulomatous disease.* Archives of Disease in Childhood, 2001. **84**(2): p. 147-151.

166. Uzel, G., et al., *Complications of Tumor Necrosis Factor-± Blockade in Chronic Granulomatous Disease—Related Colitis.* Clinical Infectious Diseases, 2010. **51**(12): p. 1429-1434.

167. Deffert, C., et al., *TNF-α blockade in chronic granulomatous disease–induced hyperinflammation: Patient analysis and murine model.* Journal of Allergy and Clinical Immunology, 2011. **128**(3): p. 675-677.e4.

168. van der Veerdonk, F.L., et al., *Anakinra for the inflammatory complications of chronic granulomatous disease.* Netherlands Journal of Medicine, 2011. **62**(2): p. 95.

169. Fernandez-Boyanapalli, R., et al., *Impaired Phagocytosis of Apoptotic Cells by Macrophages in Chronic Granulomatous Disease Is Reversed by IFN-{gamma} in a Nitric Oxide-Dependent Manner.* J Immunol, 2010. **185**(7): p. 4030-4041.

170. Leung, T., et al., *Bone marrow transplantation for chronic granulomatous disease: long-term follow-up and review of literature.* Bone Marrow Transplant, 1999. **24**(5): p. 567-70.

171. Horwitz, M.E., et al., *Treatment of Chronic Granulomatous Disease with Nonmyeloablative Conditioning and a T-Cell-Depleted Hematopoietic Allograft.* New England Journal of Medicine, 2001. **344**(12): p. 881-888.

172. Seger, R.A., et al., *Treatment of chronic granulomatous disease with myeloablative conditioning and an unmodified hemopoietic allograft: a survey of the European experience, 1985-2000.* Blood, 2002. **100**(13): p. 4344-50.

173. Gennery, A.R. and A.J. Cant, *Advances in hematopoeitic stem cell transplantation for primary immunodeficiency.* Immunol Allergy Clin North Am, 2008. **28**(439-56).

174. Martinez, C.A., et al., *Excellent survival after sibling or unrelated donor stem cell transplantation for chronic granulomatous disease.* Journal of Allergy and Clinical Immunology, 2012. **129**(1): p. 176-183.

175. Güngör, T., et al., *Successful low toxicity hematopoietic stem cell transplantation for high-risk adult chronic granulomatous disease patients.* Transplantation, 2005. **79**(11): p. 1596-606.

176. Freudenberg, F., et al., *Therapeutic strategy in p47-phox deficient chronic granulomatous disease presenting as inflammatory bowel disease.* Journal of Allergy and Clinical Immunology, 2010. **125**(4): p. 943-946.e1.

177. Majhail, N.S. and J. Douglas Rizzo, *Surviving the cure: long term followup of hematopoietic cell transplant recipients.* Bone Marrow Transplant, 2013. **48**(9): p. 1145-1151.

178. Socié, G., et al., *Nonmalignant late effects after allogeneic stem cell transplantation.* Blood, 2003. **101**(9): p. 3373-3385.

179. Sanders, J.E., et al., *Pregnancies following high-dose cyclophosphamide with or without high-dose busulfan or total-body irradiation and bone marrow transplantation.* Blood, 1996. **87**(7): p. 3045-52.

180. Ryser, M.F., et al., *Gene therapy for chronic granulomatous disease.* Expert Opinion on Biological Therapy, 2007. **7**: p. 1799-1809.

181. Hacein-Bey-Abina, S., et al., *A Serious Adverse Event after Successful Gene Therapy for X-Linked Severe Combined Immunodeficiency.* The New England Journal of Medicine, 2003. **348**(3): p. 255-6.

182. Stein, S., et al., *Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease.* Nature Medicine, 2010. **16**(2): p. 198-204.

183. Mayor, S., *Gene therapy provides temporary relief in patient with immune disorder.* BMJ, 2012. **344**: p. e1651.

184. Kang, E.M. and H.L. Malech, *Gene therapy for chronic granulomatous disease.* Methods Enzymol, 2012. **507**: p. 125-54.

185. Barde, I., et al., *Lineage- and stage-restricted lentiviral vectors for the gene therapy of chronic granulomatous disease.* Gene Ther, 2011. **18**(11): p. 1087-1097.

186. Mukherjee, S., et al., *Generation of Functional Neutrophils from a Mouse Model of X-Linked Chronic Granulomatous Disorder Using Induced Pluripotent Stem Cells.* PLoS One, 2011. **6**(3): p. e17565.

187. Zou, J., et al., *Oxidase-deficient neutrophils from X-linked chronic granulomatous disease iPS cells: functional correction by zinc finger nucleaseâ€"mediated safe harbor targeting.* Blood, 2011. **117**(21): p. 5561-5572.

188. Finn, A., et al., *Prognosis of chronic granulomatous disease.* Archives of Disease in Childhood, 1990. **65**(9): p. 942-5.

189. Arendrup, M.C., et al., *Development of azole resistance in Aspergillus fumigatus during azole therapy associated with change in virulence.* PLos one, 2010. **5**(4): p. e10080.

190. Feld, J.J., et al., *Hepatic Involvement and Portal Hypertension Predict Mortality in Chronic Granulomatous Disease.* Gastroenterology, 2008. **134**(7): p. 1917-1926.

191. The WHOQOL Group, *The World Health Organization quality of life assessment (WHOQOL): Position paper from the World Health Organization.* Social Science & Medicine, 1995. **41**(10): p. 1403-1409.

192. World Health Organization. *Preamble to the Constitution of the World Health Organization as adopted by the International Health Conference, New York, 19-22 June, 1946; signed on 22 July 1946 by the representatives of 61 States (Official Records of the World Health Organization, no. 2, p. 100) and entered into force on 7 April 1948.* 1948 23.5.12]; Available from: http://www.who.int/about/definition/en/print.html.

193. Centers for Disease Control and Prevention, *Measuring Healthy Days.* 2000: Atlanta.

194. Slevin, M.L., et al., *Who should measure quality of life, the doctor or the patient?* Br J Cancer, 1988. **57**(1): p. 109-12.

195. Chen, H.Y., D.J. Baumgardner, and J.P. Rice, *Health-related quality of life among adults with multiple chronic conditions in the United States, Behavioral Risk Factor Surveillance System, 2007.* Prev Chronic Dis, 2011. **8**(1): p. A09.

196. Cohen, J.S. and B.B. Biesecker, *Quality of life in rare genetic conditions: A systematic review of the literature.* American Journal of Medical Genetics Part A, 2010. **152A**(5): p. 1136-1156.

197. McClish, D.K., et al., *Health related quality of life in sickle cell patients: the PiSCES project.* Health Qual Life Outcomes, 2005. **3**: p. 50.

198. Varni, J.W., C.A. Limbers, and T.M. Burwinkle, *Parent proxy-report of their children's health-related quality of life: an analysis of 13,878*

parents' reliability and validity across age subgroups using the PedsQL 4.0 Generic Core Scales. Health Qual Life Outcomes, 2007. **5**: p. 2.

199. Haverman, L., et al., *Predictors of health-related quality of life in children and adolescents with juvenile idiopathic arthritis: results from a Web-based survey.* Arthritis Care Res (Hoboken), 2012. **64**(5): p. 694-703.

200. Heath, J., et al., *Self-reported quality of life in children and young people with chronic kidney disease.* Pediatr Nephrol, 2011. **26**(5): p. 767-73.

201. Levi, R.B. and D. Drotar, *Health-related quality of life in childhood cancer: discrepancy in parent-child reports.* Int J Cancer Suppl, 1999. **12**: p. 58-64.

202. Sattoe, J.N., A. van Staa, and H.A. Moll, *The proxy problem anatomized: child-parent disagreement in health related quality of life reports of chronically ill adolescents.* Health Qual Life Outcomes, 2012. **10**: p. 10.

203. Goldbeck, L., et al., *Disease severity, mental health, and quality of life of children and adolescents with asthma.* Pediatr Pulmonol, 2007. **42**(1): p. 15-22.

204. Seid, M., et al., *Disease control and health-related quality of life in juvenile idiopathic arthritis.* Arthritis Rheum, 2009. **61**(3): p. 393-9.

205. Barrera, M., et al., *Quality of life amd behavioral adjustment after pediatric bone marrow transplantation.* Bone Marrow Transplantation, 2000. **26**: p. 427-35.

206. Felder-Puig, R., et al., *Health related quality of life of pediatric patients receiving allogeneic stem cell or bone marrow transplantation: results of a longitudinal, multi-centre study.* Bone Marrow Transplantation, 2006. **38**: p. 119-26.

207. Soresina, A., et al., *The quality of life of children and adolescents with X-linked Agammaglobulinaemia.* Journal of Clinical Immunology, 2009. **29**: p. 501-7.

208. Skucek, E., et al., *Social outcome in children treated by haematopoietic cell transplant for congenital immunodeficiency.* Bone Marrow Transplant, 2011. **46**(10): p. 1314-1320.

209. Gannoni, A.F. and R.H. Shute, *Parental and child perspectives on adaptation to childhood chronic illness: a qualitative study.* Clin Child Psychol Psychiatry, 2010. **15**(1): p. 39-53.

210. de Ridder, D., et al., *Psychological adjustment to chronic disease.* The Lancet, 2008. **372**(9634): p. 246-255.

211. Taylor, S.E. and L.G. Aspinwall, *Psychosocial aspects of chronic illness*, in *Psychological aspects of serious illness: chronic conditions, fatal diseases, and clinical care.*, P.T. Costa and G.R. Vandenbos, Editors. 1996, American Psychological Association: Washington DC. p. 7-60.

212. Whittemore, R., et al., *A conceptual model of childhood adaptation to type 1 diabetes.* Nurs Outlook, 2010. **58**(5): p. 242-51.

213. Lavigne, J.V. and J. Faier-Routman, *Psychological adjustment to pediatric physical disorders: a meta-analytic review.* J Pediatr Psychol, 1992. **17**(2): p. 133-57.

214. Cadman, D., et al., *Chronic Illness, Disability, and Mental and Social Well-Being: Findings of the Ontario Child Health Study.* Pediatrics, 1987. **79**(5): p. 805-813.

215. Gortmaker, S.L., et al., *Chronic Conditions, Socioeconomic Risks, and Behavioral Problems in Children and Adolescents.* Pediatrics, 1990. **85**(3): p. 267-276.

216.	Maes, M., *Depression is an inflammatory disease, but cell-mediated immune activation is the key component of depression.* Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2011. **35**(3): p. 664-675.

217.	Howren, M.B., D.M. Lamkin, and J. Suls, *Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis.* Psychosom Med, 2009. **71**(2): p. 171-86.

218.	Dowlati, Y., et al., *A Meta-Analysis of Cytokines in Major Depression.* Biological Psychiatry, 2010. **67**(5): p. 446-457.

219.	Montinaro, V., et al., *Emotional symptoms, quality of life and cytokine profile in hemodialysis patients.* Clin Nephrol, 2010. **73**(1): p. 36-43.

220.	Juth, V., J.M. Smyth, and A.M. Santuzzi, *How Do You Feel?* Journal of Health Psychology, 2008. **13**(7): p. 884-894.

221.	Sternberg, R.J. and K. John, *Competence Considered*. 1990: Yale University Press.

222.	Granleese, J. and S. Joseph, *Reliability of the Harter Self-Perception Profile for Children and predictors of global self-worth.* The Journal of Genetic Psychology, 1994. **155**(4): p. 487-92.

223.	Adams, J.A. and S.J. Weaver, *Self-esteem and perceived stress in young adolescents with chronic disease. Unexpected findings.* J Adolesc Health Care, 1986. **7**(3): p. 173-7.

224.	Gottfredson, L., *Mainstream Science on Intelligence: An editorial with 52 signatories, history and bibliography.* Intelligence, 1997. **24**: p. 13-23.

225.	Schmidt, F.L. and J.E. Hunter, *The validity and utility of selection methods in personnel psychology: practical and theoretical implications*

of 85 years of research findings. Psychological Bulletin, 1998. **124**: p. 262-74.

226. Whalley, L.J. and I.J. Deary, *Longitudinal cohort study of childhood IQ and survival up to age 76.* BMJ, 2001. **322**(7290): p. 819.

227. Pearce, M.S., et al., *Childhood IQ and deaths up to middle age: The Newcastle Thousand Families Study.* Public Health, 2006. **120**(11): p. 1020-6.

228. Amato, M.P., et al., *Cognitive and psychosocial features of childhood and juvenile MS.* Neurology, 2008. **70**(20): p. 1891-7.

229. Potter, A.I. and P.G. Nestor, *IQ subtypes in schizophrenia: distinct symptom and neuropsychological profiles.* J Nerv Ment Dis, 2010. **198**(8): p. 580-5.

230. Gipson, D.S., et al., *Memory and executive functions in pediatric chronic kidney disease.* Child Neuropsychol, 2006. **12**(6): p. 391-405.

231. Dancey, C.P., et al., *Words fail me: The verbal IQ deficit in inflammatory bowel disease and irritable bowel syndrome.* Inflammatory Bowel Diseases, 2009. **15**(6): p. 852-857.

232. Deary, I.J., et al., *Intelligence and persisting with medication for two years: Analysis in a randomised controlled trial.* Intelligence, 2009. **37**(6): p. 607-612.

233. Dimopoulos, N., et al., *Indices of low-grade chronic inflammation correlate with early cognitive deterioration in an elderly Greek population.* Neuroscience Letters, 2006. **398**(1-2): p. 118-123.

234. Trollor, J.N., et al., *Systemic inflammation is associated with MCI and its subtypes: the Sydney Memory and Aging Study.* Dement Geriatr Cogn Disord, 2010. **30**(6): p. 569-78.

235. Karlsson, H., et al., *Association between erythrocyte sedimentation rate and IQ in Swedish males aged 18-20.* Brain, Behavior, and Immunity, 2010. **24**(6): p. 868-873.

236. Wersching, H., et al., *Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function.* Neurology, 2010. **74**(13): p. 1022-9.

237. Krabbe, K.S., et al., *Low-dose endotoxemia and human neuropsychological functions.* Brain, Behavior, and Immunity, 2005. **19**(5): p. 453-460.

238. Vitkovic, L., J. Bockaert, and C. Jacque, *"Inflammatory" Cytokines.* Journal of Neurochemistry, 2000. **74**(2): p. 457-471.

239. Cibelli, M., et al., *Role of interleukin-1β in postoperative cognitive dysfunction.* Annals of Neurology, 2010. **68**(3): p. 360-368.

240. Hein, A.M., et al., *Sustained hippocampal IL-1[beta] overexpression impairs contextual and spatial memory in transgenic mice.* Brain, Behavior, and Immunity, 2010. **24**(2): p. 243-253.

241. Gerson, A.C., et al., *Neurocognitive outcomes in children with chronic kidney disease: Current findings and contemporary endeavors.* Ment Retard Dev Disabil Res Rev, 2006. **12**(3): p. 208-15.

242. Icard, P., et al., *Cognitive improvement in children with CKD after transplant.* Pediatr Transplant, 2010. **14**(7): p. 887-90.

243. Thompson, R.J., et al., *The role biochemical and psychosocial progress in the intellectual and academic functioning of children and adolescents with cystic fibrosis.* Journal of Clinical Psychology, 1992. **48**(1): p. 3-10.

244. Kishida, K.T., et al., *Synaptic plasticity deficits and mild memory impairments in mouse models of chronic granulomatous disease.* Mol Cell Biol, 2006. **26**(15): p. 5908-20.

245. Kishida, K.T., et al., *NADPH oxidase is required for NMDA receptor-dependent activation of ERK in hippocampal area CA1.* J Neurochem, 2005. **94**(2): p. 299-306.

246. Rogers, M.H., et al., *Cognitive and behavioral abnormalities in adenosine deaminase deficient severe combined immunodeficiency.* The Journal of Pediatrics, 2001. **139**(1): p. 44-50.

247. *A Dictionary of Public Health*, J.M. Last, Editor. 2007, Oxford University Press: Oxford.

248. Newton, J. and S. Garner, *Disease registers in England: A report commissioned by the Department of Health Policy Research Programme in support of the White Paper entitled Saving Lives: Our Healthier Nation*. 2002, University of Oxford: Oxford.

249. Black, N., *High quality clinical databases: breaking down barriers.* The Lancet, 1999. **353**: p. 1205-6.

250. Eurostat. *United Kingdom and Ireland live births 2000-2009*. Number of live births (tps00111) table 11.04.2010 [cited 2010 11th April]; Available from: http://epp.eurostat.ec.europa.eu/portal/page/portal/population/data/main_tables.

251. Fletcher, R.B. and J. Hattie, *Intelligence and Intelligence Testing*. 2011, Abingdon: Routledge.

252. Galton, F., *Inquiries into the human faculty and its development*. 2nd ed. 1907, London: J.M. Dent & Co.

253. Cattell, J.M., *Mental tests and measurements.* Mind, 1890. **15**(59): p. 373-81.

254. Mackintosh, N.J., *IQ and Human Intelligence*. 1998, Oxford: Oxford University Press.

255. Spearmen, C. *General Intelligence, "Objectively Determined and Measured".* . Classics in the History of Psychology 1904 [cited 2011 10.04.2011]; Available from: http://psychclassics.yorku.ca/Spearman/index.htm.

256. Kaufman, A.S. and E.O. Lichtenberger, *Assessing Adolescent and Adult Intelligence*. 2005, John Wiley & Sons: Hoboken.

257. Homack, S.R. and C.R. Reynolds, *Essentials of Assessment with Brief Inteliigence Tests*. 2007, John Wiley & Sons: Hoboken.

258. Zhu, J., *WASI Manual*. 1999, San Antonio: Pearson.

259. Youth in Mind. *SDQ: Normative SDQ Data from Britain*. [cited 2011 17.01]; Available from: http://www.sdqinfo.org/UKNorm.html.

260. Calam, R., L. Gregg, and R. Goodman, *Psychological adjustment and asthma in children and adolescents: the UK Nationwide Mental Health Survey.* Psychosom Med, 2005. **67**(1): p. 105-10.

261. Bruce, S., et al., *Mum knows best? Psychological status in an oncology sample.* Child Care Health Dev, 2005. **31**(6): p. 643-8.

262. Ding, T., et al., *Psychological functioning of children and adolescents with juvenile idiopathic arthritis is related to physical disability but not to disease status.* Rheumatology, 2008. **47**(5): p. 660-664.

263. Glazebrook, C., et al., *Detecting emotional and behavioural problems in paediatric clinics.* Child Care Health Dev, 2003. **29**(2): p. 141-9.

264. Harter, S., *Manual for the self-perception profile for children*. 1985: University of Denver.

265. Varni, J.W. *The PedsQL Measurement Model for the Pediatric Quality of Life Inventory*. 1998-2012 11/10/12]; Available from: www.pedsql.org.

266. Varni, J.W., M. Seid, and P.S. Kurtin, *PedsQL 4.0: reliability and validity of the Pediatric Quality of Life Inventory version 4.0 generic core scales in healthy and patient populations.* Med Care, 2001. **39**(8): p. 800-12.

267. Upton, P., et al., *Measurement properties of the UK-English version of the Pediatric Quality of Life Inventory 4.0 (PedsQL) generic core scales.* Health Qual Life Outcomes, 2005. **3**: p. 22.

268. Varni, J.W., C.A. Limbers, and T.M. Burwinkle, *How young can children reliably and validly self-report their health-related quality of life?: an analysis of 8,591 children across age subgroups with the PedsQL 4.0 Generic Core Scales.* Health Qual Life Outcomes, 2007. **5**: p. 1.

269. *WHO Anthro for personal computers: Software for assessing growth and development of the world's children.* 2011, WHO: Geneva.

270. *WHO AnthroPlus for personal computers: Software for assessing growth of the world's children and adolescents.* 2009, WHO: Geneva.

271. Stanojevic, S. *Growing Lungs*. Lung Function in Growth and Aging 2011 07.08.12]; Available from: www.lungfunction.org/growinglungs.html.

272. Stanojevic, S., et al., *Reference Ranges for Spirometry Across All Ages.* American Journal of Respiratory and Critical Care Medicine, 2008. **177**(3): p. 253-260.

273. Radcliffe, J., et al., *Adjustment in childhood brain tumor survival: child, mother, and teacher report.* J Pediatr Psychol, 1996. **21**(4): p. 529-39.

274. Adeyemi, A.O., et al., *Adherence to oral antidiabetic medications in the pediatric population with type 2 diabetes: a retrospective database analysis.* Clin Ther, 2012. **34**(3): p. 712-9.

275. Mackner, L.M. and W.V. Crandall, *Oral medication adherence in pediatric inflammatory bowel disease.* Inflamm Bowel Dis, 2005. **11**(11): p. 1006-12.

276. Dobbels, F., et al., *Growing pains: non-adherence with the immunosuppressive regimen in adolescent transplant recipients.* Pediatr Transplant, 2005. **9**(3): p. 381-90.

277. Ashbee, H.R., et al., *Histoplasmosis in Europe: report on an epidemiological survey from the European Confederation of Medical Mycology Working Group.* Med Mycol, 2008. **46**(1): p. 57-65.

278. Morgan, C., et al., *Burden on UK secondary care of rotavirus disease and seasonal infections in children.* Curr Med Res Opin, 2010. **26**(10): p. 2449-55.

279. Agency, H.P. *H1N1 (2009) Pandemic archive*. 2012 11/10/12]; Available from: http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/PandemicInfluenza/H1N1PandemicArchive/.

280. Agency, H.P. *Health Protection Report Vol 6 No 35*. 2012; Available from: http://www.hpa.org.uk/hpr/archives/2012/news3512.htm#prtsss.

281. Filipovich, A.H., et al., *Impact of donor type on outcome of bone marrow transplantation for Wiskott-Aldrich syndrome: collaborative study of the International Bone Marrow Transplant Registry and the National Marrow Donor Program.* Blood, 2001. **97**(6): p. 1598-1603.

282. Moratto, D., et al., *Long-term outcome and lineage-specific chimerism in 194 patients with Wiskott-Aldrich syndrome treated by hematopoietic cell*

transplantation in the period 1980-2009: an international collaborative study. Blood, 2011. **118**(6): p. 1675-84.

283. Gennery, A.R., et al., *Transplantation of haematopoietic stem cells and longterm survival for primary immunodeficiencies in Europe: entering a new century, do we do better?* Journal of Allergy and Clinical Immunology, 2010. **126**(3): p. 602-10.

284. Gathmann, B., et al., *The European internet-based patient and research database for primary immunodeficiencies: update 2011.* Clinical & Experimental Immunology, 2012. **167**(3): p. 479-491.

285. Clarke, S.A., et al., *Clinical outcomes and health-related quality of life (HRQOL) following haemopoietic stem cell transplantation (HSCT) for paediatric leukaemia.* Child Care Health Dev, 2011. **37**(4): p. 571-80.

Appendix 1: Details of data collected

Patient details

Name

Address

Date of birth

Place of birth(if known)

Gender

Twin

Diagnostic tests performed to establish/confirm diagnosis

NBT

Flow cytometric assay

Other

None of the above

Date CGD diagnosis established:

Genetic type of CGD

X-linked

Autosomal recessive   p22/p47/p67 defect

Not known

Method used to establish genetic subtype

Molecular genetic analysis of mutation

Family history

Mothers/fathers/brothers/sisters/maternal uncle/other affected family members.

Tested: normal/affected/not tested

        If affected   carrier/CGD/alive/CGD dead

Also space for additional information/comments on pedigree

Respiratory Complications

Pneumonia	Date/organism/treatment/duration of admission
Empyema	Date/organism/treatment/duration of admission
Lung abscess	Date/organism/treatment/duration of admission
Lung function tests cap	Date/Pefr/fev1/fvc/tlc/rv/ratio of fec/fec/saturation/diffus
High resolution CT scan	Date/Report (free field) key words,
Bronchiectasis	Date of diagnosis/lobe/Type

Gastrointestinal complications

Colitis	Date/symptoms/treatment/complications
Oesopahgeal stricture	Date/symptoms/treatment/complications
Pyloric outlet obstruction	Date/symptoms/treatment/complications
Appendicitis	Date/symptoms/treatment/complications
Pancreatitis	Date/symptoms/treatment/complications
Investigations abnormality	Date/findings-normal/abnormal and location of

To include:

Ultrasound, Barium swallow, Barium meal follow through, Barium enema, White cell scan

Endoscopy/colonoscopy	Date/normal/abnormal/granulomas

Assessment of nutrition

Birth weight

Weight

Height

BMI - (Body mass index)

Original Initial Diagnosis	Oral facial granulomatosis/Crohn's/other

Liver complications

Hepatitis   Date/investigations/treatment/complications

Liver abscess   Date/investigations/treatment/complications

Surgery   Date/operation/complication/duration of admission

Other major illnesses related to CGD –

Documenting the date/location/organism/treatment/duration of hospital admission for:

Suppurative adenitis

Osteomyelitis

Septic arthritis

Sepsis

Brain abscess

Meningitis

Subcutaneous abscess

Urinary outflow obstruction/glomerulonephritis

Renal infection/abscess

Pericarditis

Chorioretinal disease

Other

Other diseases

Cancer   date of diagnosis/type

Arthritis

Discoid lupus erythematous

Systemic lupus erythematous

Other autoimmune disease   Type/Autoantibodies present

Prophylaxis

Document current/previous prophylactic agents   antibiotic/antifungal

Other specific treatment   steroids/granulocyte infusions/gamma interferon/TPN/other drugs

Bone marrow transplant/gene therapy

Date/conditioning/complications/duration of admission

Post transplant medication

Current patient status

Alive/dead/unknown

If dead   date of death/age at death/cause of death

Date of last contact with patient

Other specialists involved

Other regional centres involved