The physical health and quality of life of patients with X-linked agammaglobulinaemia in England and Wales

Volume 1 of 1

Benjamin Martin James Shillitoe

MBBS, MRCPCH

150627672

Doctorate of Philosophy (PhD)

Institute of Cellular Medicine

Faculty of Medical Sciences

Newcastle University

February 2020

Abstract

Background

Patients with X-linked agammaglobulinaemia (XLA) have absent peripheral circulating B-lymphocytes and agammaglobulinaemia caused by defects in BTK. Treatment consists of life-long immunoglobulin replacement therapy. This only contains the IgG isotype containing little or no IgA or IgM. Patients may still, therefore, experience recurrent infections and complications. Novel therapies are potentially available, most notably gene therapy and newborn screening.

I aimed to examine the clinical health outcomes and quality of life for patients with XLA in England and Wales to evaluate current practices and the potential role for novel therapies.

Methods

This is a retrospective, longitudinal observational study of patients with a definite diagnosis of XLA (BTK mutation or absent BTK expression), in England and Wales.

Retrospective clinical data were collected from patients' records, including conversion of lung function results to Z-scores. Patients and/or their families were invited to complete questionnaires on their health-related quality of life (HRQoL) and psychological health. Results were compared against UK norms and UK patients with cystic fibrosis.

Results

Fifty-four patients were enrolled in the study (21 children, 33 adults). Median age at diagnosis was 2.59 years with no statistically significant improvement seen since 1990.

Twenty-two patients (44%) had evidence of bronchiectasis on high-resolution computerised tomography. Patients with bronchiectasis were diagnosed with XLA significantly later than patients without bronchiectasis. Neither infection incidence nor IgG trough levels were associated with an increased risk of bronchiectasis.

In the absence of bronchiectasis, XLA patients had normal HRQoL results. HRQoL was strongly correlated with respiratory symptoms and lung function.

Conclusions

Recurrent respiratory tract infections and bronchiectasis remain a major burden for this cohort despite modern therapy. In the absence of bronchiectasis, patients have a normal HRQoL.

Curative therapy, such as gene therapy or bone marrow transplantation, may provide the only option for improving outcomes in XLA.

Dedication

I would like to dedicate this work to my wife, Hannah, my children, Martha and Edward and my parents to whom I owe everything.

Acknowledgements

This study was funded by the Bubble Foundation and the Jeffrey Modell Foundation with support from UKPIN.

I would like to thank all the patients and families who took part in this study.

I would like to extend my thanks to all the medical and research staff from participating centres who helped with recruitment of local patients.

Lastly, I would like to extend my thanks and gratitude to my supervisors, Professor Andrew Gennery and Professor Mark Pearce for their continuing guidance and patience throughout this PhD and my ongoing career in Paediatric Immunology.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ADA	Adenosine deaminase
ANKRD54	Ankyrin repeat domain-containing protein 54
APCs	Antigen presenting cells
ARA	Autosomal recessive agammaglobulinaemia
BAD	BCL-2 antagonist of cell death
BCAP	B cell adaptor for PI3K
BCR	B cell receptor
BLNK	B-cell linker
BMDC	BTK-deficient marrow-derived dendritic cells
BMI	Body mass index
BMX	Bone marrow tyrosine kinase on chromosome X
BP	Bodily pain
BSI	Bronchiectasis severity index
BTK	Bruton's Tyrosine Kinase
CAPS	Cryopyrin-associated periodic syndrome
CaM	Calmodulin
CARD11	Caspase recruitment domain-containing protein 11
CD79A	B-cell antigen receptor complex-associated protein alpha chain
CD79B	B-cell antigen receptor complex-associated protein beta chain
CEMA	Chronic meningoencephalitis in agammaglobulinaemia
CF	Cystic fibrosis
CGD	Chronic granulomatous disease
CIN85	CBL-interacting protein of 85 kDa
COPD	Chronic obstructive pulmonary disease
CR	Complement receptor
CRAC	Calcium release-activated calcium channels
CVID	Common variable immunodeficiency
DAG	Diacylglycerol
DC	Dendritic cell
dIgA	Dimeric IgA
ER	Endoplasmic reticulum

ESID	European Society for Immunodeficiencies
FBC	Full blood count
FcγR	Fc-gamma receptor
FEV	Forced expiratory volume
FOXO	Forkhead box transcription factors
FVC	Forced vital capacity
GH	General health
GI	Gastrointestinal
GLI	Global Lung Initiative
GSK	Glycogen synthase kinase
HADS	Hospital anxiety and depression scale
HLA	Human leukocyte antigen
HRA	Health research authority
HRCT	High resolution computerised topography
HRQoL	Health related quality of life
HSC	Haematopoietic stem cell
IBTK	Inhibitor of BTK
IG	Immunoglobulin
IFN	Interferon
IGHM	Immunoglobulin heavy constant Mu
IGLL1	Immunoglobulin lambda like polypeptide
IGRT	Immunoglobulin replacement therapy
IL	Interleukin
IP3R	IP3 Receptor
IPR(R)	Inositol 1, 4, 5 – triphosphate
IRAK1	Interleukin-1 receptor-associated kinase-1
ITAM	Immunoreceptor tyrosine-based activation motifs
ITK	Inducible T cell kinase
IV	Intravenous
LLN	lower limit of normal
LPS	Lipopolysaccharide
LRRC8A	Leucine-rich repeat containing 8 family member A
MALT1	Mucosa-associated lymphoid tissue lymphoma translocation protein 1
MAPK	Mitogen-activated protein kinase
MCS	Mental component score

MH	Mental health
MRC	Medical Research Council
Myd88	Myeloid differentiation protein 88
NFAT	Nuclear factor of activated T-cell
NF-κB	Nuclear factor-κB
NO	Nitric oxide
PAD	Primary antibody deficiency
PAGID	Pan-American Group for Immunodeficiency
PBMC	Peripheral blood mononuclear cells
PCS	Physical component score
PDK1	3-phosphoinositide-dependent protein kinase 1
PF	Physical functioning
PFT	Pulmonary function testing
PI3K	Phosphoinositide 3-kinase
PID	Primary immunodeficiency
pIgM	Polymeric IgM
pIgR	Polymeric immunoglobulin receptor
PIK3R1	Phosphoinositide-3-kinase regulatory subunit
PIN1	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
PIP3	Phosphatidylinositol 3,4,5-triphosphate
PIP5K	Phosphatidylinositol-4-phosphate-5-kinase
PKC	protein kinase C
PLCγ2	phospholipase Cγ2
PR	Physical role
RAG	Recombination activating genes
RASGRP3	RAS guanyl-releasing protein 3
RE	Emotional role
REC	Research Ethics Committee
RLK	Resting Lymphocyte kinase
ROS	Reactive oxygen species
RSES	Rosenberg Self-Esteem Scale
SC	Subcutaneous
SCID	Severe combined immunodeficiency
SDQ	Strength and difficulties questionnaire
SF	Social functioning

SF36v2	Short form 36 version 2
SGRQ	St George’s respiratory questionnaire
SH	SRC homology
sIgA	Secretory IgA
sIgM	Secretory IgM
SLP65	SH2 domain-containing leukocyte protein of 65 kDa
SYK	Spleen tyrosine kinase
TCF3	Transcription factor 3
TEC	Tyrosine kinase expressed in hepatocellular carcinoma
TFK	Tec family of kinases
TH	TEC homology
TLR	Toll-like receptor
TNF	Tumour necrosis factor
UKPID	United Kingdom Primary Immunodeficiency
UKPIN	United Kingdom primary immunodeficiency network
VT	Vitality
WASP	Wiskott-Aldrich syndrome protein
XLA	X-linked agammaglobulinaemia

Chapter 1 Introduction

1.1 Definition

XLA is an X-linked inherited primary immunodeficiency caused by defects in the BTK gene (1,2). These defects result in the dysfunctional or absent expression of Bruton's Tyrosine Kinase (BTK) (1,2). This results in a catastrophic failure of B-lymphocyte development in the bone marrow, causing a near-complete or complete absence of circulating B-lymphocytes and a resulting agammaglobulinaemia (3). The Pan-American Group for Immunodeficiency (PAGID) and European Society for Immunodeficiencies (ESID) diagnostic criteria for XLA are (4):

1) Definitive

   A male patient with less than 2% CD19+ B cells and at least one of the following:

   a) Mutation in BTK
   b) Absent BTK mRNA on northern blot analysis of neutrophils or monocytes
   c) Absent BTK protein in monocytes or platelets
   d) Maternal cousins, uncles or nephews with less than 2% CD19+ B cells

2) Probable

   A male patient with less than 2% CD19+ B cells in whom all of the following are positive:

   a) Onset of recurrent bacterial infections in the first five years of life
   b) Serum IgG, IgM and IgA more than 2SD below normal for age
   c) Absent isohemagglutinins and/or poor response to vaccines
   d) Other causes of hypogammaglobulinaemia have been excluded

3) Possible

   A male patient with less than 2% CD19+ B cells in whom other causes of hypogammaglobulinaemia have been excluded and at least one of the following is positive:

   a) Onset of recurrent bacterial infections in the first five years of life
   b) Serum IgG, IgM and IgA more than 2 SD below normal for age
   c) Absent isohemagglutinins

Congenital agammaglobulinaemia is estimated to affect 2-3 patients per million population in the United Kingdom (5). Eighty-five per cent of patients have XLA with mutations in BTK. The remaining 15% are autosomal recessive (ARA).

1.2 Discovery

In the 1930s and 1940s, it became possible to measure serum immunoglobulin levels (6,7). Shortly after this, in 1952, Colonel Ogden Bruton identified a young boy suffering from repeated severe and low-grade infections (3). He presented with a history of osteomyelitis, gastrointestinal (GI) infections, parotitis, otitis media, pneumonia and sepsis (3). At eight years old, suffering his 3rd episode of parotitis, Colonel Bruton found this young boy to be agammaglobulinaemic and went onto to treat him successfully with subcutaneous (SC) replacement immunoglobulin therapy (3,8). This was the first reported case of XLA and is widely seen as the birth of clinical immunology.

Shortly thereafter, in the 1970s, it was discovered that patients with XLA had a significant reduction in the number of circulating CD19+ B cells in the peripheral circulation (9–12). However, B cell precursors were identified in the bone marrow of these patients (13). Together, these findings suggested haematopoietic stem cells in patients with XLA could enter the B cell lineage, but that there was a block somewhere along the normal B cell differentiation pathway.

1.2.1 BTK gene

Kwan et al., reported in 1986 that the gene responsible for XLA was located on the long arm of the X chromosome at Xq21.3 to Xq22 (14). In 1993 two groups simultaneously discovered the gene responsible (BTK) and this, along with its subsequent kinase, is named after Colonel Bruton, BTK (Bruton's Tyrosine Kinase) (1,2). The BTK gene is 19 exons spanning 37 kb (15–18). X-linked females can, albeit very rarely, be affected due to skewed lyonisation (19).

There are over 800 different BTK mutations implicated in being responsible for XLA, although no single defect accounts for more than 3% of patients (20,21).

Some patients, despite a complete absence of BTK, have unusual phenotypes with higher than expected immunoglobulin and B-lymphocyte levels or reporting fewer, milder infections (22–24). However, attempts to establish a genetic/phenotype correlation have been difficult. There are no agreed definitions of what is mild, moderate or severe disease. Furthermore, many other factors can determine clinical outcome, e.g. age at diagnosis, infection rates, IgG trough levels, which need examining in large cohorts to try to establish any genetic/phenotype correlation. Such a correlation would be beneficial to clinicians to aid prognosis for patients and to help identify patients in whom radical treatments may be beneficial.

1.2.2 Autosomal recessive agammaglobulinaemia

As well as XLA, there are autosomal recessive (ARA) congenital agammaglobulinaemias. Approximately 15% of all congenital agammaglobulinaemias are autosomal recessive with known genetic defects (Table 1-1) (25).

Table 1-1 Gene mutations associated with ARA (26)

Gene Defect	Cytogenetic Location
IGHM (Immunoglobulin Heavy Constant Mu)	14q32.33
CD79A (B-Cell Antigen Receptor Complex-Associated Protein Alpha Chain)	19q13.2
CD79B (B-Cell Antigen Receptor Complex-Associated Protein Beta Chain)	17q23
IGLL1 (Immunoglobulin Lambda Like Polypeptide)	22q11,23
BLNK (B-Cell Linker)	10q23.2-q23.33
LRRC8A (Leucine-Rich Repeat Containing 8 Family member A)	9q34.11
PIK3R1 (Phosphoinositide-3-Kinase Regulatory Subunit)	5q13.1
TCF3 (Transcription factor 3)	19p13.3

The incidence of ARA is extremely low affecting approximately 1:2,000,000 births (Orphanet, ORPHA33110). 30% of ARA cases are caused by mutations in the μ heavy constant region gene, IGHM (i.e. 5% of all congenital agammaglobulinaemia) (27). Current known B cell defects are summarised in Table 1-2.

Table 1-2 Known B cell defects adapted from Hendriks et al., (28)

Disease Category	Defects		Unknown (%) (29)
	Genes involved	Proportion (%)	
p	BTK	85%	5-8%
	IGHM	3-5%	
	IGL14.1	<1%	
	CD79A	<1%	
	CD79B	<1%	
	BLNK	<1%	
Hyper IgM Syndromes	CD40L	50%	25-35%
	CD40	<1%	
	AID	15%	
	UNG	<1%	
	NEMO	5%	
	MSH6	<1%	
	INO80	<1%	
Other BCR signalling defects	CD19	Unknown	
	CD20	Unknown	
	CD21	Unknown	

1.3 B-Lymphocytes

B-lymphocytes are a vital part of the immune system. They are defined as "a population of cells that express clonally diverse cell surface immunoglobulin receptors recognising specific antigen epitopes" (30). They can be traced back more than 500 million years in the evolution of the adaptive immune system in jawed vertebrates (31). Rather than the B-lymphocytes themselves being discovered first, it was, in fact, the discovery of immunoglobulin that first prompted ideas of specific immune cells producing antibodies within the immune system (32,33). The final discovery and classification of B-lymphocytes occurred towards the end of the 1960s. By the beginning of the 1970s, using animal models and examining patients with PID, the link between these marrow-derived cells and antibody production was confirmed (34–37).

The development of B-lymphocytes begins in primary lymphoid tissue with further maturation occurring in a range of secondary lymphoid tissue. One of the primary functions of B-lymphocytes is the production of immunoglobulins by a complex process described in

further detail in section 1.5. This process first produces the isotypes IgM and then IgD. Later on in development, through antigen stimulation, variable domains of the immunoglobulin may be associated with other isotypes: IgA, IgG and IgE.

1.3.1 IgM

IgM is expressed in the early stages of B-lymphocyte development, with little somatic mutation in response to antigen stimulation. As such, this allows IgM to respond to a broader variety of antigens and is used in response to acute pathogen exposure as a first line of defence in the human immune system (38). Similar to IgG, IgM can activate complement through the binding of C1q (39). IgM is present in humans as polymeric structures (pIgM) (40). Like IgA, IgM is transported to the mucosal surfaces (e.g. respiratory tract and GI tract), via the polymeric immunoglobulin receptor (pIgR) (41). Epithelial cells express pIgR, which can bind to pIgM (42,43). The pIgM is then cleaved, leaving behind secretory IgM (sIgM) (42,43).

As will be discussed further in section 1.10.3, current immunoglobulin replacement therapy (IGRT) contains little or no IgM.

The half-life for IgM is short, at approximately 5-6 days (44,45).

IgM accounts for approximately 10% of the circulating serum immunoglobulin in humans (38).

1.3.2 IgD

Levels of circulating IgD in human serum are very low, and the function of both the free form and the membrane-bound form is poorly understood. Class-switched IgD secreting B-lymphocytes are present in the upper respiratory tract and are highly sensitive to respiratory pathogens (46). In addition, IgD class-switched B-lymphocytes are involved in the activation of innate immune cells, most notably basophils (46).

1.3.3 IgG

IgG is the principal circulating antibody in humans, with the longest half-life of all the isotypes (38). There are four different subclasses IgG1, IgG2, IgG3 and IgG4. IgG plays a significant role in various immune pathways, including activation of the complement pathway, secondary antibody response, and direct neutralisation of toxins and viruses (38).

Like IgM, IgG can activate complement through C1q binding (39). This ability varies amongst the IgG subclasses due to differences in their C1q binding (47,48). IgG3 has the highest ability, followed by IgG1 and IgG2 (47,48). IgG4 is unable to bind to C1q (47,48).

The half-life of IgG is the longest of the immunoglobulins, with approximately 3-4 weeks for IgG1, IgG2 and IgG4 and two weeks for IgG3 (44,49).

IgG accounts for approximately 75% of the circulating immunoglobulins in humans (38).

1.3.4 IgA

There are two classes of IgA; IgA1 and IgA2 (50). The predominant circulating form is IgA1. IgA1 and IgA2 are both found at mucosal surfaces (50). Circulating levels of IgA are much lower than IgG levels (51). However, this is reversed at mucosal surfaces and in secretions (51). Here, IgA plays a critical role in protecting these surfaces against pathogens by either direct neutralisation or by preventing the antigen binding to the mucosal surface (52,53). IgA exists in two forms, with circulating IgA being predominately monomeric and mucosal IgA (secretory IgA, sIgA) being dimeric (dIgA) (54). Like IgM, as discussed above, IgA is transported to the mucosal surfaces where dIgA can bind to the pIgR on the epithelial surfaces (41). Once again, similar to pIgM, the dIgA is cleaved, leaving behind the sIgA (42,43). It should be noted, as will be discussed further in section 1.10.3, that current immunoglobulin replacement therapy contains very little (if any) monomeric serum IgA and no sIgA.

Like IgM, the half-life of IgA is short, at around 5-6 days (44,45).

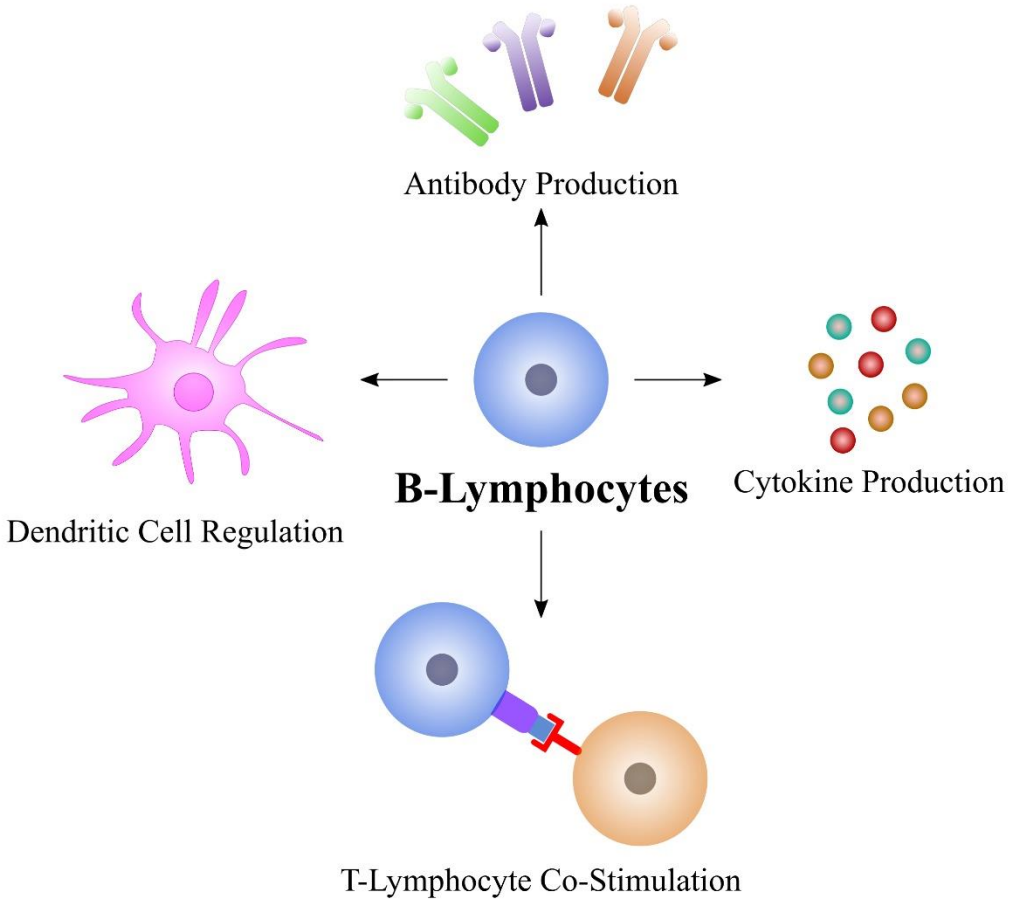
IgA accounts for approximately 15% of human circulating immunoglobulin levels (38).

1.3.5 IgE

Although found in extremely low levels, the effects of IgE are well known for its role in its reaction to allergens triggering mast cells and basophils to release histamine. IgE has an additional role in protection against parasitic worm infection (55).

1.3.6 Other functions of the B-lymphocyte

Figure 1-1 B-lymphocyte function



Aside from immunoglobulin production, B-lymphocytes have other integral roles within the human immune system (Figure 1-1). B-lymphocytes act as antigen-presenting cells (APCs), activating T-lymphocyte immune responses (56). They are also involved in the regulation of T-lymphocytes and dendritic cells through an array of immunomodulatory cytokines (IFN-γ. IL-6, IL-10) (30). It is therefore likely that an underlying B alymphocytosis would have subsequent detrimental effects on T lymphocyte-mediated immunity. Meyers et al., have added to these concerns by demonstrating a reduced frequency of regulatory T-lymphocytes in patients with XLA (57). B-lymphocytes also have an antibody-independent contribution to T-lymphocyte response to fungal pathogens (58).

As a consequence of agammaglobulinaemia, the subsequent decreased antibody/antigen interaction can have further effects on the immune system to those described above, most

notably impaired chemotaxis, decreased complement activation and reduction in the phagocytosis on pathogenic organisms (59).

It is now well understood that thymic B cells play a vital role in negative T cell selection and therefore, in their absence, there is an increased risk of autoimmune disease in congenital agammaglobulinaemia (60).

1.4 Bruton's Tyrosine Kinase

BTK is a 659 amino acid cytoplasmic tyrosine kinase with a molecular weight of 77 kDa. It is expressed at all stages of B-lymphocyte development except in plasma cells (1,2,61,62). It is also expressed in myeloid cells but not T-lymphocytes (63). It is a member of the Tec family of kinases (TFKs) which is the second largest family of mammalian cytoplasmic tyrosine kinases (Table 1-3). This family of non-receptor tyrosine kinases is strongly conserved and contributes to signal transduction pathways involving growth or differentiation factors (64,65).

Table 1-3 The Tec family of kinases

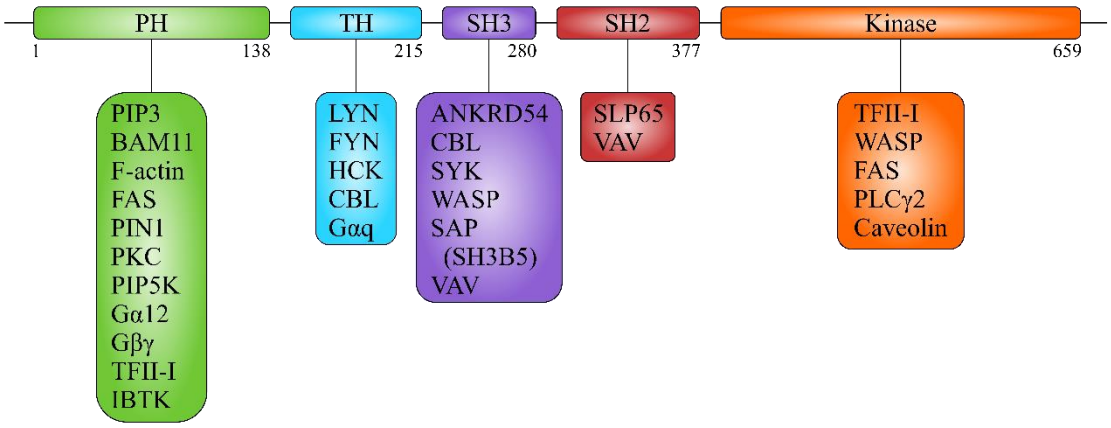
Kinase	Predominant expression
Bruton's Tyrosine Kinase (BTK)	Haematopoietic cells
Tyrosine kinase expressed in hepatocellular carcinoma (TEC)	Haematopoietic cells
Inducible T cell kinase (ITK)	Haematopoietic cells
Resting Lymphocyte kinase (RLK, also known as TXK)	Haematopoietic cells
Bone marrow tyrosine kinase on chromosome X (BMX)	Endothelial cells

These kinases all consist of a similar structure to the SRC family of kinases, i.e. with each kinase containing a C-terminal kinase, an SRC homology (SH) 2 and SH3 domain. However, they also contain a proline-rich region upstream of the SH3 domain, apart from BMX. This proline-rich region plays a vital role in the autoregulatory intramolecular interactions which occur in the SH3 domains (66). One other key difference between TFKs and SRCs is that they do not contain an N-terminal myristoylation signal or COOH-terminal negative regulatory tyrosine. They instead contain an N-terminal pleckstrin homology (PH domain) except for RLK (28). It has been demonstrated that more than one member of the TFK family

is expressed within the same cell suggesting an element of possible redundancy, potentially explaining the milder phenotype seen in BTK knockout mice where TEC can partially replace the lost BTK (67).

BTK consists of five domains, a PH domain, SRC homology 2 (SH2) domain, SH3 domain, TEC homology domain (TH) and a kinase domain (Figure 1-2). As a cytoplasmic kinase, BTK is temporarily transported to the plasma membrane upon binding of the PH domain to phosphatidylinositol 3,4,5-triphosphate (PIP_3). PIP_3 is generated by phosphoinositide 3-kinase (PI3K) activity (65). The TH domain contains a highly conserved zinc finger motif which is vital, mediating the binding and co-ordination of BTK to Zn^{2+} (65,68,69). Reliable activity and stability of BTK are entirely reliant on Zn^{2+}. Mutations in this region understandably result in an extremely unstable protein (70,71). The SH2 domain is involved in protein to protein interactions that bind to phosphorylated tyrosine (65). The exact role of the SH3 domain in BTK remains unknown, but it has been demonstrated to have autoregulatory intramolecular interactions with the TH domain in ITK (66). Y223 and Y551 are key tyrosine phosphorylation sites located in the SH3 and kinases domains (72) (Figure 1-2). Y551 is first phosphorylated by SYK or LYN during BCR signalling and promotes the catalytic activity of BTK and subsequent Y223 autophosphorylation. BTK catalytic activity activates three key signalling pathways; phospholipase C, phosphatidylinositol-3-kinase/Akt and NF-κB.

Figure 1-2 The structure of Bruton's Tyrosine Kinase and some of its interactions, adapted from Mohamed et al., (21) *ankyrin repeat domain-containing protein 54 (ANKRD54), filamentous acting (F-actin), an inhibitor of Bruton's tyrosine kinase (IBTK), phosphatidylinositol-4-phosphate-5-kinase (PIP5K), Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1), protein kinase C (PKC), phospholipase Cγ2 (PLCγ2,), SRC homology (SH), SH2 domain-containing leukocyte protein of 65 kDa (SLP65), spleen tyrosine kinase (SYK), TEC homology (TH), Wiskott-Aldrich syndrome protein (WASP)*



BTK is predominately expressed in B-lymphocytes (but not plasma cells). However, it is also expressed in all haematopoietic lineages apart from T-lymphocytes (21,62). Aside from B-lymphocytes, BTK is an expressed in numerous myeloid cells, most notably neutrophils, natural killer cells and monocytes (73–75). There is also some murine evidence for impaired osteoclast development in BTK-deficient mice (76,77). However, the clinical significance of absent BTK expression in cells other than B-lymphocytes has yet to be firmly determined.

Following downstream activation of the pre-B cell receptor (BCR), BTK plays a critical role in the mediation of proliferation and maturation of B-lymphocyte at the pre-B lymphocyte stage (20,78). This is done mainly via the promotion of calcium influx and will be discussed further in the sections below (20,78).

BTK is most recognised for its role in haematology where malignant B-lymphocytes depend heavily on BTK activity for survival. As such, BTK is a crucial target for the development of small molecular inhibitors, e.g. Ibrutinib (79). These inhibitors only target the kinase domain.

1.5 B-Lymphocyte Development

B-lymphocytes develop from haematopoietic stem cells (HSCs) through rearrangement of the immunoglobulin heavy and light chain gene segments. After recombination of the IgμH V, D and J genes, the IgμH protein is expressed on the cell surface along with the surrogate light chain (SLC) protein, as the pre-BCR (80,81). The SLC is substituting for the Ig L chains not

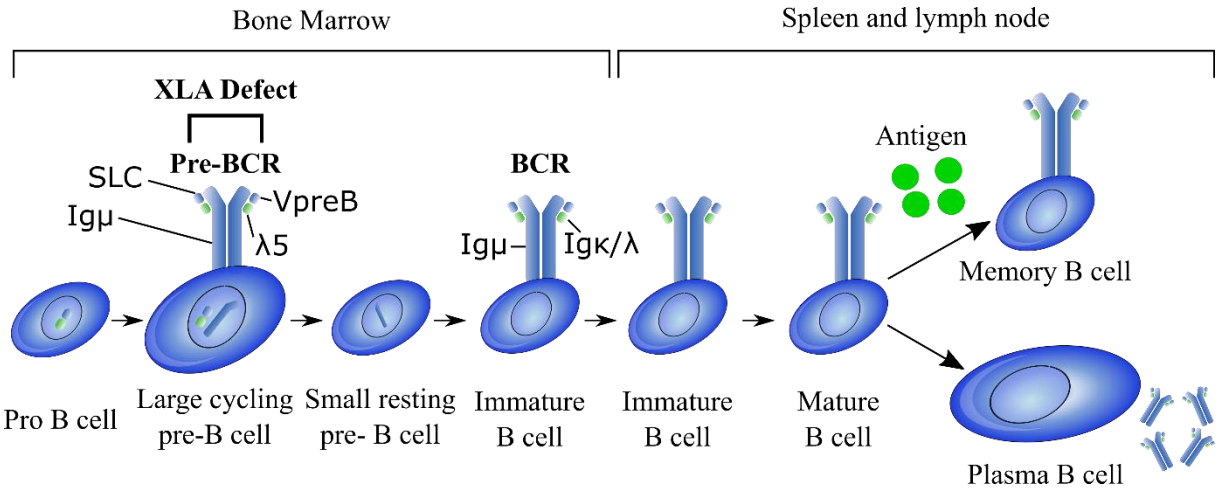
yet arranged. It is a heterodimer consisting of two germline-encoded invariant proteins, λ5 (similar to the constant region of the conventional λ) and VpreB (80,81). The pre-BCR signalling marks a vital checkpoint to test the functionality of the IgμH protein (28,82). The mechanisms initiating this pre-BCR signalling are not yet fully understood. The primary functions of this pre-BCR signalling are to inhibit further IGμH V(D)J recombination, known as allelic exclusion, clonal expansion and the triggering for B cell differentiation (82).

The rearrangement of the immunoglobulin loci is a vital part of functional B-lymphocyte development. This rearrangement process involves a purposively error-prone combination and rearrangement of the V, D and J gene segment in the H chain locus and the V and J segment in the L chain loci (83). This process ultimately results in an incredibly diverse functional repertoire of VDJ_H and V_J rearrangements encoding the B-cell receptor (BCR). The recombination activating genes 1 and 2 (RAG1, RAG2) play a vital role in instigating the initial DNA cleavage (84,85).

The pre-BCR plays a pivotal role in the maturation from the pro-B-cell to the large pre-B-cell stage (78). Pre-BCR signalling leads to a proliferation of pre-B cells and downregulation of SLC expression (82). This stage is vital to allow pre-B cells to transition from large cycling cells into small resting pre-B cells in which IGL chain recombination occurs (82). These pro–B cells are the earliest group committed to the B-cell lineage. B-lymphocyte development and survival in the periphery are, therefore, wholly dependent on BCR expression (86).

The exact arrest in XLA patients is at the transition from pro B ($c\mu^{-}SLC^{bright}$) to large pre-B I cells ($c\mu^{low}SLC^{bright}$) (87) (Figure 1-3). XLA patients have large numbers of pro-B cells resulting in an increased ratio of pro to pre-B cells (87–90). In healthy individuals, the pre-B I cells population contains large and small pre-B cells. In XLA patients, however, the pre-B I cells are predominately small, suggesting that BTK is essential for the proliferation and survival of $c\mu^{+}$ pre-B cells (28).

Figure 1-3 B-lymphocyte development, adapted from Hendriks et al., (28)



Initial studies, prior to the discovery of BTK, had identified that the underlying gene defect in XLA caused a fatal defect in the proliferation and survival of B-lymphocyte precursors (91,92). It was then further shown the earliest B-lymphocytes from carriers; CD34+. CD19+ pro-B-lymphocytes demonstrate normal, random X-chromosome inactivation (93). In addition, bone marrow from XLA patients demonstrates normal levels of CD34+, CD19+, sIg- pro-B-lymphocytes, but a significant decline in the numbers of cells expected at the next stage of differentiation (CD34-, CD19+, sIg-) (88). These findings suggested that BTK deficient haematopoietic stem cells, while being able to enter the initial B-lymphocyte lineage, are unable to proceed through normal differentiation (93).

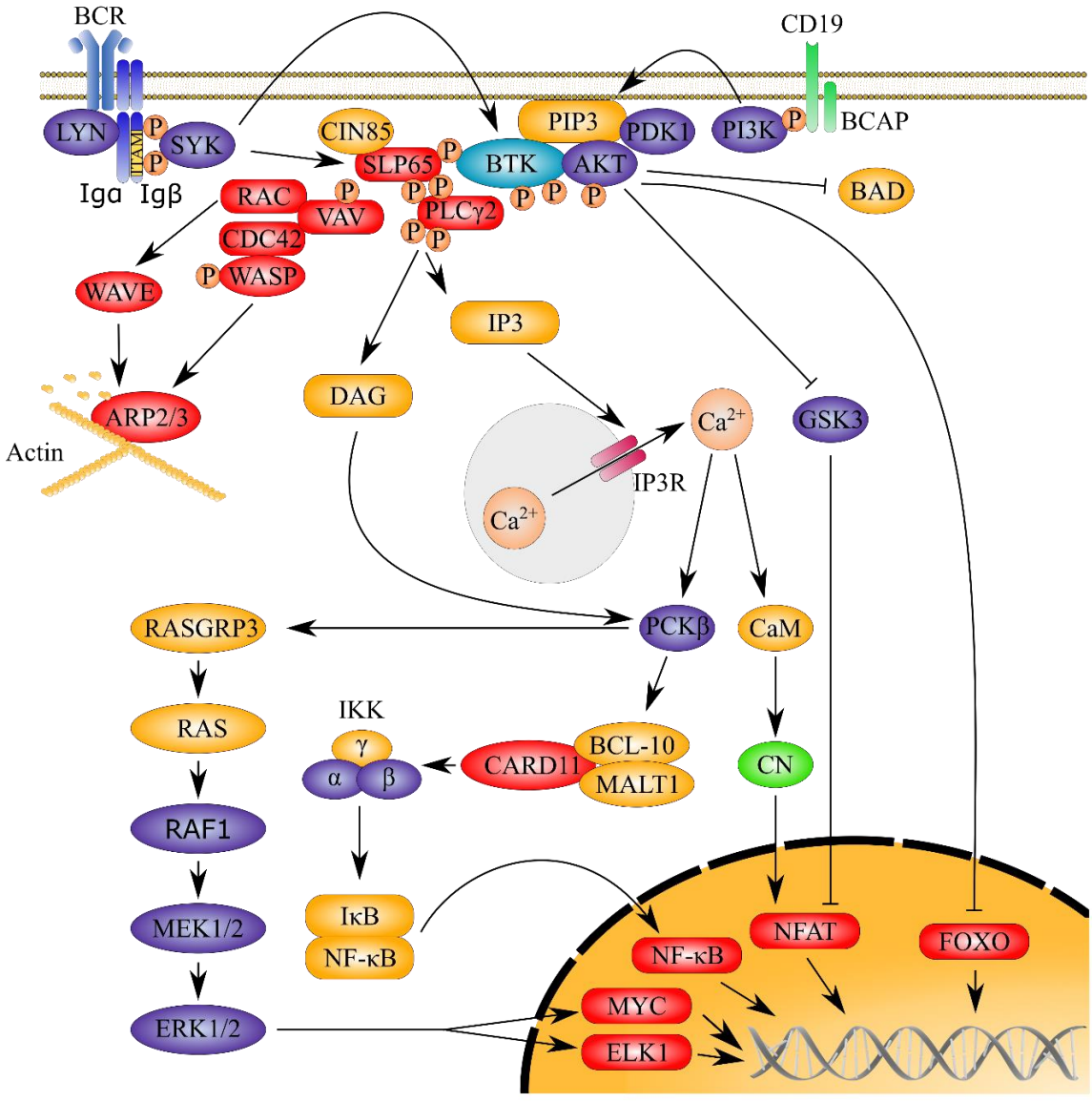
1.5.1 Role of BTK in pre-B cell and B cell Receptor Signalling

The presence of BTK and its interaction with the pre-BCR is crucial for normal B-lymphocyte development with BTK considered a sine qua non for B-cell development and survival.

Although less is known about signals mediated by the pre-BCR compared to the IgM-BCR signal, it is assumed that the pathways are similar. This is supported by evidence demonstrating that pre-BCR signalling results in the formation of a raft-associated calcium signalling molecule composed of the same tyrosine-phosphorylated signalling molecules found in BCR stimulated B-lymphocytes (94).

The activation of BTK upon BCR stimulation is dependent on two essential processes; membrane association and phosphorylation of the Y551 tyrosine (72,95–97). The membrane association of BTK is dependent on the interaction between the PH domain and the product of PI3K (72,95–97). Phosphorylation of the Y551 tyrosine by tyrosine kinases such as LYN results in BTK autophosphorylation at Y223 (72,95–97).

Figure 1-4 BTK activation downstream of the pre-BCR/BCR, adapted from Hendriks et al., (98) *B cell receptor (BCR), Diacylglycerol (DAG), Bruton's Tyrosine Kinase (BTK), inositol 1, 4, 5 – triphosphate (IP3(R)), nuclear factor of activated T-cell (NFAT), immunoreceptor tyrosine-based activation motifs (ITAM), phospholipase Cγ2 (PLCγ2), SH2 domain-containing leukocyte protein of 65 kDa (SLP65), Wiskott-Aldrich syndrome protein WASP, nuclear factor-κB (NF-κB), forkhead box O (FOXO) transcription factors; BCL-2 antagonist of cell death (BAD), B cell adaptor for PI3K (BCAP), calmodulin (CaM), caspase recruitment domain-containing protein 11(CARD11), CBL-interacting protein of 85 kDa (CIN85), glycogen synthase kinase (GSK), inhibitor of κB (IκB), inhibitor of NF-κB kinase (IKK), inositol trisphosphate (IP3), IP3 receptor (IP3R), mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1), 3-phosphoinositide-dependent protein kinase 1 (PDK1), phosphatidylinositol-3,4,5,-trisphosphate (PIP3), protein kinase C (PKC), spleen tyrosine kinase (SYK), RAS guanyl-releasing protein 3 (RASGRP3)*



BCR activation results in phosphorylation of the Ig family member Igα and Igβ cytoplasmic immunoreceptor tyrosine-based activation motifs (ITAMs) and the formation of a lipid raft-associated calcium-signalling module. This complex consists of tyrosine-phosphorylated

LYN, SYK, BLNK (SLP65), PI3K, BTK, VAV, and PLCγ2. This a crucial complex, stabilised by the C-terminal SH2 domain of PLCγ2 (65,99). Activated BTK is responsible for PLCγ2 phosphorylation at Y753 and Y759, a critical step for lipase activity (100–102). The activation of PLCγ2 results in the production of diacylglycerol (DAG) and, by cleavage of PIP_2, inositol 1,4,5-triphosphate (IP3) (65). IP3 binds to its receptor (IP3R) in the endoplasmic reticulum (ER), causing a release of intracellular Ca^{2+} stores (65). This ER store of Ca^{2+} is replenished and maintained by stromal interaction molecules, STIM1/STIM2 and calcium release-activated calcium (CRAC) channels (103). The intracellular Ca2+ activates nuclear factor of activated T-cell (NFAT) transcription factors and induces NFAT nuclear import (65). Protein kinase Cβ (PKCβ), mediated by DAG, activates the NF-κB pathway. In addition to NF- κB pathway activation, PKCβ activates mitogen-activated protein kinases (MAPK) (104,105). These MAPKs are important promotors of cell survival and cell cycle entry (104,105). PKCβ also acts as a vital feedback inhibitor of BTK activation by phosphorylation of the highly conserved S180 serine residue within the TH domain of BTK (65,106).

The IgM B-Cell Receptor (BCR) is essential for the survival of peripheral B-cells (86). Without BTK, B-cells suffer from a high rate of apoptosis, in conjunction with reduced BCR-mediated induction of the anti-apoptotic protein Bcl-xL (107,108). BTK lacking B-cells fail to induce cyclin D2 expression and, as a result, do not enter the S phase of the cell cycle (109). In addition to B-cell survival and proliferation, BCR controls integrin α4β1 (VLA-4)-mediated adhesion of B-cells to vascular adhesion molecule-1 (VCAM-1) and fibronectin via BTK (110).

In summary, upon IgM-BCR and pre-BCR stimulation, BTK plays a crucial role in the activation of PLCγ2, resulting in Ca^{2+} influx, activation of PKCβ and CaM. These processes activate four key transcription factors essential for B-cell survival, proliferation and differentiation: ELK1, c-MYC, NFκB and NFAT.

1.6 Role of BTK in myeloid cells

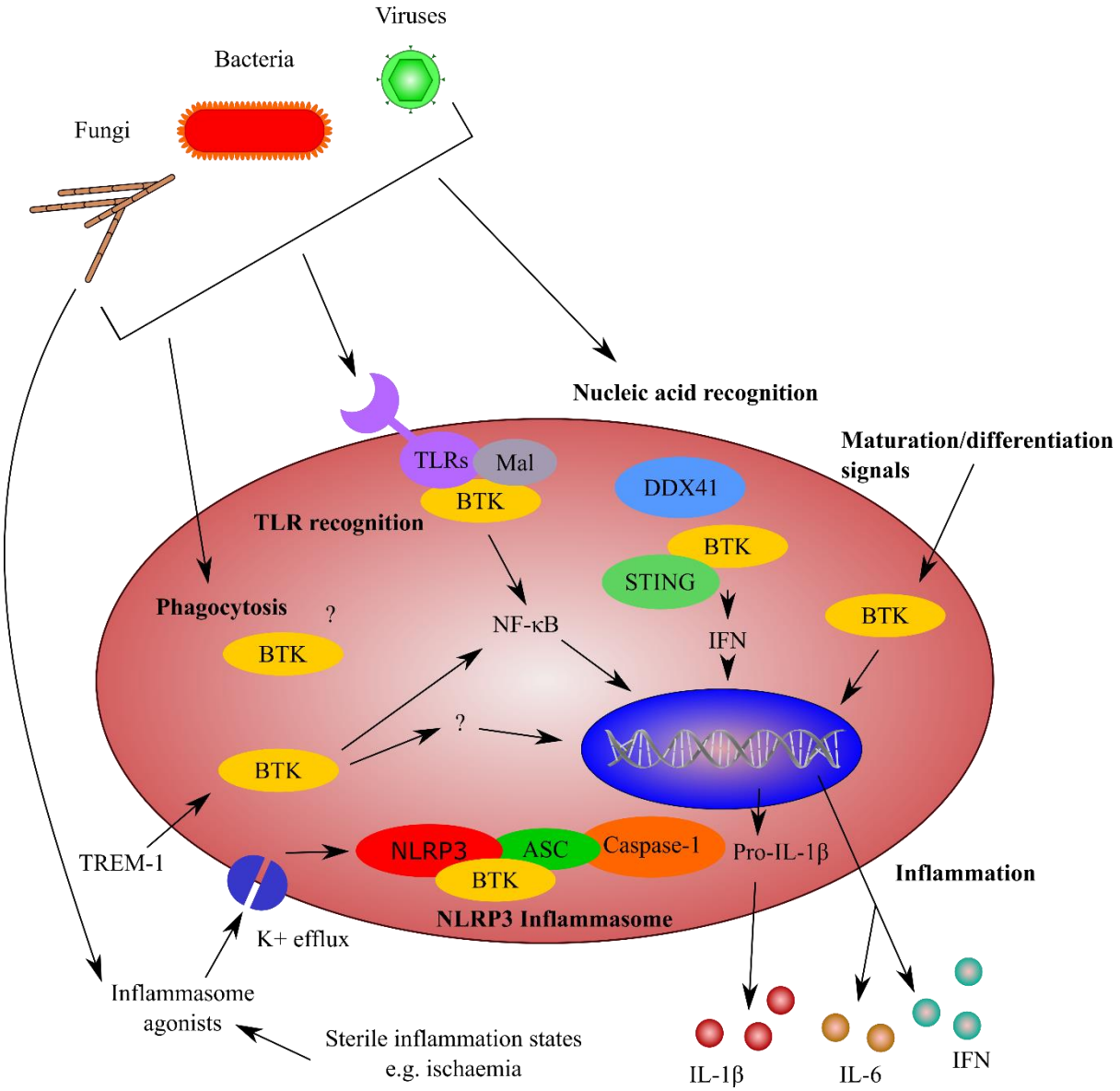
Aside from its role in the pre-BCR and BCR receptor pathways, BTK is expressed in all haematopoietic cell lineages except T cells and is involved in numerous signalling pathways as summarised in Table 1-4.

Table 1-4 Signalling pathways BTK plays a role in, adapted from Hendriks et al., (28)

Signalling Pathway	Cell types
Pre-BCR (111,112)	Pre-B cells
BCR (112–114)	B cells
CXCR4 (115)	Pre-B cells, B cells
CD38 (116)	Activated B cells
Epo-R (117)	Erythrocytes
TRAIL-R1 (117)	Erythrocytes
FcεR (118)	Mast cells, basophils
FcγR (119,120)	Myeloid cells
GPVI (121)	Platelets
IL-5R (122,123)	B cells, eosinophils, basophils
IL-6R (124)	Activated B cells, plasma cells
TLR2/4 (125,126)	Myeloid cells, B cells
TLR 7/8/9 (127–131)	Myeloid cells, B cells
CDC303 (BDCA-2) (132)	Plasmacytoid dendritic cells

While the role of BTK in the adaptive immunity is well known and is the subsequent impact on immunity self-explanatory, the effects of innate immunity are less well recognised despite work demonstrating the key roles BTK can play in innate immunity (Figure 1-5).

Figure 1-5 The role of BTK in innate immunity, adapted from Weber et al., (133)



1.6.1 Monocytes

Aside from its integral role in the development of B-lymphocytes, recent work has suggested that BTK plays a vital role in establishing the immunity function of monocytes (134). Monocytes in human XLA patients have been shown to have decreased chemotaxis, and defective *FcγR* (Fc-gamma receptors), *CR1* (Complement Receptor 1) and *CR3* (Complement Receptor 3) mediated phagocytosis when compared to healthy subjects (135). These data demonstrated that BTK deficiency causes increased susceptibility to apoptosis in monocytes (134). However, other work shows that BTK deficiency does not affect the function of monocytes or polymorphonuclear cells in XLA patients, albeit in those patients who are on treatment with intravenous immunoglobulin (IVIG) therapy (136).

1.6.2 Role of BTK in the development and function of innate immune cells

The prerequisite of BTK for B-lymphocyte maturation is well known. A role in the development of myeloid cells could also make logical sense, given their dependence on signalling from a variety of cell surface receptors (137).

In murine models, BTK deficiency was associated with reduced total numbers of monocytes and macrophages (138). Further work demonstrates these same models had an increased number of granulocytes. However, these were often immature, had reduced efficacy and had impaired recruitment of neutrophils to sites of inflammation (138,139). In human XLA patients, neutrophils have been shown to be arrested at the myelocyte/promyelocyte stage, which may go some way to explain to neutropenia often seen in XLA patients, as will be discussed in Chapter 2 (140–142). In particular, there is increased TLR and TNF induced reactive oxygen species (ROS) in XLA neutrophils (at the expense of increased neutrophil apoptosis) (73). However, like much work in this area, there are contradictory findings from other groups, although these included patients on established IGRT (136,143). Dendritic cells (DCs) in BTK deficient mice have been shown to have defects in maturation in antigen presentation (144).

Both recognition and response to a range of microbes (*Listeria monocytogenes* (145), *Staphylococcal aureus* (146), dengue virus (147), *Aspergillus fumigatus* (148)) have been shown to be dependent on BTK. The exact mechanism behind this is not fully understood but the involvement of BTK in Toll-like receptors (TLRs) (most notably TLR2 (124,128), TLR3 (147), TLR4 (125,128), TLR7/8 (128,149,150), TLR9 (145,150,151) demonstrated in human, and mouse macrophages and dendritic cells appears to be of clear significance. However, when transferring these experimental results to human XLA patients, the results seem to contradict these previous studies (143). While most BTK mutations appear to cause complete loss of function and a broadly similar phenotype across all patients, the impact of TLR signalling may have more input from genotype-phenotype correlation.

Murine models have demonstrated BTK deficiency results in delayed microfilaria clearance and decreased levels of Interleukin-12A (IL-12A), Interleukin-1 (IL-1), Tumour Necrosis Factor (TNF) and Nitric Oxide (NO) (152). Further work has demonstrated the importance of BTK in the production of TLR-induced Interleukin-10 (IL-10) and TLR3 signalling (147,153).

BTK is able to interact with TLR4, TLR6, TLR8 and TLR9 and some key proteins involved in the TLR signalling pathways; myeloid differentiation protein 88 (Myd88), Myd88 adapter-like protein (Mal) and interleukin-1 receptor-associated kinase-1 (IRAK1) (124,127). Additional work has demonstrated that BTK-deficient lymphocytes can produce higher levels of pro-inflammatory cytokines but less of the inhibitory cytokine IL-10 (154). These works on BTK-TLR interaction suggest that BTK-deficiency may have a significant impact upon immune regulation, inflammation and immune defects other than the classical antibody deficiency typically thought of in XLA.

1.6.3 BTK and in the NLRP3 inflammasome

Recently, the NLRP3 inflammasome has been shown to have a significant role in the activation of bioactive IL-1β (155,156). Furthermore, the NLRP3 inflammasome has also been implicated in pathological processes not only underlying infection but also sterile inflammatory states including myocardial infarction, stroke, Alzheimer's disease and diabetes (156). Genetic mutations in NLRP3 causes dysregulated activity of NLRP3, resulting in cryopyrin-associated periodic syndrome (CAPS) (157). The NLRP3 inflammasome consists of NLRP3, adapter ASC and caspase-1. BTK has been demonstrated to be an integral regulator in the activation of the NLRP3 inflammasome (156,158). BTK has also been shown to be essential for the NLRP3 inflammasome-dependant IL-1β release from macrophages in murine models (158). It has been demonstrated in peripheral blood mononuclear cells (PBMC) from XLA patients that NLRP3 inflammasome activity was impaired (146), which may go some way to explain some of the inflammatory symptoms that some patients appear to suffer from in this cohort. Impaired or dysfunctional NLRP3 inflammasome activity is likely to lead to immune dysregulation and has been shown to contribute to a number of human diseases including autoimmunity and malignancy (159).

1.7 BTK and autoimmunity

The few remaining B-lymphocytes XLA patients possess express immature, IgM-rich phenotypes and are enhanced for autoreactivity (160,161). As will be discussed further in chapter 2, a significant proportion of XLA patients appear to suffer from inflammatory symptoms. This could be as a result of overproduction of inflammatory cytokines in response to TLR signalling (151,162). BTK-deficient marrow-derived dendritic cells (BMDCs) in mice demonstrate increased levels of CD86 in response to lipopolysaccharide (LPS) and become antigen-presenting MHC Class-II^{high} cells at a higher rate than controls. These differences were associated with a decreased ability of these BMDCs to produce IL-10 (144). However, the impact of BTK deficiency on autoimmunity and inflammation is not clear-cut.

There is some demonstration that BTK deficiency can protect against autoreactivity, at least in murine models, which began with the first report of *Xid* mice being protected against collagen-induced arthritis (163). It is even speculated that we may soon see the introduction of BTK-inhibitors in the management of autoimmunity (164). For example, Fenebrutinib is a BTK inhibitor in phase 2 clinical trials to treat several autoimmune conditions such as rheumatoid arthritis, systemic lupus erythematosus and chronic spontaneous urticaria (ClinicalTrials.gov Identifiers: NCT02983227 and NCT03407482).

1.8 Clinical Presentation

The defects leading to XLA and ARA all block early B cell development, failing B lymphocyte maturation, absent or very low serum immunoglobulin levels and failure of specific antibody production (2). CD19+ B-lymphocyte numbers are usually less than 2% compared to 5-20% in the healthy population (63). Any B-lymphocytes that are present in the circulation demonstrate an immature phenotype with $IgM^{high}IgD^{high}$ but decreased HLA-DR, Bcl-2 and CD 21 (63). Any B-lymphocytes that were present at diagnosis usually tend to decrease even further with age (63).

The resulting agammaglobulinaemia forms the basis of the clinical phenotype. Patients typically become symptomatic after placental transferred maternal antibodies begin to decline from 4 to 6 months of age (165). Patients typically being to suffer from recurrent bacterial infections from 6 months of age once these immunoglobulin levels being to fall (166–168). Before diagnosis and instigation of treatment, patients will present with recurrent otitis, purulent rhinorrhoea, conjunctivitis, pneumonitis, diarrhoea and skin infections (166–168). They are particularly prone to encapsulated bacteria, especially *S. pneumoniae* and *H. Influnezae*¸ as well as *Giardia lamblia* (63,165,169). They are also susceptible to mycoplasma and ureaplasma, both of which are reported to have the potential to cause chronic pneumonia, arthritis, cystitis and cellulitis in this cohort as well as urethral stenosis in males (170,171).

Patients are thought to be able to combat fungi and viruses competently with the notable exception of enteroviruses (165). They are particularly susceptible to chronic enteroviral infections, which may prove fatal. (172–176). The two most common subtypes reported are Echovirus type 11 and Coxsackievirus B5 (177). Historically, the most feared complication of enterovirus infection was chronic meningoencephalitis in agammaglobulinaemia (CEMA) which is often fatal (177). It has been suggested recently that the lost role of BTK in TLR signalling may explain this particular susceptibility to enterovirus (178). Enterovirus

infection in XLA has always been a well-recognised complication historically with rates between to 15-20% in the pre IVIG era (168,179). In this era, before the introduction of IVIG enteroviral infection would often lead to chronic meningoencephalitis and often be fatal (168,176,180). The switch to IVIG/SCIG from intramuscular preparations has made a dramatic impact, but enterovirus has not disappeared in the modern era, and deaths still occur in this cohort due to enteroviral infection (142,177,181). Modern therapy will still have limitations in its treatment of enteroviral infections; infection may have started before the commencement of immunoglobulin therapy and enterovirus antibody titres in modern immunoglobulin preparations show considerable variation (182,183).

In addition to enterovirus, XLA patients may be susceptible to infections from other viruses and, in particular, have great difficulty in clearing these infections resulting in prolonged or permanent infection. XLA patients appear particularly susceptible to rhinovirus infections of the respiratory tract and often have positive respiratory samples for a prolonged period of time, up to 5 months in one case series (184). In addition, Norovirus infection of the GI tract may also play a burden for XLA patietns with one case report demonstrating a patients inability to clear a Norovirus infection (185).

Patients are usually diagnosed in early childhood, although there are case reports of patients being diagnosed well into adulthood and seemingly well despite no immunoglobulin replacement highlighting the potentially wide variation in phenotype (22).

Approximately half of all patients will have suffered a severe infection before their second birthday (63,186,187) and 10% of patients present with sepsis leading to their XLA diagnosis (167).

Neutropenia is seen in 10-25% of patients with XLA, usually at the time of their diagnosis (141,167,188,189). Interestingly, neutropenia is only very rarely seen in patients who are on immunoglobulin therapy (63). The neutropenia can be associated with Pseudomonas or Staphylococcal sepsis (141). Pseudomonas infections are well documented in XLA and one patient presented to medical services as a result of a pseudomonas liver abscess (190).

There exists a risk of vaccine-associated paralytic poliomyelitis in this cohort, although this risk has been reduced with many programs switching to the inactivated polio vaccine (191,192). A recent case report has demonstrated the potential benefit of fluoxetine in these cases (193).

1.9 Murine Models

There are two commonly used murine models for XLA. The first is *Xid*. These mice have a spontaneous mutation at a CpG site in the BTK gene resulting in a change from arginine to a cysteine at residue site 28 (R28C) (194). In humans, this identical mutation leads to a complete absence of B-lymphocytes and immunoglobulins (195). However, the affected *Xid* mice retain about half the normal number of splenic B-lymphocytes. The second model, using genetically BTK knock out mice, has a similar phenotype to the *Xid* model (126,196).

1.10 Immunoglobulin Therapy

1.10.1 History

In the initial report by Bruton, the child was treated with a subcutaneous injection of Cohn Fraction II immunoglobulin, first reported in 1944 (3,197). This process described the introduction of a novel ammonium sulphate and cold-ethanol fractional processes using large pools of human donor serum, therefore meaning immunoglobulin (Ig) products contained antibodies against a wide range of pathogens such as polio, measles and pertussis (197). This process became mainstream in the 1950s, and intramuscular injections of immunoglobulin became the standard of care for patients with antibody defects. However, these were painful, resulting in suboptimal dosages, and many severe adverse reactions were reported, such as nerve injuries. Attempts at this time to administer Cohn fraction II intravenously lead to severe anaphylactic reactions in 90% of patients due to IgG aggregates formed during the manufacturing process (198). Advances in the fractionation, purification and formulation process overcame these hurdles in the coming years using caprylate treatment and ion-exchange chromatography (199).

Further reaggregation of IgG is prevented by lowering the pH and addition of stabilisers (e.g. sucrose, maltose, fructose, proline, glycine). The significant reduction in adverse events through methods such as preventing aggregation meant intravenous immunoglobulin (IVIG) was able to be offered as the mainstay of treatment of patients who required IGRT. This switch from IM to IV preparations was made available in the 1980s with the UK switching over in 1982 (168,200). IV preparations are less painful, allowing more substantial volumes to be administered, leading to higher trough IgG levels compared to IM preparations (201,202). These higher trough levels dramatically reduced invasive infection rates and improved survival outcomes (201,202). This period coincided with the emergence of HIV, and in addition to Hepatitis C, contaminated products placed a significant burden on this cohort due to their dependency on donor blood products (203). The safety profile of donor

immunoglobulin products has dramatically improved with screening of donors and through improvements in the production process with exposure to low pH, pasteurisation, detergents and viral filtration (204). With improved production protocols, the previous risks of contamination with blood born viruses have dramatically fallen, although a small risk still exists, particularly of prion-borne disease (205,206).

An alternative route of administration is via subcutaneous injections (SCIG). This route may prove less of a burden to patients as it is often easier to self-administer at home compared to IVIG (207). It may also produce more stable IgG trough levels which would, in turn, theoretically improve clinical outcomes (207). SCIG has been available for some time, with the first case report of XLA by Bruton using the subcutaneous route as the method of administration (3). However, initial techniques were hampered by slow infusion rates which ultimately limited the total dose of available IgG. In conjunction with the fact that IgG is better absorbed from muscle than fat, initial IGRT was almost always given via the IM route (167,208). Recent improvements have meant that the limitation of slow infusion has been overcome and, as such, SCIG is now a mainstay of treatment alongside IVIG with often the only deciding factor of one over the other being patient preference (209). SCIG is often more feasible as home treatment compared to IVIG, which has been demonstrated to be cheaper, more convenient and leads to a reduction in days taken off school or work (210). Both modalities are associated with a number of side effects. IVIG may be associated with more systemic side effects, e.g. headache and hot flushes whereas SCIG may be associated with more local reactions such as redness or swelling around the injection site.

Modern IGRT is available either through the intravenous route (usually on a 3-4 weekly basis) or the subcutaneous route (usually on a weekly basis). Recently, the technique of pre-infusion of SCIg with recombinant human hyaluronidase (facilitated SCIG, fSCIg) may also offer the infusion of SCIG to be extended to 2 to 4 weeks, similar to IVIg (211).

1.10.2 General Management

Historical guidelines recommended aiming for a trough IgG level of at least 5g/l, although more recent studies advocate a higher target of 8g/l to prevent infections and improve respiratory health (212–214). Furthermore, it is now recommended that target trough IgG levels should be individualised, aiming for a level that adequately prevents infection and minimises lung function decline on an individual basis (181,215).

1.10.3 Potential Limitations

Despite advances in immunoglobulin therapy, there remain obstacles. Commercial products contain virtually no IgA or IgM. There are limited data suggesting benefit using IgM and IgA rich immunoglobulin products for a carefully selected subset of patients (216,217). However, most immunoglobulin products continue to be IgA and IgM deplete due to concerns regarding safety data from historical cohorts and their efficacy. IgM is routinely removed as it can rapidly form complexes leading to serious adverse events. The majority of products are IgA deplete as patients can develop anti-IgA antibodies which precipitate severe anaphylactic reactions (218). Also, donor serum IgA is mostly monomeric, compared to locally produced pulmonary IgA, which is polymeric and better suited to protecting mucosal surfaces (51,219). While donor IgG has had a dramatic, effect in reducing life-threatening invasive infections, IgA and IgM play a significant role in protecting the mucosal surfaces. Therefore, despite IGRT, XLA patients will remain IgA and IgG deficient throughout their lives. Without adequate replacement of these isotypes, patients are likely to continue to experience frequent mucosal infections, increasing the risk of serious complications such as bronchiectasis (220–222). Furthermore, IGRT does not attempt to compensate for other lost functions of BTK as described in this chapter. Although the exact clinical implications of the loss of these other functions are yet to be firmly established, it would be logical to conclude they must have some clinical impact.

1.10.4 Adverse Events

Coinciding with the introduction of intravenous preparation of immunoglobulin therapy came the emergence of Human Immunodeficiency Virus (HIV), and in addition to Hepatitis C, contaminated products placed a significant burden on cohorts dependent on donor blood products (203). While there are no confirmed reports of immunoglobulin transmitted HIV, contracting hepatitis C through contaminated immunoglobulin has been confirmed (223).

The contamination of blood products with blood-borne viruses and the subsequent scandals has resulted in multiple international inquiries and millions of pounds either set aside or awarded as compensation (224). Through intensive efforts to improve the immunoglobulin production processes, serious adverse effects of IGRT have become rare in modern times. These improved processes include an improved screening of donors, exposure of the product to low pH, pasteurisation, detergents and viral filtration (204). Contamination with Hepatitis C, HIV and other blood-borne viruses is now unheard of, although a small theoretical risk still exists, principally of prion-borne disease (205,206). The risk of prion-borne disease, in particular, most notably vCJD, has resulted in the decision that no immunoglobulin is

procured from UK donor pools. As of 2019, no UK patient with primary immunodeficiency on IGRT has shown clinical evidence of vCJD or shown to have abnormal prion protein in tested tissues (225).

Furthermore, there are still risks associated with IgG therapy, such as skin necrosis (226). In addition, specifically in the XLA cohort, there are reports of IVIG triggered tubulointerstitial nephritis, which can be fatal (227–230).

1.11 Haematopoietic Stem Cell Transplantation

Although Haematopoietic Stem Cell Transplantation (HSCT) is the primary treatment for many PIDs, there are limited data available examining its role in XLA. Howard et al. reported a case series in which 6 XLA patients underwent HLA matched sibling donor HSCT without a preconditioning regime (231).

In all patients, there was no donor engraftment. While these transplants resulted in no harm; there was no benefit (231). A recent case report describes successful HSCT for a patient with both XLA and Acute Myeloid Leukaemia (AML) (232). This patient underwent myeloablative conditioning, and two years, post-transplant demonstrates normal CD19, IgG, IgM and IgA levels off immunoglobulin replacement therapy (232). Colleagues in Japan have used this experience to successfully transplant an XLA patient using a reduced intensity conditioning regimen (233). This patient demonstrated normal IgG, IgM, IgA and CD19 levels 500 days post HSCT and discontinued immunoglobulin replacement therapy.

This limited literature provides some evidence that HSCT may play a role in XLA treatment in carefully selected cases, especially so in healthcare settings where lifelong immunoglobulin therapy would be prohibitively expensive and sustained follow up unfeasible. In most developed countries, the associated risks of GvHD, chemotherapy and the associated mortality, means HSCT is not routinely offered to patients with XLA in favour of immunoglobulin replacement therapy (234).

1.12 Newborn screening

T cell receptor excision circles (TRECs) are produced during normal T-lymphocyte receptor gene splicing and rearrangement. They are found only in naïve T-lymphocytes and, therefore, defects causing low or absent T-lymphocytes (most notably severe combined immunodeficiency, SCID), will concurrently have near absent or absent levels of TRECs. These can be quantified on dried blood spots taken on the Guthrie card used in newborn screening programmes (235,236).

The B-lymphocyte counterparts to TRECs are kappa-deleting recombination excision circles (KRECs), which are episcopal DNA fragment by-products that occur as a result of immunoglobulin kappa gene arrangement throughout B-lymphocyte maturation (237,238). KRECs do not replicate during mitosis and therefore, will exhibit a dilution pattern that allows a quantifiable estimation of B-lymphocyte replication (238). Regardless of the underlying genetic or molecular aetiology of their disease, patients with B-lymphocyte defects leading to absent B-lymphocytes will have low levels of or absent KRECs. This measurement can also be performed from dried blood spots on the new-born Guthrie card (238).

The potential impact of evidence thus far of national newborn screening programs for XLA is discussed in Chapter 2.

1.13 Gene Therapy

While HSCT remains the mainstay of treatment for many PIDs where a cure is the aim of treatment, gene therapy is fast evolving to become a promising alternative for certain diseases.

Patient's own hematopoietic stem cells are transduced using either lentiviral or gammaretroviral vectors containing a correct version of the affected gene. These corrected HSCs are transfused back into the patient, often with a small amount of conditioning. This modality offers the significant advantage of using the patient's own stem cells, negating the risk of GvHD and significantly reducing the amount of conditioning often used in HSCT therapy potentially offering significant safety advantages over HSCT.

The road to current clinical gene therapy has been long, and concerns remain surrounding efficacy and safety. Initial studies using gammaretroviral vectors for the treatment of X-Linked SCID resulted in high rates of malignancy caused by viral integrations within oncogenic loci (239,240). Interestingly, the similar vector designs in ADA-SCID has not shown the same adverse events to date (241).

Gene therapy for ADA-SCID using gammaretroviral vectors has shown great success and was recently approved by NICE for inclusion in the treatment of ADA-SCID in the UK (242), despite being one of the most expensive therapies ever developed (242). Despite no cases of malignancy caused by the gene therapy, research is underway developing lentiviral vectors for ADA-SCID gene therapy (242).

Significant efforts were undertaken to redesigning the gammaretroviral vector, and current gene therapy is often based on lentiviral vectors offering a potentially improved safety profile.

Although lentiviral vectors are now being implemented into clinical trials and do not demonstrate increased rates of malignancy, concerns remain regarding long-term safety and efficacy. Compared to viral vectors, methods that employ gene editing may offer greater efficacy and improved safety profile. One method of gene editing, which garners much public and press attention is CRISPR-Cas9. There are still concerns using CRISPR-Cas9, most notably that of 'off-site' cleavages, which may have mutagenic effects (243). However, while still in its infancy, recent work to repair stem cells from patients with chronic granulomatous disease (CGD) and JAK3 mutations using CRISPR-Cas9 demonstrate the potential promise this technique could have in the future (244,245).

The progress made thus far in developing gene therapy models in XLA is discussed in Chapter 2.

1.14 Outline of thesis

Chapter 2 will review the literature for current outcomes and novel treatment strategies for XLA and ARA.

Chapter 3 will detail the methodology for the study

Chapter 4 will detail the aims and hypotheses of the study

Chapter 5 will present the clinical outcomes for the study cohort

Chapter 6 will present in further detail the respiratory health outcomes for the study cohort

Chapter 7 will present the psychological health data for the study cohort

Chapter 8 will present the quality of life data for the study cohort

Chapter 9 will discuss the findings from this research project

Chapter 10 will present the conclusion from this research project

Chapter 2 Literature Review of Clinical Outcomes

This chapter will review the current and historical literature detailing clinical outcomes and health-related quality of life (HRQoL) in congenital agammaglobulinaemia and other primary antibody deficiencies (PAD). There will also be a review of the effectiveness of immunoglobulin replacement therapy in these patients. In addition, the final part of this chapter will review the current research into novel treatment and diagnostic strategies.

A literature search of MEDLINE and EMBASE was performed with the keywords "XLA", "Primary antibody deficiency", "Agammaglobulinaemia", "X-linked agammaglobulinaemia" and "BTK deficiency". Both UK and US spellings were used. The abstracts of these results were screened to include only relevant articles such as those focusing on clinical outcomes or novel therapies such as gene therapy. In addition, articles were chosen from the references from select seminal studies such as Plebani et al., and Hermaszewski and Webster (168,186).

2.1 Previous UK Cohort Studies

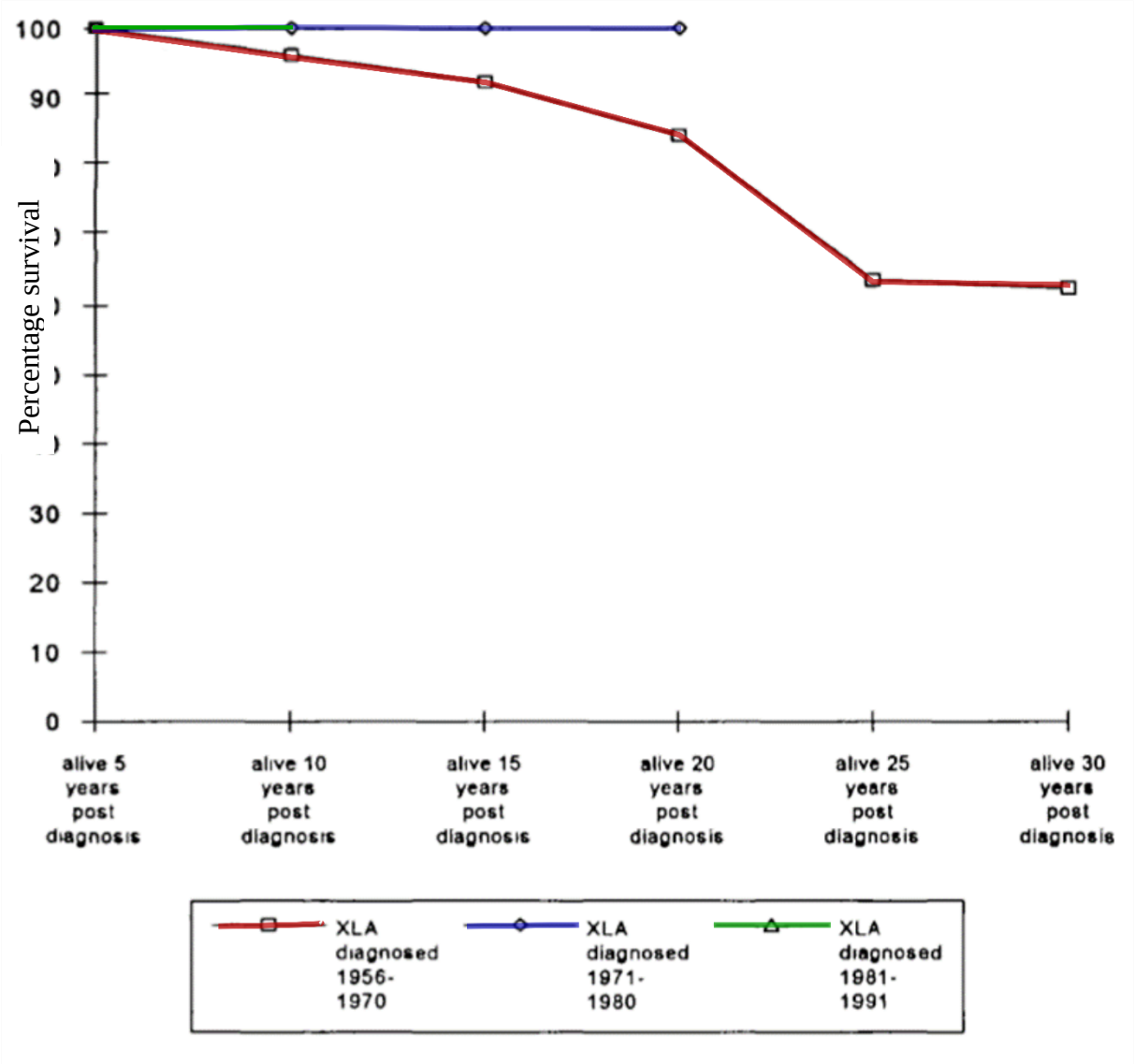
There have been only two previous studies examining outcomes in XLA in the United Kingdom. In 1971, there was a Medical Research Council (MRC) report into primary hypogammaglobulinaemia (246). However, further work has identified the diagnostic accuracy in this study to be weak with some patients in that cohort most likely suffering from some forms of severe combined immunodeficiency (SCID) (168,246). Furthermore, all patients were on (intramuscular immunoglobulin) IMIg therapy, which was the only immunoglobulin preparation available at the time, leading to suboptimal IgG trough levels and a high mortality rate (246).

In 1993, Hermaszewski and Webster carried out the second and most recent survey of XLA patients in the UK (168). Forty out of forty-four XLA patients presented with sino-pulmonary infections, and it is interesting to note that the authors noted a plateau in improvement in diagnostic delay already at this early stage in modern immunology (168). Improvement in reducing diagnostic delay and an apparent plateau in this improvement are discussed further below in this chapter. The overall 30-year survival for this cohort was 62%, although when excluding those patients born before 1971, the 20-year survival was 100% (Figure 2-1) (168).

On treatment, 36/44 patients suffered from recurrent lower respiratory infections and 36/44 experienced from chronic otitis media or sinusitis (168). The authors noted that the rate of these infections had started to decrease since the introduction of intravenous immunoglobulin

(IVIg) therapy, consistent with their overall improvements in survival (168). The results of this cohort are now however considered out of date, suffering from a legacy of historical, inadequate IMIg therapy and diagnostic inaccuracies due to the lack of genetic testing and inability to test for BTK protein expression (168). However, this seminal study set out a vital baseline for UK XLA outcomes and, along with others, helped lay the groundwork for establishing the UK primary immunodeficiency (UKPID) registry (5).

Figure 2-1 UK XLA survival 1956 – 1991 (168)



2.2 Prevalence and Incidence

With more accurately defined diagnostic criteria, and improved access to diagnostic testing, the prevalence and incidence of XLA and autosomal recessive agammaglobulinaemia (ARA)

has altered over time due to increased awareness and diagnosis. Boys, historically, who died early in infancy due to infection, may have had undiagnosed XLA. Improved awareness of XLA has now allowed these boys to be diagnosed earlier, starting IGRT earlier and not succumbing to overwhelming infection early in infancy. Furthermore, ascertaining an accurate number of cases has proven difficult until the recent introduction of national and international registries as well as important large-scale studies.

The US study of 201 XLA patients by Winkelstein et al. in 2006 estimated the US incidence of XLA to be 1:190,000 male births or 1:379,000 live births (142). The latest data from the UKPID estimates the minimum UK prevalence of XLA to be 2.41 cases per 1 million population (247). This registry estimates the minimum UK incidence to be one case per 200,000 live births (247). However, both these figures are much lower than the reported incidence of proven XLA in Argentina of 1:60,000 live births (248). Even when including only genetic proven cases, this figure is still higher than the US and UK figures at 1:115,000 births (248).

The impact of XLA and ARA in developing counties is increasingly being recognised (248–251). Cases are reported in a wide range of developing countries, with many beginning to establish national registries and publish cohort studies. Outcomes in these cohorts are invariably worse than developed nations due to difficulties in accessing health care, delays in diagnosis and unreliable access to immunoglobulin therapy. For example, in India, the mean age of diagnosis is 5.9 years, with a mean survival of 137 months (250). Nevertheless, the number of identified cases worldwide is likely to increase, as access to diagnostics and therapies increase. These nations, and methods to improve outcomes for their patients, must be born in mind when considering future research into congenital agammaglobulinaemia.

Since the beginning of the 1980s awareness of primary immunodeficiency (PID) has dramatically increased with the establishment of specialised centres and charities. Also, the availability of intravenous immunoglobulin replacement products, as will be discussed in section 2.6, led to a major step-change in outcomes for patients dependent on these products. Therefore, for the purposes of this literature review, ‘modern’ is defined as any cohort after 1980 unless otherwise stated.

2.3 Presentation

2.3.1 Age at Diagnosis and Diagnostic Delay

In large, modern cohort studies from Europe and the USA, the median age at diagnosis (and therefore instigation of therapy) ranges from 2.2 to 5.27 years (142,167,186,187). Both Plebani et al. and Winkelstein et al. demonstrated an improvement in the age of diagnosis over time (Winkelstein et al. $r = -0.43$, $p<0.0001$) (142,186). However, both authors demonstrate that this improvement has reached a plateau, with no real improvement over the last 15-20 years (142,186). There has been much effort to reduce diagnostic delay in all PIDs. As diagnostics and therapies improve and more primary immunodeficiencies (PIDs) are discovered, clinicians will be more aware and more likely to think of PID in their differentials earlier in the patient's diagnostic workup. In addition, efforts such as those by the Jeffrey Modell Foundation and their '10 warning signs of primary immunodeficiency' project, have helped to raise the awareness of PID to all healthcare professionals, aiming to reduce diagnostic delay (252,253). However, there will always be limits in reducing the diagnostic delay due to the considerably wide range of what is considered a 'normal' number of infections in the healthy child and the difficulties in identifying the child with a rare a PID against a backdrop of the healthy population. We may have, therefore, now reached the limits in reducing diagnostic delay in congenital agammaglobulinaemia (254,255). It is unclear what factors in the pre-diagnosis period increase the risk of later complications, but as discussed below, older age of diagnosis is associated with an increased risk of bronchiectasis. It is also unclear whether our current improvement in the diagnostic delay is sufficient to prevent these complications.

The age at diagnosis in developing countries or countries with less well-established immunology centres tends to be higher, ranging from 3.88 years to 7.6 years in China, Argentina and Iran suggesting a more considerable diagnostic delay than their western counterparts (191,248,251,256,257). This diagnostic delay, and therefore a delay in instigation in IGRT, could potentially lead to more complications.

There is a reported family history in XLA cases of between 15-55% (186,187,256,258). Despite these figures, patients with a family history of XLA are still rarely screened at birth or early infancy with only a third of potential patients in the 2006 USA XLA study being appropriately screened based on their family history (142). Lederman and Winkelstein both demonstrated that a family history of PID is associated with a younger age of diagnosis (median 3.5 versus 2.5 years and 5.4 versus 2.6 years respectively) (142,167). An important

area of research would be to examine those patients who were diagnosed at birth because of their family history, but such data do not yet exist.

Patients will typically become symptomatic as maternally transferred IgG level begins to wane by approximately 4-6 months of age (187). Accurate data pertaining to the clinical picture before the diagnosis of agammaglobulinaemia are lacking. Such data are difficult to validate, are subject to significant recall bias and result in uncertainties in differentiating typical childhood infections from infections that this cohort would experience. The available literature suggests 40-50% of patients presented to a medical practitioner with infections within the first 12 months of life (168,251). The diagnostic delay from the first presentation to diagnosis (and instigation of immunoglobulin therapy) is reported to be between 33 and 60 months, with developed countries having a shorter diagnostic delay (186,191,251,256). Due to the limitations in examining the pre-diagnosis period, the use of diagnostic delay is limited, and age at diagnosis is our best marker of improvements in speed to diagnosis. Future, prospective studies of newly diagnosed patients, may prove useful to help improve the accuracy of data leading up to diagnosis, but the rarity of these disorders would likely make such studies unfeasible unless a large-scale multi-national approach is taken.

2.3.2 Infections

As discussed in the introduction, the defects in these patients make them particularly susceptible to encapsulated bacteria, *G. lamblia* and enteroviruses. This is well presented within the published literature, which clearly shows that, before diagnosis, the sinopulmonary tract infections represent the most significant burden for these patients (Table 2-1). Delays in diagnosis and instigation of immunoglobulin replacement therapy may, therefore, mean that these repeated sinopulmonary tract infections are resulting in significant lung damage before the diagnosis of XLA. In the recent large cohort from Italy, 15/71 (38.5%) XLA patients had already developed chronic lung disease (CLD) before their XLA diagnosis (63% of all patients with CLD)(186). While IGRT may aid in reducing the progression of their CLD, it is unlikely to reverse it. Therefore, patients who have developed CLD before the diagnosis of their XLA are likely to have CLD for the rest of their lives

In addition, prior to treatment, patients are susceptible to severe and invasive diseases as noted by Table 2-1 reporting CNS disease and sepsis.

Neutropenia is often seen prior to or at the presentation of XLA, affecting between 11% and 22% of patients (142,248,250). Interestingly, this neutropenia is not seen in patients who are

on immunoglobulin replacement therapy (63). It has been proposed that XLA-associated neutropenia may in part be due to the limited bone marrow reserve of neutrophils seen in infants, exacerbated by the near-complete lack of B-lymphocytes (189). These episodes of neutropenia, in association with the B-lymphocyte defect, make them particularly susceptible to Pseudomonas infections. Many manuscript introductions and textbooks give this little attention but instead focus on the susceptibility to encapsulated bacteria and enterovirus infections. However, a summary of the literature clearly shows this organism to represent a significant burden to patients before their diagnosis. In addition, this organism often causes invasive disease (167), and a recent case report describes an XLA patient presenting with a pseudomonas liver abscess (190).

Table 2-1 Summary of reported presenting symptoms and organisms in congenital agammaglobulinaemia (references in brackets)

Site of infection	Percentage of patients presenting	Reported organisms
Respiratory Tract	31-76% (142,167,186,191,248,259)	*Streptococcus pneumoniae, Haemophilus influenzae, Pseudomonas species, Staphylococcal aureus, Mycoplasma pneumonia* (187,248,260)
ENT	22-75% (167,191,251)	
Sinuses	27-45% (142,186,251)	
Gastrointestinal	13-67% (142,186,251,261)	*Giardia lamblia*, Rotavirus (248)
Central Nervous System	16-21% (167,250,251)	*Haemophilus influenzae, Streptococcus pneumoniae*, Enterovirus (167,168,179,248)
Skin	21-27% (186,251)	
Arthritis	10-15% (186,251)	
Sepsis	10% (167)	*Pseudomonas species, Haemophilus influenzae, Streptococcus pneumoniae* (167,248)

2.4 Outcomes

2.4.1 Survival

Historically, in the 1950s and 1960s, the majority of XLA patients would die in early childhood due to acute or chronic infections (63). Improvements in recognition and diagnosis meant that in the 1970s, most patients were often able to survive through early childhood. However, it was unusual to reach adulthood due to chronic enteroviral infections or progressive pulmonary disease (167). The most significant difference in survival rates occurred when therapy was switched to intravenous and subcutaneous therapy in the 1980s. These modalities allowed more substantial and more frequent administrations of replacement immunoglobulin therapy resulting in higher IgG trough levels. It is generally accepted that, with modern therapy, patients should expect to survive into adulthood, likely with a near-normal life expectancy although there is no long-term follow up data of recent cohorts to confirm this fully (142,168,186,258,262).

The mortality rate of recent cohorts is now approximately 1-1.5% with many of those deaths still due to chronic enteroviral infections or hepatitis as a result of contaminated immunoglobulin products (142,186). These deaths most likely represent a legacy from patients treated with historically suboptimal therapies and from the contaminated blood products scandal in the 1980s, in which the use of contaminated blood products before 1986 lead to 4800 patients being infected with hepatitis C, of which 1200 were also infected with HIV (224). It is reported that nearly every patient with haemophilia (who are often dependant on donor blood products) treated before 1986 was infected with a blood-borne virus as a result of contaminated blood supplies (224). This scandal has led to multiple international inquiries and billions of pounds set aside or awarded for compensation (224) The remaining deaths are mainly due to chronic lung disease, and it is likely this will become the predominant cause of death in the future for the reasons discussed throughout this thesis.

In countries where clinical immunology services are still being established or where there are difficulties with access to health care, survival rates are more akin to those seen in the 1950-1980s in Europe and America. Recent data from India and Iran report 5-year survival in their XLA cohorts as 80% and 78% respectively (191,250). The most common causes of death seen in these and other developing countries are respiratory disease (both acute and chronic lung disease), sepsis and infections of the central nervous system (191,250,256,257)

2.4.2 Infections

The main aim in the management of congenital agammaglobulinaemia is to reduce infection rates, thereby reducing the subsequent risk of developing severe complications, reducing the burden of disease and maintaining a quality of life comparable to that of the background healthy population.

The recent, significant improvements in survival in western cohorts most likely reflect a dramatic reduction in invasive, life-threatening infections as a result of more timely diagnoses, the switch from intramuscular to intravenous and subcutaneous immunoglobulin therapy, improved supportive care and antibiotic prophylaxis (202)**.** In particular, since the advent of modern management, rates of invasive disease and sepsis have plummeted (142,186). In historical cohorts, enterovirus infections affected between 15% and 20% of XLA patients, often associated with inferior outcomes (168,179). Recent interrogation of the US PID registry of 390 XLA patients demonstrates 26 of those patients have suffered from meningoencephalitis, of which, 12 (3%) had confirmed enteroviral infection (177). However, all but one of these patients suffered their infection before 1995 confirming the evidence from

other cohorts that enteroviral infection on modern therapy is increasingly rare, contributing to the improvements in overall survival (177).

Although there are limited data showing incidence rates, recent studies from Italy and the USA consistently demonstrate that patients continue to experience recurrent respiratory tract infections despite adequate immunoglobulin therapy (142,186,258). In the Italian cohort of 73 XLA patients, Plebani et al. reported 37 episodes of pneumonia requiring hospital admission over a median follow-up of 7 years (186). Howard et al. report 17% of adult patients experienced at least 1 episode of otitis media in the preceding year, 80% had sinusitis, 17% had pneumonia, 39% had bronchitis, and 90% reported a recurrent cough (258).

Rates of chronic sinusitis in XLA patients remain high with reported rates of 48% - 59% despite treatment (142,186). Plebani et al. found that only the duration of follow-up was associated with an increased risk of sinusitis, and there was no correlation with IgG trough levels (186). Despite its high frequency in XLA, there are no data examining the impact of sinus infections on patients' lives, nor is there a consensus on the management of sinusitis for patients with XLA. This is especially disappointing given the potential acute complications associated with sinusitis, including intracranial abscesses (263). Earlier work has demonstrated that chronic sinusitis is often refractory to treatment and may require surgical intervention (264). Causative organisms isolated in sinusitis are likely to be those that XLA patents are particularly susceptible, such as *S. pneumoniae* and *H. influenzae*. There are likely to be limitations in the effectiveness of IGRT in treating sinusitis which may progress to chronic infection and cause long term complications (265). Most notably, there is a clear relationship between the development of sinusitis and subsequent bronchiectasis, with the sinuses acting as a clear gateway to the lung parenchyma (266). Rusconi et al. demonstrated in their antibody deficiency cohort that sinus disease could be well controlled with a medical regimen of antibiotics, steroids (intranasal/oral) and saline nasal washes, as used in the immunocompetent population (267). This recent work suggests, that active, combined therapy can be effective in the XLA cohort, and could reduce the burden sinusitis can place on patients' lives (267).

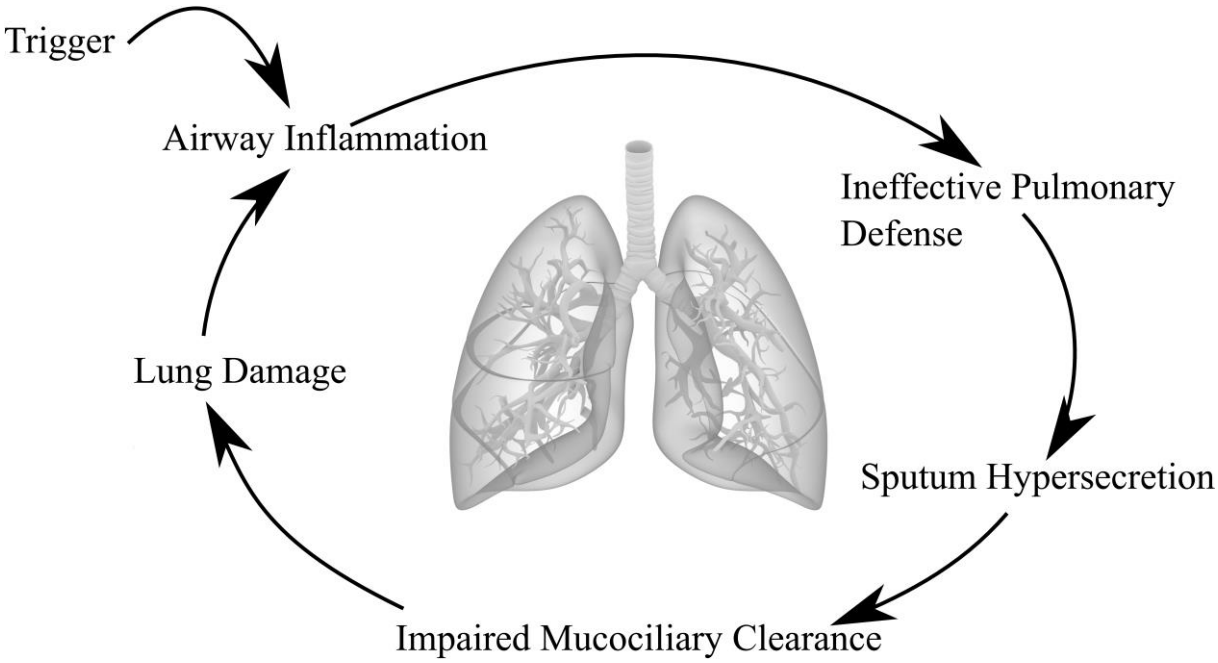
Interestingly, recurrent conjunctivitis is also commonly reported in XLA patients on treatment, affecting up to 8% of patients in previous cohorts, although this finding has yet to be repeated in modern cohorts (187).

While it is recognised that sinopulmonary tract infections are the most common infections in XLA, accurate annual infection rates are not known in large cohorts. In a recent pilot study, examining fifteen patients with congenital agammaglobulinaemia in northern England, the infection rates in adults and children post-diagnosis were quantified as 2.12 and 0.74 infections/patient/year respectively (268). The reason for this difference in infection rates in unclear but could represent a lingering effect of previous suboptimal care in the adult cohort (i.e. under-recognition of infections in their childhood). It should be noted these retrospective data are likely to be an underestimate and prospective data for CVID patietns has demonstrated an infection incidence much higher at 4.32 per year (269).

2.4.3 Pulmonary Health

The development of bronchiectasis is one of the clinician's and patient's greatest fears and is a leading cause of mortality and morbidity for XLA patients, diminishing their quality of life (186,270–272). Bronchiectasis is often progressive, refractory to treatment and may necessitate radical surgery or lung transplantation (268,273). Lung transplant is rare, but reported in XLA. However, outcomes appear poor. The addition of immunosuppression in patients with immunodeficiency will increase the risk of infection and, without correcting the underlying defect, the patient and their new lungs will still be at risk of developing bronchiectasis. A case series of 6 patietns demonstrated that 4 developed recurrent pulmonary sepsis and/or development of chronic lung disease (274). Bronchiectasis is defined as a permanent and abnormal dilatation of the bronchial airways (275). This leads to further increased susceptibility to infections with additional damage and inflammation, leading to a spiral of damage (Figure 2-2) (275)**.** In non-cystic fibrosis (CF) bronchiectasis, the 5-8 year mortality rate is reported as 10% and 13-year mortality as 30% (276–278), although the specific mortality for patients with XLA is unknown. Factors found to be independently associated with mortality in non-CF bronchiectasis are age, St. George's Respiratory Questionnaire (SGRQ) activity score (279), *Pseudomonas aeruginosa* infection and lung function (280).

Figure 2-2 The vicious circle of bronchiectasis, adapted from (281)



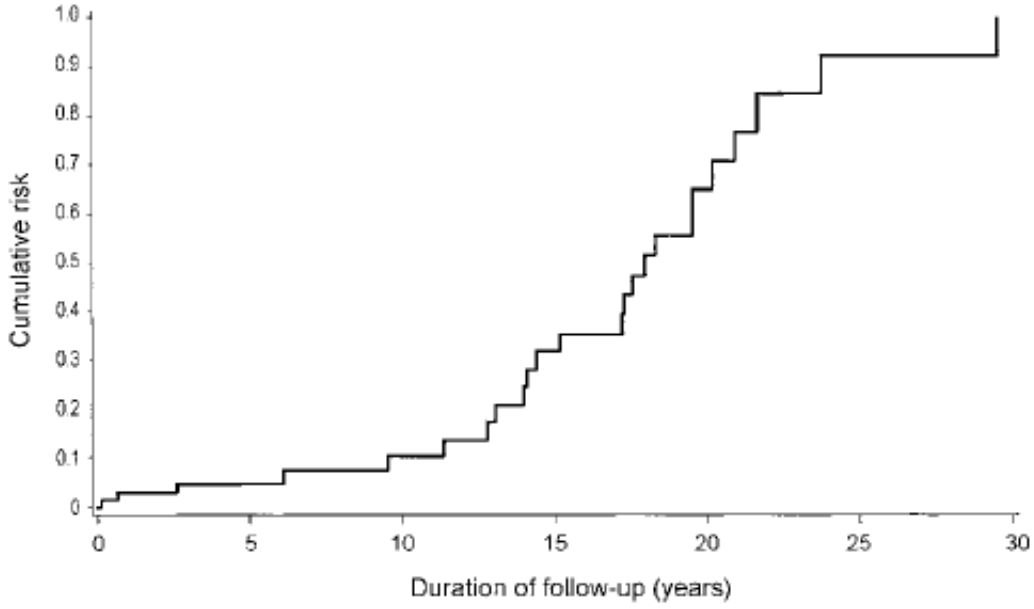
The problem of chronic lung disease in PID, especially primary antibody deficiency is well known. As soon as IV/SC immunoglobulin therapy became available, drastically reducing the frequency of severe acute infections and improving life expectancy, the potential problems of chronic lung disease in this cohort have been noted (167,168). Furthermore, it is well recognised that many patients may still develop significant lung damage despite the best current treatment (221,272,282–284). This is primarily because current IGRT products lack replacement of IgA and IgM, replacing only IgG. With IgA and IgM being important protectors of mucosal surfaces, it is perhaps logical to presume patient will still experience recurrent respiratory tract infections, even when on IGRT. IgG from IGRT is likely to reach small and large airways alike but may have a shorter half-life than serum IgG (285). Most published reports concentrate on the more common disease, common variable immunodeficiency (CVID), which has a distinctly different phenotype and genotype to XLA. Overall, rates of bronchiectasis in recent XLA cohorts range from 24% to 44% with median ages in those cohorts ranging from 9.4 years to 33 years (186,248,251,258,286).

It is logical to presume that delay in diagnosis and therefore, instigation of treatment would increase the risk of developing bronchiectasis, supported by primary antibody deficiency cohort studies (271,287). The large Italian XLA cohort found that an increasing age at diagnosis increased the risk of developing bronchiectasis before their XLA diagnosis, but did not increase the risk of developing bronchiectasis after the diagnosis of XLA (186). This may

suggest that the onset of bronchiectasis occurs due to infection pre-burden pre-diagnosis, emphasising the need for prompt diagnosis.

In this seminal study by Plebani et al. chronic lung disease was recorded in 24/73 XLA (33%) patients, and the authors calculated a 25-year post-diagnosis risk as high as 92% in their cohort from 2002 (median age 14 years, range 2-33) (186) (Figure 2-3).

Figure 2-3 Cumulative risk of developing chronic lung disease in relation to follow-up in Italian XLA patients (186)



Duration of follow-up	Cumulative risk	Standard error
5 yrs	0.05	0.03
10 yrs	0.07	0.04
15 yrs	0.32	0.08
20 yrs	0.65	0.10
25 yrs	0.92	0.07

For those patients who developed chronic lung disease after diagnosis, the only risk factor was a longer duration of follow-up. IgG trough levels, previous IM therapy or the age at diagnosis did not predict the development of chronic lung disease, once again hinting at limitations of current management (186). Indeed, the follow up in the healthy group was very short compared to the patients with bronchiectasis (5 years versus 15 years, $p<0.001$) and it would be interesting to see how the status of these healthy patients has progressed over time (186).

Interestingly, 70% of respiratory tract infections that required hospitalisation were from the nine patients who developed chronic lung disease after their diagnosis (186). Fifteen patients (21%) had developed chronic lung disease by the time of diagnosis of XLA. Compared to the

other 24 patients who also reported respiratory tract infections pre-diagnosis, these patients had a higher mean age of diagnosis (8.8 years versus 4.8 years, p=0.06). The authors chose only to analyse those patients with a presenting history of respiratory tract infections. As discussed earlier in this chapter, there are difficulties with validating presenting complaints and dealing with recall bias. These differences between patients with bronchiectasis and those without may have been different when including all 73 patients. However, the data strongly suggest that an earlier age of diagnosis and instigation of immunoglobulin therapy reduces the risk of bronchiectasis but that progression may still occur despite current therapy (186).

Howard et al. found that 13/41 US adults with XLA had chronic lung disease (mean age 30, range 21-57), mirroring the findings of Plebani et al. (258). Chronic lung disease in XLA is entirely made up of bronchiectasis. In contrast, patients with CVID can develop granulomatous-lymphocytic interstitial lung disease (GLILD), as they can develop lymphoproliferation as part of their underlying defect, which is not seen in XLA. The authors found age at diagnosis was not significantly different in those with chronic lung disease compared to those without, but this analysis excluded those patients who had an unusually late diagnosis of XLA in adulthood (258). Those with chronic lung disease reported more episodes of sinusitis and complained of more frequent shortness of breath (258). Seventy-one per cent of those with chronic lung disease reported at least one episode of sinusitis in the preceding 12 months and 61% reported a chronic cough (258). There are no data available in this cohort examining the relationship between IgG trough levels or infection incidence and the subsequent risk of developing bronchiectasis (258).

Fifteen of forty-three Argentinian XLA patients had chronic lung disease (using both chest radiography as well as computerised tomography for diagnosis), 14 of whom reported recurrent pneumonia or bronchitis (248). Recurrent lower respiratory tract infections (LRTI) and LRTI requiring hospitalisation pre-diagnosis were strongly associated with developing chronic lung disease before diagnosis ($p < 0.0001$ and $p < 0.0008$, respectively) (248). In this cohort, patients with chronic lung disease were diagnosed later than healthy XLA patients (6.5 versus 3.5 years, p=0.00001) (248). However, in this cohort, as well as further LRTI, inadequate therapy may have been a factor in the development of chronic lung disease (low IgG trough levels <400mg/dL, and the use of IMIg) (248).

A recent UK survey of 139 patients with agammaglobulinaemia (including 109 with a proven BTK genetic defect) reported that 56% developed bronchiectasis (median age 27 years, range

18-40) (286). The risk of bronchiectasis in this cohort was associated with previous pneumonia (RR 4.6, CI 1.8-11.8) and otitis media (RR 4.2, CI1.6-11.0) but not sinusitis (286). However, this was just a single reported previous episode of these infections and not the total burden of infections, nor the rates. It is therefore not possible to ascertain how the rate of infection correlates with the risk of bronchiectasis. Unsurprisingly, patients with bronchiectasis had a lower predicted FEV1 (74% versus 92%) (286).

There are no Z-scores for these results, so it is difficult to quantify the measure of the difference. Also, there are no data on changes in FEV1 over time. Using percentage predicted lung function scores rather than Z-scores has many limitations which will be discussed in further detail within the methodology section. In summary, predicted lung function scores do not take into account the normal distribution of values within a population. Therefore they cannot provide an accurate normal range of values and cannot be accurately be compared across time (288).

Forty-five per cent of patients in the UK agammaglobulinaemia survey were on subcutaneous immunoglobulin, and more patients currently on IVIg developed bronchiectasis than SCIg after adjusting for current age (RR 3.5, CI 1.2-10.1) (286). However, this analysis does not consider previous immunoglobulin routes. Bronchiectasis patients, on average, started therapy at an older age, but on multivariable analysis, only age higher than 18 years, history of previous pneumonia and intravenous therapy were significantly associated with bronchiectasis (286).

These data highlight the burden of bronchiectasis in this cohort and mirror those findings of the US and Italy (142,186). This cross-sectional data lacks detailed information on potential contributing factors to developing bronchiectasis, and it is, therefore, difficult to accurately define the risk factors for developing bronchiectasis. Furthermore, there are no data on previous IgG trough levels and how this relates to therapy, infection rates and may subsequently alter the risk of bronchiectasis,

Bacterial pathogens are repeatedly isolated from airway secretions from primary antibody deficiency patients, despite treatment. Some of these isolates are taken during asymptomatic periods (289–291) and are particularly concerning. Data from CVID patients demonstrate a ‘silent progression’ of lung disease, even in the absence of overt respiratory tract infections (292). Although largely thought to be able competently to tackle viruses, recent work has demonstrated patients with primary antibody deficiency do indeed suffer from recurrent and

persistent respiratory viral tract infections, and it is, therefore, possible that these viral respiratory exacerbations may be contributing to the development of chronic lung disease (184).

High-resolution computerised tomography (HRCT) is the gold standard for diagnosing bronchiectasis and is often used to monitor disease although there are no agreed guidelines to dictate how frequently this should occur. There needs to be a careful balance with the need to monitor lung disease and the concerns of repeated exposure to ionising radiation (293). There is increasing evidence to suggest magnetic resonance imaging (MRI) could be a viable alternative imaging modality, although there remain obstacles such as scan resolution and availability of resources (294). Both MRI and HRCT still provide some barriers to monitoring disease in infants and young children due to the need for general anaesthesia (295,296). Although limited in its ability to detect early lung disease, lung function investigations play a vital role in monitoring progression and are usually performed on an annual basis in patients with respiratory symptoms or proven lung disease (297). However, lung function can be unreliable in children less than six years of age (298).

There is currently no agreement on the frequency of HRCT employed to diagnose or monitor lung disease (299). A recent European survey showed that many clinicians agreed that adults should have a baseline HRCT scan, but there was no consensus for paediatric patients (299). There was no consensus on further imaging for follow up, but previous work would suggest HRCT every 2-4 year for patients with proven disease and less frequently for those with no disease (221,222,300,301). The need to monitor disease and not miss bronchiectasis must be balanced with radiation risk (302). This risk is particularly pertinent to children, but HRCT still plays an essential role in monitoring disease (303). Overall, children tend to be imaged less than adults, most likely due to these reasons above (299). There is a lack of local, national and European guidelines for screening and treating lung disease not just in primary antibody deficiency but PID as a whole (299).

Furthermore, there are no standardised protocols for the use of biomarkers (e.g. sputum neutrophil elastase, catalase activity, lipid peroxidation) to predict disease course, complications nor to record the presence or progression of lung disease (283,304,305). Quinti et al., have developed and validated a QoL questionnaire for CVID patients which does capture some of the impacts on QoL made by respiratory health and shows strong correlation with the SGRQ (306). However, work attempting to use this questionnaire in other PADs, including XLA, demonstrate that this tool may not capture all aspects of QoL in PAD patients

and further evaluations are needed to assess its perforamnce in PAD (307). The SGRQ could be a useful tool in this cohort (270,308) as would the bronchiectasis severity index (309). However, further work is needed to validate these tools in patients with PID or XLA. These barriers and lack of agreed guidelines have resulted in considerable variation in the monitoring and screening of bronchiectasis in primary antibody deficiency (299).

There are scant data assessing the severity or progression of bronchiectasis once it has developed in patients with XLA, despite the worsening of chronic lung disease being of paramount concern to patients as shown in cohorts of CVID (310). There are data in primary antibody deficiency to suggest that airway and systemic inflammation (as measured by CRP, IL-6 and IL-8), play a significant role in FEV1 decline and QoL in this cohort. It may be appropriate to develop strategies to reduce airway inflammation in these patients (270). It should be noted that the primary antibody deficiency cohort was heterogeneous, mainly examining CIVD patients and therefore, caution must be taken when extrapolating these results to patients with XLA (270). One further study with a small number of XLA patients found rates of decline in FEV1 in those with bronchiectasis to be more rapid than for smokers and patients with CVID (65 vs 30 and 36mL/year respectively) (215).

The Northern England pilot study showed 8/15 patients with CT-proven bronchiectasis (median age 26 years, range 5-46). Six of these patients demonstrated evidence of deterioration on either HRCT or lung function testing. This was despite adequate IgG trough levels, with two patients requiring pneumonectomies and one patient in the cohort progressing to end-stage lung failure and undergoing lung transplantation (268).

Currently, the only method to prevent bronchiectasis or reduced lung function decline is to reduce the frequency of sinopulmonary tract infections through optimisation of immunoglobulin therapy. A lower IgG trough level is associated with worsening lung disease, but there are no data to suggest an optimal level to preserve lung function (292). Previous work suggests that while increasing IgG trough levels exerts no protective effect, increasing doses of IVIg can play a protective role in reducing the decline in FEV1 (311), but this needs to be confirmed on a larger scale.

Once bronchiectasis is established, there is the potential use of inhaled corticosteroids, β agonists, prophylactic antibiotics, higher IgG trough levels, physiotherapy but minimal data support these modalities, especially in the primary antibody deficiency cohort (281,312–314). Mucous clearance is an essential part of bronchiectasis management and physiotherapy is a

standard adjunct to therapy in bronchiectasis (315,316). Mucolytics are also widely used, although the evidence base supporting their efficacy is limited. A Cochrane review in 2014 demonstrated that, while there may be some linted data supporting the use of some mucolytics such as bromhexine and erdosteine, other mucolytics should not be recommended in non-CF bronchiectasis, such as recombinant human DNase due to no evidence of benefit and potential harmful effects (317) . There are very scant data examining the use of mucolytics in children, and further work is needed to evaluate the use of mucolytics in non-CF bronchiectasis. Although a wide range of manoeuvres exists, there is a lack of published literature examining the best method and therefore no international or nationally agreed guidelines as to the best physiotherapy approach in bronchiectasis (318).

Prophylactic antibiotics may play a role in preventing infection, although practices regarding prophylactic antibiotic use in XLA vary significantly (297). A recent UK survey demonstrated that 54% of patients with agammaglobulinaemia took regular prophylactic antibiotics, even in the absence of lung disease (286). This proportion rose to 64% in those with lung disease (286). This variation in practice is confirmed in a recent European survey examining the practice of monitoring lung disease in PID (299). While many centres administer prophylactic antibiotics for patients who have developed bronchiectasis; there are few data to prove its effectiveness specifically in primary antibody deficiency or XLA (311). However, recently Milito et al., have published an RCT examining the use of prophylactic antibiotics in PAD (319). They enrolled 89 patients with PAD, assigning 44 to the treatment arm and 45 to the placebo arm (319). Treatment consisted of Azithromycin 250mg 3 times a week for 2 years (319). These data showed a clear benefit for the use of prophylactic azithromycin with the hazard risk for having an acute exacerbation in the azithromycin group of 0.5 (95% CI, 0.3-0.9; $p = 0.03$), and the hazard risk for hospitalization was 0.5 (95% CI, 0.2-1.1; $p = 0.04$) (319). The authors did note that whilst *Haemophilus influenzae* and *Streptococcus pneumoniae* were the commonest organisms isolated, as expected, there was resistance to macrolides in 25% of patients of both arms (319). The safety profile of azithromycin and placebo was comparable (319). For non-CF bronchiectasis, the most studied and effective prophylactic antibiotic is Azithromycin (250mg daily or 500mg three times a week for adults) (320). A few studies suggest clarithromycin or erythromycin as alternatives (320). However, caution must be taken when interpreting data from this heterogeneous non-CF cohort transferring these findings to the XLA cohort. Recent work demonstrates that primary antibody deficiency patients with bronchiectasis appear to have

greater airway inflammation compared to their immunocompetent counterparts despite similar severity of appearance on HRCT (270).

There are increasing data to suggest that despite more aggressive IgG therapy and the use of prophylactic antibiotics, some patients still develop bronchiectasis and that the bronchiectasis can progress further in its severity (258,321,322). However, there are scant data specifically examining XLA patients in large numbers or using more accurate measures of monitoring lung disease progression such as FEV-1 Z-Scores.

2.4.4 Autoimmunity and Inflammation

Many primary immunodeficiencies (PIDs) have an associated risk of autoimmunity and inflammatory disease (323). Inflammatory disease in XLA is conventionally thought to be infection-related, and so, with adequate immunoglobulin therapy, it has been accepted that patients with XLA are spared these complications (167,324). However, as this chapter has discussed, patients may still be experiencing recurrent infections despite modern therapy. In addition, with the potential loss of inflammatory regulation caused by the lack of B-lymphocytes, there is a possibility that this cohort may be at risk of these complications. Data from the USA demonstrates that 69% of XLA patients report at least one inflammatory symptom, 53% report multiple inflammatory symptoms and 28% of patients have been formally diagnosed with an inflammatory disorder (324).

Data from the United States Immune Deficiency Network (USIDNet) registry demonstrated 12% of XLA patients reporting arthralgia or joint swelling, with 16% having a diagnosis of arthritis (324). However, some of these may have an underlying infectious cause rather than a solely autoimmune process (e.g. Mycoplasma and Ureaplasma species) (325).

Gastrointestinal manifestations represent a significant, and perhaps under-recognized burden on this population. Further analysis of the USIDNet registry reported that 35% of XLA patients experienced gastrointestinal complications ranging from recurrent infections to inflammatory bowel disease (IBD) (326). Up to 10% of patients within this registry are formally diagnosed with IBD or enteritis (326). The most commonly reported symptoms were abdominal pain, diarrhoea, weight loss, nausea, and vomiting (326). For some patients, GI disease and/or IBD like symptoms may be the main presenting symptoms of XLA (326). Commonly isolated pathogens within stool samples in this registry are typical of the immune defects in XLA; *G. lamblia, Salmonella, Campylobacter, Cryptosporidium,* mycoplasma and enterovirus (326). How much of the GI disease in XLA is secondary to infection and how

much is due to genuine inflammatory or autoimmune processes remain unknown. IVIg has been identified as of potential benefit for the treatment of Crohn's disease and modification to standard therapy may be of some benefit in XLA patients (327,328). The microbiome is postulated to play a major role in the phenotype of IBD patients, with increasing research examining the role of potential management strategies to alter the microbiome such as probiotics, prebiotics, antibiotics and faecal microbiota transplantation (329). There are no data on the GI microbiome in XLA patients, and this would be a useful target for future research. Overall, there is scant literature examining the benefit of potential management strategies for GI disease in this cohort.

2.4.5 Malignancy

Rates of malignancy in XLA have been reported to be between 1.5 and 6%, with patients most likely to develop lymphoproliferative disorders (e.g. AML, B-precursor ALL), gastric cancer and colorectal cancer (232,330–333). This risk may be related to chronic infections, although this needs to be further studied in a large cohort with modern management and longer life expectancy (334). It is possible that, as we enter an age with increasing longer-term survival, the risk of malignancy in XLA will become more apparent.

A review of GI carcinoma in XLA reveals a young-onset (median 30 years (range 7-40)) (335). This compares to the mean age of onset of GI carcinoma in the background US population of 69 years (335). There are limited data on the exact clinical background of these patients but over half had persistent IgG trough levels <7g/L which would likely to be considered suboptimal (335). In addition, one third had proven chronic GI infection with the majority suffering from chronic atrophic gastritis (335). It should be noted that while the low IgG trough levels could represent inadequate therapy, they could also be as a result of increased GI losses or increased IgG catabolism from chronic inflammation and infection. The authors of this study and subsequent reviews have questioned whether there is a role for GI malignancy screening in this cohort (335). It may also be useful to examine baseline and serial values of faecal calprotectin in this group, for which no current data exist, as a marker of GI inflammation and risk of developing GI disease.

2.4.6 Other Clinical Complications

Approximately 3-5% of patients with XLA have large deletions at the 3' end of BTK and the closely linked TIMM8A gene (also known as DDP) (336,337). This contiguous gene deletion results in the individual having XLA Mohr-Tranebjaerg syndrome (336). The diagnosis of XLA typically precedes any deafness, and the deafness may be wrongly thought to be

secondary to recurrent otitis media. Deafness in XLA is common and most are secondary to central nervous system infections before diagnosis. Although data on recent cohorts are lacking, Lederman at al. reported 32% of the US cohort experienced hearing loss as a result of CNS infection (167).

In addition to IBD like disease seen in XLA, there is also a case report of chronic Norovirus infection in a patient with XLA (185). In addition to this case, this PhD study also revealed another XLA patient with chronic Norovirus infection. Chronic Norovirus infection is well documented in CVID (338), but not previously thought to be a problem for XLA patients due to prevailing opinion that these patients are competent to fight viral infections (with the exception of enterovirus). The potential impact of chronic Norovirus will be discussed further within the result and discussion sections of this thesis as a relatively novel finding and opinion.

Despite these well documented GI complications, data on their nutritional impact are lacking in the literature. However, recent work examining the overall nutritional status of the agammaglobulinaemia cohort found that obesity was a more pressing concern rather than faltering growth in keeping with the general population (339). Dellepiane et al. examined the Italian agammaglobulinaemia cohort and found 38% of patients were overweight or obese and 5% were underweight (339). In the US cohort, 18% of patients are obese, 32% overweight and one patient was underweight (258).

2.4.7 Quality of Life

Quality of life (QoL) is an increasingly important measure and research tool in the assessment of disease and treatment burden on patient's lives, particularly in the context of chronic disease (340). Previous research assessing QoL in primary immunodeficiencies and their treatments have played vital roles in developing optimum treatment strategies (340). The first publication of QoL in primary antibody deficiency was in 1993 with numerous studies published since (341,342). However, primary antibody deficiency covers a wide range of clinical phenotypes, and there may be significant differences in QoL amongst individual diseases. Even after grouping several disorders, the resulting sample sizes are small. Furthermore, a great deal of the published literature is mainly focused on the impact of varying immunoglobulin therapy strategies on QoL.

There are ongoing and significant efforts to improve QoL research in primary antibody deficiency and PID. For example, specific QoL surveys for PID, primary antibody deficiency

and CVID have very recently been published and have begun to be validated (306,343,344). However, these surveys are designed for heterogeneous cohorts and/or focused on CVID, which represents the biggest burden by the number of patients within primary antibody deficiencies. Furthermore, these specific tools lack the ability (currently) to compare patients with the healthy population or other disease cohorts. However, they are an important step forward in monitoring QoL in PID and could form an essential role in the ongoing clinical review of patients by measuring QoL longitudinally.

Generally, all QoL surveys in primary antibody deficiency agree that overall QoL is lower than age-matched healthy controls and even some other chronic diseases, including cancer (345). In addition, QoL appears to decline over time and the risk of developing psychological distress increases (346). However, these studies are small, often focus on CVID, and their findings, therefore, may not be entirely transferable for XLA.

Forty-one per cent of US XLA patients have not been hospitalised since their diagnosis, and 83% described their health as good or excellent, highlighting the progress that has been made in the management of this disease and PID in general (258). 86% reported missing fewer than ten days of work or school in the preceding 12 months, with 44% reporting missing none (258). Forty out of forty-one patients had adequate insurance, but nineteen of these had difficulty obtaining it (258). However, 20% of patients reported missing appointments or not keeping up with immunoglobulin therapy because of difficulties with their health care insurance (258). This highlights some of the difficulties in transferring findings from US cohorts to the UK with its nationalised health service and improved access to health care and lack of (relative) financial barriers.

American adults with XLA have lower QoL scores on the 12-item short-form version 2 (SF12v2) compared to the background healthy US population, although not statistically significant. Half of USA patients stated they have or would, in the future, undertake a prenatal diagnosis. Interestingly, in a separate questionnaire, patients reported that it was the treatment, rather than the disease that placed the highest-burden on their quality of life and daily living (258). A third of patients reported that having XLA affected their social activities, sleep or normal physical exertion (258). In addition, over half the US cohort in this study by Howard et al. reported that XLA affected their ability to travel and choose their career (258). However, the impact on employment was mainly driven by the ability and need to get adequate health insurance (258). Different findings may, therefore, be seen in the UK

population with access to universal healthcare. The second reason was the impact of the treatment (rather than being unwell) (258).

There appear to be more significant reductions of QoL in the paediatric population. Titman et al. showed that UK paediatric patients with primary antibody deficiencies have worse QoL scores compared to the healthy population and patients with diabetes mellitus (347). In particular, children scored disproportionately worse on psychological and emotional aspects, suggesting that mental and emotional health is more severely affected than physical health (347). However, when only analysing the XLA patients in this cohort, the results suggest the children scored similar scores to healthy norms, but the parents consistently reported lower scores. There were only 5 XLA patients in this study, so it is difficult to come to firm conclusions, nor is it possible to carry out statistical analyses (347). Due to the small sample sizes, the authors were unable to correlate QoL with aspects of disease and treatment burden.

Soresina et al. in their larger sample size of 25 Italian paediatric XLA patients found more statistically significant differences (348). In all domains of the PedsQL 4.0, aside from physical health, children and parents reported worse scores than healthy norms (348). Their physical health was reported as slightly lower but not statistically significant (348). Importantly, they scored consistently higher on the physical health domain than children with rheumatic arthritis (348). There was no association with QoL score and socioeconomic status or disease severity (according to clinician self-reporting) (348). These findings seem logical given children diagnosed in recent years benefit from earlier diagnoses and more aggressive immunoglobulin therapy (214), but still have to deal with the impact of treatment and from feeling 'abnormal' which is a well-recognised phenomenon for parents and children with PID (340).

A recent paper examining 60 children with primary antibody deficiency on SCIg (albeit with one XLA patient) also tried to quantify these differences in PedsQL 4.0 QoL scores (349). They did this by using Cohen's *d* effect, a commonly used method in psychology research (350). The authors found that children had medium-sized lower QoL scores than healthy norms and children with diabetes and these scores were similar to those children with cancer (349). They found that the school, social and physical subsections were affected most and the emotional subscale affected the least (349). The QoL scores in this cohort were similar to that found in other PID QoL studies helping validate the results (349). Also, the authors found that children and parents both had a low perception of the effectiveness and convenience of their treatment compared to patients with cystic fibrosis (349). The authors have grouped

both proxy and self-reports, so it is not possible to analyse any differences between children and parents (349).

The northern England pilot study showed QoL scores for eight adults with XLA, using the SF-36v2, were not statistically significantly different compared to that of the UK population, except for the subsection 'General Health' which was significantly lower compared to that of the UK population (59.5 vs 78.37, $p < 0.05$) (268), although the small sample size means these results should be interpreted with care.

Given the impact of respiratory disease in this cohort, it would be logical to presume this would contribute greatly to QoL scores, yet surprisingly this is an area rarely covered in the published literature. In 7 XLA patients, Hurst et al. have shown that SGRQ scores and SF36-v2 scores are highly correlated ($r=-0.79$, $p < 0.001$), suggesting that QoL is strongly influenced by the impact of respiratory disease and symptoms (270). The northern England pilot study showed total SGRQ scores significantly higher (i.e. worse) than the background healthy population (17.21 vs 2.72, $p = 0.005$) (268). Interestingly, the SGRQ symptom subscore for those seven patients without proven respiratory disease was still significantly higher than the background healthy population (25.08 vs 4.24, $p=0.028$) (268). In addition, the impact sub-score was also significantly higher (2.74 vs 0.00, $p = 0.043$). Although the total SGRQ score for patients without proven lung disease tended to be worse than the healthy population, this did not reach significance (8.38 vs 2.72, $p=0.116$) (268). This may indicate that patients without proven lung disease still have a significant QoL impact from respiratory symptoms because of their disease.

Although an increasingly important measure of outcome, published data on QoL in XLA is still scarce. Most current knowledge is extrapolated from small cohorts or other PIDs. Further work is needed to examine QoL in XLA, including the impact of respiratory disease.

2.4.8 Psychological Health

Congenital agammaglobulinaemia is chronic, incurable and is associated with a high risk of complications (186). Chronic disease not only affects physical health but can impact upon future emotional and social well-being (351). When patients are able to maintain a healthy emotional balance, have healthy social interactions and feel they are contributing to society, they can adjust to living with a chronic disease (352). Living with a chronic physical disorder has been shown to increase the risk of depression, anxiety, obsessive-compulsive disorder and attention deficit disorder (353,354).

In the Titman et al. study of 19 UK children with primary antibody deficiency (including 5 with XLA) parents reported significantly higher proxy rates of psychological difficulties compared to healthy children (347). The children themselves reported higher rates of psychological difficulties compared to their peers, but these differences did not reach significance (347).

In the USA, 25 adults with XLA showed good adjustment with fifteen in long-term relationships, twenty patients have graduated from college, and 20 were employed or self-employed (262). However, there is a lack of precise data on the clinical outcomes, and so it is not possible to correlate health status with psychological health or social adjustment. Within the 2006 cohort by Howard et al., patients with chronic lung disease scored significantly worse on the mental health component (258).

2.4.9 Autosomal recessive agammaglobulinaemia

XLA accounts for 85% of all congenital agammaglobulinaemia cases. Approximately 2/3rd of the remaining 15% are female, offering clues to early researchers that autosomal recessive disorders of B-lymphocyte development may also occur (25,176,355).

30% of autosomal recessive agammaglobulinaemia (ARA) cases are caused by mutations in the μ heavy constant region gene, *IGHM*, first described by Yel et al. in 1996 (27,356). Other genes in which defects in can lead to congenital agammaglobulinaemia are B-cell linker-adaptor protein (*BLNK*) (357), immunoglobulin λ-like polypeptide (*IGLL1*) (358), leucine-rich repeat contained 8 (*LRRC8A*) (359), B-Cell Antigen Receptor Complex Protein Alpha Chain (*CD79A*) (360–362), B-Cell Antigen Receptor Complex Protein Beta Chain (*CD79B*) (363,364) and in the Phosphoinositide-3-Kinase Regulatory Subunit (*PIK3R1*) (365). Due to the rarity of these individual causes of ARA, there are no sizeable detailed cohort studies. However, it is well recognised that patients with ARA tend to suffer from a more severe phenotype of agammaglobulinemia than their X-linked counterparts, often with a younger age of onset (27,366). Patients with ARA are treated similarly to XLA.

2.5 Genotype/Phenotype Correlation

There is a wide range in the clinical phenotype of XLA, even within the same family whose members share the same mutation. For example, some patients continue to be diagnosed late in adulthood, or even only after a grandson or nephew has been diagnosed with XLA (after leading a relatively healthy life) (20,22,367).

There is a wide range of BTK mutations causing XLA with over 500 different mutations now reported, and with no single mutation accounting for more than 3% of patients (368). A genotype/phenotype correlation has long been sought after to help offer a more accurate diagnosis or offer more aggressive therapy to those who might require it. Evidence for some geno/phenotype correlation is supported by the well-recognised feature that some patients with XLA can have residual circulating IgG/IgM and even a small number of circulating B-lymphocytes due to the leaky nature of their B-lymphocyte differentiation defect (369).

Efforts so far to establish an accurate and predictive clinical correlation with BTK mutation have been disappointing. A contributing factor to this is difficulty in defining severe or mild mutations in BTK. The majority of mutations in BTK result in the absence of BTK protein expression in platelets and monocytes (370–372). However, some amino acid substitutions do allow either small amounts of normal BTK protein to be produced, and some splice defects allow the BTK protein to be produced but with reduced function (373–375).

Conley and colleagues have produced most of the reliable published data examining genetic defects in XLA. Based on their expertise and experience, their definition of severe and mild mutations are shown in Table 2-2 (187). These definitions are widely used in modern studies examining genetic defects in XLA.

Table 2-2 Definitions of mild and severe mutations by Conley et al., (187)

Mild mutation	Severe mutation
Amino acid substitution at non-conserved sites	Amino acid substitution at conserved sites in other members of the Btk family (Itk, Tec)
Splice-site defects at conserved base pairs but not invariant	Frameshift mutations
	Splice site alteration at invariant sites within the first two and last two base pairs of an intron
	Premature stop codon
	Inframe deletions

From 110 US patients, 36.4% had an amino acid substation, 25.4% splice defects, 10% frame shifts and 15.5% premature stop codons (368). Using these severity definitions, Broides et al. demonstrated that the percentage of patients diagnosed with mild mutations increased with age (p=0.04) (368). In patients diagnosed in the first year of life, 29.4% had a mild mutation, whereas those diagnosed after five years, 56.2% had a mild mutation (368). The authors found a correlation between the mutation severity and plasma IgM levels (368). Patients with mild mutations were diagnosed later, had a higher percentage of circulating B-Lymphocytes and higher plasma IgM levels (368). However, there are no data correlating current clinical outcome (e.g. infection rates, complications) with mutation, and the authors concede that correlation is not strong enough to predict prognosis based on the mutation type (368).

Studies from Spain mirror these associations between mutation type and age at diagnosis, plasma IgM levels, and circulating B-lymphocytes (376). They found a trend for fewer hospital admission pre-diagnosis amongst patients with mild mutations (p = 0.04) (376). The authors note that in their 54 patients, there was a range of clinical phenotypes among patients with the same mutation, including within the same family (376).

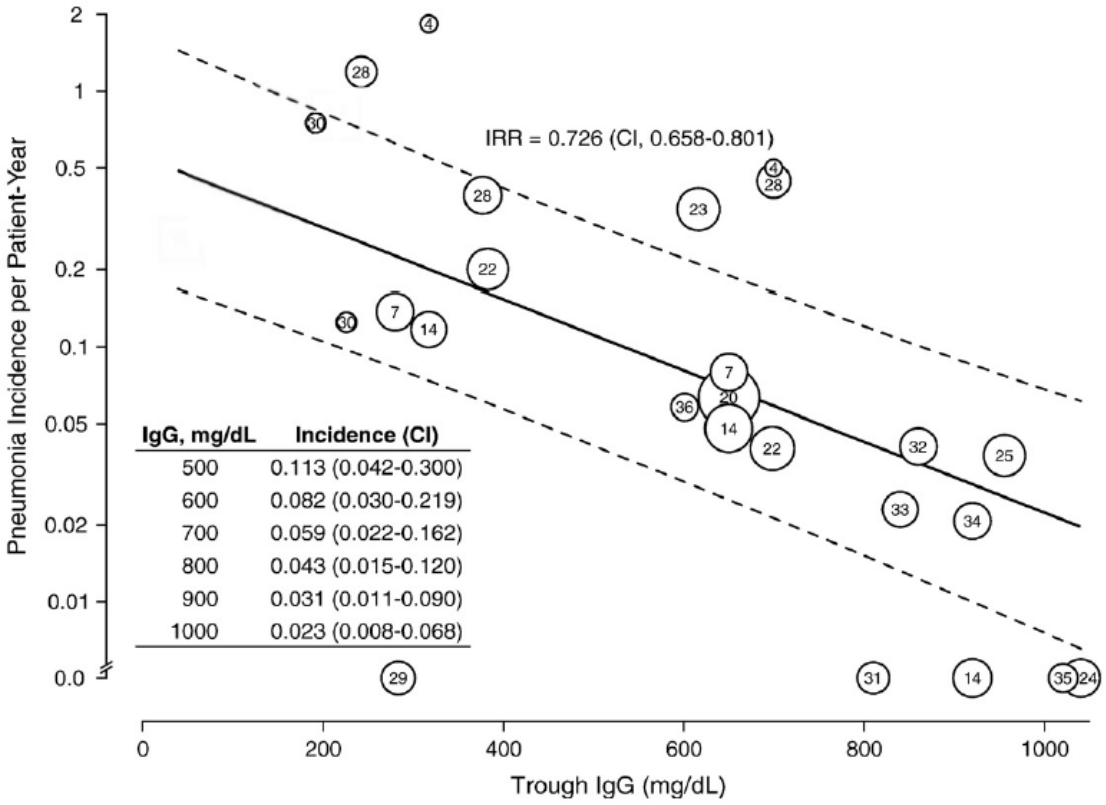
In summary, while there is not a strong genotype/phenotype correlation in XLA, there is evidence of a correlation with age at diagnosis, which may be a marker of disease severity (377–381).

2.6 Immunoglobulin Therapy

2.6.1 Effectiveness

The effectiveness of modern IVIg and SCIg perpetrations and therapies in the management of patients with antibody deficiencies is well established. A meta-analysis from 2010 examined the relationship between trough IgG levels and the incidence of pneumonia in 676 patients with antibody deficiency, including 253 patients with XLA, but only those patients on IVIG, excluding those on SCIG (382). This study found a fivefold protection against pneumonia with trough levels >10g/dL compared to 5g/dL (382), supporting contemporary guidelines which advocate the use of higher trough levels (Figure 2-4) (382).

Figure 2-4 Adapted from a meta-analysis from Orange et al. showing the effect of IgG levels (mg/dL) on pneumonia incidence per patient-year (382)**.** *Each data point corresponds to a single study. Data points are labelled by the referencing in the original article by Orange et al. Solid line shows multilevel model predictions and dashed lines indicate the 95% CI of metaregression. Abbreviations: CI, 95% confidence interval; IRR, incidence rate ration per 100mg/dL increase in trough IgG level.*



In contrast, a study examining 100 patients with CVID and 101 patients with XLA (not included in the above meta-analysis) found no significant difference in IgG trough levels between those who developed pneumonia and those that did not (222). There was an increased incidence of pneumonia with IgG trough levels below 4g/dL, a target which is not relevant to modern management of XLA (222). The major risk factors for pneumonia in this study were low IgA and IgG at diagnosis, current IgA levels <7mg/dL and those who have bronchiectasis (222). The downward spiral patients enter once they develop bronchiectasis emphasises the importance of preventing pneumonia as early as possible.

Many of these studies include only small numbers of patients with XLA or ARA. Furthermore, they concentrate solely on the number of infections that require hospital admission, which will tend to be more severe infections compared to outpatient infections. The effectiveness of IgG therapy on infections that require hospital admissions is well known. Indeed, this has been specially researched in XLA with infections requiring hospitalised falling from 0.09 per month to 0.01 month after the instigation of IVIg ($p<0.001$) (251). Data

on infections treated as an outpatient are lacking in this cohort, which may still be frequent and leading to significant morbidity for these patients.

Chan et al. found those XLA patients with IgG trough levels >700mg/dL tended to have fewer overall complications, fewer rates of bronchiectasis and better lung function (p=0.041) (256). No patients with IgG trough levels >700mg/dL had bronchiectasis or impaired lung function although it is unclear if these IgG data are cross-sectional or retrospective (256).

There is no evidence or guideline available to dictate Ig therapy once bronchiectasis has developed, although most clinicians will generally target a higher trough IgG level to prevent further sinopulmonary infections (383). In addition, patients with bronchiectasis often need higher IgG doses than those without bronchiectasis, to achieve similar IgG trough levels (181,384). Gouilleux-Gruart et al. suggest that the presence of bronchiectasis affects IgG recycling though FcRn expression and local catabolism (385) and Litzman suggests this effect could be directly proportional to the severity of bronchiectasis (386). It has also been shown that these effects in pharmacokinetics are more prominent in those patients on intravenous compared to subcutaneous therapy, highlighting a possible increased efficiency of SCIG versus IVIG (384,385).

It is possible to administer immunoglobulin with recombinant human hyaluronidase (facilitated SCIg (fSCIg)). This combination allows much larger volumes to be given at a single site (>600ml) at singe meaning fewer needles, infusions and an adverse event profile similar to SCIg with improved bioavailability (211,387).

Recent work from a French cohort, showed there were no differences in QoL when comparing PID patients on home, hospital SCIg or IVIg (388). However, they found that QoL was impaired across all subgroups compared to healthy norms (388). Although this group included five patients with XLA (of 116 patients), these findings highlight the importance that route and place of immunoglobulin therapy are unique to each patient (388). Some patients may prefer hospital-based therapy, as in this cohort. Thirteen per cent of patients changed either the route or the site of their therapy in the preceding 12 months, although there are no data to determine if this was for personal or clinical reasons (388). The overall rate of serious infections was 0.19/patient year (95% CI 0.08-0.46), which is somewhat higher than the expected rate for solely XLA patients, highlighting the heterogeneous nature of this cohort and the possible limitations in extrapolating these finds to the XLA and ARA cohorts (388).

2.6.2 Limitations

As discussed in Chapter 1, current IGRT lacks replacement of IgA and IgM, consisting only of IgG. With IgA and IgM both playing vital roles in protecting mucosal surfaces, it would be logical to presume patients will still experience recurrent infections on these sites. The most pertinent risk would be that of recurrent respiratory tract infections and the subsequent risk of bronchiectasis. Recent work from Hodkinson et al. demonstrating primary antibody deficiency patients with a low IgA and IgM are at increased risk of bronchiectasis, supports this proposed risk (389).

Patients with Hyper IgM syndrome (e.g. CD40L deficiency) have a statistically significant lower rate of non-typeable *Haemophilus influenzae* carriage compared to patients with XLA (relative risk 0.39, 95% CI 0.21-0.63) (390). Those patients with hyper IgM were able to generate antibodies against non-typeable *Haemophilus influenzae* in their saliva and blood (390). These findings were clinically significant, with hyper IgM patients suffering from fewer acute sino-pulmonary tract infections (relative risk 0.38, 95% CI 0.22-0.68) (390).

Further work demonstrates that patients with isolated IgA deficiencies who, despite normal IgG and IgM levels, experience four times as many respiratory, gastrointestinal and skin infections compared to matched healthy controls, increasing their risk of serious long-term complications (391).

Recent advances in the optimisation of immunoglobulin therapy have had a positive impact upon patient's lives, but treatment is limited in efficacy to reduce infection rates given the lack of IgA and IgM in current products. These isotypes play a major role in protecting the mucosal surfaces, most notably the sinopulmonary tract (219,390). Without their replacement, it would be logical to presume patients will continue to experience recurrent sinopulmonary infections as suggested by the evidence above (186).

2.7 Newborn Screening

Plebani et al., demonstrated that while the age at diagnosis has reduced dramatically over the preceding 20 years, this improvement is now reaching a plateau at between 2 and 3.5 years (13,14). Newborn screening, via a dried blood spot on day 5 of life, is a well-established practice, screening for life-long and potentially life-threatening conditions where early diagnosis and/or intervention has significantly improved outcome (392). Newborn screening would allow diagnosis of XLA and instigation of treatment at a pre-symptomatic phase.

Newborn screening for SCID, using TREC analysis is established or soon to start in 7 countries worldwide, including the USA, enabling diagnosis and curative treatment before serious infections develop, significantly improving outcomes (393,394). It is currently being considered for inclusion in the UK newborn screening programme (393).

A further 25 countries are carrying out pilot programmes and/or applying for newborn screening using TREC analysis to be included within the national program (394). Twelve of these counties are planning or are using a combined TREC/KREC screening program (394).

Barbaro et al. recently published the most extensive combined TREC/KREC screening study examining 58,834 newborns (395). From these, 64 were recalled with three being diagnosed with PID (395). The vast majority of these recalls were due to the abnormal KREC levels (49/64) (395). In the majority of recall cases on retesting, the low TREC/KREC levels normalised or were attributed to transient causes such as maternal immunosuppression or prematurity (395). The authors note that a correct cut-off level of KREC levels for children with congenital agammaglobulinaemia is not known and, with further studies, the cut-off KREC level could be further optimised reducing the recall rate further (395).

The authors have also have estimated that adding KREC measurement onto an existing TREC screening programme would involve minimal additional costs due to multiplex PCR reactions (<€0.10 per new-born) (395). As well as allowing the screening of XLA, a combined TREC/KREC screening programme would also allow the identification of PIDs which may otherwise be missed by a TREC analysis alone (such as late-onset ADA and Nijmegen breakage disorder) (396–398). It may also help distinguish those SCID patients with and without B-lymphocyte production, further helping the diagnostic process for these patients.

Introducing a new test into any newborn screening programme would necessitate the meeting of strict criteria, often using an amended version of the Wilson-Jungner criteria (Table 2-3)(399,400). However, the impact of pre-clinical diagnosis on later prognosis in congenital agammaglobulinaemia is unknown.

Table 2-3 UK Public Health England criteria for appraising the viability, effectiveness and appropriateness of a screening programme (400)

The condition	The condition should be judged a significant health problem
	All cost-effective primary prevention interventions have been implemented
	The natural history and implications of carriers should be understood
The test	There should be a simple, safe, precise and validated screening test.
	The distribution of test values in the target population should be known.
	The test should be acceptable to the target population.
	There should be an agreed policy on further diagnostic investigation
	Methods for the selection of genetic variants will be kept under review
The intervention	There should be an effective intervention for patients identified
	There should be agreed evidence-based policies covering which individuals should be offered interventions and the appropriate intervention to be offered.
The screening programme	There should be high-quality evidence that screening programme is effective
	There should be evidence that the complete screening programme is acceptable to health professionals and the public
	The benefits of the screening programme should outweigh any harms
	The cost should be balanced with expenditure on medical care as a whole
Implementation criteria	Clinical management should be optimised in all health care providers prior to participation in a screening programme.
	Other options for managing the condition should have been considered
	There is a plan for managing and monitoring the screening program.
	Adequate resources should be available prior to commencement
	Evidence-based information should be made available to potential participants
	Public pressure for widening the eligibility criteria is anticipated.

2.8 Gene Therapy

There are several studies demonstrating a proof of concept for the utility of gene therapy in XLA in BTK-deficient mice (401–404). However, it should be noted that BTK deficient mice tend to have a milder phenotype than their human counterparts (369). Initial retroviral vectors have demonstrated considerable and sustained B-lymphocyte function, immunoglobulin production and T-independent type II immune responses (405). Due to the concerns regarding safety and retroviral vectors, recent work has concentrated on lentiviral vectors, again showing good response and proof of concept in murine models (404,406). Despite gene therapy research in XLA beginning over a decade ago, there are currently no studies in place or planned, examining the use of gene therapy in human XLA patients.

2.9 Summary

The rarity of XLA and ARA make it extremely difficult to set up and coordinate studies with large numbers of patients. Conclusions regarding outcomes and treatment are primarily derived from heterogeneous primary antibody deficiency cohorts. While these sample sizes are often large; they regularly contain small numbers of XLA patients. These cohort studies are often made up of patients with CVID and findings from CVID; patients cannot be automatically and easily extrapolated to the management of XLA. While also an antibody deficiency, the phenotype of CVID is different from XLA and therefore subsequent outcomes are likely to be too. The age of onset is often different, and there are often other complications in CVID such as autoimmunity and or granulomatous-lymphocytic interstitial lung disease seen much less frequently in XLA or even not all (407).

The rarity of the disease often means immunology centres will only care for a minimal number, often a handful of patients. This results in difficulties in both coordinating national studies and in collecting detailed clinical information.

The XLA cohort studies that exist are mainly out of date, often confounded by many patents having received previous and inadequate IMIg therapy. The two recent and large XLA cohorts from the USA and Italy while large, are now over 15 years old. The data collection for these two studies was primarily surveys sent out to clinicians. The level of detail on these surveys is often limited, to aid input and cooperation from clinicians. For example, these two studies did not record data on previous IgG trough levels, only the most recently recorded level, from which it is not possible to analyse the relationship between IgG trough levels and complications.

As developing countries begin to establish their clinical immunology programmes, the burden of XLA and ARA worldwide is becoming ever more apparent. These countries are producing reliable cohort studies of their patients most notably from China, Iran and Argentina. However, clinical immunology is still in its infancy in these countries and treatment is not yet optimal, illustrated by their much higher mortality rates compared to Western Europe and America. Research into outcomes in XLA is vital for these countries. Access to health care and regular immunoglobulin therapy can be challenging. In some countries, it may be more effective and cheaper to offer HSCT to these patients. Although HSCT is associated with significant mortality and morbidity, newer protocols with reduced conditioning, better HLA matching, more flexible HLA matching with alternative stem cell sources (e.g. cords) has improved outcomes. However, there are many patients for whom HLA matches are unavailable.

Furthermore, it is still the prevailing opinion that the risk-benefit ratio does not swing in favour of HSCT for the management of XLA. Gene therapy may offer a promising alternative curative therapy in combination with newborn screening. This is likely to require significant research and financial investment with a subsequent high treatment cost. Due to rarity of the disorders, the business argument can be difficult to make, but the recent success of NICE approval for gene therapy in ADA-SCID offers promise these hurdles can be overcome (243).

Overall, the published literature suggests some consistent points. They tend to agree that age at diagnosis is reducing, but work from Plebani et al., (186) suggests this may be reaching a plateau. In keeping with the much broader literature on IGRT, the introduction of intravenous and subcutaneous preparations of IGRT has made life-threatening infections exceptionally rare. This improvement is also extended generally to milder infections requiring hospitalisation for the XLA cohort. However, patients still suffer from recurrent respiratory tract infections and, as such, bronchiectasis is likely to be a significant burden for this cohort. Age at diagnosis and instigation of IGRT appear to have variable effects on this outcome. Subsequent effects on QoL appear variable with adults more significantly affected than children. This may represent improved care in recent years or that many of the complications in XLA develop in late childhood and adulthood.

While there are some overarching points arising from the literature there remain some significant gaps and lack of up to date data which may have stalled further research into novel areas. There are no recent outcome data for UK patients. Accurate information on all

infections (in and outpatient), as well as lifetime IgG trough levels is lacking. With this further detailed information, it may be possible to fully assess the effect of age at diagnosis on the development of bronchiectasis. While rates of bronchiectasis in XLA are noted in the literature; there are no data examining the timing of onset, its severity and natural history in XLA. Examining other complications, notably GI disease and inflammatory symptoms, is also needed within a more extensive cohort study.

Furthermore, QoL and psychological health data are needed in a larger sample size, particularly with a focus on the impact of respiratory symptoms. Finally, attempts at genotype/phenotype correlation have so far proved fruitless. Part of this may be due to a lack of clinical information containing within these studies.

Chapter 3 Study Aims

3.1 Study Objectives

The objectives of the study were

1. To ascertain the current prevalence of complications in congenital agammaglobulinaemia, most notably that of bronchiectasis
2. To ascertain factors which increase the risk of complications in congenital agammaglobulinaemia
3. To document the progress of bronchiectasis in congenital agammaglobulinaemia
4. To quantify infection incidence in congenital agammaglobulinaemia while on treatment
5. To evaluate the QoL in patients with congenital agammaglobulinaemia
6. To evaluate the psychological health in patients with congenital agammaglobulinaemia

3.2 Hypotheses

The main hypotheses of the study were

1. Rates of bronchiectasis remain high in congenital agammaglobulinaemia despite modern diagnosis and treatment regimes
2. That early diagnosis significantly improves outcomes and reduces the risk of later complications
3. That development of bronchiectasis is not related to IgG replacement dosages or trough levels
4. That, once developed, bronchiectasis continues to progress in patients with no relation to IgG replacement dosages or trough levels
5. That patients QoL and psychological health is worse than the background healthy population and comparable to CF
6. That the prevalence of respiratory symptoms and health impacts profoundly upon a patients QoL

Chapter 4 Methodology

4.1 Design

This research project is comprised of two components:

- A single researcher review of patient medical records forming both a cross-sectional and retrospective analysis of clinical health outcomes
- A cross-sectional analysis of HRQoL and psychological health using self-report questionnaires.

This was a multi-centre site study across England and Wales.

4.2 Recruitment

4.2.1 Inclusion Criteria

All patients in England and Wales, with a definitive diagnosis of XLA and other genetically confirmed autosomal recessive agammaglobulinaemia defined as per the PAGID and ESID guidelines (4);

- Less than 2% CD19+ B cells and, at least, one of the following:
  - Mutation in BTK
  - Molecular or genetic diagnosis of autosomal recessive agammaglobulinaemia (deficiencies of μ heavy chain, μ5, Igα, Igβ, BLNK, TCF3, λ5)
  - Absent or diminished BTK protein expression
  - Maternal cousins, uncles or nephews with less than 2% CD19+ B cells

There were occasional cases where a diagnosis of XLA was made, but the criteria above not met. For example, patients with a known BTK mutation and agammaglobulinaemia but CD19+ cells > 2%, or patients with absent BTK expression but with a normal level of one or more immunoglobulin isotypes. These so-called 'leaky', atypical XLAs are increasingly being recognised (367). Such cases were reviewed on a case-by-case basis for inclusion in this study. Their exact clinical characteristics and reasons for inclusion are detailed in the results section.

4.2.2 Exclusion Criteria

Patients who lacked capacity were not recruited for this PhD research study. However, patients who lacked capacity may have been included in the United Kingdom Primary Immunodeficiency (UKPID) registry. Approval was gained to access the UKPID registry,

where there was access to limited information on the clinical status, but no data were collected on HRQoL or psychological health for these patients.

4.2.3 Ethical and HRA approvals

Written consent was obtained from patients before enrolment into the study. For patients under 16, consent was obtained from their parents or guardian and assent obtained from the young person where appropriate (Appendix A).

Ethical approval was granted by the Tyne and Wear South Research Ethics Committee (REC) on the 22nd September 2016 (REC reference 16/NE/0268). Health research authority (HRA) approval was granted on the 28th of November 2016 for sites residing in England. For sites outside England, R&D approval was sought locally at each site.

4.2.4 Identification

The United Kingdom primary immunodeficiency network (UKPIN) granted access to their national registry for data pertaining to patients with congenital agammaglobulinaemia (https://www.ukpin.org.uk/registry/registry-intro). This registry aims to collect clinical health outcome data for all patients with a PID living in the UK and was set up in 2008 (247). There are currently 35 centres which actively participate in recruiting patients and enter clinical information into this database (247). As of 2017, there were 4310 patients entered into the database (247). The UKPID registry currently has 159 alive patients with XLA entered into the database, cared for by 25 centres. Of these, 126 are cared for within England and Wales by 19 centres. Information was accessed on the initial diagnosis and latest clinical state from these data.

This information was used to approach local consultants at these nineteen centres enrolled in the UKPID registry to identify any patients who were not on this database and those with autosomal recessive agammaglobulinaemia, to consent them for more detailed clinical data recording and administration of HRQoL questionnaires. Patients and carers were given patient information sheets at routine appointments or by post. Patients were followed up at a further clinic appointment for counselling and consent for the study. All patients eligible for this study were receiving replacement immunoglobulin therapy and therefore, under the care of a specialist immunologist working for one of the above centres. It is, therefore, doubtful there were any further sources of patients eligible for inclusion in this study.

Following invitations, there were nine centres across England and Wales actively recruiting into the study. Upon their participation, it was confirmed that there would be 93 eligible

potential patients for this study. The corresponding UKPID data for these sites listed 77 eligible patients. However, the UKPID registry also included patients with probable and possible diagnoses of XLA, whereas this study only included those with a proven BTK mutation or proven absence of BTK expression.

4.3 UKPID Registry Data

While the registry attempts to encompass all patients within the UK, the depth of information is relatively shallow. Nevertheless, there were data available on the age of diagnosis, family history and current therapy. Patients identified with XLA within this registry may have also included those with a probable or possible diagnosis with XLA in addition to those with a definite diagnosis. It was not possible to differentiate between these within the registry data.

4.4 Data Collection

The following areas of research interest were chosen after discussion with patients and carers, specialists, literature review and a pilot study of congenital agammaglobulinaemia patients in the northeast of England (268).

Data collection included a combination of examining medical records and self-reporting questionnaires.

4.5 Clinical Data

Clinical data were collected from hospital notes by a single researcher and recorded via a standard proforma including infection history, IgG trough levels, IgG doses, HRCT results and pulmonary function testing (PFT) results. A copy of the standard proforma is supplied in the appendices (Appendix B).

4.5.1 Diagnosis

Age at diagnosis was defined as the age of clinical diagnosis of XLA and/or instigation of IGRT, whichever occurred earliest. Where available, the immediate full blood count (FBC), immunoglobulin levels and lymphocyte subsets before instigation of IGRT were recorded. Identification of the genetic mutation was taken from medical notes. Analysis of the BTK mutation was undertaken at centres according to local arrangements. BTK expression on monocytes was recorded from medical notes. BTK expression was undertaken at centres according to local arrangements. It was noted whether this was performed by Western blot or flow cytometry.

4.5.2 Current laboratory values

The patient's latest FBC, immunoglobulin levels and lymphocytes subsets were recorded.

4.5.3 Infections

Data regarding the number of infections were gathered from clinic letters. Infections were classified into the site of infection (e.g. respiratory tract, ENT) and whether the patient required inpatient or outpatient treatment (as a proxy marker of infection severity). These data were used to calculate the overall annual infection incidence per patient and infection incidence per patient by the site of infection. Patients with congenital agammaglobulinaemia undergo regular reviews from a specialist immunologist, usually on a 3 to 6 monthly interval. It is best practice at these reviews to document any infections the patient has experienced since the last clinic appointment as part of evaluating the effectiveness of their current IGRT. Based on the experience from a previous pilot study, documentation of infections in clinic letters was found to be of a very high standard (268). Only infections that required a course of antibiotics were recorded. If the infection was thought to be viral in origin, this was not recorded. The site of infection was documented as per the clinic letter. If this was not recorded, the anatomical site was recorded as 'not documented'. Also, infections caused by enterovirus and *Giardia lamblia*, to which this population is susceptible, were explicitly recorded.

Data pertaining to infections pre-diagnosis of agammaglobulinaemia were less frequently recorded, and it was not possible to accurately ascertain the infection incidence pre-diagnosis for the majority of patients. However, it was possible to record infections that required hospital or intensive care admission accurately. The pattern of the presentation was often recorded, e.g. recurrent chest or ear infections, and this was recorded for analysis where available and unknown if not available.

4.5.4 Nutritional status

Height and weight data were collected where available and body mass index calculated (BMI). For adults, BMI category was classified as per World Health Organisation (WHO) guidelines (408). For children, BMI scores were converted to Z-Scores and categories assigned as per the Royal College of Paediatrics and Child Health (RCPCH) growth charts using the UK90 data set(409,410). This was done using the `zanthro` and `zbmiuk` functions in STATA (411).

Table 4-1 Adult World Health Organisation BMI categories (408)

BMI	Classification
<18.5	Underweight
18.5 – 24.9	Normal weight
25.0 – 29.9	Overweight
>30.0	Obese

4.5.5 Immunoglobulin replacement therapy

Doses, route, brand and frequency of IGRT were recorded as well as the geographical location of therapy (home/hospital). These were recorded from hospital records or patient's records if self-administered. Where concurrent weight was available, doses were recorded as mg/Kg/month.

4.5.6 Treatment compliance

Treatment compliance was categorised according to the following (Table 4-2). There are no currently agreed methods for assessing treatment compliance in PID. The following were chosen after discussion with PID specialists, following experience with the pilot study (268) and based on how much data could reliability be collected from the cohort. As well as noting deviation from prescribed IGRT, it was also important to note the deviation from respiratory health monitoring, this being the major complication for this group

Table 4-2 Categorisation of treatment compliance

Category	Features
Fully compliant – All of the following	Has not missed a clinic appointment in the preceding two years to data collection Has received all IGRT on schedule in the preceding five years to data collection Has attended all investigations for IgG trough levels, HRCT and PFTs in the preceding two years to data collection
Mild issues	Has missed two or more clinic appointments in the previous two years, but receives all IGRT on time and attends for investigations including trough level measurement, HRCT and PFTs
Moderate issues– at least one of the following	Has missed three or more clinic appointments in the preceding two years to data collection

Category	Features
	Has not attended for at least one requested investigation in the preceding two years to data collection including trough level measurements, HRCT and PFTs Has stretched out IGRT interval, but to no more than one week in the preceding five years to data collection
Severe issues – at least one of the following	Has stretched out IGRT interval more than one week in the preceding five years to data collection Has missed IGRT infusion, resulting in periods without IGRT therapy

4.5.7 IgG trough levels

IgG trough levels were recorded from medical records and laboratory values. Only those values related to trough levels were recorded, i.e. with 'trough level' in the investigation request or documented in clinic letters and nursing records.

When recording and calculating the IgG trough levels for later analysis the following steps were taken

- Annual median IgG trough levels were calculated for each patient. Rather than analysing individual data points, this method would limit some of the bias from occasional outliers (these outliers are addressed in the next point). As other outcome data were calculated on an annual basis, for example, annual infection incidence, this also allowed a more straightforward analysis of these outcomes.
- To assess for variance in an individual's IgG trough levels the standard deviation (SD) was calculated across their lifetime. For each patient, the mean IgG trough level was calculated for each year, and the SD of the mean of these values was then used. This method allowed the analysis of any effect that occasional outliers might have on clinical outcomes (e.g. brief but potentially significant short periods where IgG trough levels were suboptimal). Due to the differences in the pharmacokinetics, absorption and frequency of administration between IVIG and SCIG, the trough IgG may not be completely comparable and there may be impacts upon the SD. In particular, trough IgG levels may show less deviation in SCIG due to the frequency of administration (weekly), potential for more recording of trough IgG levels (up to weekly) and absorption of IgG from the subcutaneous tissues allowing for a steadier release of IgG and serum IgG level.

However, trough IgG levels in both modalities are the currently the best measure of assessing deliverable IgG dose.

- Lifetime medians of all IgG trough levels were also calculated.
- For patients with lung disease, this was further analysed for the period before and after the diagnosis of bronchiectasis.

4.5.8 GI Disease and nutrition

Weight and height were recorded where possible, and body mass index (BMI) calculated. Data were collected on GI symptoms and whether the patient had undergone endoscopic investigations and their results.

4.5.9 High-Resolution Computerised Tomography

Sequential HRCT images were analysed for reported evidence of lung disease and subsequent progression or resolution. Interpretation of the HRCT was based on the local hospital radiologist report. It is standard practice in the reporting of HRCT scans for the reporting radiologist to compare the images to any previous scans and comment upon any progression of disease state. There were no new HRCT scans performed as part of this study.

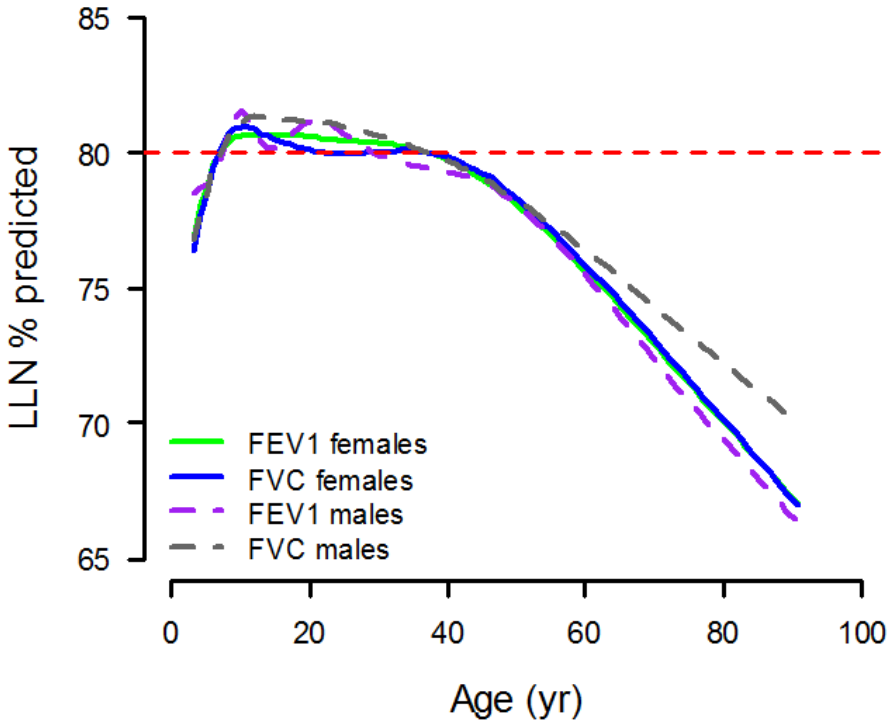
HRCT remains the gold standard for diagnosing bronchiectasis. However, it would be safe to assume that patients may have established bronchiectasis before any official diagnosis due to the interval time periods between scans. This is especially pertinent if bronchiectasis was shown on the first scan after the diagnosis of agammaglobulinaemia. These patients will likely have never had an HRCT scan performed before, and it, therefore, makes it likely the patient's bronchiectasis pre-dates their diagnosis of agammaglobulinaemia and instigation of immunoglobulin therapy. To enable consistency and integrity of data regarding the onset of bronchiectasis, any patient receiving a diagnosis of bronchiectasis within 12 months of starting immunoglobulin therapy was defined as having bronchiectasis at the time of their agammaglobulinaemia diagnosis. To further assess the impact of the lag from true bronchiectasis onset to diagnosis in some analyses, the date of diagnosis of bronchiectasis was adjusted to minus one year, minus three years and minus five years.

Where possible, it was recorded whether the HRCT was performed for routine monitoring or whether there were clinical reasons from the patient's symptoms or history to warrant a further HRCT.

4.5.10 Pulmonary Function Testing

Sequential historical lung function test results were analysed for changes in FEV1 (forced expiratory volume in 1 second), FVC (forced vital capacity) and FEV1/FVC ratio. These values are adjusted for height. It is standard clinical practice for lung function tests to report the values as a 'percentage of predicted' (e.g. an FEV1 of 1.5 compared to a predicted median population value of 2 would equal 75% predicted). It has been a standard part of clinical practice to define the lower limit of normal (LLN) in pulmonary function as 80% of the predicted value. This practice arises from a paper by Bates and Christie (412): "a useful general rule is that a deviation of 20% from the predicted normal value probably is significant". This recommendation, with little clinical evidence, was seemingly adopted without question across medical practice. However, this rule is only valid if the scatter around the predicted value is proportional to that value which, in PFTs, it has shown not to be (288). The reality is that over a broad age range, the LLN is well below this 80% predicted value line (288) (Figure 4-1).

Figure 4-1 The lower limit of normal (LLN) for FEV1 and FVC expressed as a percentage of the GLI-2012 predicted values in the 3-95 year age range. Taken from (288)



In 2012, the Global Lung Initiative (GLI) published extensive data on the normal distribution of lung function values in healthy individuals across a range of ages and races. These values are adjusted for height, sex and race. The LLN in respiratory medicine is the 5th percentile (i.e. 90% of the healthy population), equal to a Z-Score of -1.64. The Z-score is defined as

the measure of the standard deviation from the population mean or predicted score and is calculated from the following formula:

$$Z = \frac{x - \mu}{\sigma}$$

Where Z = the Z-Score, x = the observed value, μ = the mean of the sample or population and σ = the standard deviation of the sample or population.

To further emphasise the flaws in accepting 80% predicted as the LLN, the LLN using a Z-Score of -1.64 in a 3, 20 and 80-year-old white female is 74%, 80% and 66% of the predicted value respectively. The predicted values and Z-Scores from this GLI dataset have been well validated (413,414). For the present study, raw lung function data were recorded and inputted into supplied licensed software from GLI, calculating the Z-Score and whether lung function was normal/abnormal according to these figures (415).

4.5.11 Bronchiectasis Severity

The two most widely used tools to assess the severity of bronchiectasis are the bronchiectasis severity index (BSI) (309) and the FACED (416) score. Both scores were developed simultaneously as areas of research into predicting long-term outcomes in bronchiectasis (309,416,417). The BSI, comprising of HRCT score, FEV1, MRC dyspnoea score (418), bacterial colonisation, prior hospital admission and exacerbations is a sensitive tool for predicting future hospital admissions and mortality (Table 4-3, Table 4-4, Table 4-5) (309). The FACED score, comprising of FEV1, age, *P. aeruginosa* colonisation, radiological extension and dyspnoea accurately predicts mortality (Table 4-6, Table 4-7) (416). Recent work has demonstrated that prediction of mortality over a more extended time period (15 years) is more accurate in the FACED scoring system, which has the added benefit of being a simpler scoring system (417). However, it does not make any prediction for hospital admissions. Both tools still require validation in independent cohorts, but current consensus suggests that the two should play complementary roles in research and clinical practice (417). As such, all patients with bronchiectasis were assessed using both tools where sufficient data were available.

Table 4-3 Bronchiectasis Severity Index (BSI) (309)

Severity criteria	0 points	1 point	2 points	3 points	4 points	5 points	6 points
Age	<50		50-69		70-79		80+
BMI kg/m2	≥18.5		<18.5				
FEV1 % predicted	>80%	50-80%	30-49%	<30%			
Hospital admissions in the past 2 years	No					Yes	
Exacerbation frequency in last 12 months	0-2		3 or more				
MRC dyspnoea score (Table 4-4)	1-3		4	5			
Colonisation status	Not colonised	Chronic colonisation		*P. aeruginosa* colonisation			
Radiological severity	<3 lobes involved	3 or more lobes or cystic changes					

Table 4-4 MRC Dyspnoea scale (418)

Grade	Degree of Breathlessness
1	I am not troubled by breathless except on strenuous exercise
2	I get short of breath when hurrying on a level or when walking up a slight hill
3	I walk slower than most people on the level. I have to stop after a mile or so or stop after 15 minutes walking at my own pace
4	I have to stop for breath after walking 100 yards or after a few minutes on level ground
5	I am too breathless to leave the house, or I get breathless when dressing/undressing

Table 4-5 Calculation of final BSI Score (309)

BSI Score		Outcome	Predicted incidence
0-4 points	**1 year**	Mortality	0-2.8%
		Hospitalisation Rate	0-3.4%
	4 year	Mortality	0-5.3%
		Hospitalisation Rate	0-9.2%
5-8 points	**1 year**	Mortality	0.9-4.8%
		Hospitalisation Rate	1-7.2%
	4 year	Mortality	4-11.3%
		Hospitalisation Rate	9.9-19.4%
9+ points	**1 year**	Mortality	7.6-10.5%
		Hospitalisation Rate	16.7-52.6%
	4 year	Mortality	9.9-29.2%
		Hospitalisation Rate	41.2-80.4%

Table 4-6 FACED score (416)

Component	Score
(F)EV1	>50% = 0 points ≤50% = 2 points
(A)ge	≤ 70 years = 0 points >70 years = 2 points
(C)hronic colonisation	No pseudomonas = 0 points Pseudomonas = 2 points
(E)xtension	<2 lobes affected = 0 points ≥2 lobes affect = 1 points
(D)yspneoa	No dyspnoea = 0 points MRC Score ≥2 = 2 points

Table 4-7 FACED scoring and bronchiectasis severity (416)

Score	Severity
0-2 points	Mild bronchiectasis
3-4 points	Moderate bronchiectasis
5-7 points	Severe bronchiectasis

4.5.12 Sputum microbiological data

Microbiological data from the most recent sputum sample was recorded, including organism and antimicrobial sensitivities and resistance.

4.6 Genetic Data

Genetic mutation data were gathered from clinical information received from the patient's local NHS genetic testing laboratory. There are no established guidelines for analysing a genotype/phenotype correlation in XLA, and so several methods were used:

- First, patients were grouped per type of mutation: missense, splice site, frameshift/in-frame deletions/insertions and others (including mutations in the promotor or start codon).
- Secondly, patients were grouped per domain site of mutation (i.e. PH, TH, SH3, SH2 or kinase domain).
- Thirdly, patients were grouped as severe or mild as per categories first proposed by Conley et al. (Table 4-8) (368).

Table 4-8 Mutation severity classification in XLA from Broides et al. (368)

Mild mutation	Severe mutation
• Amino acid substitution at non – conserved sites • Splice-site defects at conserved base pairs but not invariant	• Amino acid substitution at conserved sites in other members of the BTK family (ITK, TEC) • Frameshift mutations • Splice site alteration at invariant sites within the first two and last two base pairs of an intron • Premature stop codon Inframe deletions

- Fourthly, mutations were scored for predicted severity using a variety of genetic mutation prediction software packages as described in further detail below.
- To assess predicted mutation severity, all mutations were inputted through VarSome (https://varsome.com), which processes mutations through a number of packages simultaneously. Mutations were analysed against the hg19/CRCh37 human reference genome (419) and the NM_000061.2 BTK gene transcript (420). The chosen packages were SIFT (421), Polyphen2 (422), PROVEAN (423), MutationTaster (424) and FATHMM (425). Splice site mutations are also scored using Database Splicing

Consensus Single Nucleotide Variant (dbscSNV) (426). These were chosen as some of the most widely used tools in the scientific literature with high sensitivity and specificity and were able to calculate converted rank scores (427). Mutations were then analysed as per their converted rank score for each tool and as per their predicted pathogenicity category from each mutation prediction tool (definitely disease-causing, probably disease-causing, probably not disease-causing and definitely not disease-causing). These definitions are taken from and are in agreement with the joint consensus recommendation from American College of Medical Genetics and Genomics (ACMG) (428) .

- The mutations were also analysed using DANN, a pathogenicity scoring methodology based on deep neural networks, with scores ranging from 0 – 1 with 1 being the most damaging (429).

4.6.1 Disease severity

There are no agreed disease severity classification scales for XLA or other primary antibody deficiencies. After discussion with immunology specialists, the following categories were devised for this study.

Table 4-9 XLA severity classification

Disease severity	Clinical characteristic
None	No end-organ damage and No recurrent infections
Mild	Recurrent infections but no end-organ damage
Moderate	End organ damage such as bronchiectasis or Recurrent hospital admissions for infections
Severe	Surgical intervention for end organ damage or Severe bronchiectasis defined as • BSI Score >8 • FACED Score >4 • FEV1 <50% predicted Or undergone HSCT

4.7 Health-Related Quality of Life

HRQoL is a multidimensional concept measuring physical, psychological and social wellbeing to assess the impact of a patient's illness and its treatment on their QoL (430).

While QoL and HRQoL are often used interchangeably in the literature, they are two different concepts. HRQoL focuses on the impact of the patient's illness and its treatment on their QoL. QoL is a much broader concept, assessing non-health related factors on a patient's overall QoL. This study only assessed health-related quality of life. HRQoL should be measured by the patient's/family's perspective and not biased or influenced by opinions of the health care team. Tools measuring HRQoL must adhere to this definition.

HRQoL was recorded with a variety of self-reporting questionnaires either by the patient or by their carer/parent. These self-reporting questionnaires and were handed to patients and/or their carers during clinic appointments. Patients were also offered the opportunity to complete these at home and post back with provided stamped self-addressed envelopes. The questionnaires were not completed on the day of immunoglobulin infusions to limit bias from any adverse reactions to the treatment. The data collected were compared against published population norms and other disease cohorts as described below.

4.7.1 Comparator groups

All HRQoL data and psychological health data were compared against UK norms and against patients with cystic fibrosis where data was available (431–440).

CF was chosen as a comparator, being a chronic, incurable condition complicated by recurrent infections and for which respiratory symptoms and bronchiectasis place a major burden. It also has some comparisons in that the age of onset of bronchiectasis is young, as has also been shown in the existing literature for patients with XLA.

4.7.2 Short Form 36 Version 2

The short form 36 version 2 (SF36v2) is a well-recognised tool for measuring the quality of life in adolescents and adults and has been used extensively when researching patients with immunodeficiency and a range of other diseases groups (258). The SF36v2 was used for patients over 16 years of age. It comprises of 36 questions, of which the results are inputted through the provided licensed software to calculate scores for 8 subdomains and 2 overall scores (441). There are 4 subdomains related to physical health: physical functioning (PF), physical role (PR), bodily pain (BP), general health (GH), all combining to calculate the physical component score (PCS) (441). These are scored out of 100, with a higher score indicating a higher HRQoL. These data were compared against normative UK data (431) and for UK patients with CF (432).

A copy of the SF36v2 can be found in the appendices (Appendix C).

4.7.3 PedsQL *4.0*

The PedsQL 4.0 generic core scale questionnaire is a well-recognised self-reporting tool for measuring HRQoL (348,442). It has been well validated, shown to be reliable and able to differentiate between healthy children and those with chronic diseases (442). It has been used previously to study the quality of life in patients with primary antibody deficiency (PAD) (347). It comprises of 23 questions within four subdomains: physical, emotional, social and school functioning. These combine to give an overall score, a physical health summary score and a psychosocial health summary score (433). The higher the value in each these domains and overall score indicate a higher HRQoL. There are altered versions of the questionnaire for children and young people, depending on their age and a separate one for parents. The questionnaire was given to children aged 5 to 16 and parents of children aged 2-16. The data were compared against UK population norms (433) and patients with CF (434).

A copy of the PedsQL 4.0 can be found in the appendices (Appendix C)

4.7.4 St. George's Respiratory Questionnaire

SGRQ is a widely used tool for recording HRQoL in regards to respiratory health in adults (435). It comprises of 50 questions within 3 domains; symptoms, activity and impacts (psychosocial), contributing to an overall total score. It was initially designed for chronic obstructive pulmonary disease (COPD) and asthma but has since been well validated in bronchiectasis (443) including a direct correlation with mortality (444). It correlates strongly with other markers of disease activity such as FEV1, cough, breathlessness, 6-minute walk test as well as other measures of HRQoL such as the SF36v2 (279). It has also been shown to be reliable studying patients with primary antibody deficiency (270). The patient's answers are inputted into the supplied excel spreadsheet, calculating the scores for the subdomains and overall total score (435). These data were compared against UK healthy norms data (435) and patients with CF (436).

A copy of the SGRQ can be found in the appendices (Appendix C).

4.8 Psychological Impact

4.8.1 Strengths and Difficulties Questionnaire

The strength and difficulties questionnaire (SDQ) was used for children aged 4-16 years old. The SDQ is a well-recognised measure of social, emotional and behavioural difficulties in children and is a widely used tool for screening for psychological difficulties in childhood (445). It is validated in a range of chronic diseases such as arthritis (446) and cancer (447). It

has also been used in studies of patients with immunodeficiency (347). It is designed so carers of children aged 4-16 years old and children over the age of 11 can complete the questionnaire. There are altered versions of the questionnaire for children and young people, depending on their age and a separate one for parents. The tool consists of 4 areas; conduct, emotion, hyperactivity and peer relationships, which can then be combined to give a total difficulty score (445). The scores were also used as a screening tool for detection of emotional, conduct, hyperactivity and any psychiatric disorder as either unlikely, possible or probable. These total scores are generated using a pre-prepared STATA do file provided by the authors of the SDQ (448) (Appendix C). These were compared against published normative UK data (449).

A copy of the SDQ can be found in the appendices. (Appendix C).

4.8.2 Short Form 36 v2

As well as a physical component the SF-36 also contains a mental component with four domains; emotional role (RE), mental health (MH), vitality (VT) and social functioning (SF), combing to calculate the mental component score (MCS). As discussed previously, the SF-36v2 is a well-validated tool for examining physical and mental health and has been used extensively in research involving patients with immunodeficiency (258). The results for the adult patients were compared against published normative data and patients with CF (431,432).

A copy of the SF36v2 can be found in the appendices (Appendix C).

4.8.3 Hospital and Anxiety Depression Scale

The hospital anxiety and depression scale (HADS) is a frequently used scoring system designed specifically to avoid including symptoms associated with medical conditions and is validated in both adolescents and adults (12 years and over) (450). It can be used to both screen for disease and to assess severity. It comprises of 14 questions, 7 assessing for anxiety and 7 assessing for depression each answered on a 4-point scale (0-3). The maximum score for each domain is 21, with a higher score indicating more severe disease. It has been shown to be valid in individuals with or without psychological problems (450). These scores can be assigned categories or compared against populations norms using the raw scores (437) (Table 4-10).

Table 4-10 Categories for Depression and Anxiety domains of the HADS (451)

Category	Score
Normal	0-7
Mild	8-10
Moderate	11-14
Severe	15-21

Results were compared against UK male population norms (437), UK males with cystic fibrosis (438).

A copy of the HADS can be found in the appendices (Appendix C).

4.8.4 Rosenberg Self-Esteem Scale

The Rosenberg Self-Esteem Scale (RSES) is a widely used tool in clinical research to ascertain self-esteem (452). It was initially designed for adolescents but has been well validated in the adult population (453). The RSES was used in participants aged 12 and upwards. The RSES comprises of 10 statements with participants asked to rate their level of agreement with each one on a 4-point scale ranging from strongly agree to strongly disagree. The questionnaire combines to give a total score of 30, with less than 15 representing low self-esteem, 15-25 being normal and more than 25 signifying high self-esteem (453). Raw scores were compared against UK male CF patients (440) and UK normative male data (439).

A copy of the RSES can be found in the appendices (Appendix C).

4.9 Cost and Impact of treatment

Cost of IGRT was calculated using the latest values supplied by the 2017-2018 British national formulary (BNF) (454). These are only the costs for the immunoglobulin product itself and do not account for the costs of other consumables or staff.

Patients were asked to record the number of days they had taken off work or full-time education in the preceding 12 months as a direct consequence of their illness (e.g. hospital appointments, acute illness, infusions).

Parents of affected children were also asked to record the number of days they had taken off work in the preceding 12 months to look after their child due to a direct consequence of their child's disease or treatment.

4.10 Analysis

Statistical analysis was done using STATA v15.1 (www.stata.com). A previous pilot study recruited 15 patients with congenital agammaglobulinaemia in the northern region of the UK (268). The UKPIN registry currently has 159 alive patients in the UK with XLA. Previous studies in PID have found a high participant rate from patients (455,456).

Based on the previously published pilot data from Newcastle (268), the following sample size calculations were done

- To detect a statistically significant difference in age at diagnosis of 4 years between those with and without bronchiectasis would require a total sample size of 58 (significance level of 0.05 and 80% power)
- To detect a statistically significant difference of 2 in the physical component score of the SF36v2 compared to a published healthy norm reference value would require a sample size of 46 (significant level 0.05 and 80% power). 2 is the current minimally clinically important difference (MCID) in the PCS calculated by the team who designed the SF36v2 (441).
- To detect a statistically significant difference of 5.16 in the parent total score of the PedsQl 4.0, a sample size of 61 would be required. 5.16 is the MCID calculated by Hillard et al. examining young people with diabetes (457)

Variables were assessed for normality using skewness and kurtosis assessment, and the null hypothesis that the data were normally distributed was rejected if the p-value was <0.05. Parametric data are displayed as mean and standard deviation (SD) and non-parametric data as the median and interquartile range (IQR).

For the psychological health and quality of life data where data are compared against published norms, means were compared using a one-sample t-test. For non-parametric data, the data were compared using the sign test. A p-value <0.05 was considered statistically significant.

For patients with bronchiectasis, a number of outcome variables (e.g. IgG trough levels, infection incidence), data and averages were subcategorised as either before or after the diagnosis of bronchiectasis. This was assessed for a number of reasons. Firstly, the presence of bronchiectasis confounds many of these variables. For example, bronchiectasis itself predisposes to infections and clinicians are likely to target higher IgG trough levels in patients with bronchiectasis. Separating the two time periods also allows a meaningful comparison of

these patients in their 'healthy' phase to patients who currently do not have lung disease. There are still some limitations to this method as will be discussed further in the discussion chapter, most notably the lag time from true bronchiectasis onset to the radiological diagnosis of bronchiectasis. As discussed previously, the time of onset of bronchiectasis as adjusted to ascertain if this affected variables. Despite the limitations, these steps were felt to the most reliable and accurate method available.

Spearman's correlation was used to analyse correlations between continuous variables and results are presented as Spearman's rho correlation coefficient and p-values.

Risk of developing bronchiectasis was modelled using a time to event analysis. The time variable was taken from birth (age 0). This was chosen as pathogenicity, and the risk factor leading to the development of bronchiectasis was the number of infections. Patients were born with the predisposing risk factor (their XLA) to developing respiratory tract infections, and so they were deemed exposed to risk from birth. Patients without bronchiectasis were censored at the time point that their clinical data was collected (right censored). The final model was a cox regression and variables were analysed as follows

- Categorical variables were analysed individually using the log-rank test of equality
- Continuous variables were analysed using a univariate Cox proportional hazard regression

Lung function results were analysed using repeated measures multilevel models (Stata's `xtmixed` command) with observations over time (age at time lung function testing performed, level 1) nested within study participants (level 2). This was done using growth-curve models. The model included a random effect for time and fixed effects for baseline covariates. Again, similar for the above Cox regression, time is taken as from birth for the aforementioned reasons. Covariates were inputted as unstructured.

Chapter 5 Clinical Results

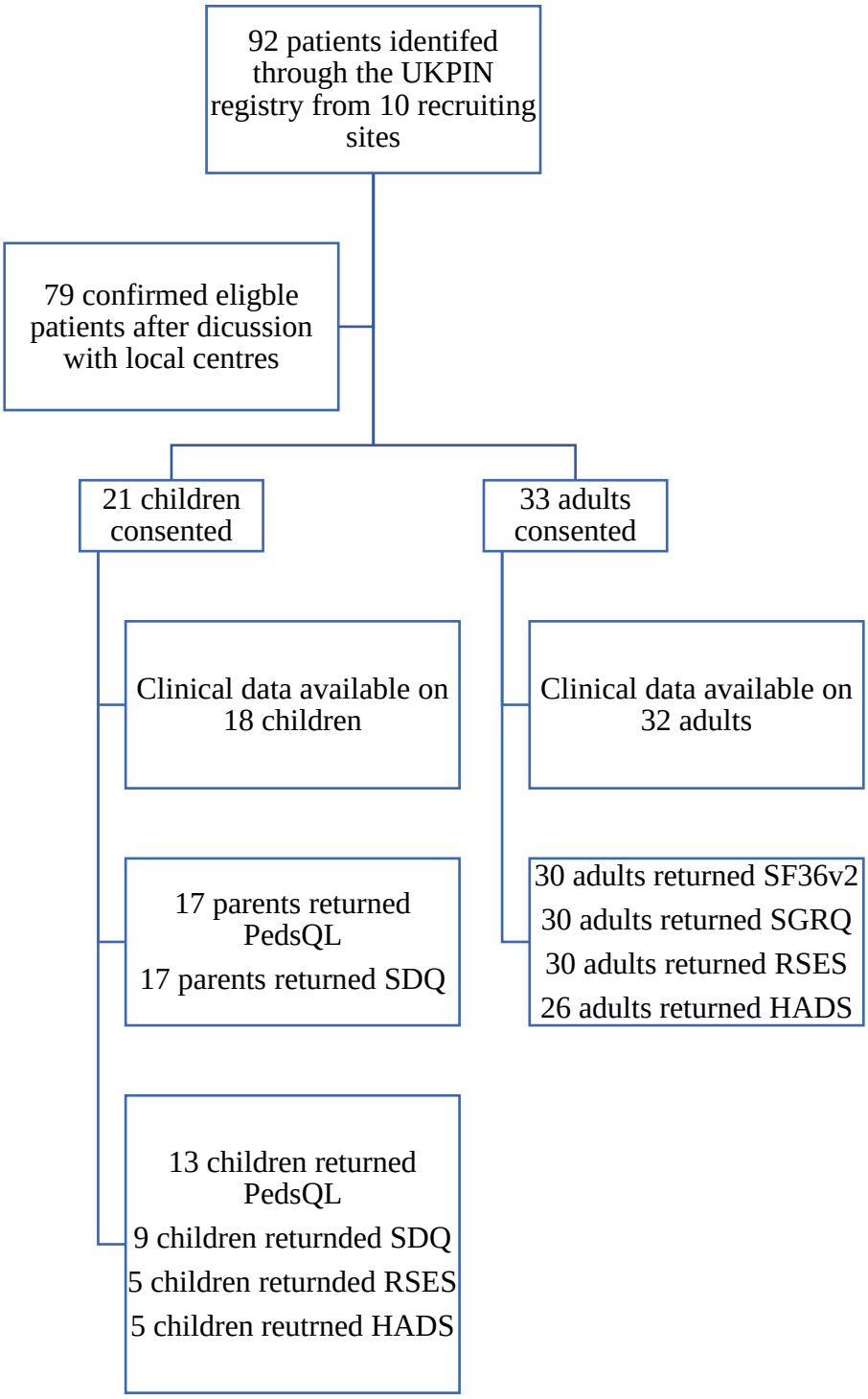
This chapter will detail the clinical outcomes for this XLA cohort. Outcomes regarding respiratory health outcomes are introduced in this chapter but will be covered in more detail in Chapter 6.

5.1 Recruitment

One-hundred and thirty-two patients, cared for by ten centres in England and Wales, were identified using the UKPID registry data. These registry data included patients with definite, probable and possible XLA according to the PAGID and ESID guidelines (4). The UKPID registry data were used to identify and invite centres to participate in the study. Ten centres replied and actively recruited into the study. From these centres, 92 eligible patients were identified using the UKPID registry data, of which 79 were confirmed by local PIs as being definite XLA patients. From these, 53 (67%) patients were successfully contacted and recruited into the study. A summary flow chart of study recruitment is shown in (Figure 5-1).

5.1.1 Recruitment

Figure 5-1 Study recruitment



Patients were recruited from nine centres in England and one centre in Wales (Figure 5-2, Figure 5-3).

Figure 5-2 Study recruitment sites

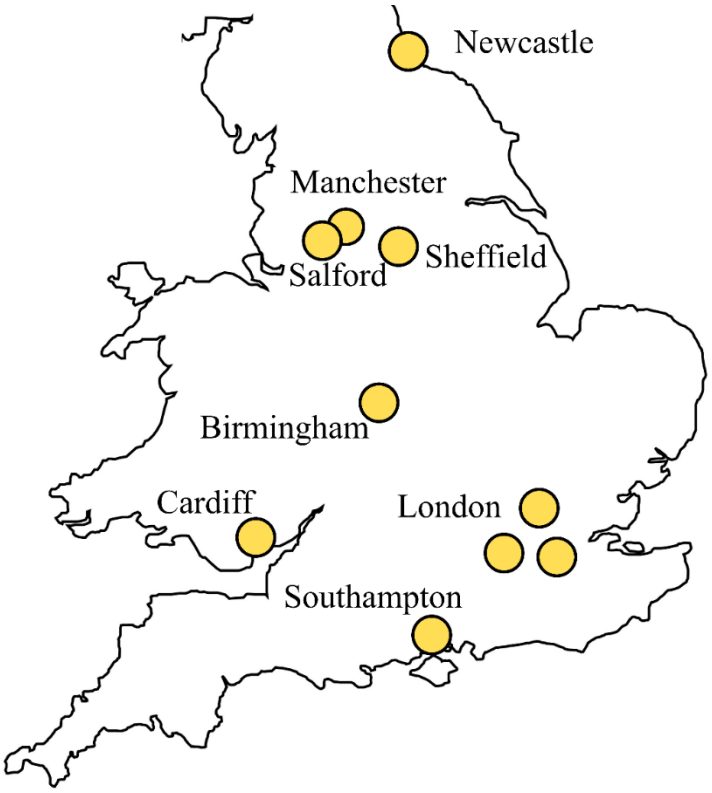
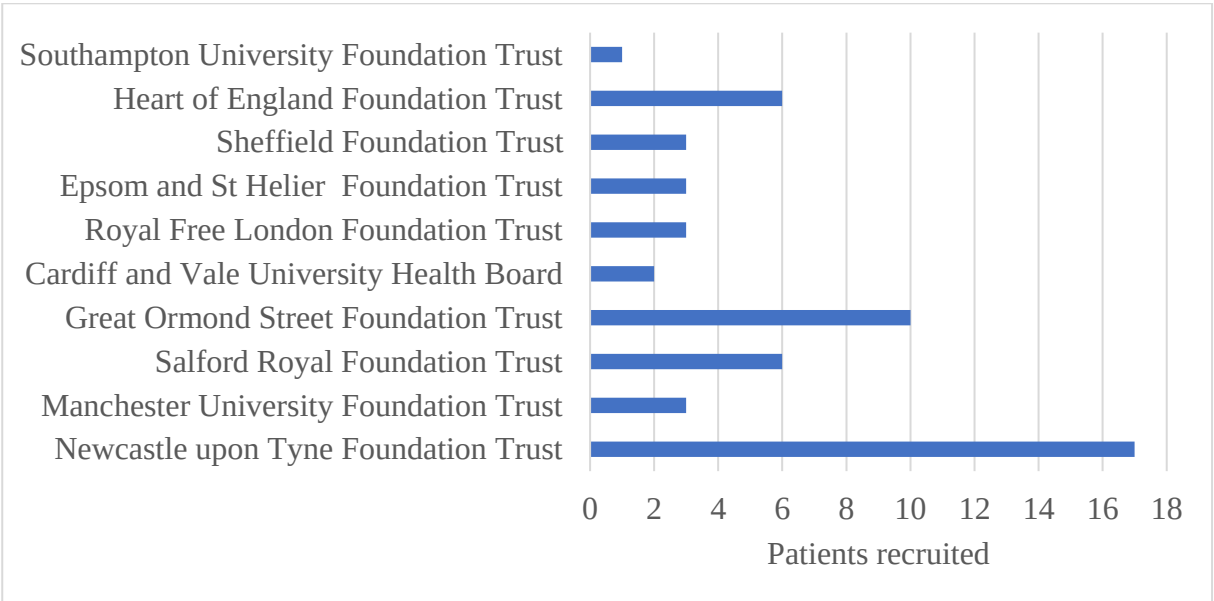


Figure 5-3 Centre recruitment



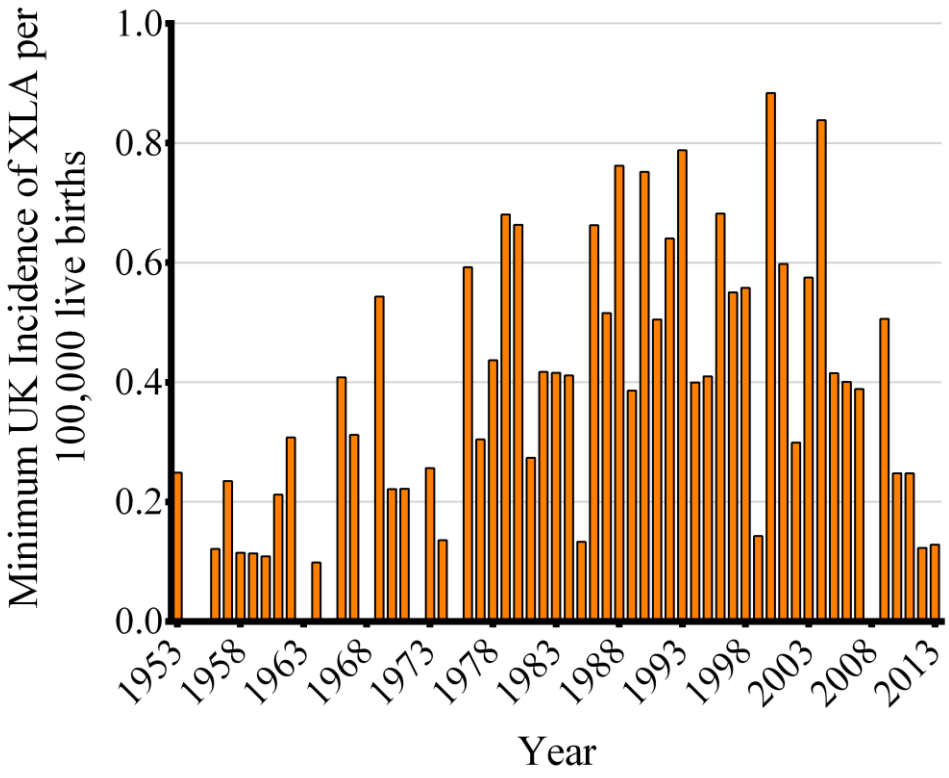
5.2 UKPID Registry Data

Before describing the XLA cohort data collected for this PhD research study, I will first report on the data made available from the UKPID registry. The UKPID registry is limited in its depth of data but does aim to include all patients with a diagnosis of PID in the UK

5.2.1 Incidence and Prevalence

Data were extracted from the UKPID registry in July 2017. One hundred and sixty-four patients were recorded as having a diagnosis of XLA. As of July 2017, 159 were alive; three have died since the inception of the registry (2008), and two were lost to follow up. Based on the July 2017 Office of National Statistics (ONS) UK population estimate of 65.6 million (458), this equates to a minimum UK prevalence of 2.42 cases per million population. Based on these registry data, the UK annual mortality rate of XLA is 0.24%. The mean incidence from 1990-2010 was 0.50 cases per 100,000 UK live births. This incidence has increased with time (Spearman $\rho = 0.349$, $p = 0.010$) (Figure 5-4).

Figure 5-4 UK Incidence of XLA 1952-2013 (UKPID Registry Data)

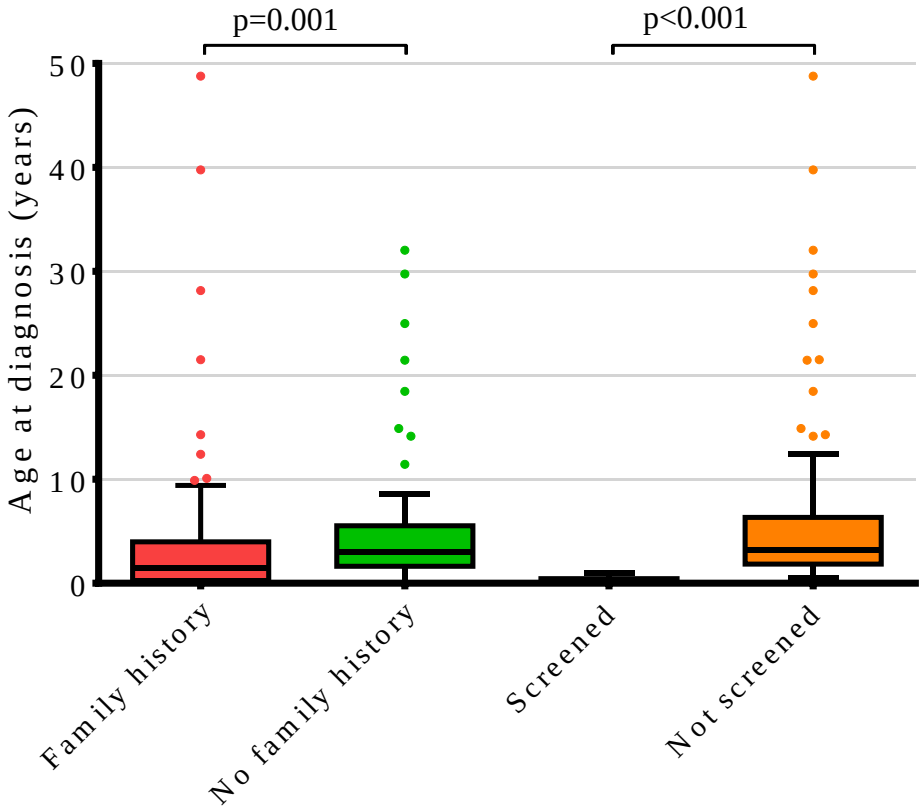


5.2.2 Diagnosis

Age at diagnosis was defined as the age at which IGRT starts, or the clinical diagnosis of XLA was made, whichever was earlier. Age at diagnosis was available for 140 patients. The median age at diagnosis in the UKPID registry data is 2.12 years (IQR 0.60 – 4.80). There

was a positive family history in 43 of 128 cases (33.4%) where data were available. Those with a family history of XLA had a statistically significantly lower median age of diagnosis of 1.47 years (IQR 0.31 -3.96) versus 2.99 years (IQR 1.68 – 5.51), p = 0.001. However, the UKPID registry does not collect data on whether the patient was screened asymptomatically based on family history. An approximate proxy was calculated by classifying those with a diagnosis of less than 12 months, and positive family history as being screened asymptomatically. In addition, any child with a diagnosis of less than 6 months was deemed as being diagnosed asymptomatically. Six months was chosen as a suitable cut off as this is the age that levels of maternally transferred immunoglobulin have a clinically significant decline and patients with congenital agammaglobulinaemia tend to become symptomatic (165). Excluding those that were likely screened due to family history, the median age of diagnosis was 3.25 years (IQR 1.91 – 5.97) (Figure 5-5).

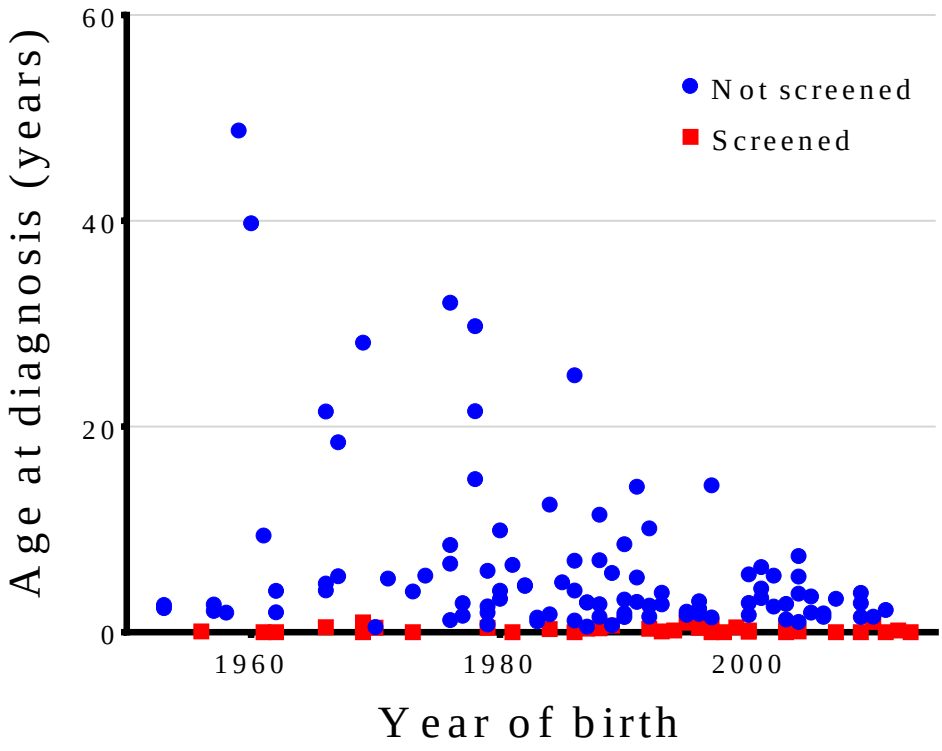
Figure 5-5 Age of diagnosis in XLA in the UKPID registry



Of the 48 patients, 15 patients were diagnosed at birth, and 33 patients were diagnosed as adults, with the oldest age of diagnosis being 39.77 years of age. Age of XLA diagnosis (and therefore instigation of IGRT) has improved over time (Spearman's ρ = -0.242, p = 0.004) (Figure 5-6). This remains the case when excluding those with a family history (Spearman's

ρ = -0.258, p = 0.037) and those who with a family history and likely asymptomatically screened (Spearman's ρ = -0.270, p = 0.006).

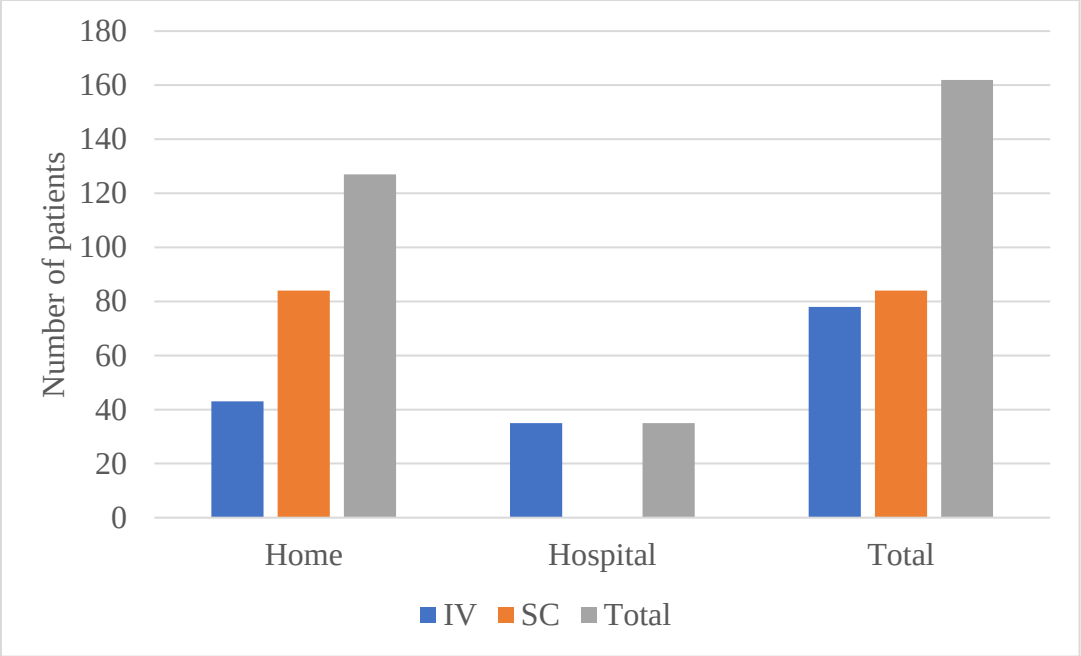
Figure 5-6 Age at diagnosis over time (UKPID registry data)



5.2.3 Immunoglobulin therapy

There was a very slight preference for subcutaneous therapy, with 51.22% of XLA patients receiving their IGRT via this route (out of 164 available patients) (Figure 5-6). There is a strong preference to receive IGRT at home with 78.40% of patients receiving their therapy at home (out of 162 available patients). All patient's receiving subcutaneous therapy did so at home, and fifty-five per cent of patients on IVIg did so at home.

Figure 5-7 Site and route of IGRT



Privigen was the commonest IV product, accounting for 33.75% of all IV products prescribed (Table 5-1). Hizentra and Subcuvia account for the commonest SC immunoglobulin products accounting for 30.95% and 29.86% of all SC products, respectively (Table 5-2)

Table 5-1 IV immunoglobulin products

Product	Number (n)	Percent (%)
Privigen	27	33.75
Flebogamma	12	28.75
Kiovig	3	11.25
Flebogamma 5%	8	10.00
Vigam	5	6.25
Gammaplex	3	3.75
Octagam	3	3.75
Intratect	1	1.25
Pentaglobin	1	1.25
Total	63	100

Table 5-2 SC Immunoglobulin products

Product	Number (n)	Percent (%)
Hizentra	26	30.95
Subcuvia	25	29.76
Subgam	25	29.76
HyQvia	3	3.57
Vivaglobin	3	3.57
Gammanorm	2	2.38
Total	84	100

Table 5-3 Dosage and dosing interval period of IGRT (median (IQR))

	Dose (mg/Kg/month)
Intravenous (n = 72)	568 (476 – 748)
Subcutaneous (n = 67)	504 (436 - 668)
All (n = 139)	544 (456 – 700)
	Dosing interval (days)
Intravenous (n = 66)	21 (21 – 21)
Subcutaneous (n = 9)	14 (14 – 21)
All (n = 75)	21 (21 – 21)

As expected, patients on SCIG receive their therapy more frequently than those on IVIG (Table 5-3). Patients on IVIG did have a significantly higher monthly equivalent dose than those on SCIG ($p = 0.039$).

5.3 PhD XLA Data

I will now describe the data collected exclusively for this PhD research project. Fifty-four patients were recruited, of which clinical data were available for 50. The ongoing results presented here and in Chapter 6, pertain to those 50 patients. For analysis where data were not available for these 50 patients, this will be clearly stated in the denominator. The remaining four patients provided QoL data only, with no clinical data available at the time of recruitment.

5.4 Data coverage

The total follow-up of study participants was 1015.57 patient-years (Table 5-4). 56% (571.18 patient-years) of follow up data pertaining to infection rates, lung function data, HRCT and other data gathered from medical notes and clinic letters was collected. This equated to a median data collection of 73% of follow up data collected per patient. For paediatrics this was 100% and adults 54% (p = <0.001). Fifty-three per cent of all potential IgG trough levels were available for data collection (542.34 patient-years). This equated to a median IgG trough level collection of 76% per patient. For paediatrics, this was 100% and adults 55% (p=0.000). The better data collection in paediatrics representing better note keeping in recent years and the ease of collecting recent clinical notes versus historical notes (up to 60 years in some cases). In addition, it is more likely that adults will have transferred care over their lifetime through numerous hospitals, increasing the difficulty in tracing and obtaining notes.

Table 5-4 Total follow up and available data collected (patient-years)

	All	Paediatric	Adult
Follow up (patient years)	1015.57	106.38	909.19
Median follow up (patient years) (IQR)	20.68 (8.63 – 29.86)	5.28 (1.76 – 10.43)	27.28 (20.85 – 35.18)
Available clinical information accessed (years) (percentage of all follow up)	571.18 (56%)	90.75 (85%)	480.43 (53%)
Median clinical information coverage per patient (%) (IQR)	73% (45 – 100)	100% (100 - 100)	54% (31 – 77)
Available IgG trough levels accessed (years) (percentage of all follow up)	542.34 (53%)	98.37 (92%)	443.97 (49%)
Median IgG trough levels coverage per patient (%) (IQR)	76% (37 - 100)	100% (100 - 100)	55% (32 – 77)

5.5 Demographics

The median age of the cohort at the time of data collection and analysis (May 2018) was 26.87 years (IQR 11.33 – 36.58) with a median follow up of 20.81 years (IQR 8.63 – 29.86). Thirty-six percent (n = 18) of patients were aged 18 or less. With the Great North Children's Hospital (GNCH) and Great Ormond Street Hospital (GOSH) being the tertiary centres for paediatric immunology in the UK, 72% of paediatric patients were recruited from these two centres. The median age of the paediatric patients was 7.2 years (IQR 3.30 – 12.49) with a

median follow up of 5.28 years (IQR 1.53 – 9.53). The median age of the adult patients was 34.05 years (IQR 28.34 – 40.75) with a median follow up of 27.28 years (IQR 21.14 – 35.30). All but three patients of the cohort were Caucasian.

Table 5-5 Baseline demographics of the study cohort

Demographics	
Age (years) (median (IQR))	26.87 (11.33 – 36.58)
Paediatric	7.20 (3.30 – 12.49)
Adult	34.05 (28.34 – 40.75)
Follow Up (years) (median (IQR))	20.60 (8.63 – 29.86)
Paediatric	5.28 (1.53 – 9.53)
Adult	27.28 (21.14 – 35.30)
Age Group (N (%))	
Paediatric	18 (36%)
Adult	32 (64%)
Family History (N (%))	
Yes	24 (48%)
No	26 (52%)

5.6 Diagnosis

I first present the data pertaining to factors and clinical phenotype pre-diagnosis and directly prompting the diagnosis of XLA. Where available, I also present laboratory and immunological data in these patients before diagnosis and commencement of IGRT.

5.6.1 Age

The median age of diagnosis was 2.59 years (IQR 0.94 – 5.36) (Table 5-6). This has significantly improved over time (Spearman's $\rho = -0.367$, $p = 0.009$) (Figure 5-8). Excluding those who were screened at birth due to a family history of XLA (therefore relying on clinical findings for diagnosis, the median age at diagnosis was 2.97 (IQR 1.25 – 5.96). This has tended to improve with time, but this correlation did not reach statistically significant (Spearman's $\rho = -0.287$, $p = 0.069$) (Figure 5-8).

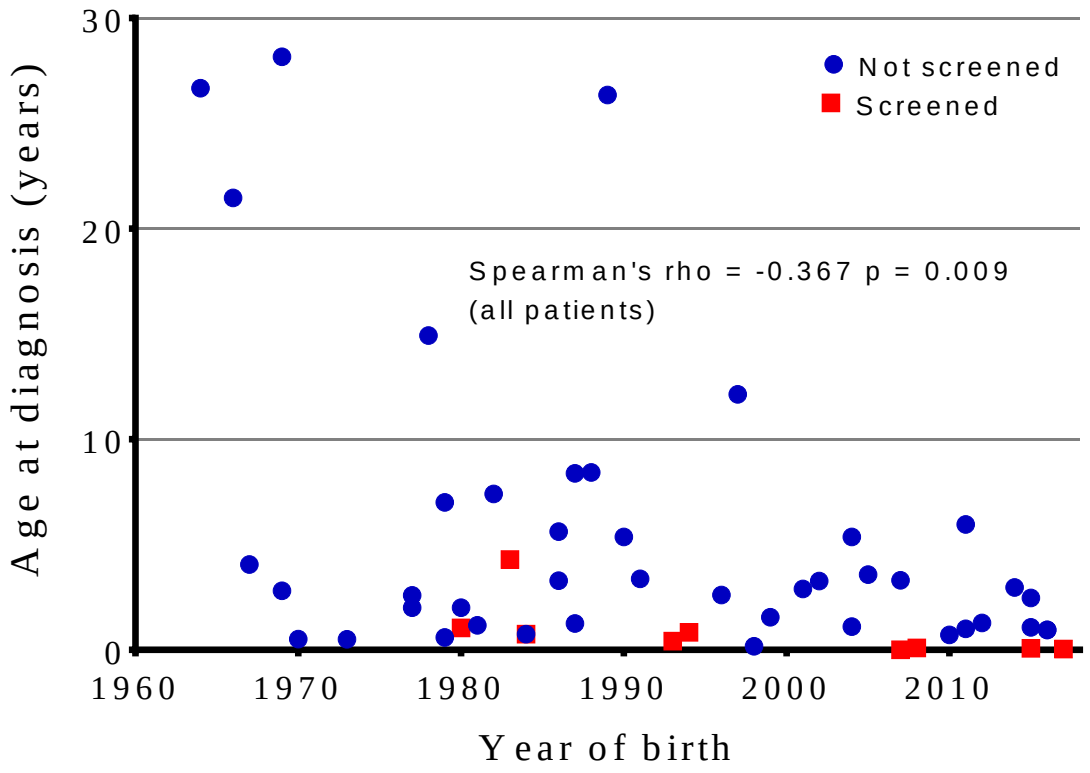
The median age of diagnosis for the paediatric patients is 1.19 years (IQR 0.71 – 3.27) and for adults 3.04 (IQR 1.10 – 7.89) ($p= 0.036$). For patients who were screened because of a

previously known family history of XLA or suspected XLA, the age at diagnosis is significantly lower at 0.42 years (IQR 0.07 – 0.83 p = <0.001).

Table 5-6 Variables and age at diagnosis (years, median (IQR))

Variable	Age at diagnosis	p value
Age at diagnosis for cohort	2.59 (0.94 – 5.36)	
Screened based on family history		
Yes (n = 9)	0.42 (0.07 – 0.83)	<0.001
No	2.97 (1.25 – 7.00)	
Current age group		
Paediatric	1.19 (0.71 – 3.27)	0.036
Adult	3.04 (1.10 – 7.89)	

Figure 5-8 Age of diagnosis over time



5.6.2 Immunology at diagnosis

Immunoglobulin levels at diagnosis and prior to commencement of IGRT were available for 19 patients. The median IgG level at diagnosis was 0.17 g/L (IQR 0 – 4.00). Ten out of these nineteen patients had detectable IgG levels at diagnosis (median IgG level 3.7 g/L (IQR 2.42 – 7.34). The median IgA level at diagnosis was 0 g/L (IQR 0 – 0), with only four patients

having detectable IgA at diagnosis (median IgA level 0.22 g/L (IQR 0.06 – 1.23). The median IgM level at diagnosis was 0 g/L (0 – 0.09) with seven patients having detectable IgM levels at diagnosis (median IgM level 0.17 g/L (IQR 0.08 – 0.38).

The presence or absence of any of the immunoglobulin isotypes was not associated with significant differences in age of diagnosis. There was no correlation with circulating immunoglobulin levels and age of diagnosis (Table 5-7). It should be noted the lower limit of detection does differ between different assays between different centres and within centres with changing assays over time. This will have affected the number of patients with detectable immunoglobulin. In general, assays have improved over time and the lower limit of detection has decreased over time.

Table 5-7 Immunoglobulin levels at diagnosis and age of diagnosis (years, median (IQR))

Detectable Immunoglobulin level or not	**Age at diagnosis**	**p-value**
Detectable IgA (n = 4)	6.87 (3.32 – 10.26)	0.110
No detectable IgA (n = 15)	2.89 (1.07 – 3.30)	
Detectable IgM (n = 7)	3.30 (1.07 – 5.37)	0.866
No detectable IgM (n = 12)	2.75 (1.19 – 6.68)	
Detectable IgG (n = 10)	4.47 (1.07 – 8.38)	0.286
No detectable IgG (n = 8)	2.75 (1.87 – 3.13)	
Correlation of Immunoglobulin level with age at diagnosis/p-value		
IgG	*r = 0.027, p = 0.915*	
IgM	*r = -0.047, p = 0.850*	
IgA	*r = 0.418, p = 0.075*	

The lymphocyte subsets at diagnosis were available for 15 patients and are shown in Table 5-8.

Four patients had a history of neutropenia prior to or at the time of diagnosis. Three of these patients presented with pseudomonas skin infection. No patient with normal neutrophil counts reported pseudomonas skin infection. The median CD19/20 count was 0 cells/mcL (IQR 0.00 – 0.01) where data were available. Four patients had detectable CD19/20 cells at diagnosis (median level 10 cells/mcL (IQR 10 – 20). There was no statistically significant association between having detectable CD19/20 cells at diagnosis and detectable levels of

IgA, IgM or IgG ($p = 0.111$). It should be noted that the lower limit of detection of CD19/20, neutrophils and serum immunoglobulins may differ between different centres within centres over time with changing and improving assays. In general, as assays have improved over time the lower limit of detection have improved over time. For example, at the Newcastle upon Tyne hospitals laboratories, the lower limit of neutrophil detection is 0.01×10^9/L and current flow cytometry can detect CD19 levels below 10 cells/mcL)

Table 5-8 Lymphocyte subsets of patients at diagnosis (prior to the instigation of IGRT) (cell/mcL, median (IQR))

	Cell count	Percentage
CD3 + T cells	3852 (2850 – 5650)	94 (93 – 95)
CD4 + T cells	1090 (492 – 2637)	67 (55 – 70)
CD8 + T cells	1418 (270 – 1650)	27 (24 – 38)
CD16/56 + NK Cells	363 (212 – 480)	8 (5 – 50)
CD 19/20 + B cells	0 (0 – 0.1)	0 (0 – 0.1)

The presence or absence of detectable levels of CD19/20 lymphocytes at diagnosis was not significantly associated with a difference in age at diagnosis or correlation (Table 5-9).

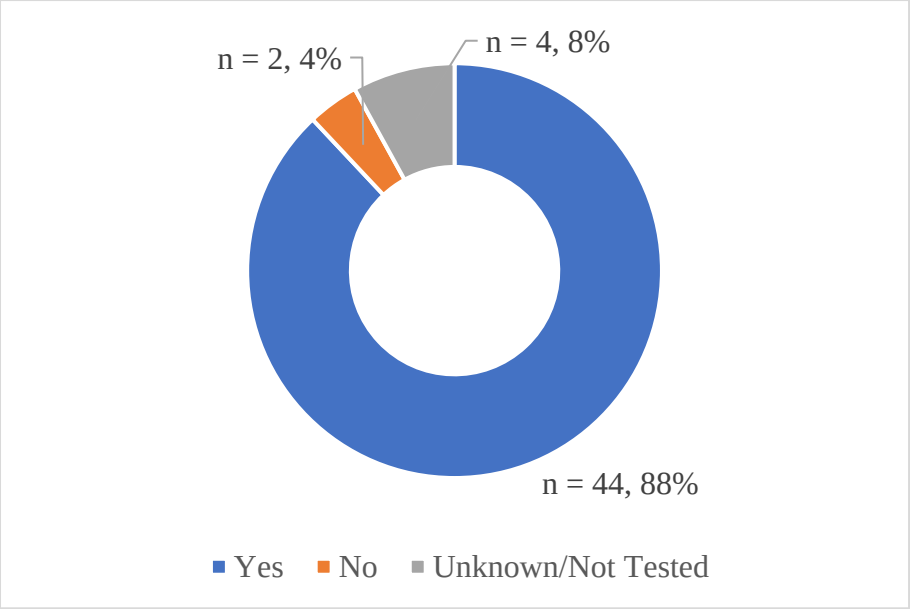
Table 5-9 Relationship of CD19/20 levels at diagnosis and age at diagnosis (years, median, IQR)

CD19/20	Age at diagnosis	p-value
Present	2.02 (1.01 – 4.46)	0.396
Absent	1.19 (0.08 – 2.68)	
Correlation of CD19/20 (cells/µL) with age at diagnosis	Spearman's rho = 0.189, p = 0.556	

5.6.3 Genetic mutations and BTK expression

A genetic defect was found in 44 out of 50 (88%) patients, and BTK expression was absent in 19, reduced in 2, normal in 6, and not tested in 23 (Figure 5-9). In all patients with normal BTK expression, or where BTK expression was not tested, a BTK mutation was found. The exact genetic defect was not available for eight out of the 44 patients recorded as having a proven genetic defect in BTK. In two cases, this was due to missing/unavailable genetic results but there was a consistent recording of these cases having a proven genetic defect documented in the clinical records. For the remaining cases, this was due to patients being diagnosed based on absent CD19/20 lymphocytes and a family member with a known BTK mutation. In these cases, the patient was not retested, presuming they had the same mutation as the index case. However, I was unable to recruit or find the index case to ascertain the exact mutation.

Figure 5-9 Genetic mutations (n, %)



BTK expression data were available for 27 patients (54%). It was absent in 19 (70%), reduced in two and normal in six. In one of the patients with initial apparent normal BTK expression, further testing was undertaken, and it was found to be dysfunctional.

There was no significant different difference in the age at diagnosis comparing the presence or absence of proven genetic mutation (p = 0.909), nor on the BTK expression status (p = 0.332). There may be potential differences between hospitals due to differing assays which may affect these results.

Table 5-10 Relationship of BTK expression and genetic mutation on age at diagnosis (years, median (IQR))

	Age at diagnosis	p-value
Genetic Defect		
Yes (n = 44)	2.53 (0.88 – 5.79)	0.909
No (n = 2)	3.04 (2.80 – 3.29)	
Unknown/Not tested (n = 4)	2.08 (1.07 – 3.98)	
BTK Expression		
Normal	5.64 (1.07 – 12.14)	0.332
Absent	2.80 (1.55 – 5.36)	
Reduced	1.05 (1.00 – 1.11)	
Not tested	1.28 (0.51 – 4.05)	

BTK mutations are described in more detail in section 5.14 within this chapter.

5.6.4 Clinical Presentation

There was detailed information on clinical presentation available for 43 patients (86% of the cohort). There was a recorded family history of XLA in 24 (48%) patients. Nine patients (21%) were screened due to a known or suspected family history of XLA. They were all asymptomatic at the time of diagnosis.

All but one of the remaining patients presented with infection(s) (34 patients, 79%). Two patients who could have been potentially screened due to positive family history were not, and were diagnosed once they became symptomatic with recurrent infections. One patient was diagnosed with XLA during diagnosis and treatment of acute lymphoblastic leukaemia (ALL).

Fifteen out of the 43 for whom data were available (35%) presented with repeated severe/life-threatening infections, defined as either meningitis, osteomyelitis or infections requiring surgical intervention or ITU stay. Five patients (10%) required at least one ITU stay prior to diagnosis. Fifty-one per cent of patients presented with a history of either recurrent upper respiratory tract infections (URTIs), lower respiratory tract infections (LRTIs) or otitis media (OM). (Table 5-11). Three patients required surgical interventions for an infection prior to their XLA diagnosis. One patient underwent a tonsillectomy; one patient required surgical intervention for pseudomonas skin infection, and a further patient required a below-knee amputation caused by osteomyelitis following a traumatic injury.

Table 5-11 Presenting Symptoms. (Totals add up to more than fifty and percentage to more than 100% as some patients presented with more than infection)

Presenting Symptoms	Number (Percentage) of patients presenting with (total available = 43)
Recurrent lower respiratory tract infections	17 (40%)
Screened on family history	9 (18%)
Sepsis	7 (16%)
Skin Infection	6 (14%)
CNS Infection	4 (9%)
Recurrent Otitis Media	3 (7%)
Eye infections	3 (7%)
Osteomyelitis	2 (5%)
Infective Arthritis	2 (5%)
Recurrent upper respiratory tract infections	2 (5%)
GI Infection	2 (5%)
Chickenpox	2 (5%)
TB	1 (2%)
Malignancy	1 (2%)

Excluding those that were screened asymptomatically, for 18 patients (36% of the cohort) there was enough information available to calculate pre-diagnosis annual infection incidence. The median annual infection incidence pre-diagnosis was 1.11 infections per year (IQR 0.36 – 3.90). The median respiratory infection incidence pre-diagnosis was 0.24 per year (IQR 0 – 1.63). The median number of infections reported before XLA was diagnosed in those without a family history was 4.5 (IQR 2 - 7).

For organisms cultured before the diagnosis of XLA (n = 21), Streptococcus pneumoniae was the most frequent organism isolated (33% of organisms) (Table 5-12). Pseudomonas aeruginosa infections accounted for 60% of skin infections. Where organism data were available, Streptococcus pneumoniae accounted for all the cases of meningitis.

Table 5-12 Organisms cultured in pre-diagnosis infections

Site of infection	Cultured organism (n)
Skin	Staphylococcal aureus (n = 2), Pseudomonas aeruginosa (n = 3)
Meningitis	Streptococcus pneumoniae (n = 3)
Sepsis/Bacteraemia	Streptococcus pneumoniae (n =1), Neisseria meningitides (n = 1), Haemophilus influenzae (n = 2), Pseudomonas aeruginosa (n = 1), Staphylococcal Albus (n = 1)
Osteomyelitis	Streptococcus pneumoniae (n =1), Pseudomonas aeruginosa (n = 1)
Lower respiratory tract	Streptococcus pneumoniae (n =2)
Gastrointestinal	Rotavirus (1), Campylobacter (1)
Arthritis	Haemophilus influenzae (n = 1)

5.7 Current Immunology

I will now present data pertaining to the period after diagnosis of XLA and commencement of IGRT (Table 5-13). It should be noted that the lower limit of detection may differ between laboratories due to differing assays. I did not have access to the lower limit of detection of the differing assays but, for reference, the lower limit of detection of IgA, IgM and IgG is 0.05g/L at the Newcastle upon Tyne Hospitals laboratories.

Thirty-nine out of forty-seven patients (83%) have current undetectable IgA levels and IgM levels (Table 5-13). For those patients with detectable levels the median IgA level was 0.26 g/L (IQR 0.055 – 0.675) and median IgM levels of 0.035 g/L (IQR 0.0 – 0.2). Forty out of fifty (80%) patients have undetectable (<2%) circulating B-lymphocytes. For those patients with detectable levels the median CD19/20 count was 12.5 cells/mcL (0%) (IQR 1 – 60). Patients who currently have some detectable CD19/20 cells were not statistically more likely to have circulating IgM levels ($p = 0.051$), or IgA ($p=0.217$). Patients with detectable IgA were more likely to have circulating IgM levels ($p = 0.02$).

There were no significant differences between adults and children and their current CD19/20 count ($p = 0.089$), IgA levels ($p = 0.181$), or IgM levels ($p = 0.515$).

There were six (12% of the cohort) patients with normal BTK expression on monocytes (all on flow cytometry), their median CD19/20 count was 0 (IQR 0 – 1). There were two (6%) patients with present, but reduced BTK expression; their median count CD19/20 was 86.5 (IQR 0 - 173). There was no significant difference in patients current CD19/20 count based on their BTK expression status ($p = 0.478$).

Table 5-13 Current immunology and laboratory values

Variable		
Immunoglobulins (g/L, median (IQR)		
IgA	0 (0 – 0.00)	
IgM	0 (0 – 0.00)	
IgG	9.7 (8.4 – 11.29)	
Adult Lymphocyte subsets	Cells/mcL	Percentage
CD3	1801 (807 - 2454)	87 (80-91)
CD4	1010 (661 – 1230)	58 (49 – 61)
CD8	490 (350 – 972)	30 (26-35)
CD19/20	0 (0 - 0)	0 (0 – 0)
CD16/56	253 (183 - 315)	13 (7 – 19)
Paediatric Lymphocyte subsets	Cells/mcL	Percentage
CD3	3158 (2358 – 4986)	93 (91 – 95)
CD4	1628 (1049 – 2533)	63 (51 – 69)
CD8	670 (350 – 1418)	27 (24 – 37)
CD19/20	0 (0 - 0)	0 (0 – 0)
CD16/56	214 (164 – 341)	6 (5 – 8)
Full Blood Count		
Haemoglobin (g/L)	141 (123 – 152)	
Platelets (x 10^9/L)	313 (254 – 359)	
White cell count (x 10^9/L)	8 (6.5 – 9.6)	
Neutrophils (x 10^9/L)	4.3 (4.0 – 5.8)	
Lymphocytes (x 10^9/L)	2.3 (1.6 – 2.9)	
BTK Expression		
Normal	6 (12%)	
Absent	20 (40%)	
Reduced	2 (4%)	
Not tested	22 (44%)	
Genetic defect		
Yes	44 (88%)	
No	2 (4%)	
Not tested	4 (8%)	

5.8 Infections on IGRT

Infections labelled as 'respiratory tract' are a combination of lower respiratory tract, otitis and sinus infections. Infection of some sites is so rare that displaying incidence does not portray any useful information. As such, some values for some infection sites are displayed as raw overall totals for illustration purposes. For statistical analyses, the infection incidence is still being compared.

The overall median annual infection incidence on IGRT was 1.11 infections/year (IQR 0.69 – 1.87). The median annual infection incidence in paediatric patients (1.38 (IQR 0.91 – 3.00)) tended to be higher than adult patients (0.91 (0.65 – 1.51)) but did not quite reach statistical difference, $p = 0.053$.

The majority of these were infections of the respiratory tract with an annual infection incidence of 0.90 (0.53 – 1.88). Paediatric patients have a significantly higher respiratory tract infection incidence than adults ($p = 0.034$). Due to increased awareness of PID in recent years and improvements in management, this difference could merely represent a decrease in the threshold for prescribing antibiotics in recent years. To ascertain if this might be true, I examined the infection data for only the last 18 years for both current paediatric patent and current adults (i.e. 1999 onwards). However, even when only analysing recent infection incidence (and therefore, potentially recent antibiotic prescribing practices), current paediatric patients still have a high respiratory tract infection incidence compared to adults over that same period (median 1.24 vs 0.72 infections per year, $p = 0.034$). I then went on to further only analyse infections recorded in childhood to ascertain if it was paediatricians perhaps had a lower threshold to prescribe antibiotics than their adult colleagues did (as oppose to antibiotic prescribing practices changing over time). I, therefore, compared the infection incidence under the age of 18 for current paediatric patients and for current adult patients, where data were available. This showed that current paediatric patients have a higher respiratory tract infection incidence compared to the childhood of current adult patients (median 1.24 infections per year versus 0.48, $p = 0.001$). These differences, therefore, most likely represent differences and changes in paediatric clinical practice, which will be discussed in further detail in the discussion chapter

Table 5-14 Annual infection incidence (median (IQR))

	All Patients (n=50)	Paediatric	Adult	p value
All infections	1.11 (0.69-1.87)	1.38 (0.91-3.00)	0.91 (0.65-1.51)	0.053
Respiratory Tract infections	0.80 (0.53-1.87)	1.24 (0.70-1.96)	0.75 (0.41-1.25)	0.034
Lower Respiratory infections	0.78 (0.52-1.65)	1.24 (0.70-1.96)	0.63 (0.27-1.25)	0.012
Sinus infections	0.00 (0.00-0.03)	0.00 (0.00-0.00)	0.00 (0.00-0.08)	0.003
Otitis infections	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00 (0.00-0.02)	0.355
	Totals			
Skin infections	37	22	15	0.211
Eye infections	25	7	18	0.698
GI infections	17	2	15	0.357
CNS infections	0	0	0	NA
Bone and Joint infections	0	0	0	NA
Sepsis	1	0	1	0.466
GU infections	11	2	9	0.255
Other infections	4	1	3	0.724

The median number of total infections per patient was nine (IQR 4 – 15, minimum 0, maximum 54) for the overall cohort since diagnosis. Respiratory tract infections accounted for the majority of these with a median value of seven infections per patient. There were 603-recorded infections for the cohort with respiratory tract infections accounting for 85% (n = 511). The number of sinus infections is surprisingly low and not reported in children. This is most likely due to an underreporting of sinus infections or related symptoms due to their perceived benign nature. Patients may also be self-treating these, and not reporting their symptoms to their immunologist.

There were a small number of UTIs (2 in children, 9 in adults). XLA and PAD patients are not usually thought of as being susceptible to UTI infections and these infections may simply reflect the normal background rate of UTIs although future work should seek to confirm this.

The vast majority of these infections were treated as an outpatient, with inpatient treatment only accounting for 10 of all the infections recorded. Four (18%) patients had an ITU/HDU admission on IGRT that was related to infection.

There was no correlation with the lifetime median infection incidence and the lifetime median IgG trough level (rho = 0.174, p = 0.242).

5.8.1 Infections pre versus post instigation of IGRT

There were eighteen patients where data were available and who were not screened asymptomatically. The median overall annual infection incidence before diagnosis (and instigation of IGRT) was 1.11 (0.36 – 3.90) compared to their annual infection incidence on IGRT of 1.12 (IQR 0.63 – 1.89), p = 0.557 (Table 5-15).

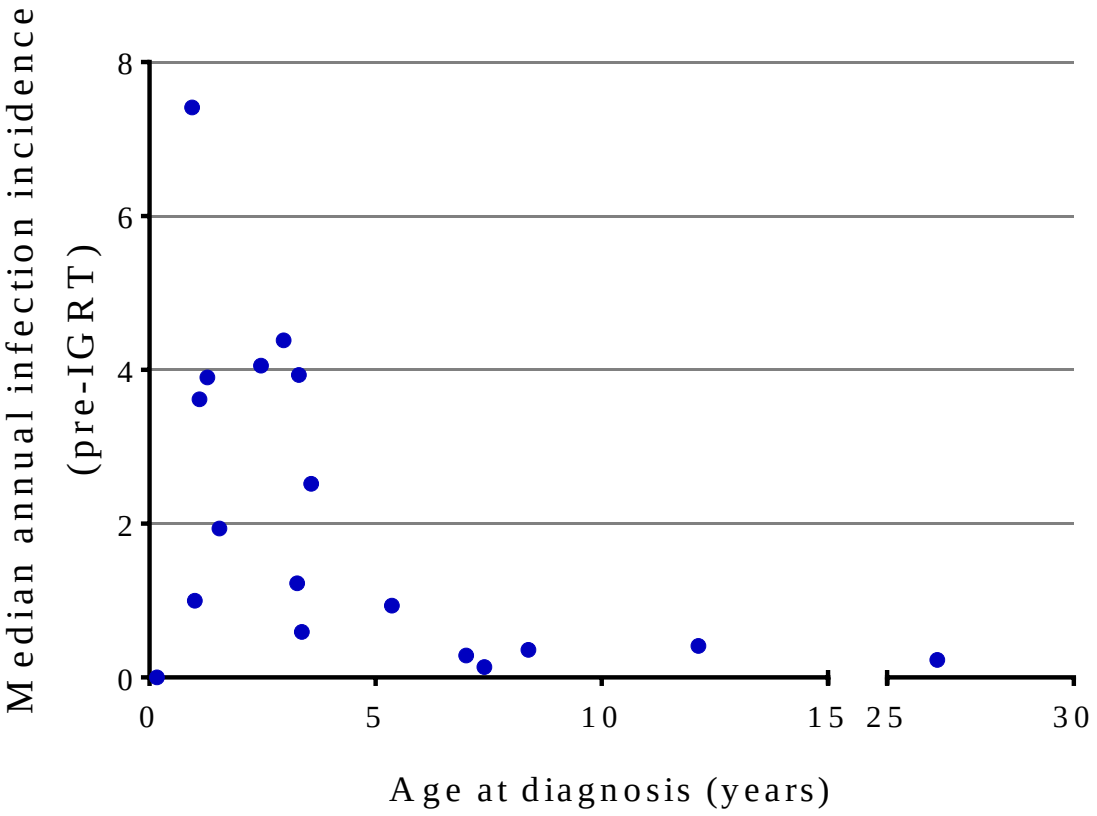
There were significantly fewer central nervous system infections, invasive musculoskeletal infections and recorded sepsis on IGRT compared to without. However, these events were still rare, both pre and post-diagnosis.

Table 5-15 Annual infection incidence pre and post XLA diagnosis (median (IQR))

	Pre diagnosis	Post diagnosis	p-value
All infections	1.11 (0.36 – 3.90)	1.12 (0.63 – 1.89)	0.557
Respiratory tract infections	0.90 (0.00 – 2.03)	0.86 (0.54 – 1.89)	0.723
Lower respiratory tract infections	0.90 (0.00 – 2.03)	0.79 (0.54 – 1.89)	0.723
Sinus infections	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.158
Otitis infections	0.00 (0.00-0.56)	0.00 (0.00 – 0.00)	0.078
	Totals		
Skin infections	8	7	0.200
Eye infections	0	2	0.158
GI infections	2	0	0.317
CNS infections	4	0	0.046
Bone and Joint infections	5	0	0.046
Sepsis	7	1	0.009

For those patients whose pre-diagnosis data collection is considered to be 100%, increasing age of diagnosis was associated with a lower pre-diagnosis infection incidence (excluding those who were screened asymptomatically) (Spearman's rho = -0.490, p = 0.039) (Figure 5-10). Translating this to clinical practice, this likely represents that those with more frequent infections (and potentially a more severe phenotype) are more likely to be investigated for PID earlier than those with fewer infections.

Figure 5-10 Pre-diagnosis median annual infection incidence and age at diagnosis



5.8.2 Chronic sinusitis and conjunctivitis

Eleven patients (22%) reported are diagnosed with recurrent sinusitis, and six patients (12%) are diagnosed with recurrent conjunctivitis. There was a significant association between the two comorbidities (p = 0.017).

At last follow-up, patients with sinusitis were significantly older than those without (36 years versus 20 years, p = 0.003). There were no significant differences in current age for patients with or without chronic conjunctivitis (p = 0.355). There were no significant differences for age at diagnosis or lifetime median IgG trough levels for patients with chronic sinusitis compared to those without. The same was true when comparing those patients with and without chronic conjunctivitis.

Table 5-16 Demographics of patients with and without chronic sinusitis or chronic conjunctivitis (median (IQR))

	Chronic sinusitis	None	p-value
Age (years)	36.22 (29.15 – 38.98)	20.07 (7.89 – 34.35)	0.025
Age at diagnosis (years)	1.25 (0.83 – 7.00)	2.80 (0.94 – 5.36)	0.815
Lifetime median IgG trough level (g/L)	9.88 (9.37 – 10.48)	9.03 (7.90 – 10.60)	0.567
Latest IgA level (g/L)	0.00 (0.00 – 0.04)	(0.00 – 0.00)	0.341
Latest IgM level (g/L)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.905
	Chronic conjunctivitis	**None**	**p-value**
Age (years)	31.88 (19.88 – 40.34)	25.44 (9.22 – 36.41)	0.355
Age at diagnosis (years)	1.55 (1.04 – 3.37)	2.70 (0.89 – 5.49)	0.754
Lifetime median IgG trough level (d/dL)	10.48 (8.10 – 11.51)	9.34 (7.90 – 10.50)	0.469
Latest IgA level (g/L)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.292
Latest IgM level (g/L)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.292

5.9 Comorbidities and Complications

Past medical history and data pertaining to co-morbidities was available for 50 patients and is summarised in Table 5-17.

Table 5-17 A summary of current comorbidities in this XLA Cohort

Comorbidity	Number of patients (%)
Sinopulmonary disease	
Bronchiectasis	22 (44%)
Abnormal HRCT (Not Bronchiectasis)	9 (18%)
Chronic sinusitis	11 (22%)
Gastrointestinal disease	
Chronic non infective diarrhoea	5 (10%)
Chronic Infective diarrhoea	3 (6%)
Inflammatory bowel disease	3 (6%), 1 of whom is Crohn's
Underwent endoscopy	10 (20%)
Musculoskeletal	
Arthritis	4 (8%)
Fatigue/arthralgia	3 (6%)
Malignancy	1 (2%)
Psychiatric/Neuro	
Memory disturbance	3 (6%)
Anxiety	6 (12%) – 1 of whom have resolved
Depression	7 (14%) - 2 of whom have resolved
Epilepsy	2 (4%)
Deafness	5 (10%)

5.9.1 Respiratory Disease

Twenty-two (44%) of patients had proven bronchiectasis on HRCT, and a further nine patients (18%) had abnormal HRCT scans (Table 5-17). Sixty-three per cent of adults have a diagnosis of bronchiectasis and 11% of children have a diagnosis of bronchiectasis. The median age of onset was 21.97 years. Four patients have required surgery for their lung disease.

The number of patients with a formal diagnosis of chronic sinusitis is surprisingly low given the background literature. All of these patients were diagnosed with chronic sinusitis based on persistent clinical symptoms. No patient had a CT or other imaging of their sinuses.

The respiratory health of the cohort will be discussed further in Chapter 6.

5.9.2 Gastrointestinal disease

Ten (20%) patients report at least one GI symptom necessitating endoscopy, of which one has resolved (Table 5-18). Two patients have confirmed infective (norovirus) diarrhoea with nutritional compromise. One of these patients has undergone haematopoietic stem cell transplantation in a bid to clear the norovirus infection, and the second has undergone assessment for HSCT. The second has undergone assessment for HSCT and is currently deciding whether to go ahead with the procedure. He currently requires supplemental gastrostomy feeding to maintain adequate nutrition but is maintaining adequate IgG trough levels on standard therapy. In contrast to the first patient undergoing HSCT, this second patient is able to maintain IgG trough levels on standard therapy, and his IgG dose has not had to be increased after the development of the norovirus infection. The failure to clear norovirus in this patient by conventional and other novel therapies has been described previously (185).

One patient has confirmed Crohn's disease, which is currently well controlled, and he is the maternal uncle to patient 24. Patient 24 has chronic, non-infectious diarrhoea and has been described as IBD (inflammatory bowel disease) like disease, but with normal histology, although it should be noted no biopsies were taken from the terminal ileum. A further one patient has been described as having IBD like disease.

Table 5-18 GI disease in XLA cohort

Patient ID	Details	Histology	Resolved or Current
4	Non-infectious colitis	Normal	Resolved
17	Chronic norovirus infection, chronic enteropathy, poor weight gain, NG feeding.	Chronic enteropathy with duodenal villous blunting, marked increased intraepithelial lymphocytes and lymphocytosis in lamina propria. Colonic biopsies showed increased epithelial lymphocytes	Current. Has undergone HSCT
22	Chronic non-infectious diarrhoea	Normal	Current
23	Poor growth. "IBD like disease" – improvement with infliximab. Treatment for confirmed Giardia	Duodenal villous blunting	Current – Infliximab and Giardia treatment has resulted in the beginning of good growth and improvement of symptoms
24	Chronic non-infectious diarrhoea. Related to Patient ID 26	Normal (no terminal ileum samples)	Current
25	Persistent Nausea, previous diarrhoea	Upper OGD Normal	Current
26	Crohn's disease	Initial - Pancolitis, most recent shows remission	Current

Patient ID	Details	Histology	Resolved or Current
30	Stool urgency and diarrhoea – Irritable bowel syndrome	2014 - normal	Current
36	Chronic Norovirus, On PEG supplemental feeds	Villous Blunting, Some ulcers in the small bowel	Current. Considering HSCT.
37	Chronic abdominal pain and non-infectious diarrhoea	Normal	Current
38	Previous Giardia Infection	None done	Resolved
50	Deranged liver function tests. Thought to be non-alcoholic fatty liver disease (NAFLD)	None done	Current

Further analysis was carried out on ten of the patients with GI disease. No further analyses were carried out for the patient with deranged liver function tests (patient 50) or the patient with a previous Giardia infection (patient 38). For patient 50, it was felt the deranged liver function was most likely due to non-alcoholic fatty liver disease (NAFLD), unrelated to the XLA.

There were no significant differences in the demographics, current immunology values or clinical therapy between those currently with and without GI disease.

Table 5-19 Characteristics of those with and without current GI disease (median (IQR))

Variable	No GI disease	GI disease	p-value
Age (years)	28.34 (10.65 – 36.41)	21.38 (11.33 – 41.15)	0.717
Age group			
Paediatrics	14	4 (22%)	1.000
Adult	26	6 (19%)	
Age at diagnosis (years)	2.85 (1.09 – 5.66)	0.77 (0.17 – 3.58)	0.099
Follow up (years)	20.81 (5.28 – 30.31)	20.88 (9.53 – 27.63)	0.645
Lifetime IgG Trough (g/dL)	9.07 (7.83 – 10.50)	10.5 (9.89 – 11.40)	0.066
Latest IgG trough (g/dL)	9.56 (8.54 – 11.25)	11.32 (8.18 – 13.60)	0.190
Latest IgA level (g/dL)	0 (0 – 0)	0 (0 – 0)	0.137
Latest IgM level (g/dL)	0 (0 – 0)	0 (0 – 0)	0.620
Latest CD19/20 (cells/µL)	0 (0 – 0)	0 (0 – 0)	0.382
Current IGRT			
SCIg	20	5	0.264
IVIg	19	4	
SCIg/IVIg	0	1	
Previous IM therapy	8	1	0.665

Variable	No GI disease	GI disease	p-value
BTK expression			
Normal	6	0	1.000
Absent	18	1	
Reduced	2	0	
Genetic Defect			
Yes	33	10	1.000
No	2	0	
BMI			
Grade 2 Thinness	2	0	1.000
Grade 1 Thinness	4	1	
Normal Weight	13	5	
Overweight	14	4	
Obese	2	0	

Twenty of the forty-five patients for whom BMI data were available were overweight (44%) (Table 5-20). Eighteen (40%) were a healthy weight and seven (16%) were underweight. Only one of the 13 paediatric patients were overweight versus 19 (59%) of the adult patients ($p = 0.002$). There was no statistically significant association with BMI category and the presence of XLA related GI disease ($p = 1.000$). The median BMI for adults was 23.63 (IQR 21.00 – 27.12). The median BMI Z-score for children was -0.14 (IQR -0.73 – 0.71).

Table 5-20 BMI status for the cohort

	Paediatrics	Adults	Total
Grade 2 Thinness	2	0	2
Grade 1 Thinness	1	4	5
Normal Weight	9	9	18
Overweight	1	17	18
Obese	0	22	2

5.9.3 Musculoskeletal disease

Seven patients had chronic muscle and joint pains, arthritis or muscle and joint-related fatigue. Four of these patients have a formal diagnosis of arthritis. All of these four patients had previously confirmed mycoplasma infection of the affected joints.

A further three patients with significant muscle aches and/or fatigue had also been diagnosed with fibromyalgia. There were no reported previous joint or bone infections in these three patients.

5.9.4 Malignancy

Only one patient had a reported history of malignancy. This patient developed acute lymphoblastic leukaemia (ALL) at the age of seven years. During his treatment and recovery, he demonstrated a persistent agammaglobulinaemia, which was later proven to be XLA. He has an older brother with XLA, recruited to this study, who has no history of malignancy.

5.9.5 Memory disturbances

Three (6%) patients had reported problems with their memory, warranting specialist referral with a neurologist. Their current median age was 38.63 years (IQR 31.11 – 48.76). One of these patients had depression and a further one has anxiety. The third patient had a history of recurrent echovirus encephalitis. The supervising medical team presumed that the memory problems were related to the echovirus infection

5.9.6 Deafness

Five (10%) patients have at least unilateral deafness, all of whom it is presumed this is secondary to infection. Three of the five had meningitis prior to the diagnosis of XLA, and the remaining two had recurrent ear infections. No patient with deafness or within the whole cohort has mutations at the 3' end of *BTK* and the closely linked *TIMM8A* gene associated with both XLA and deafness (337).

5.9.7 Other complications

Two patients have epilepsy, one of whom is a patient with recurrent echovirus encephalitis. In this case, it is highly suspected that the epilepsy is due to the underlying infection.

5.10 Disease Severity

There was sufficient clinical detail for 50 patients to ascertain a disease severity as per the process described in the methods chapter. Fifty – four per cent of patients have moderate or severe disease severity. After Bonferroni (Holm) correction, patients with moderate disease are significantly older than those patients with mild disease ($p = 0.006$). There were no statistically significant differences in age at diagnosis across the disease severity groups.

Table 5-21 Disease severity by current age and age at diagnosis (years, median (IQR))

Disease severity	Number of patients	Current age	Age at diagnosis
Asymptomatic	3 (6%)	21.30 (2.40 – 30.06)	3.28 (0.94 – 12.14)
Mild	19 (38%)	12.83 (6.34 – 32.03)	1.28 (0.75 – 3.58)
Moderate	19 (38%)	34.35 (24.66 – 41.15)	2.80 (1.04 - 7.00)
Severe	9 (18%)	31.11 (19.88 – 48.11)	2.60 (1.25 – 14.92)
p value		0.031	0.622

5.11 Immunoglobulin Therapy

Immunoglobulin replacement therapy data were available for 49 patients. One patient, with a proven BTK genetic defect, recurrent infections and mild hypogammaglobulinaemia had not yet been started on IGRT at the time of the study.

5.11.1 Route of IGRT

Fifty-one per cent of patients (n = 25) were receiving their IGRT intravenously, and 47 per cent (n = 24) via the subcutaneous route. There is one child on both IV and SC immunoglobulin therapy. This patient has persistent norovirus infection and is undergoing HSCT. His enteropathy was severe enough that he required high doses of combined SCIg and IVIg to maintain adequate IgG trough levels.

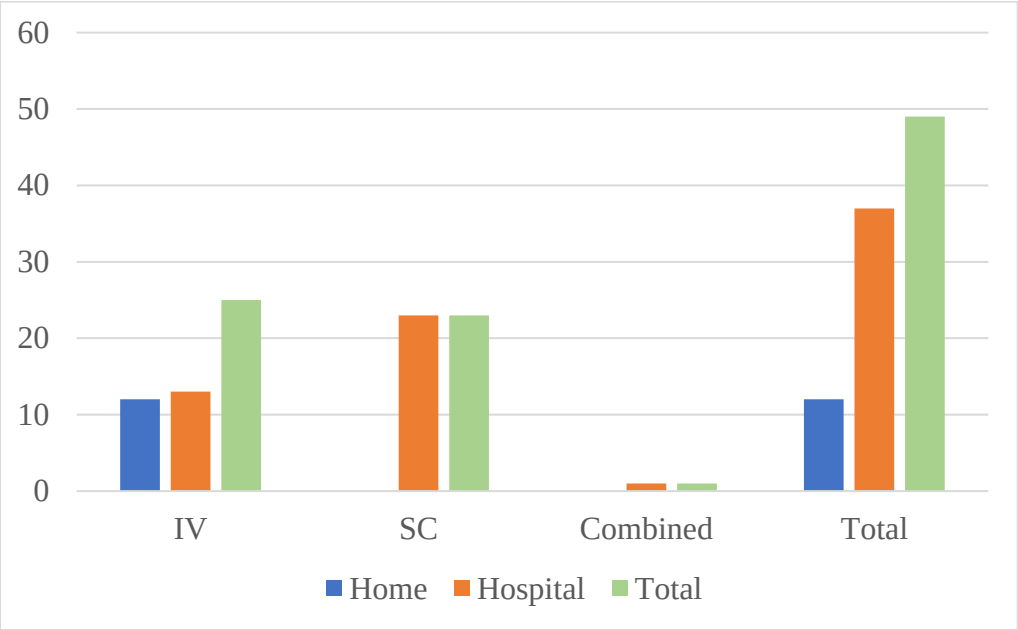
A significantly larger proportion of children received SCIg versus IVIg compared to adults (p = 0.001). 82% of paediatric patients received their therapy via the SC route versus 29% of adults. Despite this, there was no significant difference between the age groups as to how many receive their therapy at home (p = 0.330). This lack of significant difference was likely since 55% of adults who do receive their IGRT intravenously still being able to administer this at home, under their own or a relative's cannulation. None of the four children who received IVIg were able to do this at home, due to patient and family preference.

As expected, significantly more patients on SCIg received their therapy at home compared to IVIg (p < 0.001). All patients on SCIg received their therapy at home versus 48% of IVIg.

Nine (18%) patients have received intramuscular IGRT previously. These patients are significantly older than patients who have never received IM IGRT (median age 40.34 years versus 20.08 years, p = 0.0002). Having IM IGRT previously was not associated with a significant difference in lifetime median infection annual incidence (0.81 infections/year with

previous IM therapy versus 1.25 infection per year, p = 0.134). It was not associated with disease severity, p = 0.100.

Figure 5-11 Site and route of IGRT



Privigen is the commonest IV preparation, accounting for 36% of all products (Table 5-22 IV immunoglobulin products). Hizentra accounts for the commonest SC immunoglobulin products accounting for 50% of all SC products (Table 5-23).

Table 5-22 IV immunoglobulin products

Product	Number (n)	Percent (%)
Privigen	9	36
Flebogamma	8	32
Kiovig	5	20
Gamunex	3	12
Total	25	100

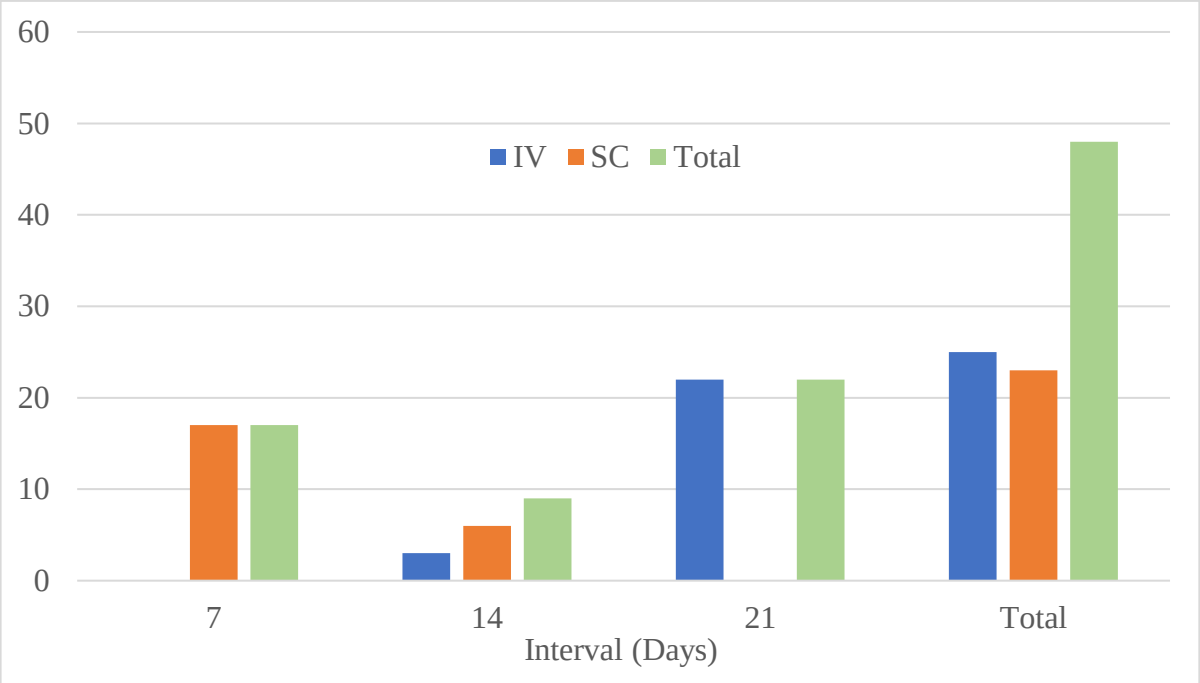
Table 5-23 SC immunoglobulin products

Product	Number (n)	Percent (%)
Hizentra	11	50
Subcuvia	6	27
Subgam	4	18
Cuvitru	1	5
Total	22	100

5.11.2 Dosing of IGRT

The median IGRT dose for the entire cohort was 568mg/Kg/month (IQR 488 – 678, n = 44). For IVIg, the median dose was 533mg/Kg/month (IQR 489 – 644). For SCIg, the median dose was 593mg/Kg/month (IQR 447 – 678). These were not significantly different (p = 1.000). The median interval between doses was seven days (IQR 7 - 21, n = 46) (Figure 5-12). For IVIg, the median interval was 21 days (IQR 21-21) and for SCIg 7 days (IQR 7-14). This was significantly different (p = 0.000).

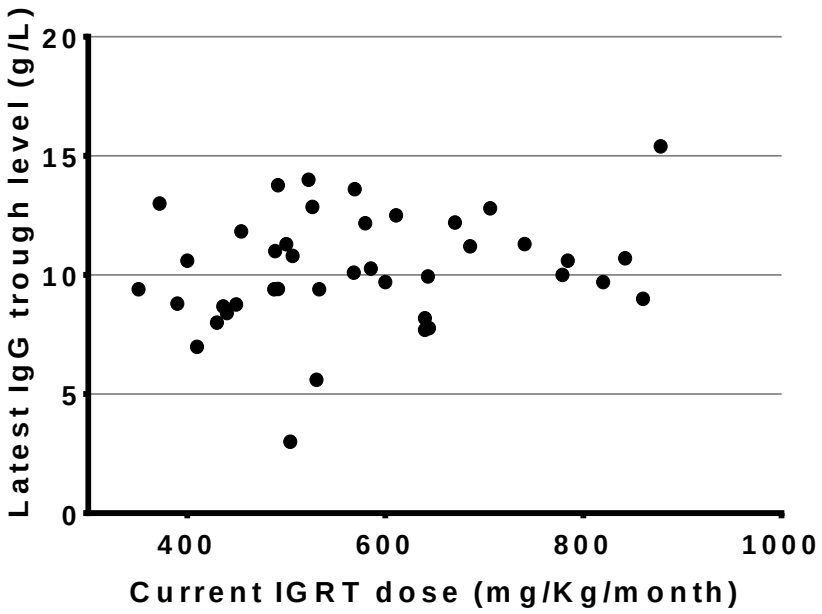
Figure 5-12 Frequency of IGRT



The median last recorded IGRT dose for the entire cohort was 568mg/Kg/month (IQR 490 – 656, n = 44). This is does not significantly correlate with the last recorded median IgG trough levels (10.0 g/L, IQR 8.70 = 12.17) (Spearman's rho = 0.269, p = 0.085) (Figure 5-13). This lack of correlation is likely to be due to a number of reasons; route of administration and

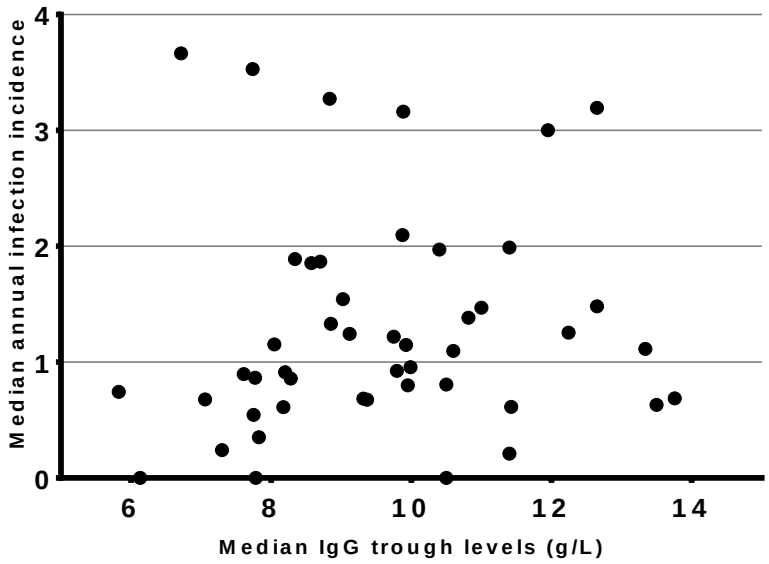
potential different in pharmacokinetics and IgG metabolism between patients. This lack of correlation adds further argument to the individual of IgG doses based on clinical symptoms rather than a blanket target as supported by others (207).

Figure 5-13 Current IGRT dose and IgG trough level



The lifetime median IGRT dose was 568mg/Kg/month (IQR 490 – 656). This did not correlate with lifetime median IgG trough levels (9.37 g/L, IQR 8.05 – 10.82) (rho = 0.083, p = 0.600) or median lifetime infection incidence (rho = 0.206, p = 0.181). Lifetime median IgG trough levels were not significantly associated with median lifetime annual infection incidence (rho = 0.174, p = 0.242) (Figure 5-14).

Figure 5-14 Median lifetime IgG trough levels and median lifetime median annual infection incidence



The last recorded IGRT dose for current paediatric patients is significantly higher than adults (median 640 mg/Kg/month, IQR 585 – 741 versus 522 mg/Kg/month, IQR 449 – 600, p = 0.012). Their last recorded IgG trough levels were not significantly different (p = 0.857).

Last recorded IgG trough levels did not significantly differ by current administration route (p = 0.078).

After Bonferroni (Holm) correction, patients with moderate disease (as defined by my classification) have significantly lower last recorded IgG trough levels than those with mild disease (p = 0.006). There was no significant difference for median lifetime IgG trough levels across disease severity (p = 0.340) (Table 5-24).

Table 5-24 Disease severity and IgG trough levels (g/L, median (IQR))

Disease severity	Number of patients	Last recorded IgG trough level	Median lifetime IgG trough level
Asymptomatic	3 (6%)	9.40 (6.93 – 11.20)	7.78 (6.12 – 9.31)
Mild	19 (38%)	11.15 (10.27 – 12.80)	9.84 (8.34 – 11.40)
Moderate	19 (38%)	9.40 (8.40 – 10.10)	9.75 (8.18 - 10.50)
Severe	9 (18%)	10.00 (8.00 – 14.80)	8.28 (7.61 – 11.40)
p value		0.047	0.340

There was no correlation with the standard deviation of the yearly mean IgG trough levels with annual infection incidence or annual respiratory infection incidence. Having spent a longer time on IGRT also did not correlate with the standard deviation. There was no significant difference in the lifetime IgG trough SD based on current IGRT route, therapy compliance or disease severity (Table 5-25). This suggests that therapy compliance and route do not significantly affect variation in IgG trough levels. In turn, any variations in IgG trough levels do not appear to affect clinical phenotype or disease severity.

Table 5-25 Association of the standard deviation of median annual IgG trough levels on outcomes

	Correlation of SD of the median annual IgG trough levels	p-value
Lifetime yearly infection incidence	Rho = 0.186	0.217
Life time yearly respiratory tract infection incidence	Rho = 0.084	0.581
Time on IGRT	Rho = 0.100	0.509
	SD (Median, IQR)	
Current IGRT route		
Subcutaneous	1.31 (0.92 – 1.74)	0.215
Intravenous	1.47 (0.90 – 2.14)	
Mixed	6.73 (NA)	
Therapy Compliance Issues		
None	1.44 (0.97 – 2.14)	0.210
Mild	0.92 (0.90 – 1.21)	
Moderate	1.77 (1.74 – 2.51)	
Disease Severity		
Asymptomatic	0.92 (0.79 – 1.66)	0.513
Mild	1.40 (1.12 – 2.36)	
Moderate	1.39 (0.88 – 2.09)	
Severe	1.51 (0.97 – 2.51)	

5.12 Prophylactic antibiotics

Twenty-one patients (42%) are on prophylactic antibiotics. Nine (32%) patients without bronchiectasis are on prophylactic antibiotics. Twelve (55%) patients with bronchiectasis are on prophylactic antibiotics. There was no significant association with the use of prophylactic antibiotics and the presence or absence of bronchiectasis (p = 0.111). Azithromycin accounts for the most commonly prescribed antibiotics for prophylaxis, accounting for 62% of all prophylactic antibiotics. There were no significant differences in antibiotics choice between those with and those without bronchiectasis (p = 0.296) (Table 5-26).

Table 5-26 Prophylactic antibiotic use in cohort (n, %)

Antibiotics	All (n= 21)	Bronchiectasis (n= 12)	No Bronchiectasis (n= 9)
Amoxicillin	2 (10%)	0 (0%)	2 (22%)
Azithromycin	13 (62%)	8 (67%)	5 (56%)
Co-Trimoxazole	4 (19%)	2 (17%)	2 (22%)
Doxycycline	2 (10%)	2 (17%)	0 (0%)

Accurate dosing data were available for 18 patients. The majority of patients took a daily regimen (61%) versus a three-time a week dosing regimen (usually Monday, Wednesday and Friday). The majority of patients (80%) took their prophylactic antibiotics all year round as opposed to just over the winter period. The choice of antibiotics did not significantly affect dosing choices ($p = 0.457$), or whether it was just given over winter ($p = 1.000$) (Table 5-27).

Table 5-27 Dosing of prophylactic antibiotics (n, %)

	All	Amoxicillin	Azithromycin	Co-Trimoxazole	Doxycycline
Daily	11 (61%)	2 (100%)	5 (45%)	2 (66%)	2 (100%)
Three time as week	7 (39%)	0 (0%)	6 (55%)	1 (34%)	0 (0%)
Winter only	4 (20%)	0 (%)	3 (25%)	1 (34%)	0 (0%)

There are no significant differences in the median lifetime annual infection incidence for patients who are currently on prophylactic antibiotics versus those who are not ($p = 0.558$), and this remains true when only analysing respiratory tract infections ($p = 0.467$) (Table 5-28). There continue to be no significant differences when comparing those with and without bronchiectasis (Table 5-28).

Table 5-28 Median lifetime annual infection incidence and the use of prophylactic antibiotics (median (IQR))

All infections	On prophylactic antibiotics	No prophylactic antibiotics	p-value
All Infections			
Whole Cohort	1.09 (0.81 – 1.87)	1.15 (0.62 – 1.91)	0.558
Bronchiectasis	1.00 (0.80 – 1.41)	1.05 (0.63 – 1.85)	1.000
No Bronchiectasis	1.38 (0.86 – 1.89)	1.19 (0.35 – 1.97)	0.440
Respiratory Tract infections			
Whole Cohort	0.91 (0.54 – 1.87)	0.73 (0.45 – 1.76)	0.467
Bronchiectasis	0.86 (0.52 – 1.18)	0.78 (0.55 – 1.65)	0.895
No Bronchiectasis	1.38 (0.76 – 1.89)	0.69 (0.18 – 1.88)	0.280

The data on the use of prophylactic antibiotics only pertains to the patient's current clinical status. It was not possible to accurately determine when prophylactic antibiotics were started and how long patients have been prescribed them. This, therefore, introduces some limitations and confounding in the analysis above to determine the effectiveness of prophylactic antibiotics in XLA.

To address some of these limitations, I also analysed only the last single year of available data for annual infection incidence, where I was able to accurately determine if prophylactic antibiotics were used that year.

Table 5-29 Number of infections for last year of available data collection, comparing the use of prophylactic antibiotics

	On prophylactic antibiotics	No prophylactic antibiotics	p-value
All infections			
All	1 (0 – 2.5)	0 (0 – 1)	0.096
Bronchiectasis	1 (0 – 3)	0.5 (0 – 2.5)	0.653
No Bronchiectasis	1 (1 – 1)	0 (0 – 1)	0.089
Respiratory tract infections only			
All	1 (0 – 1.5)	0 (0 -1)	0.108
Bronchiectasis	1 (0 – 2)	0.5 (0 – 2)	0.822
No Bronchiectasis	1 (1 – 1)	0 (0 – 1)	0.060

Just looking at the last single year of available infection and prophylactic antibiotic data, there are no significant differences in the number of overall infections or respiratory tract infections comparing those on prophylactic antibiotics and those who are not. There remained no significant differences when analysing according to the current presence of bronchiectasis or not.

5.13 Treatment compliance

Using the criteria I defined in the methodology, 19% of patients (n = 9) were defined as having current or previous mild and moderate issues with treatment compliance (from a total of 48 patients for whom data was available). There was no association between treatment compliance and disease severity (p = 0.092) (Table 5-30). There were no significant differences or therapy compliance comparing adults to children (p = 0.253). Although, it should be noted there is a trend towards more issues with compliance and worsening disease severity with no patient with asymptomatic disease or mild severity reporting issues with treatment compliance. Further details on compliance were not available so it is not possible to ascertain the time frame of these issues and its relationship to disease severity. It is therefore not possible to ascertain if worsening treatment compliance is associated with increased disease severity or vice versa.

Table 5-30 Treatment compliance (n)

Compliance/Disease Severity	None	Mild Severity	Moderate	Severe
No issues	2	18	12	7
Mild	0	0	5	1
Moderate	0	0	2	1
Significant issues	0	0	0	0

5.14 Genetics

A genetic defect was found for 44 patients (88%). Two patients underwent genetic testing, but no defect has been found. For the remaining four patients, I could find no evidence of genetic testing being carried out. Out of the 44 patients with a genetic mutation in BTK, basic data on the mutation was available for 37 of them, of which precise data were available for 31. Seven patients are recorded as having a BTK mutation but no details available. The most common reason for this was being diagnosed on the basis on absent B-lymphocytes and a

positive history of a family member with a proven BTK mutation, but where I was unable to recruit the family member and ascertain the exact mutation. There were two patients (brothers) who are recorded as having a BTK mutation in the clinic notes but I was unable to find the original genetic report.

5.14.1 Mutations

Null mutations accounted for 68% of mutations versus 32% missense mutation. Premature stop codons were the most prevalent type of mutation, accounting for 40% of patients in whom data was available.

Table 5-31 Mutations in the cohort (* denotes mutations not previously described in the literature)

Codon change	Protein change	Position	Domain	Mutation
c.238C>T* (n = 2)	P80A	Exon 3	PH	Amino acid substitution at non conserved sites
c.1000T>C*	Y334H	Exon 12	SH2	AA substitution at conserved sites in Btk family
c.1070_1071delAGinsTCT*	E357Vfs*4	Exon 12	SH2	Frameshift Mutation
c.1275C>A	Y425X	Exon 14	Kinase	Premature Stop Codon
c.1349+4A>G* (n = 2)		Intron 14		Splice defects at conserved invariant base pairs
c.1566+1G>A (n = 2)		Intron 15		Splice defects at first/last intron base pairs
c.1684_1685delCGinsTA*	R562X	Exon 17	Kinase	Premature Stop Codon
c.1691C>A* (n = 2)	S564Y	Exon 17	Kinase	Amino acid substitution at non conserved sites

Codon change	Protein change	Position	Domain	Mutation
c.1733_1735dupCTG*	S578_D579 insA	Exon 17	Kinase	Inframe deletions
c.1750+1G>A		Intron 17		Splice defects at first/last intron base pairs
c.1580_1584delGTTT		Exon 16	Kinase	Frameshift Mutation
c.1889T>C	M630T	Exon 18	Kinase	AA substitution at conserved sites in Btk family
c.1902G>A (n = 2)	W634X	Exon 18	Kinase	Premature Stop Codon
c.309+2T>C*		Intron 4		Splice defects at first/last intron base pairs
c.43C>T	Q15X	Exon 2	PH	Premature Stop Codon
c.700C>T	Q234X	Exon 8	SH33	Premature Stop Codon
c.710delA*	K237Rfs*4 0	Exon 9	SH33	Frameshift Mutation
c.756G>A	W252X	Exon 9	SH33	Premature Stop Codon
c.763C>T (n = 2)	R255X	Exon 8	SH33	Premature Stop Codon
c.778C>T	Q260X	Exon 9	SH33	Premature Stop Codon
c.82C>T	R28C	Exon 2	PH	AA substitution at conserved sites in Btk family
c.863G>A	R288Q	Exon 10	SH2	AA substitution at conserved sites in Btk family

Codon change	Protein change	Position	Domain	Mutation
c.866G>T*	S289L	Exon 11	SH2	AA substitution at conserved sites in Btk family
c.952T>C	S318P	Exon 11	SH2	AA substitution at conserved sites in Btk family
Deletion of Promotor in Exon 1* (n = 2)		Exon 1	PH	
Duplication of exons 6 -18 creates a frameshift mutation resulting in a stop Codon*		Exons 6-18		Premature Stop Codon
Frame Shift --> Stop Codon				

Figure 5-15 Mutation types (n)

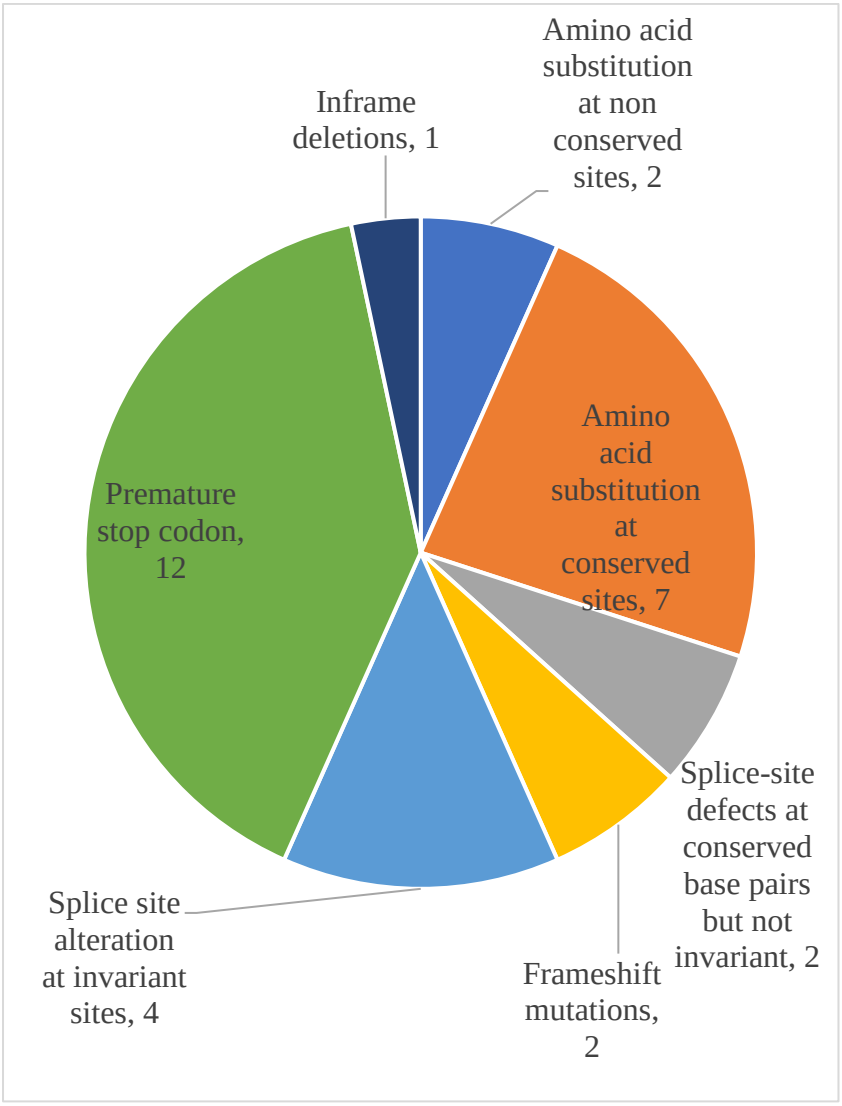
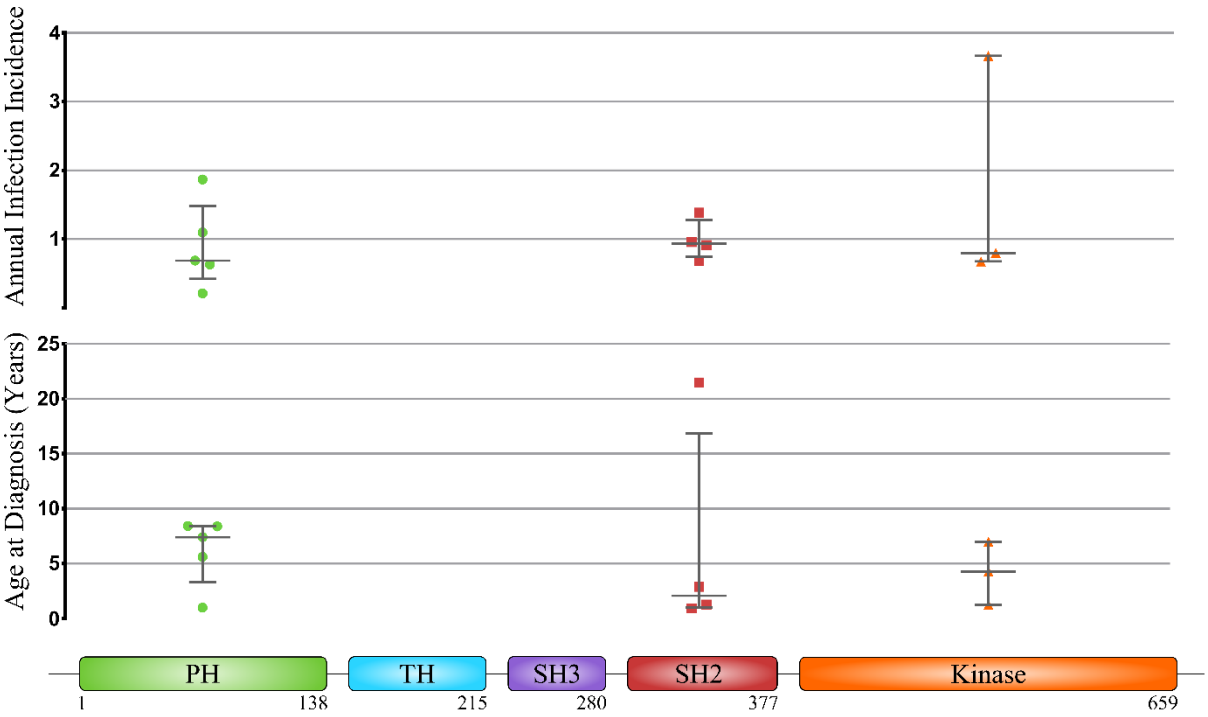


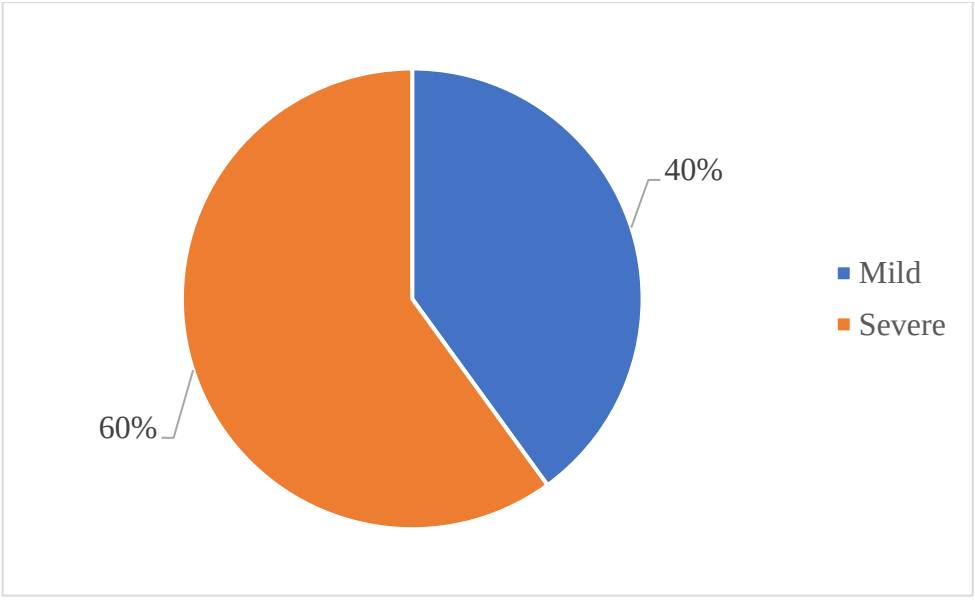
Figure 5-16 - Age at diagnosis and lifetime annual infection incidence by mutation protein domain site for missense mutations



For missense mutations, there were no significant differences for median lifetime annual infection incidence ($p = 0.763$) or age at diagnosis ($p = 0.572$) by protein domain of the underlying genetic mutation.

Using the definitions from Broides et al., (368) 40% had a mild mutation versus 60% with a severe mutation (Figure 5-17).

Figure 5-17 Mutation severity as per Broides et al., (368) **classification**



5.14.2 Correlation with age at diagnosis and disease severity

To assess for clinical phenotype and genotype correlation, mutation severity, type and location were assessed against age at diagnosis and disease severity (Table 5-32). Disease severity was assessed as per the methods section. Proposing that milder disease would present later, age at diagnosis could be used as a proxy for disease severity, at least in the initial phase. Patients who were screened asymptomatically because of their family history were excluded from these analyses, but the index case in the family with the same mutation could be used as they were diagnosed on clinical grounds and not because of family history.

Disease severity was not significantly associated with mutation severity (p = 1.000), the protein domain of the mutation (p = 0.792) or the mutation type (p = 1.000). Age at diagnosis was not significantly effect by mutation severity (p = 0.113), protein domain of the mutation (p = 0.657) or the mutation type (p = 0.827) (Table 5-32).

Table 5-32 Association between mutation and age at diagnosis and disease severity. (Excluding patients screened who screened asymptomatically due to family history) (years, median (IQR))

	Age at Diagnosis	p-value	Disease Severity p-value
Broides et al., Mutation Severity (368)			
Mild	2.29 (0.51 – 5.36)	0.113	1.000
Severe	3.82 (2.47 – 8.38)		
Protein Domain for Missense mutations			
PH Domain	7.41 (5.61 – 8.38)	0.657	0.792
TH Domain	N/A		
SH3	N/A		
SH2	2.09 (1.11 – 12.18)		
Kinase	4.12 (1.25 – 7.00)		
Mutation Type			
Missense	2.89 (1.25 – 7.00)	0.827	1.000
Null	3.30 (2.00 – 5.96)		

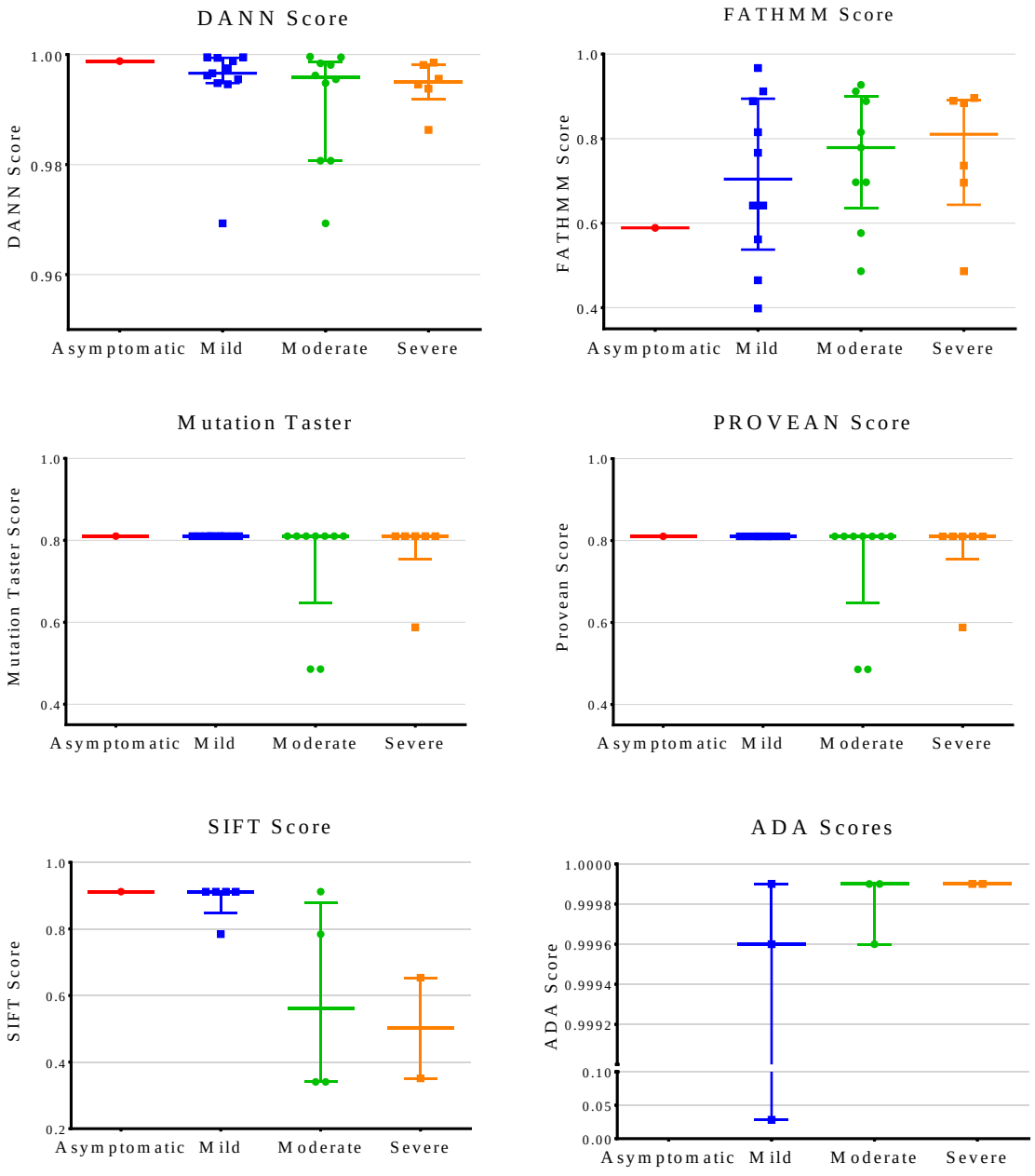
To further assess any correlation with the genetic mutation and clinical phenotype, mutations were analysed as per a number of pathogenicity prediction scores as defined in the methodology. None of the chose mutation pathogenicity programs demonstrated a correlation with the prediction score

and the age at diagnosis. Aside from the PROVEAN program, no program demonstrated a correlation with the median lifetime annual infection incidence. The PROVEAN program demonstrated a significant and moderate correlation with increasing severity as per their algorithm and the median lifetime annual infection incidence ($\rho = 0.464$, $p = 0.011$) (Table 5-33).

Table 5-33 Correlation of mutation prediction algorithm scores and clinical phenotype (rho, p-value)

Mutation prediction algorithm	Correlation with Age at diagnoses	Correlation with infection incidence
DANN	$\rho = 0.001$, $p = 0.965$	$\rho = -0.236$, $p = 0.228$
FATHMM	$\rho = 0.023$, $p = 0.913$	$\rho = -0.022$, $p = 0.914$
Mutation Taster	$\rho = -0.185$, $p = 0.366$	$\rho = 0.001$, $p = 0.965$
PROVEAN	$\rho = -0.074$, $p = 0.705$	$\rho = 0.464$, $p = 0.011$
SIFT	$\rho = -0.157$, $p = 0.625$	$\rho = -0.265$, $p = 0.066$
ADA Score	$\rho = 0.632$, $p = 0.093$	$\rho = -0.069$, $p = 0.872$

Figure 5-18 Disease severity and mutation prediction scores



There were no significant differences amongst increasing disease severity and DANN scores ($p = 0.561$), FATHMM scores ($p = 0.743$), Mutation Taster scores ($p = 0.460$), PROVEAN scores ($p = 0.757$), SIFT scores ($p = 0.115$) or ADA scores ($p = 0.323$) (Figure 5-18).

For my final analysis of any genotype and phenotype correlation, I have analysed the genetic defects for associations with circulating immunoglobulin levels at diagnosis and latest clinic appointment to investigate any association with a genetic defect and the immunological phenotype. There are no differences in immunological phenotype at either diagnosis or latest clinic based on mutation type, protein severity or mutation severity. This suggests that residual levels of BTK function and circulating peripheral immunoglobulin levels (and

therefore a theoretically milder clinical phenotype) cannot be predicted by the underlying BTK mutation (Table 5-34).

Table 5-34 Immunological phenotype by mutation type and classification (g/L) (median (IQR))

	Diagnosis IgG	Diagnosis IgA	Diagnosis IgM	Current IgA	Current IgM
Protein Domain for missense mutations					
PH Domain	3.4 (2.9 – 4)	0 (0 – 0.1)	0 (0 – 0.24)	0 (0 – 0.17)	0 (0 – 0.07)
TH Domain	N/A	N/A	N/A	N/A	N/A
SH3	N/A	N/A	N/A	N/A	N/A
SH2	0 (0 – 0)	0 (0 – 0.02)	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)
Kinase	N/A	N/A	N/A	0.04 (0 – 0.05)	0 (0 – 0.12)
p value	0.076	1.000	0.414	0.263	0.408
Mutation severity (Broides et al., (368)**)**					
Mild	2.42 (0 – 7.5)	0 (0 – 0.34)	0.17 (0 – 0.38)	0 (0 – 0)	0 (0 – 0)
Severe	0.14 (0 - 3.4)	0 (0 – 0)	0 (0 – 0.02)	0 (0 – 0)	0 (0 – 0)
p value	0.623	0.659	0.122	0.417	0.914
Mutation type					
Missense	2.9 (0 – 3.4)	0 (0 – 0.02)	0 (0 – 0)	0 (0 – 0.05)	0 (0 – 0.04)
Null	0.14 (0 – 4.4)	0 (0 – 0)	0 (0 – 0.09)	0 (0 – 0)	0 (0 – 0)
p value	0.859	0.552	0.474	0.101	0.459

5.15 Cost of therapy

The mean annual cost of the immunoglobulin products per patient is £24, 171 (SD 9270). Being a weight-dependent product, the mean annual cost is higher for adults compared to paediatrics (£18, 276 vs £26, 137, p = 0.0253) (Table 5-35).

The mean annual cost is higher for those patients on intravenous versus subcutaneous therapy (£26, 085 vs £19, 280, p = 0.0161).

Table 5-35 Cost of immunoglobulin therapy (£, mean (SD))

	Mean annual cost	p-value
All	£24, 171 (9270)	
Paediatrics	£18, 276 (13614)	0.025
Adults	£26, 137 (6528)	
Subcutaneous	£19, 280 (6503)	0.016
Intravenous	£26, 084 (8403)	
No Bronchiectasis	£21, 839 (2974)	0.181
Bronchiectasis	£26, 037 (6192)	

5.16 Summary of Clinical Results

- The current median age of diagnosis for XLA in England and Wales was 2.97. This has improved with time. However, when excluding those patients who were screened because of family history (and therefore relying upon clinical judgment alone), this trend does not reach statistical significance.
- Bronchiectasis is a significant complication affecting nearly half the cohort. This will be discussed in detail in the next chapter.
- Twenty per cent of patients have GI complications, requiring endoscopic investigations. These are a mixture of infectious and inflammatory (IBD/IBD like) diseases.
- Patients still experience recurrent infections on IGRT, the vast majority of which are of the respiratory tract.
- Twenty-two per cent of patients have chronic sinusitis.
- Infection incidence is not significantly associated with IGRT dose, methods of delivery or IgG trough levels.
- There is no correlation with gene mutation and clinical phenotype using a variety of analysis methods.
- Eighteen per cent of patients have severe disease.
- The degree of disease severity is not associated with infection incidence, IgG trough levels or treatment compliance.

Chapter 6 Respiratory Health Results

This chapter will examine the respiratory health of XLA patients. As discussed in the literature review, the primary concern for clinicians is the development of bronchiectasis as a result of repeated infections. This is especially pertinent given the potential limitations of current therapy discussed in this thesis thus far. Each potential risk factor for developing bronchiectasis will first be described within its section. The major determining factors will then be included in a time to event analysis.

Progression of disease will be analysed through lung function results, HRCT results and the need for any surgical interventions.

The primary respiratory outcome in this cohort is the development, or not, of bronchiectasis. As such, much of the comparisons and analysis within this chapter will be between these two subgroups to determine what, if any, are the determining risk factors for developing bronchiectasis in XLA

6.1 Data Quality

Approximately half of all potential follow up data was available and collected for both patients (n = 22) with and without bronchiectasis (n = 28) (Table 6-1). The median data coverage tended to be higher for patients without bronchiectasis, although this only reached statistical significance for IgG trough data. These differences are because patients without bronchiectasis tend to be younger (35.29 years versus 13.41 years) and, as such, it was easier to get access to their full clinical records.

Table 6-1 Data attainment by the presence of bronchiectasis

	Bronchiectasis	No bronchiectasis	p-value
Follow up (patient years)	568.77	446.80	
Available clinical information (years, %)	321.37 (57%)	249.81 (56%)	
Median clinical information coverage per patient (%, IQR)	51% (45 – 82)	100% (54 – 100)	0.072
Available IgG trough levels (years, %)	264.61 (47%)	277.73 (62%)	
Median IgG trough levels coverage per patient (%, IQR)	58% (31 – 78)	99 (55 – 100)	0.012

6.2 Respiratory Disease

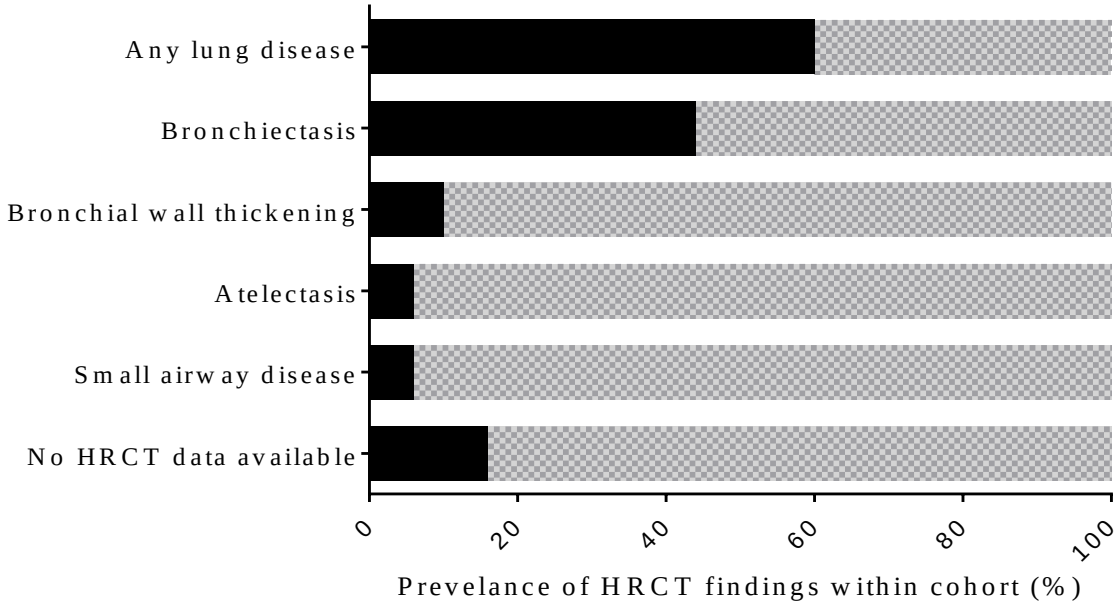
At the time of final data analysis (August 2018), 22 patients (44%) had bronchiectasis on HRCT. A further nine patients (18%) had abnormal findings on HRCT (Figure 6-1).

Four of these nine patients have bronchial wall thickening, three have small airway disease, and one has atelectasis. While these findings could demonstrate post-infection changes and are therefore potentially reversible; they are also important potential precursors to bronchiectasis. None of these eight patients has yet demonstrated a return to normality on HRCT. The remaining patient previously had scarring on his HRCT because of repeated infections pre-diagnosis of his XLA resulting in a pneumonectomy in later adult life. This necessitated further surgery at 32, with residual abnormal findings on his HRCT, but no formal diagnosis of bronchiectasis. Overall, 31 (62%) have either bronchiectasis or abnormal findings on HRCT.

Eight out of the fifty-one patients (16%) have never had an HRCT performed. At last follow up, five of these were children under the age of five, where performing an HRCT would likely require a general anaesthetic or sedation. It would, therefore, not be clinically justified to perform an HRCT in these patients unless there was a clear clinical indication. The median age of the remaining three patients at last follow up was 21.30 years.

Twenty out of thirty-two (63%) adults have bronchiectasis, and two out of 18 paediatric patients (11%) have bronchiectasis ($p = 0.001$).

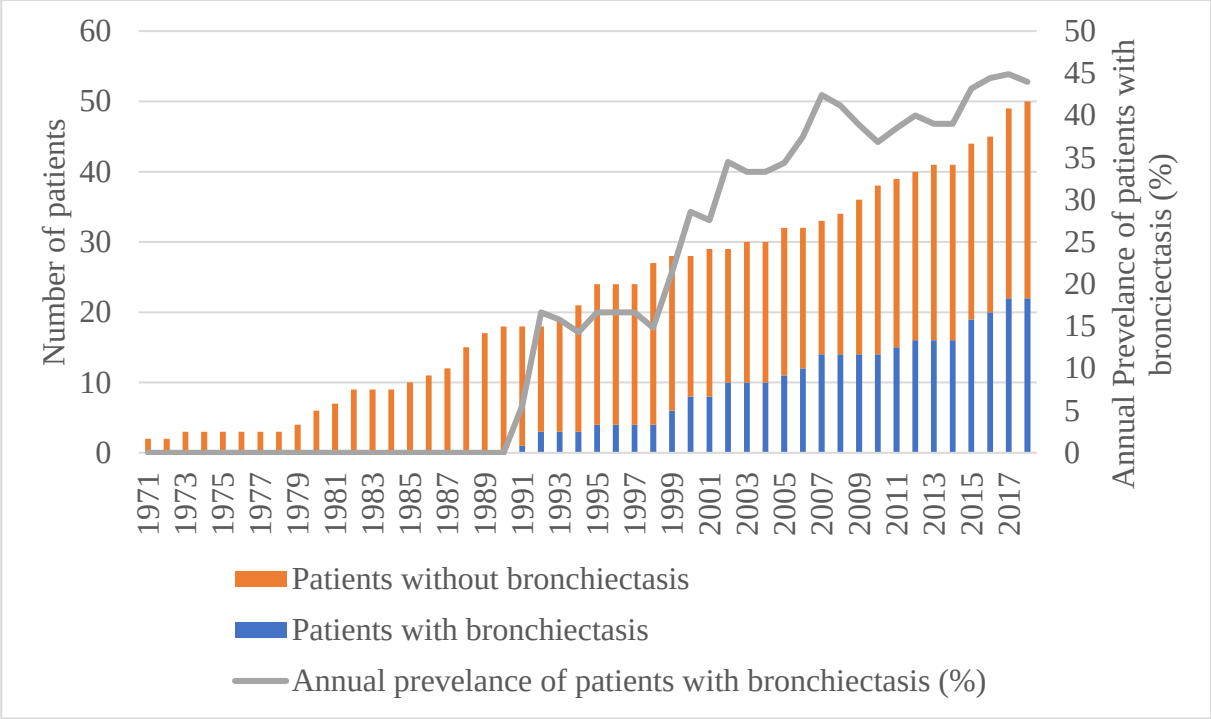
Figure 6-1 Prevalence of abnormal HRCT findings on latest HRCT



6.3 Prevalence of Bronchiectasis

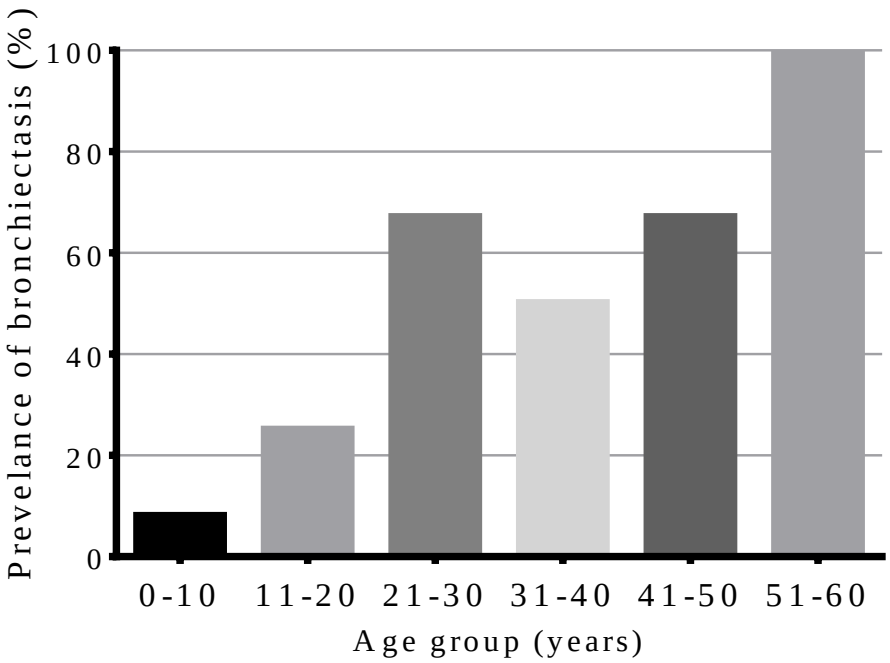
The prevalence in 2018 of bronchiectasis within the XLA cohort in England and Wales was 44%. By looking back over time and diagnosis of bronchiectasis, it is possible to estimate the prevalence of bronchiectasis each year over time (Figure 6-2). It would be expected that the prevalence of bronchiectasis would increase over this time, with an increasing number of patients now living long enough to develop the complication. However, it may also be expected that if more recent improvements in care were decreasing the risk of bronchiectasis and overall survival continues to improve, then the prevalence would, at some moment in time, begin to plateau and decrease. As of 2018, demonstrated in Figure 6-2, there is no plateau in the annual prevalence of bronchiectasis within the XLA cohort.

Figure 6-2 Annual prevalence of bronchiectasis within the XLA cohort



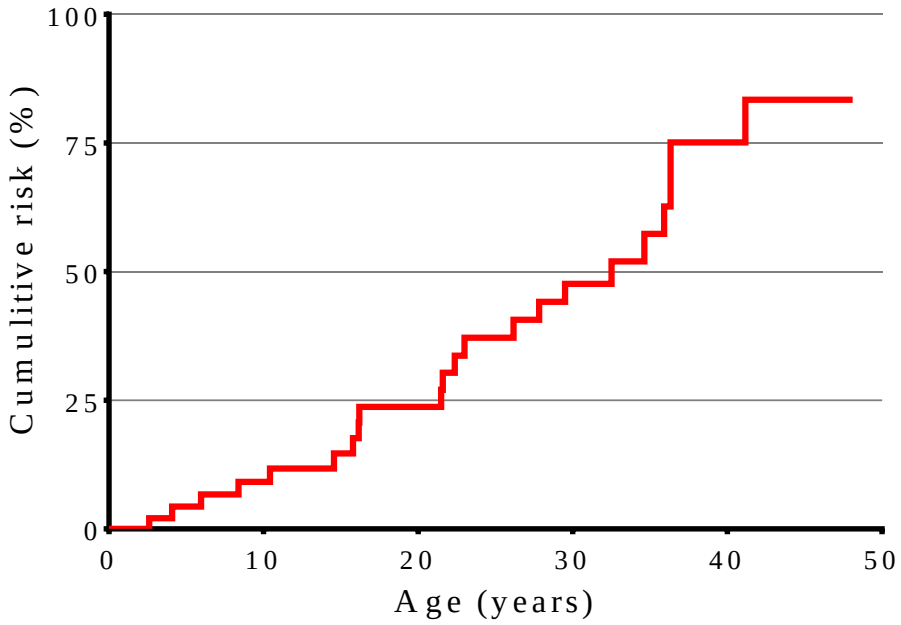
The main risk factor for non-CF bronchiectasis (irrespective of the underlying diagnosis) is the burden of repeated infections (275). Age, therefore, is a useful proxy for infection burden as a measure of risk for developing bronchiectasis over time. This may overcome some of the limitations in trying to record and ascertain true infection incidence and take into account the burden of subclinical infections. Figure 6-3 demonstrates an apparent increasing bronchiectasis prevalence within the cohort with increasing age groups with 62% of patients over the age of 30, demonstrating bronchiectasis on their latest HRCT.

Figure 6-3 Prevalence of bronchiectasis by age group



Kaplan-Meir analysis demonstrates a 50% risk of developing bronchiectasis by 32.50 years (95% CI 22.37 – 36.44) (Figure 6-4).

Figure 6-4 Kaplan-Meir cumulative risk plot over time (age)



Prevalence of bronchiectasis is not significantly different amongst the centres according to where the patient is currently being treated (Table 6-2) (p = 0.130). Data on previous centres, the patient may have been treated at was not available.

Table 6-2 Prevalence of bronchiectasis amongst the recruiting centres

Centre	Number and proportion of XLA patients with bronchiectasis
Birmingham Heartlands	4, 66%
Epsom & St Helier	3, 100%
Great Ormond Street	1, 13%
Manchester Royal	0, 0%
Newcastle	9, 53%
Royal Free Hospital	1, 33%
Salford Royal Hospital	3, 50%
Sheffield Hospital	1, 33%
University Hospital Wales	0, 0%

6.4 Current Demographics

Patients with bronchiectasis were significantly older than patients without bronchiectasis and had a significantly longer follow up period (Table 6-3)

XLA patients who were smokers or ex-smokers were significantly more likely to have bronchiectasis. All current and ex-smokers had bronchiectasis.

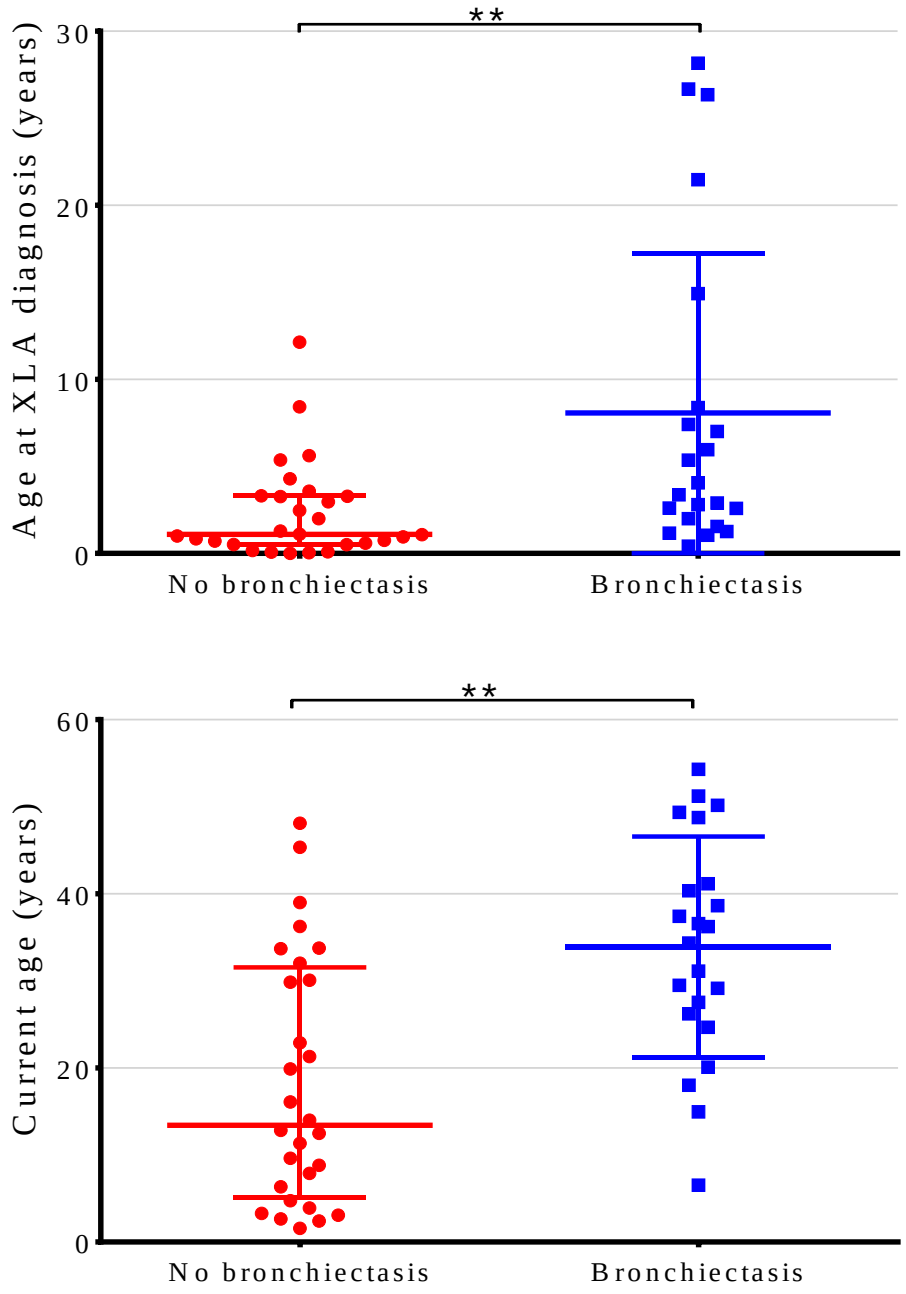
Patients with bronchiectasis were not more likely to have received previous intramuscular immunoglobulin therapy (IMIg), report a history of serious disease pre-diagnosis of XLA or more likely to have been screened asymptomatically (Table 6-3)

Table 6-3 Baseline characteristics of those patients with and without bronchiectasis (median (IQR))

	Bronchiectasis	No Bronchiectasis	p-value
Age (years)	35.29 (26.12 – 41.15)	13.41 (5.55 – 31.05)	0.001
Follow Up (years)	25.59 (20.60 – 35.18)	10.43 (4.40 – 26.60)	0.012
Smoking (n)			
Smoker	2	0	0.006
Ex-smoker	4	0	
Non-Smoker	16	26	
Ever IM therapy (Yes, %)	23%	14%	0.481
Screened (Yes, %)	9%	25%	0.266
Serious disease pre XLA diagnosis (Yes, %)	47%	33%	0.505

Patients with bronchiectasis were diagnosed with their XLA significantly later than those without (3.71 years versus 1.09 years $p = 0.002$) (Figure 6-5). However, due to improvements in reducing the age of diagnosis over time (demonstrated in Chapter 5), patients without bronchiectasis were also significantly younger at last follow up. It is, therefore, possible that patients who currently do not have bronchiectasis have not yet had enough time (and therefore, exposure to risk) to develop bronchiectasis.

Figure 6-5 Age at diagnosis and current age. ** p <0.005. (Scatterplot, median and IQR)



6.5 Age of onset

The gold standard for diagnosis of bronchiectasis is by HRCT. Age of onset is defined as the time of the first HRCT to detect bronchiectasis.

Seventeen patients had bronchiectasis diagnosed on their first HRCT (77% of all patients with bronchiectasis). There will be a lag from the actual clinical onset of bronchiectasis to diagnosis, due to delays in recognising symptoms and arranging an HRCT to diagnose bronchiectasis. Therefore, as described in the methods, to compensate for some of this lag,

patients who had bronchiectasis demonstrated on their first HRCT and within 12 months of their XLA diagnosis, it was assumed their bronchiectasis onset was present at the time of their XLA diagnosis. In this cohort, three patients fulfilled these criteria. As such, for all future analyses, the onset of bronchiectasis for these patients was equal to their XLA diagnosis.

For the 14 patients who were diagnosed with bronchiectasis on their first HRCT, but more than 12 months after their XLA diagnosis, the median age of bronchiectasis onset was 24.58 years (IQR 15.67 – 34.63). The median follow up time from their XLA diagnosis to bronchiectasis onset for these 14 patients was 13.77 years (IQR 4.70 – 21.00). No patient was diagnosed with bronchiectasis before the diagnosis of his XLA.

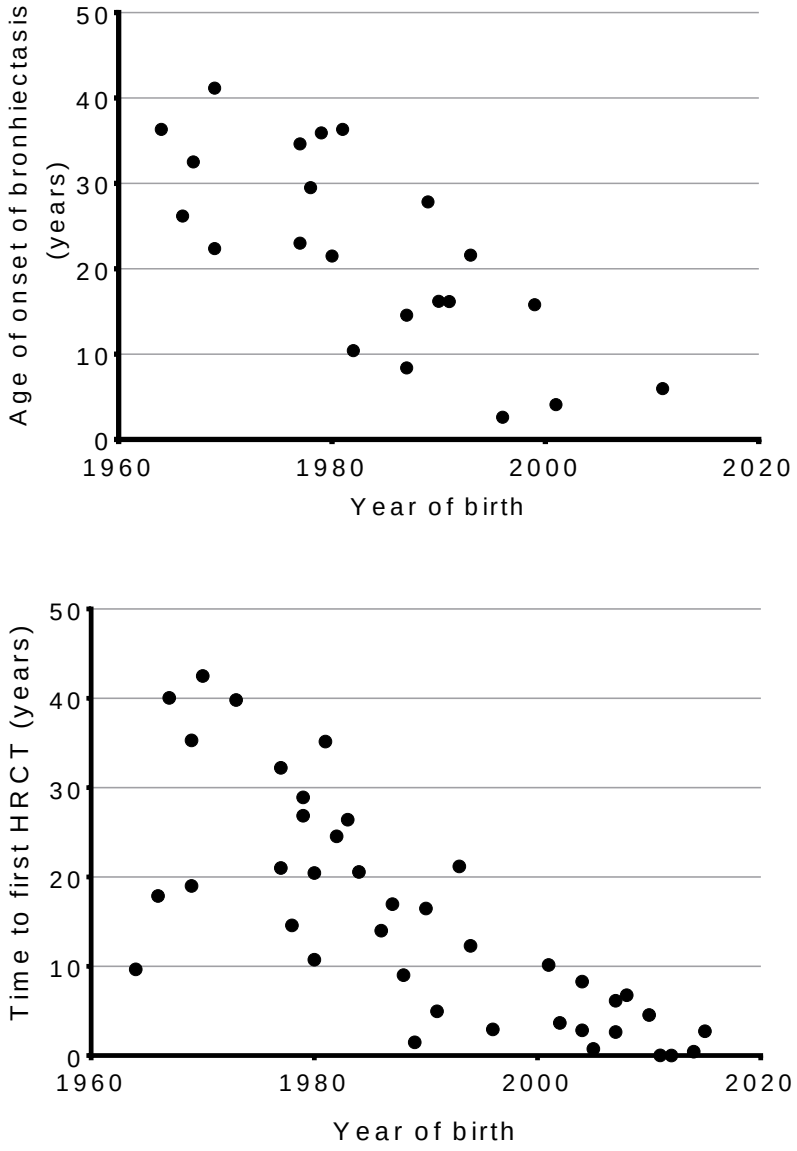
The median age of onset of bronchiectasis was 21.97 years (Table 6-4). The median time from XLA diagnosis to the onset of bronchiectasis was 13.15 years. Patients with bronchiectasis are significantly older and have a significantly longer follow up (Table 6-4).

Table 6-4 Age at and time to diagnosis and onset of bronchiectasis (years, median (IQR))

Variable	Bronchiectasis	No Bronchiectasis	p-value
Age of onset	21.97 (14.55 – 32.51)		
Follow up period until bronchiectasis	13.15 (3.00 – 21.00)		
Bronchiectasis on first HRCT (n)	8		
Current Age	35.29 (26.12 – 41.15)	13.41 (5.55 – 31.05)	0.001
Current Follow up	25.59 (20.60 – 35.18)	10.43 (4.40 – 26.60)	0.012
Age at diagnosis	3.71 (2.00 – 8.38)	1.09 (0.55 – 3.30)	0.002
Time from XLA to first HRCT	18.44 (9.90 – 26.73)	7.52 (2.78 – 17.27)	0.055

Age at diagnosis of bronchiectasis has significantly decreased over time (Spearman's rho = -0.758, $p < 0.001$) (Figure 6-6). Rather than the actual onset of bronchiectasis decreasing over time, this most likely represents a greater awareness for bronchiectasis and easier access to HRCT. This supported by decreasing time to first HRCT after diagnosis of XLA over time (Spearman's rho = -8.05, $p < 0.001$) (Figure 6-6).

Figure 6-6 Age of onset of bronchiectasis and time to first HRCT over time



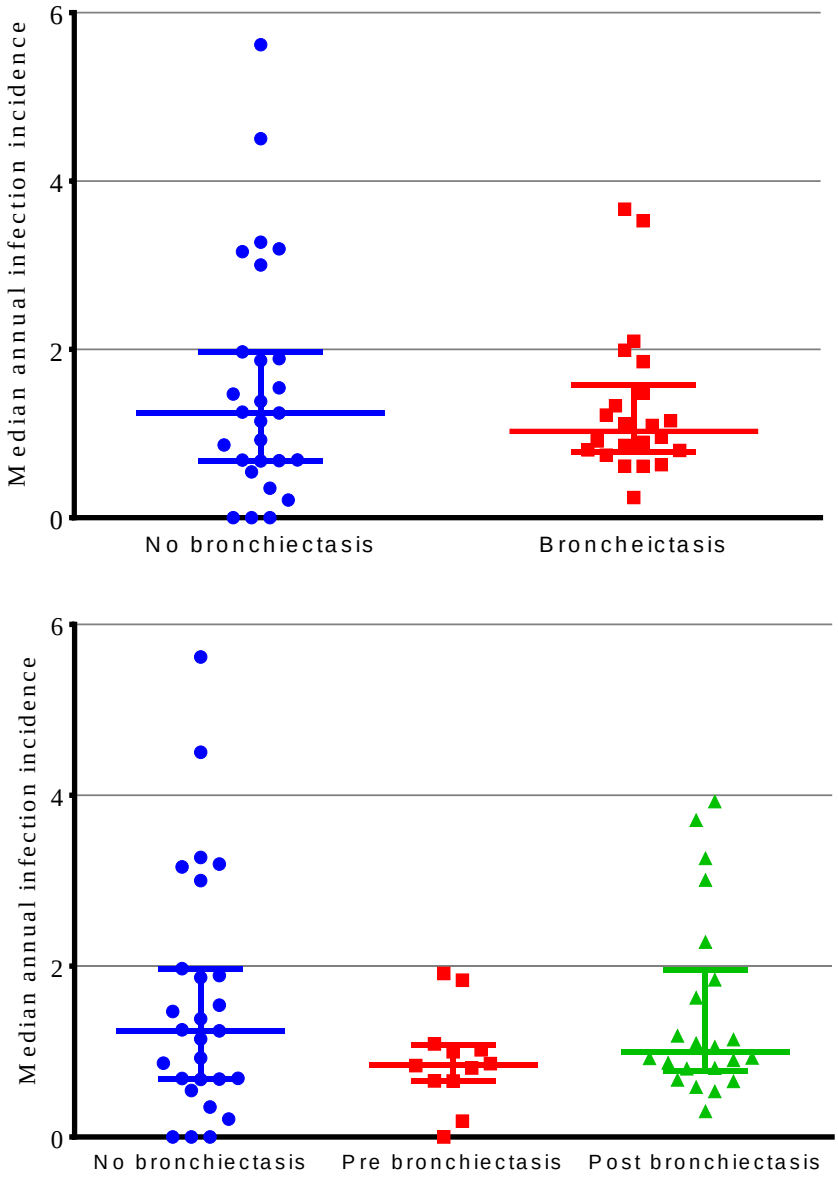
6.6 Infections on IGRT

Similar to the previous chapter, aside from infections of the respiratory tract, the overall raw number of infections recorded is displayed for illustration purposes due to the rarity of these infections. Where comparing for statistically analyses, it is the annual infection incidence being compared unless stated otherwise

There were no significant differences in the lifetime median annual infection incidence comparing patients with and without bronchiectasis (1.03 versus 1.24) ($p = 0.856$) (Table 6-5) (Figure 6-7).

As bronchiectasis itself will increase the susceptibility to infections, infections were separated before and after the development of bronchiectasis. The median annual infection incidence for current healthy patients was first compared to patients with bronchiectasis, but only in their pre-bronchiectasis period (1.24 versus 0.85, p = 0.206). For patients with bronchiectasis, their pre bronchiectasis period was then compared to their post bronchiectasis period and found no significant differences in annual infection incidence (0.85 versus 0.99, p = 0.346) (Figure 6-7) (Table 6-5).

Figure 6-7 Annual infection incidence. (Vertical scatterplot, median and IQR)



With respiratory tract infections being the main infection risk, these had a further analysis. No significant differences were found in median annual respiratory tract infection rates for

patients currently with or without bronchiectasis (0.80 versus 0.88, p = 0.856) (Table 6-5). There were no differences in current healthy patients compared to patients with bronchiectasis in their pre-bronchiectasis period values (0.88 versus 0.74, p = 0.201). There were no significant differences when comparing median annual respiratory tract infection rates in patients' pre-bronchiectasis periods compared to their post bronchiectasis periods (0.74 versus 0.85, p = 0.347) (Table 6-5) (Figure 6-8).

As discussed within the methodology, the diagnosis of bronchiectasis is made on HRCT. It is, therefore, possible that due to the interval between HRCT scans, that there is a lag from true clinical onset of bronchiectasis to the HRCT diagnosis. The analysis of infection incidence data was therefore done with adjustments made to the age of bronchiectasis diagnosis of minus 1, 3 and 5 years. In each adjustment, no significant differences were found.

Figure 6-8 Annual respiratory tract infection incidence. (Vertical scatterplot, median and IQR)

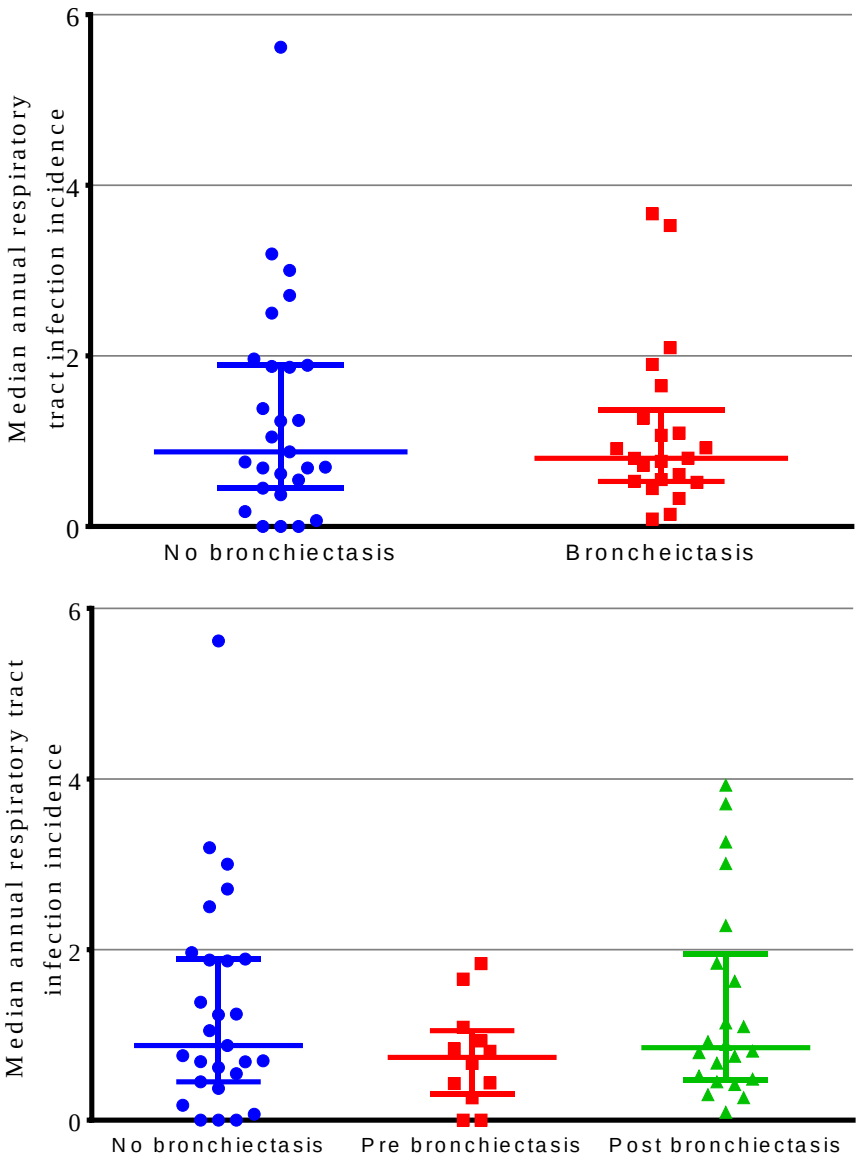


Table 6-5 Annual infection incidence (median (IQR)). ([$]Bronchiectasis versus none [#]pre versus post bronchiectasis [^]no bronchiectasis versus pre-bronchiectasis)

	No Bronchiectasis	Ever Bronchiectasis					
		Total	p value$	Pre-Bronchiectasis	Bronchiectasis	p value#	p value^
All infections	1.24 (0.68-1.97)	1.03 (0.80-1.48)	0.856	0.85 (0.66-1.06)	0.99 (0.80-1.84)	0.347	0.206
Respiratory tract infections	0.88 (0.45 - 1.89)	0.80 (0.53 - 1.27)	0.856	0.74 (0.35 - 1.01)	0.85 (0.48 - 1.85)	0.347	0.201
Lower respiratory infections	0.76 (0.34-1.87)	0.79 (0.53-1.27)	0.904	0.74 (0.35-1.01)	0.80 (0.48-1.84)	0.410	0.377
Sinus infections	0.00 (0.00-0.03)	0.00 (0.00-0.04)	0.836	0.00 (0.00-0.00)	0.00 (0.00-0.04)	0.084	0.056
Otitis infections	0.00 (0.00-0.00)	0.00 (0.00-0.06)	0.123	0.00 (0.00-0.11)	0.00 (0.00-0.00)	0.047	0.102
Totals							
Skin infections	8	9	0.474	3	6	0.791	0.518
Eye infections	9	16	0.278	0	16	0.158	0.166
GI infections	5	2	0.549	2	0	0.158	0.884
CNS infections	0	0	NA	0	0	NA	NA
Bone and Joint infections	0	0	NA	0	0	NA	NA
Sepsis	1	0	0.367	0	0	NA	0.505
GU infections	6	5	0.620	4	1	0.256	0.357
Other infections	3	1	0.397	0	1	0.317	0.236

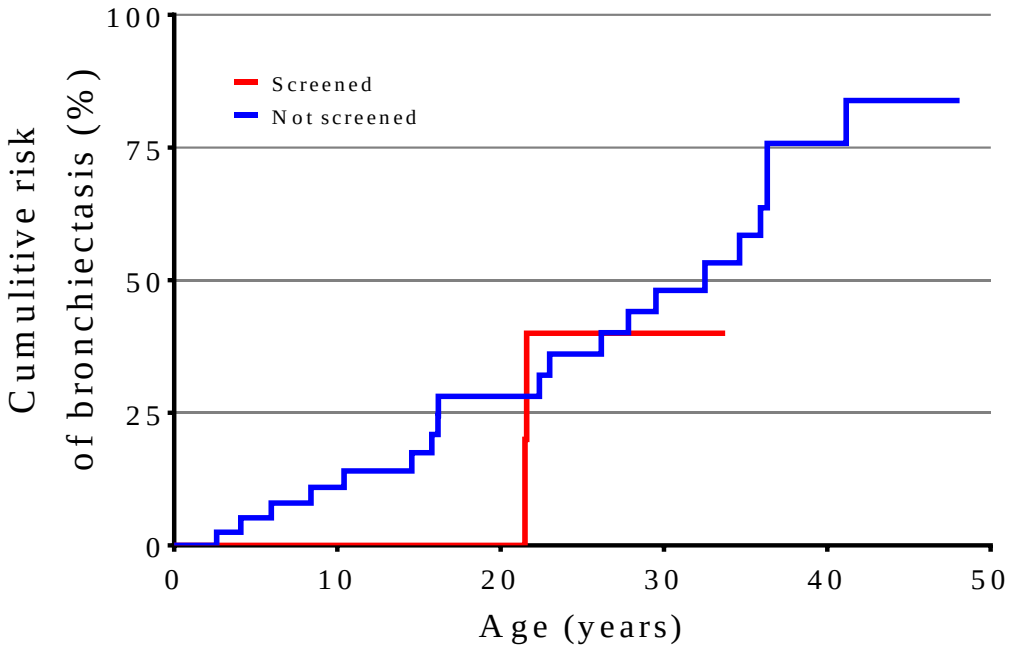
6.7 Time to event analysis

A Kaplan-Meir failure plot demonstrates a 50% risk of bronchiectasis by 32.51 years. The analysis above demonstrates that patients without bronchiectasis were diagnosed with XLA significantly younger than patients with bronchiectasis. However, they were also significantly younger and may not have had enough time (and therefore, exposure to infection) to develop bronchiectasis at last follow up. Also, the previous analysis has demonstrated that there were no significant differences in infection rates for patients with and without bronchiectasis. Data are now presented from a time to event analysis to analyse further the potential risk factors leading to the development of bronchiectasis. Individual factors were first assessed by univariate analyses (log-rank test of equality for categorical variables and Cox proportional hazard model with a single predictor for continuous variables) before integrating to a final time to event analysis

6.7.1 *Asymptomatic screening*

First, patients were analysed based on whether they were screened asymptomatically due to family history (a proxy for newborn screening). This did not show any significant differences in the development of bronchiectasis (p = 0.621).

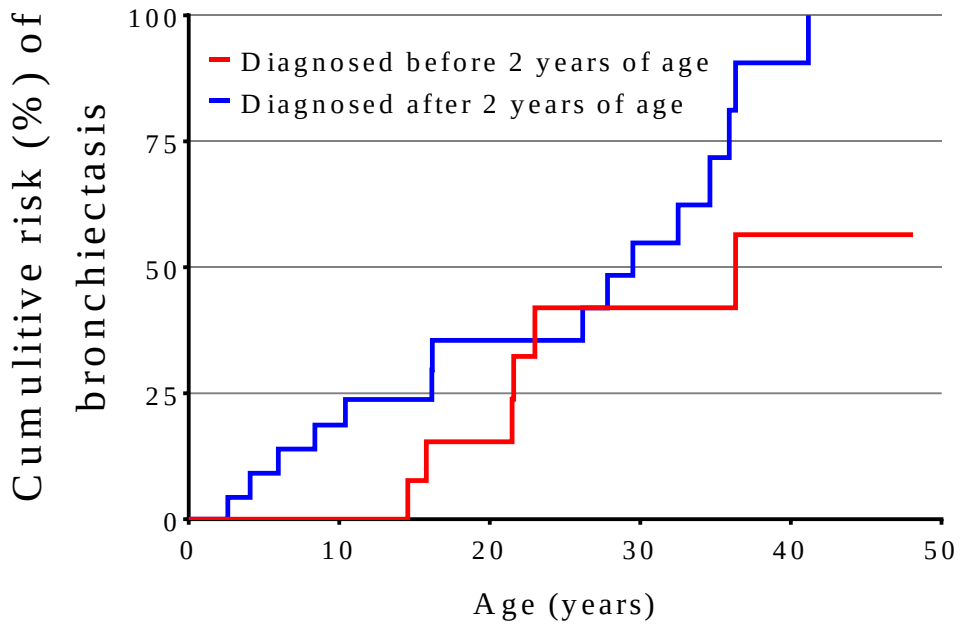
Figure 6-9 Kaplan-Meir failure plot for bronchiectasis by asymptomatic screening



6.7.2 *Age of diagnosis*

Age of diagnosis was analysed in two ways. It was modelled as a continuous variable (Table 6-6). Due to the small sample size, it was also analysed a categorical variable, representing early or late diagnosis. The current median age of diagnosis in this study was 2.60 years which is tending to improve with time, as shown in Chapter 5. After discussion with immunology specialist and general paediatricians regarding what would be considered an early diagnosis, a cut off of 2 years was chosen. This was felt to be an ambitious but realistic target. Targeting an earlier diagnosis based on clinical diagnosis was felt to be unrealistic given the difficulties facing clinicians in identifying the child with antibody deficiency against the backdrop of the healthy background population and the normal pattern of childhood infections. Being diagnosed before the age of two was associated with a decreased risk of developing bronchiectasis but did not reach statistical significance (p = 0.071) (Figure 6-10).

Figure 6-10 Kaplan-Meir failure plot for bronchiectasis by early or late diagnosis



6.7.3 *Continuous variables*

The following were analysed by Cox proportional hazard model.

Table 6-6 Univariate analyses for continuous variables on the risk of bronchiectasis

Variable	Hazard ratio	p-value	95% Confidence Interval
Age	0.952	0.047	0.910, 0.999
Lifetime median IgG trough level	0.938	0.563	0.754, 1.167
Lifetime median annual respiratory tract infection incidence	1.604	0.064	0.973, 2.645
Age at Diagnosis	1.01	0.745	0.966, 1.05
First recorded FEV Z Score	0.684	0.037	0.478, 0.978

6.7.4 *Time to event model*

The variables were then included into a final time to event analysis. Due to the small sample size, as described previously, age at diagnosis was not included. Instead, age at diagnosis was coded as a categorical variable based on a cut-off of 2 years.

Table 6-7 Final multivariate time to event analyses model

	Hazard ratio	p-value	95% Confidence interval
Age	0.940	0.066	0.881, 1.00
Diagnosed before 2 years of age	0.588	0.514	0.120, 2.895
Lifetime median annual respiratory tract infection incidence	1.197	0.691	0.493, 2.903
Lifetime median IgG trough level	0.837	0.310	0.593, 1.180
First recorded FEV Z Score	0.716	0.249	0.406, 1.262

As demonstrated in Table 6-7**,** in the multivariate analysis, there was no significant association between the variables and the risk of bronchiectasis. Unexpectedly, increasing age was close to being significantly associated with a decreasing risk of bronchiectasis. However, due to better access to HRCT, the formal age of diagnosis of bronchiectasis has

decreased in recent years. This likely means it is difficult to analyse the risk of bronchiectasis with age thoroughly.

Patients diagnosed at under two years of age were also found to have a significantly lower risk of developing bronchiectasis in the univariate but not multivariate analysis. As described early, patients with an earlier diagnosis are also more likely to be diagnosed more recently. Therefore, they will also benefit from overall improvements in clinical care for PID (459).

6.8 Immunology

There were no statistically significant differences in latest IgA, IgM or CD19/20 B-lymphocyte numbers investigations between patients with and without bronchiectasis (Table 6-8). However, patients with bronchiectasis had lower current IgG trough level than those without bronchiectasis (p = 0.040).

Table 6-8 Latest immunology investigations (median (IQR))

	Bronchiectasis	No bronchiectasis	p-value
IgA (g/L)	0 (0 - 0)	0 (0 – 0)	0.948
IgM (g/L)	0 (0 – 0)	0 (0 – 0)	0.134
Trough IgG (g/L)	9.40 (8.40 – 10.10)	10.80 (9.40 – 12.50)	*0.040*
CD19/20 (cells/mcL)	0 (0 – 0)	0 (0 – 0.5)	0.356

There was no statistically significant difference in the initial diagnostic immunology investigations between patients with and without bronchiectasis. These data were available for 19 patients (Table 6-9).

Table 6-9 Initial diagnostic immunology investigations (median (IQR))

	Bronchiectasis	No bronchiectasis	p-value
IgA (g/L)	0 (0 – 0.1)	0 (0 – 0)	0.389
IgM (g/L)	0 (0 – 0.17)	0 (0 – 0.08)	0.919
Trough IgG (g/L)	1.21 (0 – 2.90)	0.17 (0 – 5.87)	0.524
CD19/20 (cells/mcL)	0.01 (0 – 0.01)	0 (0 – 0.1)	0.699

6.8.1 *BTK expression*

Patients with bronchiectasis were not more likely to have absent BTK expression compared to patients without bronchiectasis (p = 0.565).

Table 6-10 BTK expression in the two groups

	Bronchiectasis (n)	No bronchiectasis (n)	p-value
Absent	10	9	0.565
Reduced	0	2	
Normal	3	3	

6.8.2 *Genetic mutations*

There were no significant differences in the number of patients with bronchiectasis amongst difference classifications of mutation severity/type (Table 6-11).

Table 6-11 Rates of bronchiectasis amongst different mutation severity classifications

	Bronchiectasis (n)	No bronchiectasis (n)	p value
Broides et al., Mutation Severity (368)			
Mild	4	8	0.504
Severe	12	14	
Protein Domain for Missense mutations			
PH Domain	3	5	1.000
TH Domain	0	0	
SH3	3	3	
SH2	2	3	
Kinase	4	5	
Mutation Type			
Missense	6	6	0.725
Null	10	16	

6.9 Microbiology

Sputum microbiology within the last 12 months was available for 34 patients (24 with bronchiectasis and ten without). There were no significant differences in the growth cultures

between those with and those without bronchiectasis (p = 0.330). The overall majority of cultures grew H. influenzae. (76%) (Table 6-12).

Table 6-12 Latest sputum microbiology

Organism	All (n)	Bronchiectasis (n)	No bronchiectasis (n)
H. Influenzae	26	20	6
Moraxella	2	0	2
Regional flora	2	2	2
No growth	2	2	0

Focusing on H. influenzae, 66% of the sputum samples taken above were during a presumed acute infective episode. The remaining 34% were taken because of ongoing monitoring or investigation of chronic symptoms. Twenty-three percent of H. influenzae cultures were resistant to either amoxicillin or co-amoxiclav, although no H. influenzae was resistant to both (Table 6-13). H. influenzae resistance to doxycycline was low at 8% with 85% reporting as being sensitive to doxycycline.

Table 6-13 Sputum samples antibiotic sensitivities

Antibiotic	Sensitive (n, %)	Resistant (n, %)
Amoxicillin	12 (46%)	6 (23%)
Co-amoxiclav	6 (23%)	6 (23%)
Doxycycline	22 (85%)	2 (8%)

6.10 High-Resolution Computerised Topography

6.10.1 *Monitoring of lung disease*

The median time from XLA diagnosis to first HRCT was 6.45 years at a median age of 11.57 years (Table 6-14). For patients who currently had bronchiectasis their median time to first HRCT after diagnosis was 8.34 years and for those without 6.08 (p = 0.756). The age at first HRCT was 18.21 years and 7.53, respectively (p = 0.031). Again, patients without bronchiectasis were younger and therefore may have benefited from easier access to HRCT in recent years.

Table 6-14 Timing and intervals for HRCT (years, median (IQR))

	All	No bronchiectasis	Bronchiectasis	p value
Age of first HRCT	11.57 (5.74 – 27.64).	7.53 (4.17 – 18.51)	18.21 (8.60 – 35.84)	0.031
Time to first HRCT from XLA diagnosis	6.45 (2.06 – 19.16)	6.08 (2.68 – 13.14)	8.34 (1.34 – 20.72)	0.756
Interval period between HRCT	8.05 (5.69 – 14.70)	14.70 (6.41 – 19.20)	7.75 (4.16 – 8.82)	0.063

Excluding those patients who have never had an HRCT, the median number of HRCT scans per patient over the available data period was one. For patients, without bronchiectasis, this was also one and with bronchiectasis, three ($p = 0.017$). The median interval between HRCT for those who have more than one HRCT was 8.05 years. This was not significantly different comparing those with and without bronchiectasis, but patients with bronchiectasis tended to have more frequent HRCT than those patients without bronchiectasis (Table 6-14).

6.10.2 *Progression of bronchiectasis on HRCT*

Excluding those patients whose bronchiectasis progressed to such a degree of severity, they required cardiothoracic surgery; three patients demonstrated a progression of their bronchiectasis on HRCT. This is over a median time of 11.38 years from diagnosis of bronchiectasis to the time of data collection. A further three patients required surgery because of the degree of progression of their bronchiectasis. These patients will be discussed in further detail in 6.14.3. Therefore, 16 patients (73% of those with bronchiectasis) demonstrated no progression of their lung disease on HRCT.

6.11 Lung Function

For lung function analysis, data were first presented regarding monitoring in XLA. To assess disease severity and progression of lung disease based on lung function, the first recorded and latest lung function test results were compared. Changes in lung function over time are then analysed using a multilevel mixed model analysis.

There were lung function test results available for 32 patients, with 211 results available for these patients.

The median time from XLA diagnosis to first lung function testing did not differ significantly for patients with and without bronchiectasis (10.88 versus 8.76 years, p = 0.637) (Table 6-15). The age of first lung function testing, however, was significantly younger for those patients without bronchiectasis (10.30 versus 19.48 years old, p = 0.02). As described previously, patients without bronchiectasis are more likely to have been diagnosed more recently and therefore benefiting from improved clinical care to perform lung function testing at earlier ages (460). The median time between lung function testing was 5.40 years between the two groups and was not significantly different.

Table 6-15 Frequency and timing of pulmonary function testing (years, median (IQR))

	All patients	Patients with bronchiectasis	Patients with no bronchiectasis	p-value
Median LFTs per patient	4 (2 – 6.5)	5 (3 – 8)	3 (1 – 5)	0.0382
Time to First LFT	9.14 (5.08 – 20.34)	10.88 (4.12 – 20.55)	8.76 (6.01 – 16.68)	0.637
Age of first LFT	16.67 (9.48 – 22.55)	19.48 (12.87 – 34.03)	10.30 (6.75 – 17.52)	0.020
Frequency of LFT	5.40 (2.61-8.97)	6.65 (2.61-12.08)	5.51 (3.00-9.25)	0.493

Patients with bronchiectasis had significantly lower first recorded FEV1, FVC and FEV: FVC Z-scores compared to patients without bronchiectasis (Table 6-16). In keeping with their diagnosis, the latest FEV1, FVC and FEV: FVC Z-scores are also significantly lower for patients with bronchiectasis compared to those without.

Table 6-16 First and latest pulmonary function. (median (IQR))

	All Patients	Bronchiectasis	No bronchiectasis	p-value
FEV1				
Starting FEV1 % predicted	82.08 (70.67 - 97.15)	71.66 (53.05 - 79.92)	97.15 (88.31 - 102.75)	0.000
Latest FEV1 % predicted	85.87 (67.25 - 101.54)	74.43 (44.69 - 89.15)	94.43 (82.08 - 102.75)	0.003
Starting FEV1 Z-score	-1.47 (-2.50 - -0.25)	-2.41 (-3.82 - -1.63)	-0.25 (-1.02 - 0.21)	0.000
Latest FEV1 Z-score	-1.23 (-2.73 - 0.13)	-2.26 (-3.97 - -0.93)	-0.45 (-1.37 - 0.21)	0.004
FVC				
Starting FVC % predicted	90.80 (75.38 - 99.45)	76.70 (68.91 - 88.04)	99.18 (91.42 - 104.56)	0.002
Latest FVC % predicted	89.68 (76.37 - 104.56)	79.02 (61.11 - 89.68)	96.77 (91.21 - 104.73)	0.007
Starting FVC Z-score	-0.80 (-2.10 - -0.04)	-2.00 (-2.65 - -1.02)	-0.07 (-0.73 - 0.37)	0.002
Latest FVC Z-score	-0.87 (-1.95 - 0.37)	-1.64 (-3.08 - -0.87)	-0.26 (-0.68 - 0.39)	0.001
FEV: FVC Ratio				
Starting FEV:FVC Z-score	-0.81 (-2.24 - 0.72)	-1.41 (-2.73 - -0.33)	-0.32 (-1.48 - 1.01)	0.028
Latest FEV:FVC Z-score	-0.99 (-2.28 - 0.40)	-2.26 (-2.74 - -0.28)	-0.40 (-1.10 - 0.80)	0.032

Patients with bronchiectasis have significantly worse lung function compared to patients without bronchiectasis for FEV1, FVC and FEV: FVC ratio. Also, the first recorded lung function scores are significantly worse for patients with bronchiectasis (Table 6-16). Due to limitations in data collection, some of these first recorded results may have been at a time when the patient already had bronchiectasis. Therefore, analyses were then carried out only using starting lung function results in the documented absence of bronchiectasis (Table 6-17).

Table 6-17 First recorded lung function Z-score results in the absence of bronchiectasis (median (IQR))

	No Bronchiectasis	Bronchiectasis	p-value
ZFEV1	-0.25 (-1.02 – 0.21)	-2.38 (-3.26 - -1.47)	0.002
ZFVC	-0.07 (-0.73 – 0.37)	-1.92 (-2.88 - -0.66)	0.013
ZFEV: FVC	-0.32 (-1.48 – 1.01)	-0.97 (-2.70 – 0.11)	0.137

Initial ZFEV1 and ZFVC scores were still significantly lower for patients who would later go on to develop bronchiectasis compared to those who are currently healthy, even when strictly only including those who did not have bronchiectasis and presumed, clinically, to have normal lung health.

There were no significant differences between the first and latest FEV1 Z-scores (Figure 6-11), FVC Z-scores (Figure 6-12) or FEV: FVC Z-scores (Figure 6-13) indicating an element of stability in lung disease in patients both with and without bronchiectasis. This change in lung function over time will now be analysed in more detail in a multilevel mixed model analysis.

Figure 6-11 First and last recorded FEV1 Z-scores. (Scatterplot, median and IQR).
Dotted line represents the lower limit of normal

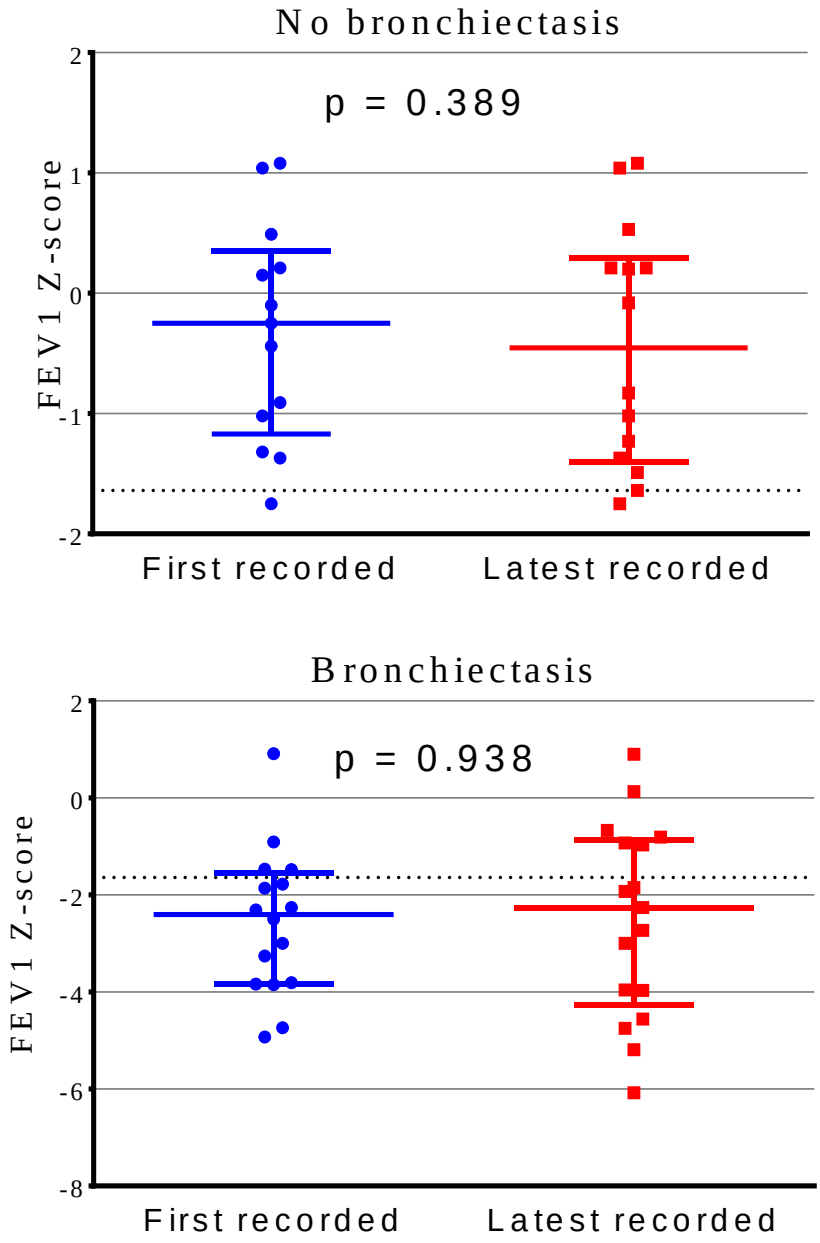


Figure 6-12 First and last recorded FVC Z-scores. (Scatterplot, median and IQR).
Dotted line represents the lower limit of normal

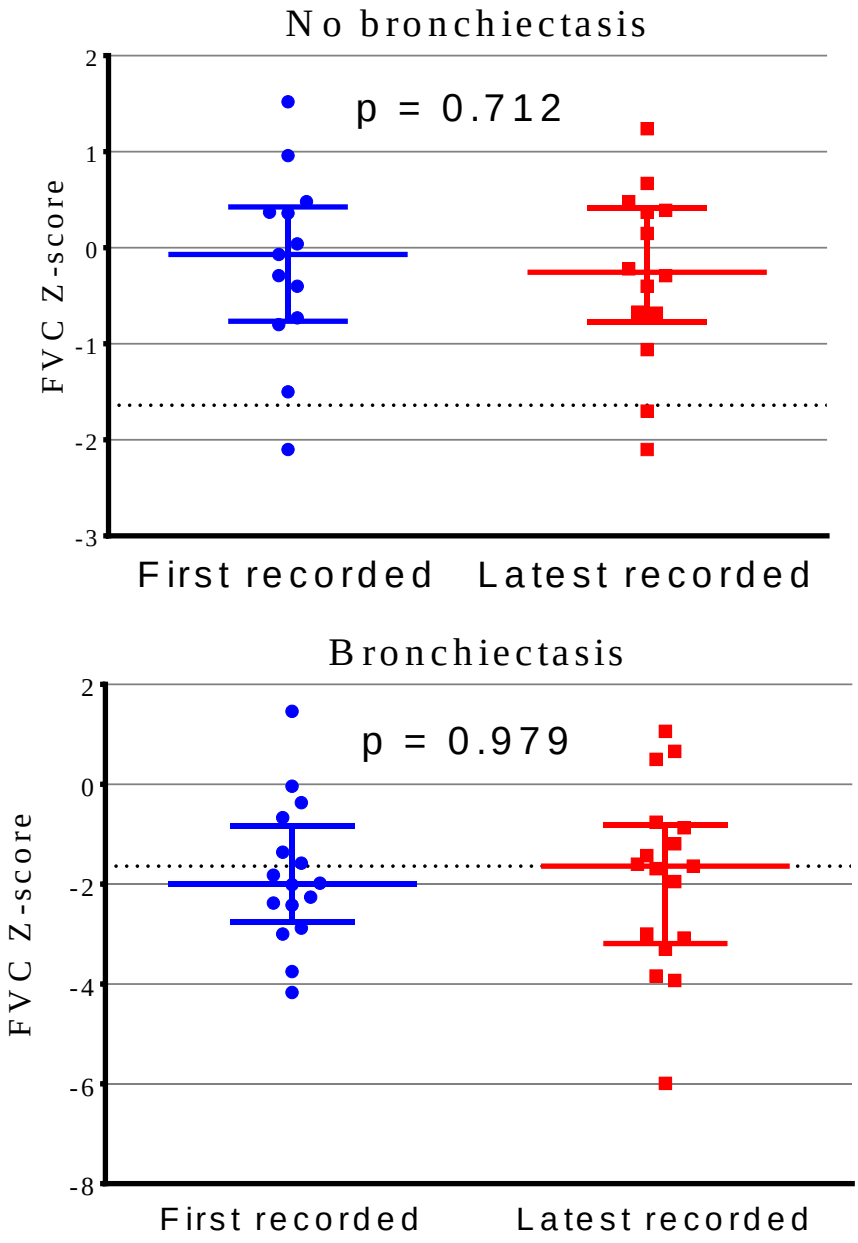
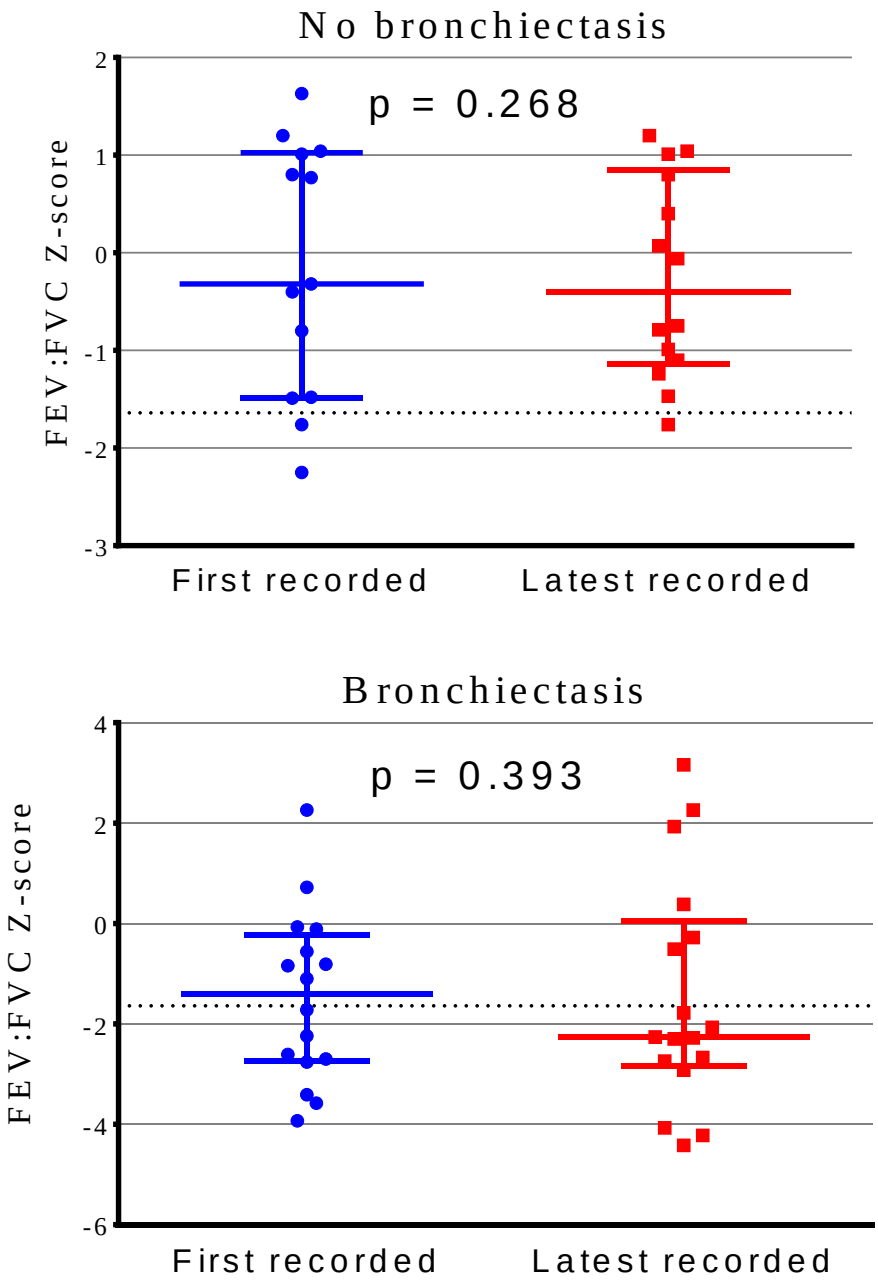


Figure 6-13 First and last recorded FEV1: FVC Z-scores. (Scatterplot, median and IQR). *Dotted line represents the lower limit of normal*



6.11.1 *Longitudinal Data*

A multilevel mixed model analysis was used to analyse the longitudinal changes in lung function over time (age) (Table 6-18). The model below was based on the hypothesis that increasing age and an increasing number of infections would be associated with a declining lung function. An additional hypothesis proposed that delayed diagnosis may result in increasing lung damage due to repeated infections while not being not on IGRT. Therefore,

age at diagnosis was also included in the model. Proposing that any decline in lung function may not be linear, a quadratic function of age was also added to give a second-degree polynomial. The model below converges after two iterations. On average, the presence of bronchiectasis was associated with an FEV1 Z score 1.11 lower than those patients without bronchiectasis. An increasing lifetime median annual respiratory tract infection incidence was also associated with lower FEV1 Z scores. Neither age nor the quadratic function of age had a significant coefficient. This would be in keeping with the simpler analysis above, suggesting a degree of stability in lung function over time in this cohort. Increasing age at diagnosis was not associated with decreasing FEV1 Z-scores.

Table 6-18 Multilevel mixed model analysis of FEV1 Z-scores

	Coefficient	p value	95% CI
Age	0.00	0.875	-0.08, 0.09
Age (Quadratic)	0.00	0.353	0.00, 0.00
Bronchiectasis	-1.11	0.009	-1.96, -0.28
Median respiratory tract infection incidence	-0.73	0.001	-1.18, -0.28
Age at diagnosis	-0.04	0.630	-0.90, 1.49
Random effects			
	Estimate		95% CI
SD (Age)	0.09		0.06, 0.12
SD (_cons)	1.76		1.25, 2.47

6.12 IGRT therapy

6.12.1 *Place and site of therapy*

There were no significant differences in route of IGRT (p = 0.442) or place of IGRT (p = 0.650) based on the presence of bronchiectasis or not.

6.12.2 *Doses and IgG trough levels*

There were no significant differences between current IGRT doses or lifetime IgG trough levels for patients with and without bronchiectasis (Table 6-19). However, patients with bronchiectasis currently have significantly lower IgG trough levels compared to patients without bronchiectasis (9.40 g/L versus 10.80 g/L, p = 0.040).

As discussed in Chapter 5, the standard deviation of the annual mean IgG trough levels was also analysed for any association with the development of bronchiectasis as a proxy for treatment compliance/stability. There were no significant differences in the standard deviation of yearly median IgG trough levels for patients with and without bronchiectasis.

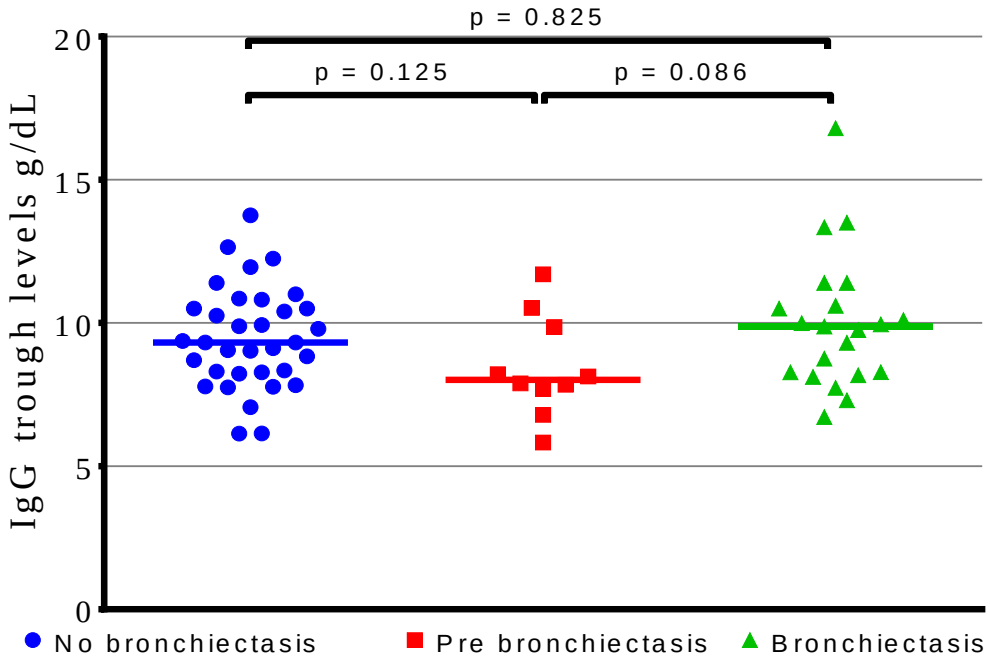
Table 6-19 Latest IGRT doses (mg/Kg/month), median lifetime and latest IgG trough levels (g/L) and SD of annual median IgG trough levels (median (IQR))

	Bronchiectasis	No bronchiectasis	p-value
Current IGRT dose	532 (469 – 644)	596 (490 – 696)	0.572
Latest IgG trough levels	9.40 (8.40 – 10.10)	10.80 (9.40 – 12.50)	0.040
Lifetime median IgG trough level	9.30 (8.05 – 10.60)	9.37 (8.34 – 10.82)	0.662
SD of yearly median IgG trough levels	1.44 (1.04 – 2.14)	1.44 (0.92 – 2.09)	0.939

While analysing patients who currently have bronchiectasis, but in their 'pre-bronchiectasis period, their IgG trough levels are not significantly different from patients who currently have no bronchiectasis ($p = 0.125$) (Figure 6-14). After patients develop bronchiectasis, their IgG trough levels tended to be higher (8.01 g/L versus 9.88g/L), but these did not reach statistical significance ($p = 0.086$).

As for the infection incidence data, the analysis of IgG trough data was done with adjustments made to the age of bronchiectasis diagnosis of minus 1, 3 and 5 years. In each adjustment, no significant differences were found.

Figure 6-14 IgG levels according to lung disease status. (Scatterplot, median and IQR)



6.13 Other therapies

Twenty percent of patients with bronchiectasis were receiving physiotherapy as part of their current therapy, either directed or self-administered. Seven percent of patients without bronchiectasis also received some form of physiotherapy.

Twelve patients (55%) of those with bronchiectasis are currently prescribed prophylactic antibiotics. Prophylactic antibiotics were discussed in Chapter 5 (5.12).

6.14 Bronchiectasis severity

6.14.1 *Bronchiectasis severity index (BSI)*

Figure 6-15 BSI for the cohort

BSI Score	Number (n)	Per cent
0-4	19	86%
5-8	3	14%

Eighty-six percent of patients with bronchiectasis had a BSI score of 0-4, and 14% had a score 5-8. A BSI score of 0-4 is predicted to have a 4-year mortality of 0 -5.3% and a score of 5-8 is predicted to have a 4-year mortality of 4 – 11.3% (309,417). As expected, an

increased BSI score correlate strongly with worsening lung function ($\rho = -0.690$, $p = 0.002$). BSI scores did tend to increase with age, although this correlation was not statistically significant ($\rho = 0.400$, $p = 0.067$).

6.14.2 *FACED scoring of bronchiectasis severity*

Table 6-20 FACED bronchiectasis severity scores

Bronchiectasis Severity	Number	Percentage
Mild	17	77%
Moderate	4	18%
Severe	1	5%

Seventy-seven percent of patients with bronchiectasis had mild bronchiectasis as scored by the FACED bronchiectasis severity score (Table 6-20). Twenty-three percent had moderate or severe bronchiectasis. Similar to the BSI, an increasing FACED score was associated with worsening lung function ($\rho = -0.734$, $p = 0.001$). However, FACED scores were not correlated with age ($\rho = 0.147$, $p = 0.515$). BSI scores and FACED scores strongly correlated ($\rho = 0.538$, $p = 0.010$).

6.14.3 *Surgical intervention*

Three patients have required surgical intervention for their bronchiectasis. Two patients have had pneumonectomies at ages 8 and 18 years. One further patient has required a bilateral lung transplant at the age of 32. These patients are defined as having severe disease as per the definitions in Chapter 4. As analysed in Chapter 5, patients with severe disease have no significant difference in clinical or immune phenotype.

6.15 Conclusions

- Bronchiectasis remains the major complication for XLA patients, affecting approximately half the cohort
- There have been significant improvements in monitoring for bronchiectasis, demonstrated by a significant decline in the age of bronchiectasis onset
- There were no significant associations with IgG trough levels or infection incidence and the risk of developing bronchiectasis.

- Being diagnosed before the age of 2 years may be associated with a significantly lower risk of developing bronchiectasis.
- The prevalence of bronchiectasis within the XLA cohort has not yet begun to reduce
- Initial lung function testing for patients who would later go on to develop bronchiectasis was significantly lower than patients who have no current evidence of bronchiectasis. This may suggest a significant amount of lung damage has occurred before the diagnosis of XLA or shortly after that. It may be that, for these patients, the development of bronchiectasis is almost inevitable.
- For a small number of patients, their bronchiectasis severity progresses to such a degree that radical intervention is needed.
    - However, for the majority of patients, lung function remains stable over time, albeit remaining abnormal in those patients with bronchiectasis.

Chapter 7 Results – Psychological Health

This chapter will present the data pertaining to the psychological health and its related quality of life in the XLA cohort.

7.1 Diagnosis

Twelve patients (24%) have a history of a formally diagnosed mental health condition, of which six have resolved. At last follow up, five patients reported a history of anxiety, five reported depression and two reported anxiety and depression. Although direct access to mental health records was not possible, three of these patients report their mental health issues are as a direct result of concerns regarding their prognosis and current clinical status.

7.2 SF36v2 MCS scores

SF36v2 contains domains scoring the quality of life-related to psychological health and mental health wellbeing (441). These scores were compared against UK norms (431) and UK patients with CF (432). Comparisons were also made between XLA patients with and without bronchiectasis. There are four subdomains; energy/vitality, social functioning, role (mental), mental health. These domains are common across both version 1 and version 2 of the SF36. Version 2 of the SF36 combines these domains to produce an overall MCS. The UK CF data derives from version 1 of the SF36, and there is, therefore, no MCS available for this dataset.

The SF36v2 was filled by 30 adults (91% of the adult study participants).

7.2.1 SF36v2 MCS scores versus UK norms and CF patients

Scores for the SF36v2 subdomains and overall MCS for the cohort versus UK norms and patients with cystic fibrosis are shown in Table 7-1. Only mean and standard deviation (SD) values were available for the UK and CF data. However, presuming these data were normally distributed, the corresponding median value would be similar to the mean and therefore could be compared against the median values for the XLA patients. The mean and SD for the XLA data is also presented for comparison. There were no significant differences in any domain comparing XLA patients against UK norms. There were no significant differences comparing XLA patients against UK CF patients, except for the mental role subdomain scores, which were significantly higher for XLA patients.

7.2.2 SF36v2 scores for patients with bronchiectasis

There was no difference between XLA patients with bronchiectasis and those without aside from social functioning scores, which were significantly lower for XLA patients with bronchiectasis (Table 7-2). There were no significant differences in the SF36v2 psychological health-related QoL scores comparing XLA patients with bronchiectasis against healthy UK male norms and UK male CF patients. Scores for the mental health subdomain did tend to be lower for XLA patients with bronchiectasis compared against UK male CF patients, but this did not reach statistical significance.

Table 7-1 SF36v2 MCS scores for the cohort (median (IQR)) compared to UK norms and UK patients with cystic fibrosis (mean (SD)). [$] Data not available

Component	XLA patients	XLA patients	Male UK norms	p-value	Male UK CF patients	p-value
Energy/Vitality	62.50 (43.75-68.75)	55.06 (21.89)	60.81 (18.93)	0.591	62.2 (21.6)	0.591
Social Functioning	100.00 (75.00-100.00)	80.95 (25.50)	84.71 (22.56)	0.679	81.9 (22.5)	0.679
Role-Mental	91.67 (83.33-100.00)	84.92 (21.18)	88.08 (19.91)	0.883	78.0 (35.6)	0.021
Mental Health	67.50 (60.00-80.00)	69.29 (17.27)	74.32 (17.24)	0.321	75.5 (18.4)	0.107
MCS	50.74 (42.86-55.38)	47.50 (10.03)	51.16 (9.34)	0.307	$	N/A

Table 7-2 SF36v2 MCS scores for the cohort (median (IQR)), comparing bronchiectasis versus no bronchiectasis, UK norms and UK CF patients. [$] Data not available

Component	**Bronchiectasis**	**No bronchiectasis**	**p-value**	**Versus male UK norms**	**Versus male UK CF patients**
Energy/Vitality	50.00 (43.75- 62.50)	62.50 (46.88 - 71.88)	0.394	0.277	0.277
Social Functioning	75.00 (62.50- 100.00)	100.00 (93.75 - 100.00)	0.045	0.275	0.275
Role-Mental	91.67 (83.33- 100.00)	91.67 (79.17 - 100.00)	0.910	0.972	0.273
Mental Health	65.00 (55.00- 80.00)	72.50 (65.00 - 85.00)	0.342	0.151	0.054
MCS	46.59 (44.29 - 54.72)	52.47 (42.58 - 54.30)	0.612	0.173	$

7.2.3 Association of SF36v2 mental health scores and disease severity

There were no significant differences in SF36v2 psychological health scores across disease severity groups. Patients with severe disease tended to report lower scores in all domains, particularly mental health, but these differences did not reach statistical significance (Table 7-3).

Table 7-3 Short Form 36 version 2 scores and disease severity (median (IQR))

	Asymptomatic	Mild	Moderate	Severe	p-value
Energy/Vitality	81.25 (62.5 – 100.00)	62.50 (31.25 – 62.50)	56.25 (50.00 – 75.50)	40.63 (18.75 - 62.50)	0.377
Social Functioning	93.75 (87.50 – 100.00)	100.00 (100.00 – 100.00)	87.50 (75.00 – 100.00)	31.25 (23.00 – 68.75)	0.132
Role-Mental	95.84 (91.67 - 100.00)	83.33 (75.00 – 91.67)	100.00 (83.3 – 100.00)	83.33 (50 – 91.67)	0.443
Mental Health	95.00 (90.00 – 100.00)	65.00 (65.00 – 70.00)	72.50 (65.00 – 85.00)	57.50 (40.00 – 67.50)	0.057
MCS	57.89 (53.22 – 62.55)	42.86 (43.30 – 52.24)	50.74 (45.85 (55.47)	45.44 (31.81 – 50.99)	0.249

7.2.4 Correlation of SF36v2 psychological health scores and clinical status

There was no correlation between the patient's current overall mental component score and their clinical status or phenotype (Table 7-4).

Table 7-4 Correlation of short-form 36 version 2 MCS and clinical outcomes and phenotype. Spearman's rho and p values shown.

	Spearman's ρ	p-value
Age	0.239	0.297
Age at diagnosis	0.003	0.991
Annual infection incidence	-0.188	0.414
Latest FEV1 Z-Score	0.191	0.513

7.3 SDQ Scores

The SDQ was filled in by nine children and seventeen parents (81% of paediatric patients). SDQ scores were compared against healthy UK male normative data (449). SDQ scores were not compared for those children with and without bronchiectasis due to the small numbers with bronchiectasis who completed the SDQ (n = 1). Excluding this one patient did not make any differences to the results.

7.3.1 SDQ scores versus UK norms

There were no significant differences in the SDQ subdomains or the total SDQ score compared to healthy UK male norms. This applied to both self-reported and parent-reported scores (Table 7-5)

For the UK norm data, only mean and standard deviation (SD) values were available. However, presuming these data were normally distributed; it has been assumed the median value is similar to the mean and therefore can be compared against the median for the XLA data. The mean and SD values for the XLA data is included for comparison.

Table 7-5 SDQ scores for the cohort (median (IQR)) versus UK norms (mean (SD)) (449)

Component	Cohort (median, IQR)	Cohort (mean, SD)	UK norms	p-value
Self-Scores				
Emotional Score	2.00 (1.00 - 4.00)	2.43 (2.07)	2.6 (1.9)	0.513
Conduct Score	1.00 (1.00 - 3.00)	1.43 (1.72)	2.4 (1.7)	0.513
Hyperactivity Score	4.00 (3.00 - 5.00)	3.71 (1.38)	3.9 (2.2)	0.439
Peer Score	0.00 (0.00 - 3.00)	1.14 (2.04)	1.6 (1.4)	0.582
Prosocial Score	8.00 (7.00 - 9.00)	8.43 (0.98)	7.5 (1.7)	0.509
Impact Score	0.00 (0.00 – 0.00)	0.29 (0.76)	0.3 (0.8)	0.492
Total Score	9.00 (5.00 – 14.00)	8.71 (4.46)	10.5 (5.1)	0.634
Parent Scores				
Emotional Score	2.00 (1.00 - 4.00)	2.58 (1.93)	1.8 (2.0)	0.329
Conduct Score	1.00 (1.00 - 4.00)	2.33 (2.50)	1.7 (1.8)	0.924
Hyperactivity Score	4.50 (4.00 - 7.00)	4.75 (2.18)	4.0 (2.7)	0.924
Peer Score	1.00 (0.00 - 2.00)	1.25 (1.60)	1.5 (1.7)	0.288
Prosocial Score	9.00 (7.00 - 9.00)	8.5 (1.51)	8.3 (1.6)	0.505
Impact Score	0.00 (0.00 – 1.00)	0.58 (1.00)	0.5 (1.2)	0.397
Total Score	10.00 (7.00 - 15.00)	10.92 (5.71)	9.1 (6.0)	0.396

7.3.2 SDQ prediction of disorders

Using the SDQ score results, 24% of patients are likely to have a hyperactivity disorder. Thirty per cent are predicted to possibly or probably have a conduct disorder and 18% to possibly or probably have an emotional disorder (Table 7-6).

Table 7-6 Likelihood of emotional, conduct and hyperactivity disorders on the SDQ

	Unlikely	Possible	Probable
Hyperactivity Disorder	13 (76%)	4 (24%)	0 (0%)
Conduct Disorder	12 (71%)	4 (24%)	1 (6%)
Emotional Disorder	14 (82%)	2 (12%)	1 (6%)

7.3.3 Comparing SDQ scores between children and parents

The total SDQ score was highly correlated between parent proxy and child self-reported scores ($\rho = 0.654$, $p = 0.021$) (Table 7-7 Correlation between child and parent SDQ Score.). However, correlations for the subdomains were inconsistent. There was a strong correlation for the emotional and peer scores (although the peer score did not reach statistical significance).

Table 7-7 Correlation between child and parent SDQ Score.

SDQ Component	Correlation (Spearman's rho)	p-value
Emotional Score	0.955	0.003
Conduct Score	-0.440	0.383
Hyperactivity Score	0.647	0.165
Peer Score	0.801	0.056
Prosocial Score	-0.426	0.399
Impact Score	0.633	0.178
Total Score	0.654	0.021

7.3.4 Correlation of SDQ score and clinical status

There were, largely, no significant correlations between self or parent-reported total SDQ score and clinical status. However, FEV1 scores were negatively associated with a worsening total SDQ score. This did not reach statistical significance for self-reported scores but did so for parent proxy scores (Table 7-8).

Table 7-8 Correlation of SDQ score and clinical outcomes and phenotype. Spearman's rho and p values shown.

	Spearman's ρ	p-value
Self-scores		
Age	0.281	0.378
Age at diagnosis	0.512	0.676
Annual infection incidence	0.133	0.732
Latest FEV1 Z-Score	-0.800	0.104
Parent scores		
Age	0.068	0.842
Age at diagnosis	-0.117	0.765
Annual infection incidence	0.333	0.381
Latest FEV1 Z-Score	-0.900	0.037

7.4 Anxiety

Twenty-six patients filled out of the HADS screening tool for anxiety (79% of eligible patients) (451). These results were compared against healthy UK norms (437) and UK male CF patients (438).

Table 7-9 HADS-anxiety outcomes for the cohort

HADS-anxiety outcome	Number of patients (n, %)
Normal	18, 69%
Borderline	3, 12%
Abnormal	5, 19%

Nineteen percent screened positive for anxiety on the HADS-A questionnaire with a further three patients reporting a borderline score (Table 7-9). This compares against 12.5% of healthy UK male norms who screen positive on the HADS-A and 13.9% who screen as borderline (437). 11.5% of UK male CF patients screen positive on the HADS-A and 18.6% screen borderline (438). There were no significant differences in the raw HADS-A scores versus UK male norms or UK male CF patients (Table 7-10).

Table 7-10 Raw HADS-A Scores versus UK male norms (median (IQR)) (437) **and UK male CF patients (mean (SD))** (438)

	XLA Patients	UK male norms	p value	UK male CF patients	p-value
HADS-A Score	5 (4 – 9)	5 (2 – 8)	0.316	5.7 (3.9)	0.809

There were no differences in the HADS-A screening outcomes between those with and without bronchiectasis, p = 1.000 (Table 7-11). There were no significant differences in the raw HADS-A scores between the two groups; median score 4 versus 5, p = 0.142.

Table 7-11 HADS-anxiety outcomes comparing those with and without bronchiectasis (n, %)

HADS-anxiety outcome	Bronchiectasis	No bronchiectasis
Normal	9. 64%	8, 73%
Borderline	2, 14%	1, 9%
Abnormal	3, 22%	2, 18%

There is a strong and significant correlation between a worsening HADS-A raw score and a worsening overall mental component score from the SF36v2, $\rho = -0.610$, p = 0.003.

There was no correlation between HADS-A raw scores and clinical status or phenotype (Table 7-12).

Table 7-12 Correlation of HADS anxiety score and clinical outcomes and phenotype. Spearman's rho and p values shown

	Spearman's ρ	p-value
Age	0.147	0.483
Age at diagnosis	0.037	0.862
Annual infection incidence	0.123	0.566
Latest FEV1 Z-Score	0.260	0.350

7.5 Depression

Twenty-six patients filled out of the HADS screening tool for depression (79% of eligible patients) (451). These results were compared against healthy UK norms (437) and UK male CF patients (438).

7.5.1 HADS depression scores

Only one patient (4%) screened positive for depression on the HADS-D questionnaire with a further two patients scoring borderline (Table 7-13). This compares against 6.9% of UK male norms who screen positive on the HADS-D and 8.5% who screen as borderline (437).

Table 7-13 HADS-D outcomes for the cohort

HADS-depression outcome	Number of patients (n, %)
Normal	23, 88%
Borderline	2, 8%
Abnormal	1, 4%

3.1% of UK male CF patients screen positive on the HADS-A and 10% screen borderline (438). There were no significant differences in the raw HADS-D scores versus UK male norms or UK male CF patients (Table 7-14).

Table 7-14 Raw HADS-D Scores versus UK male norms (median (IQR)) (437) **and UK male CF patients (mean (SD))** (438)

	XLA Patients	UK male norms	p value	UK male CF patients	p value
HADS-D Score	2 (0 – 5)	3 (1 – 6)	0.664	3.4 (3.3)	0.422

There was no difference in the HADS-D outcomes between those with and without bronchiectasis, p = 0.487 (Table 7-15). However, no patient without bronchiectasis screened a positive or borderline for depression. In addition, patients with bronchiectasis had a significantly higher HADS-D raw scores compared to those without; 4 versus 0, p = 0.017.

Table 7-15 HADS-depression outcomes comparing those with and without bronchiectasis (n, %)

HADS-depression outcome	Bronchiectasis	No bronchiectasis
Normal	11, 79%	11, 100%
Borderline	2, 14%	0, 0%
Abnormal	1, 7%	0, 0%

Similar to the HADS-A scores, a worsening HADS-D score was strongly and significantly associated with a worsening overall mental component score form the SF36v2, $\rho = -0.728$, $p < 0.001$.

There was no correlation between HADS-D raw scores and clinical status or phenotype (Table 7-16).

Table 7-16 Correlation of HADS depression score and clinical outcomes and phenotype. Spearman's rho and p values shown

	Spearman's ρ	p-value
Age	0.270	0.192
Age at diagnosis	0.265	0.201
Annual infection incidence	0.361	0.083
Latest FEV1 Z-Score	0.324	0.239

7.6 Rosenberg Self Esteem Scale

Thirty participants filled in the RSES (452). Six patients were aged 12-18 and twenty – four were over eighteen years of age. Fifty-six percent had bronchiectasis (compared to forty-four percent of the entire cohort). Eighty-three percent of the cohort reported normal or high self-esteem on the RSES (Table 7-17).

Table 7-17 RSES outcomes for the cohort

RSES outcome	Number of patients (n, %)
Low self-esteem	5, 17%
Normal self-esteem	16, 53%
High self-esteem	9, 30%

7.6.1 RSES scores for the cohort versus UK norms and CF patients

While rates of low esteem were low, and most patients recorded high self-esteem, raw RSES scores for XLA patients were significantly lower than UK healthy male norms (439) and UK male CF patients (440) (Table 7-18).

Table 7-18 RSES Scores (median (IQR)) versus UK male norms (439) **and UK male CF patients** (440) **(mean (SD))**

XLA	UK Norms	p value	CF patients	p value
26.50 (18 – 29)	31.68 (5.67)	<0.001	33.94 (5.1)	<0.001

7.6.2 RSES scores for patients with bronchiectasis

There were no significant differences in the rates of levels of self-esteem between patients with and without bronchiectasis (p = 0.163, Table 7-19). There were no differences in their raw RSES scores (27 versus 26, p = 0.158). However, no patient without bronchiectasis reported low self-esteem versus 29% of patients with bronchiectasis doing so on the RSES.

Table 7-19 RSES outcomes for bronchiectasis and no bronchiectasis

RSES outcome	Bronchiectasis	No bronchiectasis
Low self-esteem	5, 29%	0, 0%
Normal self-esteem	4, 24%	5, 38%
High self-esteem	8, 47%	8, 62%

7.6.3 Correlation of RSES score and clinical status

There was no correlation between RSES scores and clinical status or phenotype (Table 7-20). There was strong positive correlation between RSES scores and SF36v2 MCS scores (Spearman's rho = 0.670, p = 0.001).

Table 7-20 Correlation of RSES score and clinical outcomes and phenotype. Spearman's rho and p values shown

	Spearman's ρ	p-value
Age	0.077	0.716
Age at diagnosis	0.009	0.966
Annual infection incidence	-0.246	0.246
Latest FEV1 Z-Score	0.262	0.345

7.7 Summary

- Quality of life related to mental health for adult XLA patients, as measured by the SF36v2, were broadly comparable to both UK healthy norms and patients with cystic fibrosis. For patients with bronchiectasis, these scores tended to be lower than those patients without bronchiectasis, with the social functioning subdomain being significantly lower.
- SDQ scores for paediatric XLA patients were comparable to UK healthy norms.
- A higher proportion of XLA patients screened positive for anxiety disorders on the HADS-A questionnaire (19%) compared to UK male norms (12.5%) and UK male CF patients (11.5%).
- There is a strong correlation between a worsening score on the HADS anxiety screening questionnaire and quality of life-related to psychological health as measured by the SF36v2.
- There were no differences in the proportion of XLA patients who scored positive on the HADS-D questionnaire (4%) compared to UK male norms (6.9%) and UK male CF

patients (3.1%). However, all XLA patients who score borderline or positive on the HADS-D had bronchiectasis.

- There is a strong correlation between a worsening score on the HADS depression screening questionnaire and quality of life-related to psychological health as measured by the SF36v2.
- Seventeen percent of the cohort reported low self-esteem on the RSES. Raw RSES scores for XLA patients were significantly lower than UK norms and CF patients.
- Aside from parental proxy total SDQ scores and latest FEV1 Z-Score, there were no correlations with any of the measures of mental health-related quality of life and clinical phenotype.

Chapter 8 Results – Quality of Life

This chapter will present the data pertaining to the quality of life-related to physical health. In addition, it will focus on the impact of respiratory healthy specifically on patients' quality of life.

8.1 Paediatric Quality of Life Scores (PedsQl 4.0)

A total of 17 parents and 13 children filled out the PedsQl 4.0 (81% of enrolled patients) (442). Results were compared against healthy UK norms (433) and UK cystic fibrosis patients (434). It was not possible to separate the group based on the presence of bronchiectasis or not due to the small numbers of paediatric patients with bronchiectasis (n = 1). Excluding this one patient made no significant difference to the results.

8.1.1 *Parent and child correlation of the PedsQl 4.0 scores*

The overall total score correlated strongly and significantly for parent and child reports (Table 8-1). In addition, the social score subdomain also strongly correlated between the child and parent reports. The remaining subdomains did tend to correlate, but not reaching statistical significance.

Table 8-1 Correlation between Parent and Child PedsQl 4.0 Score. Shown as coefficient, p-value

Peds QL4.0 Component	Spearman's rho	p-value
Psychosocial Score	0.463	0.130
Physical Score	0.510	0.090
Emotional Score	0.215	0.502
Social Score	0.835	0.001
School Score	0.397	0.201
Total Score	0.654	0.021

8.1.2 *Paediatric quality of life scores versus UK norms and CF patients*

There were no significant differences in any of the subdomains nor the total PedsQl 4.0 score for XLA patients compared to healthy UK norms or UK CF patients (Table 8-2). This applied to both self-reported scores and parent proxy scores. Only mean values were available for the UK and CF data. However, presuming these data were normally distributed, the corresponding median value would be similar to the mean and therefore could be

compared against the median values for the XLA patients. The mean and SD for the XLA data is also presented for comparison.

Table 8-2 PedsQl 4.0 Scores (median (IQR)) versus UK norms (mean (SD)) (433) **and CF patients** (434) **(mean (SE))**

	UK Cohort (median, IQR)	UK cohort (mean, SD)	UK Norms	p value	CF Patients	p value
Self-Scores						
Psychosocial Score	78.33 (63.33-85.00)	71.67 (20.58)	80.50 (14.06)	0.249	72.3 (1.47)	0.552
Physical Score	87.50 (82.14-87.50)	80.63 (16.39)	86.08 (14.06)	0.551	79.2 (1.64)	0.194
Emotional Score	70.00 (60.00-80.00)	67.50 (20.03)	76.99 (18.43)	0.173	70.7 (1.92)	0.649
Social Score	95.00 (70.00-100.00)	86.5 (23.34)	86.85 (16.86)	0.972	78.8 (1.73)	0.216
School Score	70.00 (50.00-80.00)	61.00 (23.55)	77.29 (16.92)	0.194	65.6 (1.86)	0.551
Total Score	80.43 (71.74-88.04)	74.78 (18.22)	82.25 (13.09)	0.311	74.7 (1.42)	0.345
Parent Scores						
Psychosocial Score	81.67 (67.50-87.50)	79.44 (15.25)	79.00 (14.70)	0.875	73.3 (1.23)	0.346
Physical Score	85.94 (73.44-90.63)	77.08 (18.62)	84.99 (16.08)	0.753	79.3 (1.52)	0.530
Emotional Score	75.00 (65.00-85.00)	76.11 (19.49)	74.67 (17.67)	1.000	68.0 (1.55)	0.387
Social Score	95.00 (80.00-100.00)	92.78 (12.53)	84.62 (17.24)	0.080	81.7 (1.53)	0.131
School Score	70.00 (55.00-82.50)	69.44 (19.44)	77.72 (18.50)	0.084	68.8 (1.65)	0.937
Total Score	79.89 (67.93-89.13)	78.62 (15.50)	81.12 (13.85)	0.480	75.3 (1.22)	0.433

8.1.3 *Correlation of paediatric quality of life scores and clinical outcomes*

There were no differences in the total PedsQl 4.0 score for both self and total scores across disease severity groups (p = 0.635 and 0.300 respectively).

There was no significant correlation with the paediatric QoL scores and current age, age at diagnosis or annual infection incidence for self or parent reports (Table 8-3). There was a strong negative correlation with the patient's current respiratory health as measured by their latest FEV1 Z-score for both parent and self-score. This did not reach statistical significance for self-score but did so for parent score (Table 8-3).

Table 8-3 Correlation of PedsQl 4.0 scores and clinical outcomes and phenotype. Spearman's rho and p values shown

	Spearman's ρ	p-value
Self PedsQl 4.0 total score		
Age	0.280	0.378
Age at diagnosis	0.152	0.676
Annual infection incidence	-0.133	0.732
Latest FEV1 Z-Score	-0.800	0.104
Parent PedsQl 4.0 total score		
Age	0.068	0.842
Age at diagnosis	-0.117	0.765
Annual infection incidence	0.333	0.381
Latest FEV1 Z-Score	-0.900	0.037

8.1.4 *Paediatric quality of life scores by site and route of IGRT*

Children's self-reported PedsQl 4.0 tended to lower in all domains for those on IVIG compared to SCIG, although the numbers receiving IVIG was very small (n = 2), and these differences did not reach statistical significance (Table 8-4). There were no differences noted in the parent proxy scores for the two groups. One child was receiving both IVIG and SCIG and omitted from this initial analysis

Similarly, children's self-reported scores for hospital therapy tended to be lower than those receiving home therapy, again with small numbers (3 patients receiving hospital therapy) and did not reach statistical significance. All three patients receiving their therapy in hospital were receiving IV therapy (including the two from the above analysis and the one patient

receiving both IVIG and SCIG). There were no differences notes in the parent proxy scores for the two groups.

Table 8-4 PedsQl 4.0 score by route and site of IGRT (median (IQR))

	Intravenous (n =2)	Subcutaneous (n=14)	p value	Hospital (n = 3)	Home (n = 13)	p value
Self Scores						
Psychosocial	50.83 (25.00 – 76.70)	80.00 (71.67 – 88.33)	0.142	63.33 (25.00 – 76.67)	80.00 (71.67 – 88.33)	0.086
Physical	73.44 (59.38 – 87.50)	87.50 (71.88 – 93.75)	0.552	87.50 (59.38 – 87.50)	87.50 (71.88 – 93.75)	0.724
Emotional	50.00 (40.00 – 60.00)	70.00 (65.00 – 90.00)	0.142	60.00 (40.00 – 70.00)	70.00 (65.00 – 90.00)	0.649
Social	65.00 (30.00 – 100.00)	100.00 (95.00 – 100.00)	0.385	70.00 (30.00 – 100.00)	100.00 (95.00 – 100.00)	0.156
School	37.50 (5.00 – 70.00)	75.00 (50.00 – 80.00)	0.182	50.00 (5.00 – 70.00)	75.00 (50.00 – 80.00)	0.104
Total Score	58.70 (36.96 – 80.43)	81.52 (76.09 – 90.22)	0.242	71.74 (36.96 – 80.43)	81.52 (76.09 – 90.22)	0.139
Parent Scores						
Psychosocial	87.50 (83.33 – 91.67)	81.67 (73.33 – 91.67)	0.500	83.33 (68.33 – 91.67)	81.67 (73.33 – 91.67)	1.000
Physical	76.56 (65.63 – 87.50)	85.94 (81.25 – 90.63)	0.615	65.63 (46.88 – 87.50)	85.94 (81.25 – 90.63)	0.243
Emotional	92.50 (90.00 – 92.50)	75.00 (65.00 – 80.00)	0.180	90.00 (70.00 – 95.00)	75.00 (65.00 – 80.00)	0.362
Social	90.00 (80.00 – 100.00)	100.00 (100.00 – 100.00)	0.513	90.00 (80.00 – 100.00)	100.00 (100.00 – 100.00)	0.283
School	80.00 (75.00 – 85.00)	70.00 (55.00 – 90.00)	0.502	75.00 (45.00 – 85.00)	70.00 (55.00 – 90.00)	1.000
Total Score	83.70 (77.17 – 90.22)	82.61 (79.35 – 89.13)	1.000	77.17 (60.87 – 90.22)	82.61 (79.35 – 89.13)	0.606

8.2 Adult quality of life scores – Short form 36 version 2

HRQoL for adults was measured using the SF36v2 (441). This was filled by 30 adult participants (91% of enrolled adults). Their results were compared against healthy UK males norms (431) and UK male patients with CF (432). There are four subdomains; physical function, role (physical), general health and pain. These domains are shared across both version 1 and version 2 of the SF36. Version 2 of the SF36 combines these domains to produce an PCS. The UK CF data derives from version 1 of the SF36 and there is, therefore, no PCS available for this dataset.

8.2.1 *Adult XLA quality of life scores versus UK norms and CF patients*

The XLA patients had significantly lower scores for the pain and general health subdomains of the SF36v2 compared to UK healthy male norms (Table 8-5). There were no significant differences in the physical function, pain role or the overall PCS compared to UK norms.

XLA patients had significantly better scores in the physical role component compared to CF patients. They also tended to have higher physical function scores, but this did not reach statistical significance. There was no other significant difference in the QoL scores compared to CF patients.

Only mean values were available for the UK and CF data. However, presuming these data were normally distributed, the corresponding median value would be similar to the mean and therefore could be compared against the median values for the XLA patients. The mean and SD for the XLA data is also presented for comparison.

8.2.2 *Adult XLA quality of life scores in bronchiectasis*

Patients with bronchiectasis scored statistically significant lower scores for all domains of the SF36v2 apart from the physical role domain, compared to patients without bronchiectasis (Table 8-6). Patients with bronchiectasis had statistically significantly lower scores in the general health subdomain compared to UK norms. Their scores were broadly comparable to UK CF patients aside from the pain subdomain. This tended to be lower for XLA patients with bronchiectasis compared to CF patients but did not reach statistical significance.

8.2.3 *Adult quality of life scores by route and site of IGRT*

There were no statistical differences in SF36v2 scores when comparing either route or site of IGRT (Table 8-7).

8.2.4 *Correlation of adult quality of life scores and clinical outcomes*

Patients with severe disease had significantly worse overall SF36v2 physical component scores compared to patients with moderate or mild disease (Figure 8-1).

Figure 8-1 Short form 36 v2 physical component scores by disease severity. * = p <0.05 (median, IQR)

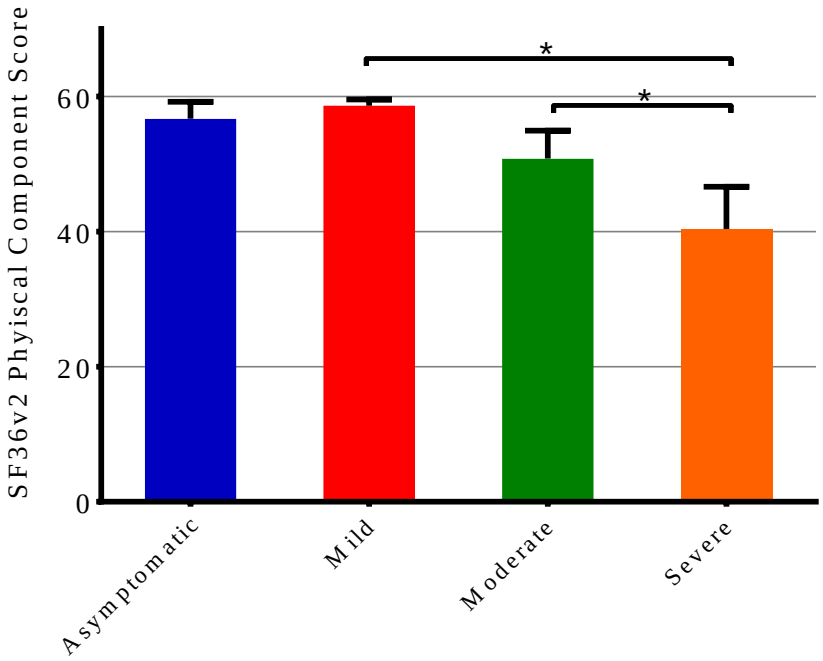


Table 8-5 SF36v2 physical health scores for the cohort (median (IQR)) compared to male UK norms (431) **and UK patients with cystic fibrosis** (432) **(mean (SD)). $(Data not available)**

	UK XLA patients (median, IQR)	UK XLA patients (mean, SD)	UK Norms	p-value	CF patients	p-value
Physical Function	100.00 (85.00-100.00)	88.57 (19.63)	89.76 (18.78)	0.253	82.4 (20.7)	0.061
Role-Physical	93.75 (87.50-100.00)	86.90 (18.53)	89.01 (21.09)	0.351	75.00 (22.5)	0.028
Pain	74.00 (62.00-100.00)	75.76 (24.63)	81.25 (22.21)	0.197	84.2 (19.6)	0.165
General Health	42.00 (27.00-62.00)	47.24 (26.39)	70.86 (20.29)	0.002	46.8 (24.0)	0.935
PCS	52.65 (47.88-56.71)	51.24 (8.77)	50.63 (9.41)	0.322	$	$

Table 8-6 SF36v2 physical health scores for patients with bronchiectasis compared against the rest of the cohort (median (IQR)), UK norms and cystic fibrosis patients (p values shown). $ Data not available

	Bronchiectasis	No Bronchiectasis	Versus XLA patients with No Bronchiectasis	Versus UK Norms	Versus CF Patients
Physical Function	90.00 (85.00- 90.00)	100.00 (100.00 - 100.00)	0.049	0.699	0.504
Role-Physical	87.50 (81.25- 93.75)	93.75 (87.50 - 100.00)	0.066	0.599	0.460
Pain	74.00 (72.00- 100.00)	73.00 (62.00 - 84.00)	0.021	0.054	0.054
General Health	42.00 (22.00- 52.00)	71.00 (42.00 - 100.00)	0.035	0.002	0.196
PCS	48.47 (48.08- 52.05)	56.23 (53.25 - 59.21)	0.014	0.600	$

Table 8-7 SF36v2 physical health scores by route and site of IGRT (median (IQR))

	Intravenous (n = 21)	Subcutaneous (n = 9)	p value	Hospital (n = 10)	Home (n = 20)	p value
Physical Function	100.00 (85.00 – 100.00)	100.00 (90.00 - 100.00)	1.000	95.00 (85.00 – 100.00)	100.00 (90.00 - 100.00)	0.479
Role-Physical	100.00 (87.50 – 100.00)	87.50 (81.25 – 93.75)	0.087	100.00 (93.75 – 100.00)	90.63 (84.38 – 96.88)	0.231
Pain	84.00 (62.00 – 100.00)	72.00 (61.00 – 100.00)	0.617	84.00 (84.00 – 100.00)	72.00 (61.50 – 100.00)	0.450
General Health	42.00 (22.00 – 67.00)	42.00 (22.00 – 52.00)	0.768	67.00 (37.00 – 82.00)	42.00 (22.00 – 47.00)	0.265
PCS	55.62 – 47.88 – 58.64)	49.57 (48.08 – 53.25)	0.696	54.95 (47.89 – 58.95)	50.81 (47.98 – 56.50)	0.598

The overall SF36v2 physical component score had a significant negative correlation with the patient's annual infection incidence and a significant correlation with their current respiratory health as measured by their current FEV1 Z-score (Table 8-8).

Table 8-8 Correlation of short-form 36 version 2 physical component scores and clinical outcomes and phenotype. Spearman's rho and p values shown

	Spearman's ρ	p-value
Age	-0.113	0.626
Age at diagnosis	-0.282	0.216
Annual infection incidence	-0.473	0.030
Latest FEV1 Z-Score	0.697	0.006

8.3 St George's Respiratory Questionnaire

Quality of life directly related to patient's respiratory health was measured using the SGRQ (435). A higher score depicts a higher impact on a patient's QoL in that domain. This was filled in by 30 adult participants (91% of enrolled adults). Their results were compared against healthy UK male norms (435) and patients with CF (436).

Only mean values were available for the UK and CF data. However, presuming these data were normally distributed, the corresponding median value would be similar to the mean and therefore could be compared against the median values for the XLA patients. The mean and SD for the XLA data is also presented for comparison.

8.3.1 *SGRQ scores versus UK norms and CF patients*

XLA patients had significantly worse QoL scores related to respiratory health in all domains of the SGRQ compared to UK healthy norms (Table 8-9). There were no differences in their scores compared to cystic fibrosis patients.

8.3.2 *SGRQ scores in bronchiectasis*

XLA patients with bronchiectasis had significantly worse SGRQ scores in all domains compared to XLA patients without bronchiectasis and healthy UK norms (Table 8-10). Their scores were not significantly different compared to CF patients except for the symptom score domain, which was significantly worse in XLA patients with bronchiectasis.

Table 8-9 XLA SGRQ scores (median (IQR)) compared against UK healthy norms (mean, 95% CI) and cystic fibrosis patients (mean (SD))

	UK XLA patients (median, IQR)	UK XLA patients (mean, SD)	UK Norms	p-value	CF patients	p-value
Symptom Score	39.43 (19.67 – 66.54)	44.48 (31.17)	12 (9-15)	0.001	35.29 (19.3)	0.274
Activity Score	18.47 (12.17 – 35.60)	28.53 (27.17)	9 (7 -12)	0.005	28.90 (25.2)	0.394
Impact Score	14.42 (3.89 – 25.13)	19.21 (18.00)	2 (1 -3)	0.001	18.60 (14.6)	0.689
Total Score	21.06 (11.44 – 33.30)	26.16 (20.90)	6 (5-7)	0.011	24.50 (16.8)	0.484

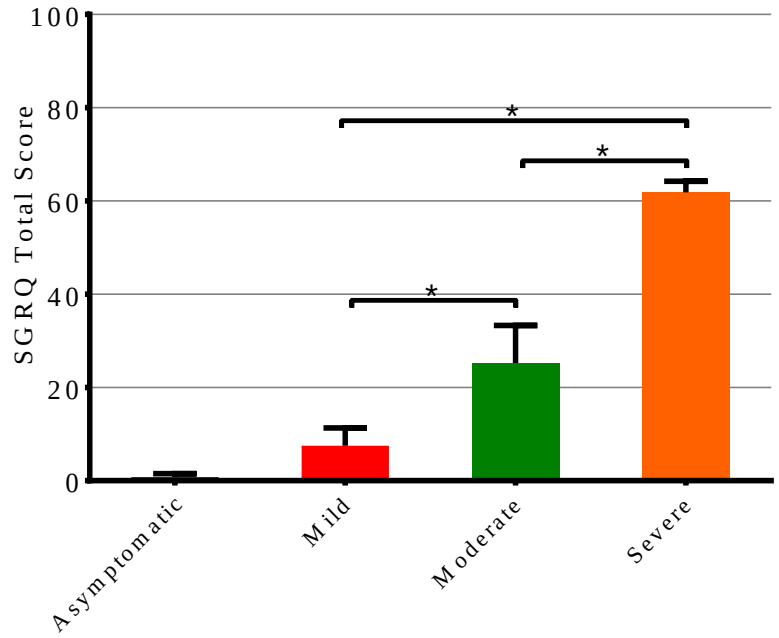
Table 8-10 St George's respiratory health questionnaire scores for patients with bronchiectasis compared against the rest of the cohort (median (IQR)), UK norms and cystic fibrosis patients (p values shown)

	Bronchiectasis	No Bronchiectasis	Versus XLA patients with No Bronchiectasis	Versus UK Norms	Versus CF Patients
Symptom Score	58.58 (32.33- 77.57)	12.37 (0.00 - 34.29)	0.004	0.002	0.023
Activity Score	35.60 (18.47- 54.43)	5.96 (0.00 - 12.17)	0.011	0.003	0.345
Impact Score	22.29 (14.42- 41.44)	0.00 (0.00 - 5.52)	0.001	0.002	0.173
Total Score	32.58 (21.06- 49.44)	6.37 (1.52 - 14.00)	0.002	0.002	0.446

8.3.3 *Correlation of SGRQ with clinical outcome*

SGRQ total scores worsened significantly with worsening disease severity (Figure 8-2). There were only two patients in the asymptomatic group who returned an SGRQ questionnaire, and their total scores were therefore not statistically significant than other groups.

Figure 8-2 St George's respiratory questionnaire total scores by disease severity. * = p <0.05 (median (IQR))



The total SGRQ score did not correlate with the patient's current age nor their age at diagnosis. It did correlate significantly and strongly with their annual infection incidence and, as expected, their current lung function results (Table 8-11). It also correlates strongly with patient's overall HRQoL as measured by the physical component score of the SF36v2.

Table 8-11 St George's respiratory questionnaire total scores and clinical outcomes and phenotype. Spearman's rho and p values shown

	Spearman's ρ	p-value
Age	0.152	0.523
Age at diagnosis	0.206	0.384
Annual infection incidence	0.640	0.002
Latest FEV1 Z-Score	-0.666	0.009
SF36v2 PCS	0.697	0.006

8.4 Summary

- HRQoL scores for paediatric XLA patients are comparable to UK healthy norms and CF patients.
- HRQoL scores for paediatric XLA patients largely correlate well for self-reported and parent-reported scores.
- Respiratory health, as measured by lung function, correlates strongly with HRQoL in paediatric patients
- HRQoL in adult patients was broadly comparable to UK healthy norms apart from the general health domain, which was worse in XLA patients.
- HRQoL in adult patients with bronchiectasis was significantly worse than patients without bronchiectasis
- HRQoL directly related to respiratory health was significantly worse in XLA patients compared to healthy norms and comparable to CF patients.
- Having bronchiectasis results in a significantly worse respiratory-related HRQoL and for the symptom score domain was significantly worse than CF patients.
- HRQoL related to respiratory health, worsened with disease severity, increasing annual infection incidence and was strongly correlated with lung function.
- The respiratory health QoL scores correlated strongly with the overall HRQoL scores measured by the SF36v2, implying respiratory health plays a significant factor in determining the patients overall HRQoL.

Chapter 9 Discussion

9.1 Summary

This analysis of 53 patients with a definite diagnosis of XLA, demonstrates that the vast majority of patients suffer at least one chronic complication as a result of their XLA. The majority of these are bronchiectasis with 44% developing bronchiectasis at the time of data collection.

9.2 Diagnosis

9.2.1 Genetic testing

This study only included those patients with a definite diagnosis of XLA as per the ESID and PGAIID guidelines, i.e. absent BTK expression or a proven BTK mutation (4). The number of XLA patients is significantly lower than the number of patients with agammaglobulinaemia in the UKPID registry (n = 204) (247). Not all of these are male, and many of these patients most likely have only a probable or possible diagnosis, i.e. lacking a genetic mutation. A recent survey of antibody patients in the UK found 109 patients with a BTK mutation from a total of 130 male patients with agammaglobulinaemia (286). From personal correspondence with PIs across the UK, I am aware of a further 15 patients in Northern Ireland and 14 patients in Scotland with a proven BTK mutation who are not in this data set.

Several patients had been initially diagnosed as other PADs, usually CVID based on a negative genetic mutation testing in BTK. These tests were often done shortly after the discovery of the BTK gene and improvements have been made in genetic testing since (461). These patients then went onto to have BTK mutation testing redone more recently and were found to have disease-causing mutations in their BTK gene and their diagnosis reclassified as XLA. This reclassification is essential, giving patients a definitive diagnosis and offering a significantly different prognosis (CVID vs XLA) and is vital for them and family members regarding family planning. These few examples therefore also raise the possibility of other PAD patients in the UK (most likely CVID), being misdiagnosed based on previous negative testing for BTK mutations. Studies using whole genome and whole exome sequencing in patients initially diagnosed with CVID have discovered patients with BTK mutations who had previously had normal results using historical Sanger sequencing techniques (462). It is therefore vital that as new genetic techniques become available or improvements are made in currently available techniques that patients without a genetic diagnosis, who may have had

previously had BTK mutations ruled out and fit phenotypically with XLA should have their DNA retested for BTK mutations.

21% of those patients tested had apparent BTK expression on monocytes despite BTK mutations and phenotype consistent with XLA. There may be some patients with a label of antibody deficiency in the UK who have not had genetic testing for XLA because they have normal BTK expression and the (incorrect) belief this rules out XLA. This may be especially pertinent in more historic cohorts where access to genetic testing was limited. These data demonstrate that normal BTK expression does not rule out XLA and all patients with a phenotype consistent with XLA should undergo genetic testing for XLA.

9.2.2 Age

Reducing the age of diagnosis is a major driver for many clinicians working in primary immune deficiency, meaning that supportive or curative therapy can be instigated as soon as possible before complications develop. This theory may hold true for XLA patients, with starting IGRT as soon as possible, before patients either succumb to life-threatening infections or by reducing the number of severe respiratory tract infections and thereby reducing the risk of bronchiectasis.

The median age of diagnosis in this XLA cohort was 2.59 years and had been improving with time. This age of diagnosis is nearly one year younger than the Italian cohort by Plebani et al., analysed in 2000 (186). Similar to the Plebani et al., data it would appear that these improvements in age at diagnosis are reaching a plateau. The median age of diagnosis since 1990 is very young at 1.28 years, but with no significant trend in reduction over time (Spearman's rho = -0.270, p = 0.191). Much of this reduction in time to diagnosis may be, in part, due to this cohort having much higher survival rates and therefore being able to pass the BTK mutations on. Offspring would, therefore, be able to be diagnosed at birth. This is partially supported by the result that after 1990 the median age of diagnosis for those who were not screened was 2.60 years.

Clinicians are likely reaching their limit in being able to clinically recognise and diagnose XLA in the absence of a positive family history. Infants often only become symptomatic after the age of 6 months once maternally transferred antibody levels have fallen, and they have presented with recurrent infections and an astute clinician has considered the possibility of a primary immune deficiency. However, it can be challenging to identify the child with a

PID against a backdrop of the healthy background population experience typical childhood infections. It may be that a general paediatrician may only encounter a new presentation of PID once or twice in their career.

It is therefore likely that, if clinicians wish to reduce the age of diagnosis even further, newborn screening would be the only feasible way of attaining this.

9.2.3 Clinical Presentation

Nine patients were screened while asymptomatic due to family positive family history of XLA. However, two patients, where a family history of XLA was known, were not screened and only diagnosed once they had become symptomatic. It is vital that, during booking of pregnant women, an accurate family history is taken. Hopefully, as awareness of PID grows through initiatives such as the Jeffrey Modell Foundation, diseases like XLA will be more readily remembered and appropriate action, such as screening, taken at the birth of newborns (253).

Several patients (15 out 43) presented with repeated serious or invasive infections defined as either meningitis, osteomyelitis or ITU stay. Five of these patients required ITU admissions due to life-threating infections. These data highlight that not only do patients present with repeated mild infections but approximately a third of patients present with potentially life-changing or even life-threatening disease. While all these patients have ultimately survived these infections, I did not have access to death record data, and it is likely a number of XLA patients have died due to infections before the diagnosis of their XLA was made. It is also possible there are some patients who died due to infections, in whom a diagnosis of XLA has never been made.

Again, due to increased awareness if PID, many UK ITU departments do readily investigate children presenting with severe and invasive infections for PID especially those who have succumbed to infections they have been vaccinated to (with XLA patients being unable to mount appropriate responses to the vaccine programme). However, this will not change the course of the initial severe infection(s). Only newborn screening and early instigation of IGRT could potentially prevent these severe infections. As will be discussed later, the fact that the number of serious infections was vanishingly rare once IGRT once started, instigation of IGRT while asymptomatic would likely prevent nearly all serious and life-threatening infections in this cohort.

The remaining patients presented with milder, but repeated infections, the majority being lower respiratory tract infections as expected (40%). There was also a significantly higher number than expected of patients (14%) presenting with repeated skin infections. The majority of causative organisms were encapsulated bacteria, again as expected for this disease. The number of *Pseudomonas aeruginosa* infections at presentation was surprisingly high (n = 5) and is not typically thought of as an organism XLA patients would be particularly susceptible to. One explanation may be due to the neutropenia seen in 10- 25% of XLA patients before IGRT is started (141). Four patients in this cohort had neutropenia at diagnosis; however I was unable to find neutrophil counts at diagnosis for the patients who had Pseudomonas infections at diagnosis, but I theorise these patients did indeed have neutropenia before diagnosis rendering them susceptible to Pseudomonas infections.

The exact cause of neutropenia in XLA patients is currently unknown and is rarely seen once IGRT is started (63). In this cohort, no patient developed neutropenia once IGRT had started. The exact reason is unknown, but neutrophil maturation in XLA patients has shown to be arrested at the myelocyte/promyelocyte stage (140–142). One potential theory is that, before IGRT is started, the stress on the bone marrow caused by severe or repeated infections leads to a degree of bone marrow suppression and neutropenia, which is then relived once IGRT is started and the number of serious infections is reduced (189). However, this is only conjecture and would need to be studied accurately to fully ascertain the reasons for neutropenia in XLA patients before IGRT is started.

9.3 Infections on Immunoglobulin replacement therapy

Overall, the data in this cohort demonstrates that XLA patients receive one course of antibiotics for infection once every 18 months. The vast majority of these (85%) are respiratory tract infections. Otitis and sinus infections account for the small number of remaining infections. There are a handful of occasional infections of other sites, most notably of the GI tract.

Respiratory tract infection comprising the majority of infections while on IGRT does not come as a great surprise given the limitations of IGRT as described throughout this thesis. The lack of replacement IgA and IgM appears to still make XLA patients susceptible to respiratory treat infections.

What is perhaps more concerning, is the lack of a difference in infection rates before and after treatment is instigated (1.11 versus 1.12 infections per year). While the number of severe and invasive infections become vanishingly rare after IGRT is started, the annual incidence of respiratory tract infections remains largely the same, 0.90 versus 0.86 infections per year. Caution must be taken with these results as data before IGRT was limited, with data only available for 18 patients, and limitations relating to the recording of infection data discussed further in this chapter. Nevertheless, these data strongly suggest that while IGRT makes significant impacts on serious and life threatening infections, its impact on milder, more indolent infections, particularly of the respiratory tract, appear limited. Again, this supports the theoretical concerns regarding the lack of IgA and IgM in current products and the limited ability of current IGRT to protect mucosal surfaces against infections.

Paediatric patients had significantly higher respiratory tract infection rate than adults while on IGRT. There are likely to be several reasons for this. The threshold to treat infections may be less in children compared to adults, amid worry of missing actual bacterial infections. This may be further exacerbated by recent attempts to improve the diagnosis and treatment of sepsis across the UK (463). Even healthy children experience more respiratory tract infections than adults, with normal healthy children reporting between 6-10 viral colds per year (464). While XLA patients will be perfectly capable of combating viral infections, it can be challenging to differentiate between viral and bacterial infections in children, and it is understandable for clinicians to overtreat in this age group so as to not miss significant bacterial chest infections. This apparent difference in respiratory tract infections between adult and paediatric XLA patients is most likely a bias due to these natural differences in the number of viral infections the two age groups experience per year and not a reflection of a different XLA phenotype between children and adults.

However, it should be highlighted that the retrospective nature of this data collection, in addition to relying on patient's recognition of their symptoms and seeking medical attention, is likely to have resulted in an underestimation of the infection burden in these patients. Two prospective studies calculated a much higher respiratory infection burden, with Ponsford et al., reporting a mean respiratory exacerbation of once every 6 days in PAD patients versus once every 6 weeks in controls (269,465). These two studies also highlight the significant burden of viral infections in respiratory infections for PAD patients, potentially accounting for up to 56% of exacerbations (269,465). Viral infections have perhaps not been thought of

as major drivers of respiratory infections in XLA patients, but it is likely they are and, in addition, potentially increasing the risk bronchiectasis.

9.4 Bronchiectasis

9.4.1 Rates and onset

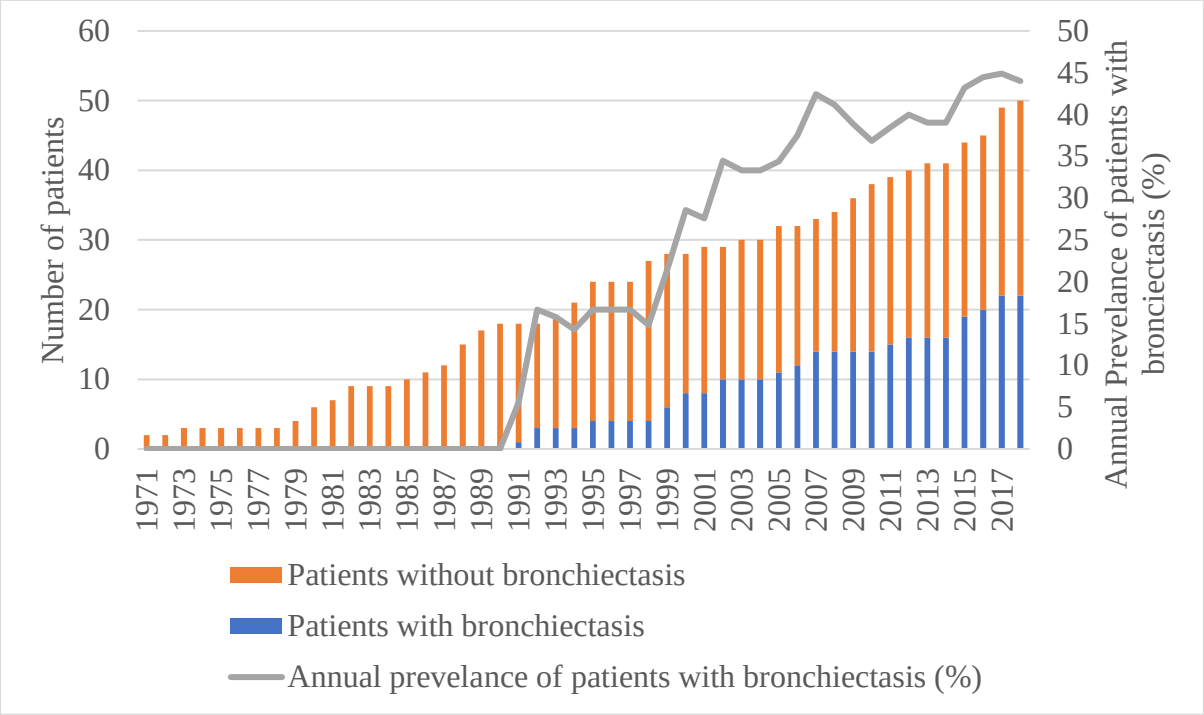
The rate of bronchiectasis of 44% in this cohort tallies well with a recent survey of UK primary antibody patient (107 of whom with presumed XLA), which reported 56% of patients had bronchiectasis (286). This rate increases to 62% for patients over 30. These data correlate well with the large study from Plebani et al., (186). Interestingly, although the Italian data is 18 years older than this UK study, the rates of bronchiectasis are remarkably similar, although there were fewer patients with bronchiectasis detected before their XLA diagnosis in these UK data.

Three patients had bronchiectasis diagnosed on their first HRCT after their XLA diagnosis and within 12 months of their XLA diagnosis. As discussed within this thesis, as bronchiectasis is a radiological diagnosis, the exact lag from the actual onset of bronchiectasis to its detection on HRCT is unknown and dependent on many factors including clinical preference and access to perform HRCTs. However, a pragmatic decision to classify any patient who had bronchiectasis detected on their first HRCT, and within 12 months of their XLA diagnosis, as having bronchiectasis at the time of their XLA diagnosis was felt to be reasonable. It is possible, even probable, that their bronchiectasis disease predates the clinical diagnosis of their XLA by some time, although no one in this cohort had a formal diagnosis of any chronic lung disease before the diagnosis of their XLA. However, even in worse case scenarios, presuming these three patients did have bronchiectasis before their XLA was diagnosed, these numbers are still better than the cohort data from Plebani et al. where 15 out of 71 had a diagnosis of chronic lung disease before their XLA (186). This England and Wales cohort is significantly more recent than the Italian cohort, and awareness of PID and PAD has significantly improved since then resulting in quicker routes to diagnosis. It may also be possible that improvements and increase uptake in vaccination programmes since then has resulted in less carriage by the population of pathogens XLA patients are susceptible to (e.g. *Streptococcus pneumoniae).*

A further nine patients (18%) have abnormal changes on their HRCT, the majority of which is bronchial thickening. Whilst these changes are reversible; they are important precursors of bronchiectasis.

Figure 9-1 attempts to estimate the prevalence of bronchiectasis within the XLA cohort over time. While there will be increasing numbers simply as the cohort becomes older, so too will new younger patients (presumably without bronchiectasis) be entering the cohort. This figure demonstrates that over the past decade, the prevalence of bronchiectasis has remained stable at approximately 50-55%. This study suggests that the risk of developing bronchiectasis has not decreased over this, and I, therefore, do not expect this number to decrease. It is possible that the prevalence of bronchiectasis increases as the current cohort becomes older. This effect will not have been seen before as, before the availability of IVIg in the 1980s, the poor survival rate of XLA did not allow a long enough survival to see these effects (168).

Figure 9-1 Annual prevalence of bronchiectasis within the XLA cohort

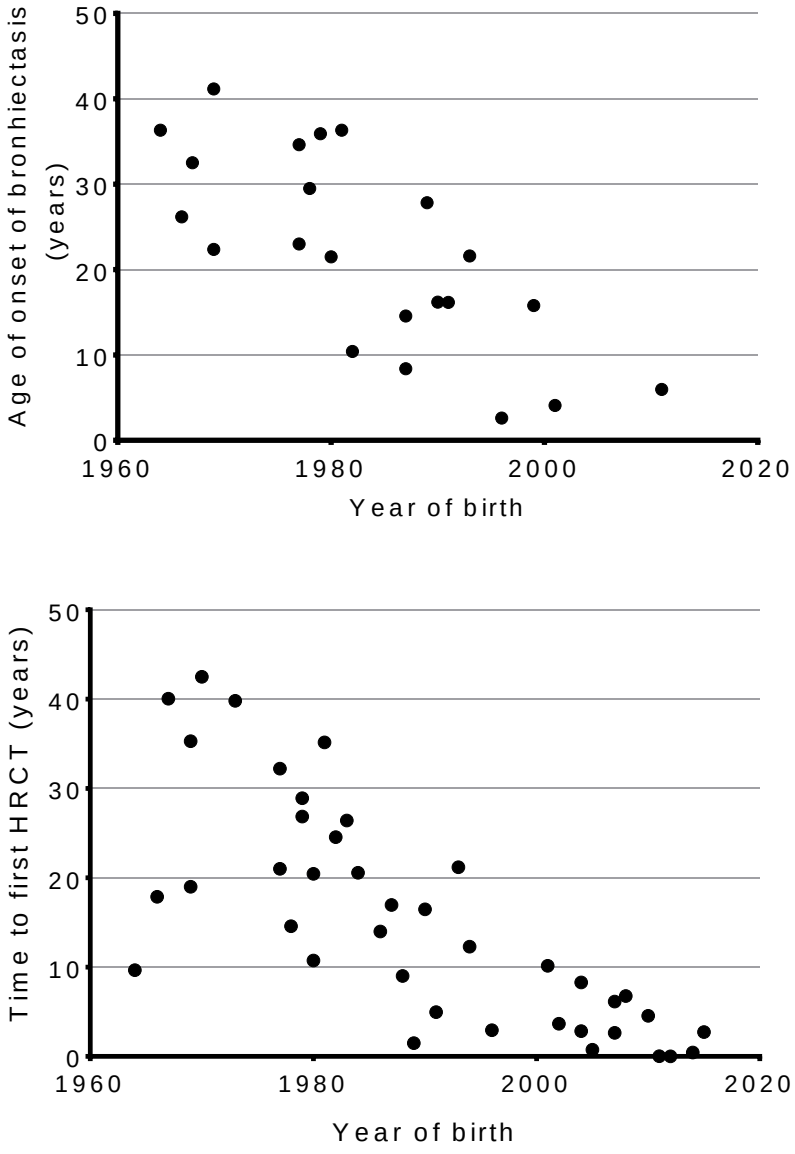


These data along with others strongly suggests that a significant proportion, possibility even the majority of XLA patients should expect to develop bronchiectasis as some point in their adult life and likely in early adulthood rather than later. No previous study has attempted to document the longitudinal progression of bronchiectasis in this cohort or other cohorts of PAD patients, given the difficulties of setting up a large enough longitudinal study with such rare diseases. Although this study was limited by using retrospective data, it does give some insight into the patterns of onset of bronchiectasis in this cohort and its progression.

Diagnosis of bronchiectasis is made by HRCT, and due to increased availability and access to HRCT, it is challenging to give an accurate analysis of the exact age of onset of

bronchiectasis. The current age of onset of bronchiectasis was 21.97 years, which would be considered extremely young for developing for such a complication. However, as Figure 6-7 demonstrates, the age of onset of bronchiectasis has decreased over time. This demonstrates easier and quicker access to HRCT over time. If we take a pragmatic cut-off of 1990 to represent when HRCT became more readily available, the age of onset of bronchiectasis was 10.87 years which is particularly concerning and strongly suggests that disease phenotype and infection incidence early in life needs to be aggressively monitored and treated. It may also demonstrate that much lung damage has occurred even before diagnosis, and it may be inevitable for some patients to develop bronchiectasis.

Figure 9-2 Age of onset of bronchiectasis and time to first HRCT over time



9.4.2 Risk factors

While on initial analyses age at diagnosis does appear to be younger for patients without bronchiectasis, as discussed previously, due to reductions in age at diagnosis over time, patients without bronchiectasis are also younger and therefore may not have enough time (as a proxy for exposure to infections) to develop bronchiectasis.

Although the overall numbers are small, the time to event model attempts to overcome some of these confounding factors. Interestingly, this model showed that increasing infection incidence did not increase the risk of bronchiectasis. This tallies with the simple analysis of comparing infection incidence for those without and with bronchiectasis, which found no significant difference (1.24 versus 0.84 infections per year). Again, these findings suggest a somewhat inevitability of bronchiectasis.

Being diagnosed before the age of two was associated with a decreased risk of developing bronchiectasis on univariate analysis but did not reach statistical significance (log-rank $p = 0.071$). There was no significant decreased risk of developing bronchiectasis for early diagnosis on the multivariate analysis. Being diagnosed asymptomatically due to screening was not associated with a significantly decreased risk, but it should be noted that the numbers who were screened were small ($n = 9$). However, these findings suggest that early diagnosis and early instigation of IGRT can reduce the risk of bronchiectasis, and it is possible that newborn screening could make even further reductions. This may also suggest that much of the risk for developing bronchiectasis occurs in the pre-diagnosis period. Infections in this pre-IGRT period may be more severe due to the lack of any immunoglobulin and are a significant driver in developing further bronchiectasis. These could cause an element of sub-clinical lung damage which increases the risk of later developing bronchiectasis. Furthermore, there may be some patients who have developed significant enough damage before their XLA diagnosis, and that the development bronchiectasis is inevitable. This theory is further supported by the data demonstrating that patients who later went to develop bronchiectasis had significantly lower lung function scores at diagnosis than patients who have not developed bronchiectasis.

In summary, this study is too small fully ascertain if younger age at diagnosis, while asymptomatic, significantly reduces the risk of bronchiectasis. However, the early onset of bronchiectasis within this cohort, strongly suggests a high burden of infection within early childhood is a significant contributor to the development of bronchiectasis. Furthermore, the

time to event analysis suggest a reduced risk for those patients diagnosed at less than two years. Taking these two findings together suggests that the infection load and path to bronchiectasis occurs early on life and diagnosis. It may be, for patients diagnosed after the age of 2 years, the path to bronchiectasis has already begun and potentially inevitable. If this were correct, future practice should aggressively target high IgG trough levels in children and lends strength to the argument for prophylactic antibiotics for all XLA patients, including those without apparent bronchiectasis. However, it may still be the likely outcome for patients diagnosed early to still develop bronchiectasis, all be it at a later time onset than the current cohort, as these data demonstrate that patients continue to report respiratory tract infections throughout their life.

9.4.3 Progression

There were a small number of patients whose bronchiectasis became very severe, very quickly necessitating pneumonectomy and, in one case, a bilateral lung transplant. There is a case report of another patient with XLA in whose bronchiectasis severity necessitated a lung transplant (273). The long-term outcome for this patient is difficult to predict with his underlying PID now exacerbated by the need to take immunosuppression for their transplant. Presumably, their risk of re-developing bronchiectasis is still quite high as their risk factors (having XLA, the limitations of IGRT) remain.

For the remainder of patients, however, the HRCT and pulmonary function data suggest the severity of their lung disease is relatively stable. 73% of patients demonstrated no progression of bronchiectasis on HRCT, and the multilevel mixed model analysis demonstrated that lung function is not significantly declining over time. This model did note that increasing respiratory infection incidence did result in a declining lung function over time, which makes logical sense. This finding emphasises the need to carefully monitor and quickly and aggressively treat chest infections.

While the progression for many appears stable, it should be noted that severity for a significant proportion remains high even in those who have not required surgery. The median last recorded FEV1 Z-score was -2.26, and 14% have a bronchiectasis severity index (BSI) score of 5 or higher. This BSI score would estimate four-year mortality of 4-11.3% (309,417)

9.5 Monitoring of Respiratory Disease

As discussed above, while the onset of bronchiectasis is potentially inevitable and perhaps only modifiable by very early diagnosis, the progression of bronchiectasis can be kept at bay, most importantly by reducing the number of respiratory tract infections. It is therefore vital that patients have regular and accurate monitoring of their lung health and respiratory tract infections treated early and aggressively. Both lung function and HRCT play a significant role in the ongoing monitoring of lung disease.

Monitoring for the development and progression of bronchiectasis should be paramount in the management of XLA. Early detection will allow more judicious use of prophylactic antibiotics, use of physiotherapy and stricter control of IgG trough levels. These data also show that the onset the bronchiectasis may be very young, and this will pose some challenges in the diagnosis of bronchiectasis as it requires HRCT. As well as balancing the risk of radiation exposure, in children, there is the added challenge of getting children to lie still. This will often need oral sedation, and may often need general anaesthetic, and these factors will need to be taken into account in the balancing the risk ratio of deciding when to perform an HRCT.

The underlying pathological process underpinning bronchiectasis probably starts long before any clinically significant declines in lung function can be detected. Biomarkers, such as sputum neutrophil elastase, catalase activity or lipid peroxidation offer the promise of safe, minimally invasive techniques to detect the onset of bronchiectasis earlier, but the clinical implementation of these seems far away (304,305). MRI would offer a safe alternative to HRCT (295,296). However, even if this reached clinical practice, it is doubtful there would be enough availability within the NHS (or indeed any health care system) to offer regular MRI as the primary means of diagnosing bronchiectasis.

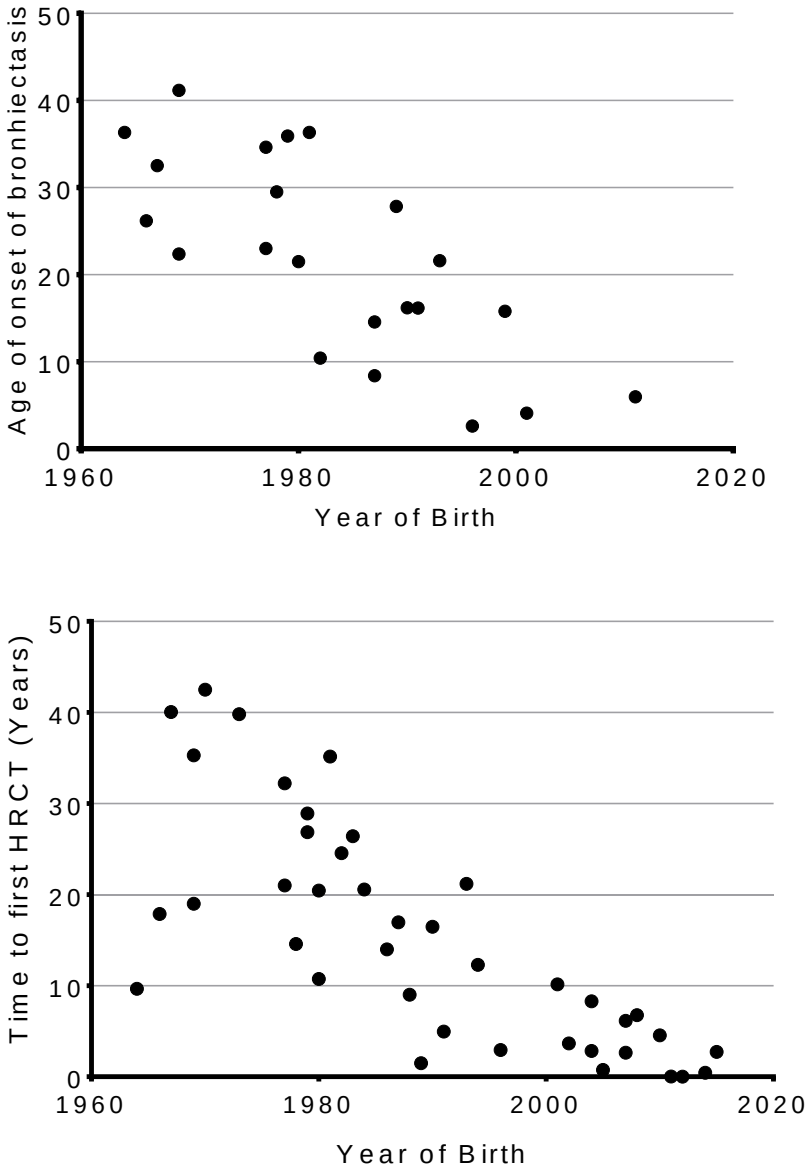
The lung function data in this study demonstrates that PFT can play a role in the detection of significant lung disease. Lung function can be reliably performed in children as young as five years of age (298). To both aid the early diagnosis of bronchiectasis and to reduce the burden of HRCT, I would recommend that all patients have annual lung function if asymptomatic. This is significantly more frequent than current practice with these data demonstrating a median interval of 5.4 years between lung function testing. While most patients do not demonstrate a progression of their bronchiectasis, up to 25%, do, with a small but significant number progressing to such a degree to require radical surgery or lung

transplantation. I would therefore recommend that patients with severe bronchiectasis or bronchiectasis, which demonstrates progression either clinically or on HRCT have lung function performed every six months.

A prosed framework for the timing of HRCT scans in adults could be; five-yearly in patients with no lung disease or clinical concerns, 2-3 yearly in patients with stable and mild bronchiectasis and 1-2 yearly in patients with severe or progressing bronchiectasis. There should also be proactive efforts to perform HRCT at any points in between these intervals if there any clinical concerns. These proposals are consistent with a recent European survey, in which most experts reported they recommend HRCT be performed every 2-4 years in patients with lung disease and less frequently in those without (299).

Access to HRCT has dramatically improved over recent years. Figure 9-3 clearly demonstrates that the age at which first HRCT is performed has significantly decreased in recent years. Allowing for that age at diagnosis has also decreased over time; the time from diagnosis to HRCT has also decreased over time.

Figure 9-3 Age of onset of bronchiectasis and time to first HRCT over time



9.6 Immunoglobulin Therapy

Immunoglobulin replacement therapy can be administered via the subcutaneous or intravenous routes. Both options are available to be administered at home or hospital with advantages and disadvantages to each.

These data demonstrated there were no significant differences in quality of life scores when comparing IV or SC IGRT or when comparing home against hospital therapy. QoL scores did tend to be lower for children who received their therapy via the IV route, but these numbers were small and did not reach statistical significance. There will be several factors

influencing the decisions as to where and how IGRT is administered. These will be individual to each patient and their family, for example; clinical indications, venous access and compliance. Furthermore, as the patient's social and clinical circumstances alter over time, likely, the route and site of IGRT may also have to be flexible and change and adapt as per the patient's needs. Appropriate training, support and flexibility of immunoglobulin services must be therefore be maintained to meet patient's needs.

However, as these data demonstrate, infection-related complications still represent a significant burden for this cohort. Although there are no data available to assess the impact of increasing IGRT doses on the chronic infection/colonisation suggested in this discussion, a more tightly controlled and aggressive targeting of IGRT dosing may be enough to significantly reduce the significance breakthrough infection which may, in turn, help delay the onset of end-organ damage or even reduce its prevalence. Stubbs et al., have demonstrated that with current IGRT practices in the UK, lung damage still occurs demonstrated by deteriorating lung function (286). One potential adjunct to treatment may be the targeted use of regular hospital admissions and administration of IV antibiotics, similar to the practice for CF patients (466).

Immunoglobin products are expensive. The annual cost of immunoglobulin for PID in England in 2015/16 was £40 million with an average annual cost of £12, 614 (467). By recording the individual preparations used by patients this study was able to more accurately calculate the current costs of IGRT for XLA patients. The current annual mean cost in this cohort for 2018 is £24, 171. Ignoring the effects of inflation, this would equate to a cost of £1, 205, 550 over a 50-year period. These costs do not include those attributed to staff costs, training, time or consumables associated with IGRT.

Increasing demand and variable supply place continuous pressures on the immunoglobulin service for the UK (467). This is further confounded by the fact that no immunoglobulin products are sourced from UK donors due to the theoretical risk of transmitted prion disease following the mad cow disease outbreak in the UK (467). Furthermore, the Brexit process may place even further pressures on this service with the potential need for new agreements and regulatory approvals (467). The secure availability of IGRT of XLA patients and all PID patients should be a priority for ministers involved in the negation processes surrounding Brexit.

9.6.1 IgA and IgM enriched products

As discussed throughout this thesis, that fact that current IGRT only replaces IgG potentially places patients at significant risk of still developing infections and their subsequent complications, something that this project strongly suggests is occurring. In addition, IgA and IgM play major roles in immune modulation and regulation. Their loss may lead to defects in these areas, as potentially suggested by these data. Again, these defects would not be compensated for with current immunoglobulin products. One option, therefore, maybe the introduction of IGRT products which contain IgA or IgM.

Fresh frozen plasma has historically been suggested as a replacement for IGRT, starting in 1975, when the currently available option of IM-IGRT also had the added limitation of limited volumes (468)**.** Infused FFP can result in significant levels of IgG, IgA and IgM in patients (469). However, to be used a long-term replacement therapy and achieve satisfactory trough levels, it has been to given twice weekly, which is not feasible or convenient enough for patients. Interestingly there is, however, reported use of FFP as an adjunct therapy in 2 patients with PID and relapsing *Campylobacter jejuni* infection (470). This was given for between 2 and 4 weeks and resulted in complete remission for the two patients (470).

There are some IgA/IgM enriched IGRT products available most notably Pentaglobin, Trimodulin and IgAbulin.

Pentaglobin contains 72% IgG, 12% IgM and 16% IgA respectively and is given as an IV preparation (471). There are some data available to suggest that Pentaglobin has better opsonic activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia Coli* compared against IVIG (472,473). There is no reported use of Pentaglobin as the standard IGRT in patients in PID. However, there are again case reports of using Pentaglobin as an adjunct therapy in successfully treating patients with hypogammaglobinaemia and chronic *Campylobacter jejuni* GI infections (470).

Trimodulin contains 21% IgA and 23% IgM and 56% IgG (474). A recent phase II trial examining patients with severe community-acquired pneumonia demonstrated that the use of Trimodulin resulted in less infection-related adverse events when compared to placebo (474). It also demonstrated improved outcomes in patients with higher CRPs and lower initial IgM

levels (474). However, there is no work demonstrating the effectiveness of Trimodulin in PID patients.

IgAbulin is an oral preparation of an IgA. It has shown to be effective in preventing necrotising enterocolitis in neonates and has been used in treatment for children with chronic diarrhoea (475,476). There are no data for the use of IgAbulin in patients with PID.

It should be noted that all the above preparations derive their IgA from plasma. As discussed in the introduction, serum IgA is predominately monomeric (477), in contrast to locally produced IgA from mucosal surfaces which is mainly dimeric (54). It is feasible, however, that the administration of serum-derived monomeric IgA may still be of benefit for patients with PAD or PID. There is some work demonstrating that serum-derived monomeric IgA and IgM can bind recombinant secretory component and form secretory IgA (sIgA) and secretory IgM (sIgM) (478). There is no human work to establish its effectiveness published yet.

There are some early data describing the feasibility of nebulised IgG, IgM and IgA allowing direct topical therapy to the lung tissue (479). This has been shown to be feasible in primates and, in mice, that the administration of nebulised IgG appeared to offer protection against pneumococcal pneumonia (479). However, as described above, the derived IgA is likely to be monomeric rather dimeric. It is unclear if this therapy could replace the immunomodulatory functions of IgA and IgM lost in XLA. There are no clinical data available to determine if this modality is feasible or effective for XLA patients.

IgA and IgM enriched products being used as the standard IGRT product for patient with XLA may be worth serious consideration. In the meantime, however, for those patients with chronic, potentially serious infections (for example to two patients with chronic norovirus infection, once of which is undergoing HSCT), using adjunct IgA/IgM enriched products should be thought as a potential treatment modality before consideration of more radical options, most notably HSCT.

9.7 Adjunct therapies

9.7.1 Prophylactic antibiotics

The use of prophylactic antibiotics was variable, either in the presence of bronchiectasis or not. This study was not designed nor powerful enough to evaluate the effectiveness of

prophylactic antibiotics in bronchiectasis. However, there is a large body of work clearly demonstrating their effectiveness in non-CF bronchiectasis (466). While there needs to be a balance against increasing the risk of resistant organisms (as demonstrated by the microbiology data in this study), all efforts should be made to reduce the risk of progression of bronchiectasis, and it should be standard practice that all XLA patients with bronchiectasis should be on prophylactic antibiotics. Relying on reported infection rates to decide on whether prophylactic antibiotics should be used is unreliable. As discussed earlier, it is possible that these patients are chronically infected and chronically damaging their airways. The fact they have developed enough infections to develop bronchiectasis should be enough to warrant prophylactic antibiotics. It is unclear if prophylactic antibiotics play a role in the management of XLA in the absence of bronchiectasis.

There is surprisingly little evidence on the use of prophylactic antibiotics in PID patients with bronchiectasis. There is one recent RCT of 89 patients with PAD which demonstrated a significant reduction in the number of exacerbations and hospitalisation for those patients on prophylactic azithromycin versus placebo (319). Aside from unknown effectiveness, a significant argument against the widespread use of prophylactic antibiotic use is the potential risk of antibiotic resistance, although little data are examining this. In the UK, there is currently an RCT assessing the effects of prophylactic flucloxacillin in newly diagnosed patients with cystic fibrosis (ISRCTN 18130649). This study should present some necessary data on the impact of prophylactic antibiotic use on antimicrobial resistance.

These data have shown that bronchiectasis remains a major burden for XLA patients and that respiratory tract infections are still common. The role of prophylactic antibiotics for all XLA patients should be explored further to determine if this has a place in the management of XLA, including in the absence of bronchiectasis.

9.8 Other complications

Whilst the risk of bronchiectasis has been known for some time, this study highlights the prevalence and severity of other complications such as gastrointestinal disease. It is especially important to note that these complications may be due to immune dysregulation rather than from an infectious component (e.g. the IBD like phenotype seen in XLA patients). It is likely that the XLA phenotype includes a degree of immune dysregulation given the phenotype described in these data and the wide-ranging role of B-lymphocytes and BTK as

described in the introduction. It is vital to note these complications. as they are unlikely to be amenable to current therapy, consisting of IgG replacement.

IgA and IgM play vital roles as anti-inflammatories and in maintaining immune system homoeostasis between commensal microorganisms and pathogens at mucosal surfaces (480,481). The loss of these immune regulators at mucosal surfaces may go some way to explain the IBD like phenotype seen in XLA patients. It may also play a role in the lungs of XLA patients where the burden of immune dysregulation and inflammation could be as important as the burden of infection in determining long term respiratory health. Again, the lack of IgA and IgM replacement in current immunoglobulin products mean that these defects will still be present in XLA patients on current therapies.

9.8.1 GI

A significant proportion of patients have or still are suffering from GI symptoms/complications, most of whom warranted endoscopic investigation.

There were three patients (6%) with confirmed IBD or IBD-like disease, which tallies well with the US data (326). The exact pathogenesis behind the link of XLA and IBD is poorly understood, but the potential loss of inflammatory regulation caused by the lack of B-lymphocytes may go some way to explain this. Standard practice for the management of IBD in this XLA cohort was similar to that of general IBD patients, namely polymeric diet and the consideration of steroids or biologics such as Infliximab.

Of note are the two patients with chronic norovirus infections, one of whom has been published as a case report prior (185). Due to the persistent symptoms and ongoing nutritional compromise, both of these patients were recently referred for HSCT, one of whom has successfully undergone bone marrow transplant. Persistent norovirus infection is well associated with CVID but, aside from the aforementioned case report, has not previously been reported in XLA (338). The underpinning mechanism explaining why XLA patients would be unable to clear Norovirus infection is unclear, but these two cases and two other international cases (personal communication) further highlight the immune defects in XLA aside from a pure antibody deficiency. In particular, the lost role of B-lymphocytes acting as antigen-presenting cells, and the lost role of BTK in cells other than B-lymphocytes may be important factors. Of particular concern in Norovirus infection in PID, is its refractory nature to standard and novel therapies. As shown during this PhD, the resulting enteropathy can be

severe and it was, for this reason, radical options such as HSCT were considered. The patient in this cohort offered HSCT is now 18 months post HSCT with good immune reconstitution and normal vaccine responses off IGRT.

Although controversial, this case demonstrates that carefully selected and targeted use of HSCT may play a role in XLA and is discussed further within this chapter.

9.8.2 Musculoskeletal

The proportion of patients with a formal diagnosis of arthritis or inflammatory arthritis symptoms is mirrored strongly by the USA registry data (13% vs 12%) (324). Interestingly, however, the four patients with a formal diagnosis of arthritis all reported previous confirmed episodes of mycoplasma infections of the affected joints. There were no reported cases of de novo joint inflammation in this cohort in the absence of previously infected joints.

There were three other patients with non-specific but significant muscle aches or fatigue who have been given the diagnosis of fibromyalgia but not a formal inflammatory disorder.

Previous data have raised the concern of inflammatory joint conditions in XLA through some process of inflammatory deregulation as a result of a lack of B-lymphocytes (324). These data find no strong evidence for an increased risk of inflammatory joint disorders in XLA, although as described in the GI complications section, it may well be this cohort is still at risk of other inflammatory conditions. The inflammatory joint conditions reported in other cohorts might be due to missing data regarding previous infections of the affected joints and incorrect diagnosis.

9.8.3 Malignancy

There was only reported case of malignancy in this cohort, a case of acute lymphoblastic leukaemia (ALL), at the age of 7 years when he was also diagnosed with XLA.

As discussed in Chapter 2, previous cohort data have found potentially higher rates of malignancy than the background population with 1.5 – 6% of patients reporting a history of malignancy (332). The majority of reported malignancy is either lymphoproliferative (largely AML or B-cell precursor ALL) or solid tumours of the GI tract (330,332). It is postulated that the risk of the latter is directly related to chronic inflammation as a result of recurrent infections (334). Indeed, in this cohort, no solid GI malignancies were reported, although I am aware through personal communications and the UKPIN registry of a UK

patient over the age of the 50 years old who has previously had colon cancer. This data set has not included data on deceased patients, and it is possible cases of malignancy have therefore been missed if the patient succumbed to their illness. Efforts are currently underway to try and access data relating to deceased patients.

There has been previous discussions and concerns regarding the risk of haematological malignancy in XLA, possibly through impaired or absent BTK playing a role in the development of ALL (482). Mary Conley has previously concluded that if there is an increased risk of haematological malignancies in XLA, this increased risk is likely to be minimal, and these UK data would agree with that conclusion (482). However, it may take larger cohort than this one to ascertain this risk fully. Again, through personal communications from European colleagues, there remain ongoing concerns regarding the risk of haematological malignancies in XLA, and further observational work in this area is probably warranted but would likely need European wide data to assess this fully.

9.8.4 Neurodegeneration

Although not included within this cohort, I am aware of two UK patients with XLA who have severe neurodegeneration with no apparent cause. While enterovirus is a well-recognised, all be it historical, cause of CNS disease in this cohort, no pathogen has been identified in either of these patients (172). Although there are no published data on the phenomena on unexplained neurodegeneration in XLA patients, through this PhD study, I have become aware of several XLA patients in the UK, Europe and USA with this phenomenon. There are plans to study this group in more detail to ascertain a cause. Potential explanations would be a viral infection that has not been tested for (or needs invasive brain biopsy to isolate), or a neurodegenerative condition associated with their BTK mutation.

9.9 Genetic Mutations

Although there are no formally agreed methods for assessing mutation severity nor clinical severity in XLA, the methods described here present some of the most detailed work seen in this cohort. A variety of methods were used to assess mutation severity, including those suggested by Broides et al. (368). Also, I assessed severity based on mutation type and position as well using a variety of mutation prediction score algorithms.

Although the sample size is small, the level of detail allowed assessment of disease severity based on several variables including complications, infection incidence and age at diagnosis.

Age at diagnosis may be a useful proxy for disease severity with the presumption that milder disease presents later on. Supporting this, are the data from this thesis that demonstrated a lower annual infection incidence pre-diagnosis was associated with a higher age at diagnosis. Whether or not these patients do indeed continue to have milder disease and fewer complications, is currently unknown although these data in this data suggest age at diagnosis bases on clinical findings may not have a significant impact on long term clinical outcomes.

Allowing for the limitations above; however, the data here suggest there is no correlation between gene mutation and clinical phenotype. Therefore, attempting to stratify disease risk or treatment strategies cannot be based on the patient's BTK mutation.

9.10 Psychological and emotional health

Overall rates of depression and anxiety, as well as psychological related QoL scores were broadly comparable against the UK healthy population. However, it should be noted, all patients who scored abnormal scores in the HADS depression scoring tool had bronchiectasis.

One unusual symptom reported in this cohort was the difficulties with memory. These have been severe enough to warrant neurological investigation although these have all been shown to be normal. In one of the patients, this is having a significant impact on their work. This is unreported in the current literature, and the exact mechanism and relationship to their XLA are unknown. One explanation may be that these symptoms are on the milder end of the unexplained neurodegeneration seen in other XLA patients. Another potential explanation is this symptom as a manifestation of an underlying psychological disease, although none of these patients had a formal diagnosis of anxiety or depression and their HADS anxiety and depression screening scores were normal.

It may be prudent to regularly screen patient with XLA for depression and anxiety, and develop access to psychological support within clinical services.

9.11 Quality of life

The main pertinent finding from the HRQoL data was that, in the absence of bronchiectasis, HRQoL is broadly comparable to the background healthy population. However, in the presence of bronchiectasis, HRQoL is significantly reduced and comparable to patients with CF. Furthermore, respiratory symptoms, lung function and SGRQ all correlate strongly with the overall HRQoL, demonstrating that respiratory health is a significant determinant of

HRQoL in XLA. These findings add further strength to the argument that prevention of lung disease should be a major focus of management in XLA given these data suggest in the absence of lung disease XLA patients can have normal physical health and a normal quality of life.

9.12 Strengths

This is the most extensive review of XLA patients in England and Wales and the first in the modern era. The inclusion criteria of genetic proven or functionally proven (absent BTK expression) also significantly decreases the diagnostic heterogeneity of the cohort compared to other studies examining primary antibody deficiency. Other XLA studies may have included patients with agammaglobulinaemia incorrectly diagnosed as XLA if not necessitating genetic diagnosis or absent BTK as inclusion criteria.

The dedication of a 3-year PhD research project and one researcher has allowed a depth of retrospective data collection not seen in any previous XLA cohort study. Notably, previous studies examining PAD or XLA have not been able to examine separately the pre and post bronchiectasis periods in those patients who would later go onto develop bronchiectasis. This has allowed the analysis of patients with bronchiectasis to see if their initial post-diagnosis, 'non-bronchiectasis' period, was any different to patients who remain without bronchiectasis.

Converting lung function scores to correct Z-scores is also a significant strength of this study. As discussed within the methodology, using percentage predicted values to compare ages and analyse initial longitudinal fashion is inaccurate.

9.13 Limitations

9.13.1 Sample size

During this study, I was unable to recruit any patient with autosomal recessive agammaglobulinaemia due to a lack of definitive diagnosis. Although I came across several patients have a diagnosis of ARA, I found no patient with a proven molecular or genetic diagnosis explaining their ARA and, as such, were not included in this study.

XLA is a rare disease, and the resulting sample size of this study is small, although it still provides useful and reliable descriptive statistics on outcomes in XLA patients. In particular, this limits the ability to carry out a robust statistical analysis on the factors leading to complications, most notably bronchiectasis. Similarly, the small differences in the HRQoL

scores are also limited by the small sample size, and interpretation of these must be taken with caution. However, small sample sizes are a constant limitation in the study of rare diseases and a balance must be struck between carrying out a detailed analysis on a strictly selected but small group, against carrying out a larger analysis on a more heterogeneous cohort with potentially less detail recorded. Despite its small size, the level of detail contained by these data, does not exist anywhere else for XLA. Furthermore, with the future plan to extend this study to Northern Ireland and Scotland, the projected samples size will be 80-100 and, whilst still small, will make this one of the largest and detailed XLA studies to date.

9.13.2 Missing data

The main limitation of this study is its retrospective component, small sample size and the problem of missing data. The missing data mainly pertains to patients diagnosed before 1999, with many paediatric patients having 100% data available for this study. This is undoubtedly a potentially significant area of bias with older patients potentially developing more severe disease as a result of inadequate, historical management plans.

However, this limitation could not be overcome with this study design. A prospective study could aim to overcome these biases; however, such a study is unlikely for such a rare disease as XLA. Although, if studied as part of a broader primary antibody deficiency study, this may be feasible in the future.

9.13.3 Infection data

The recording of infection incidence relied solely on the recording of infections at the patient's routine clinic appointments. This itself is subject to recall bias from the patients at the time, and the onus is on the clinicians to review infections and note them at clinical reviews, although this should be standard practice.

With any patient with an underlying susceptibility to infection, there may always be a bias from clinicians to overtreat infections with antibiotics on the presumption that is bacterial in origin on the fear of missing these infections. However, the low gross number of infections in this cohort suggests this is not the case, but it is possible even these small numbers represent an 'overtreatment' of self-limiting viral infections. These biases are likely to be consistent throughout the cohort and therefore, comparisons within the cohort should still be valid.

Due to the retrospective nature data, it was not possible to fully ascertain the onset of infection symptoms and the timing from any delay to start of antibiotic treatment.

9.13.4 Lag on the actual onset of bronchiectasis to CT detection

The onset of bronchiectasis was defined as the first detection on HRCT. It is challenging to ascertain what the interval is between the exact onset of bronchiectasis and its detection on HRCT. Bronchiectasis itself is an entirely radiological diagnosis; the exact clinical onset of the disease is yet to be established. Non-invasive testing such as lung function may aid clinicians in hinting at the development of bronchiectasis and prompting HRCT. However, the current diagnoses of bronchiectasis is entirely dependent on the timing of HRCT which may be routine or not dependent on the clinician's opinion of the patient's risk based on their clinical phenotype. The periods in this study, pre and post bronchiectasis, will, therefore, be subject to a degree of bias due to the lag from actual disease onset to radiological diagnosis which itself is subject ultimately to the clinician's decision to perform the HRCT. However, adjustments to the age of bronchiectasis diagnosis of minus 1, 3 and 5 years made no significant differences to the results.

9.14 Recommendations for clinical practice

9.14.1 Diagnosis

While BTK expression is often absent/reduced and quicker than genetic testing, this is not always the case. In addition, as mentioned previously, knowing about a genetic mutation can offer patients and families more certainty about their diagnosis and is essential for family planning not only for the patient but also potentially the extended family.

Therefore, all patients with XLA should be offered genetic testing for BTK mutations. In addition, as mentioned previously, Sanger sequencing technique has improved, and BTK mutations may have been missed on older assays, as demonstrated by a number of patients within this cohort who were initially labelled as CVID due to negative BTK mutation testing but subsequently relabelled as XLA when repeating genetic testing was performed (461,462). This is important both for counselling for the patient and wider family, but also to offer accurate prognosis and better-tailored treatment.

As next-generation sequencing becomes more available and cheaper, we may reach a point soon where all patients with PID undergo next generating genetic testing.

9.14.2 Follow up and monitoring

It is clear from this work and others that despite modern therapies, awareness of PID and access to resources, XLA is not a benign condition and needs regular monitoring to optimise care.

Part of these regular reviews should include an accurate review of infections over the preceding 12 months, chronic respiratory and sinus problems, nutrition, pulmonary health and impact upon the patients and their families' lives.

A proposed standard approach could an "annual MOT" for all patients with XLA where infections over the past 12 months are recorded along with IgG trough levels, and IGRT dosing can be reviewed. Patients should be asked explicitly about chronic cough and sinus disease that may not have been treated with antibiotics and therefore easily missed. Height and weight should be recorded. In addition, if old enough, patients should have an annual PFT performed and reviewed at this point. It may also be prudent to routinely record patients HRQoL at these reviews using the tools or similar used in this project. Some of this could be done with the support of the UKPIN and ESID registries which would have the added benefit of providing an accurate repository of data for further follow up of these patients.

This MOT and ongoing clinical review of XLA patients, should have involvement of the MDT, particularly ENT, respiratory medicine and radiology. This intensification of current follow up practice will enable earlier detection of complications and optimisation of therapies including novel options brought by other members of the MDT.

One of the overarching aims of these reviews would be to optimise therapy so as to prevent the development of complications but also to improve the detection of bronchiectasis, which largely relies on HRCT imaging. Regular PFT and a low threshold to image will aid the early diagnosis of bronchiectasis and enable earlier intensification of therapy to help prevent progression. Regular microbiological testing of respiratory samples will also help monitor for infection and guide antimicrobial choices to optimise respiratory health.

Further work into biomarkers of lung disease (e.g. sputum neutrophil elastase, catalase activity, lipid peroxidation (283,304,305)), may enable quicker and less invasive detection for bronchiectasis development which currently relies heavily on the use of HRCT, which carries the risk of ionising radiation. Methods to reduce the reliance on these imaging modalities would be of great benefit, particularly for the paediatric population.

For newly diagnosed patients, paediatric patients or those with more severe complications, it may be prudent to offer more frequent follow-up, e.g., every six months.

It may also be prudent to measure IgG trough levels more than once a year. Twice a year would likely be sufficient for most patients unless there are clinical concerns about the effectiveness of treatment or compliance.

If a patient develops bronchiectasis, there should be an intense effort to optimise treatment early to halt the progression of their bronchiectasis. As these patients are tending to develop bronchiectasis at a relatively early age, it may also be appropriate to offer all XLA patients with newly diagnosed bronchiectasis an initial review with a respiratory specialist, ideally somebody with an interest in PID. Early optimised of bronchiectasis therapy, e.g. prophylactic antibiotics, physiotherapy and smoking cessation are very likely to have significant improvements on later outcomes. The majority of patients may not need further respiratory review, but for any patients with abnormal lung function, or progressing disease, it would be appropriate to offer them regular review with a respiratory specialist.

There is a potential for XLA patients to be 'out of sight, out of mind' with the presumption that once they are on their IGRT, all that is required is a quick check-up and occasional HRCT and lung function testing performed, with the expectation that these patients have good clinical and HRQoL outcomes. These data clearly demonstrate that the perception that XLA is a benign disease needs to urgently change. The dissemination of these data will hopefully educate clinicians that XLA is a serious condition and can have early progression to end organ damage despite current therapies. These patients still require regular and detailed follow up until which point a cure or more definitive treatment is introduced for this cohort.

9.15 Implications for patients

This research clearly shows that XLA places a significant burden on their lives and that of their families. The introduction of IVIg/SCIg and the presumption this will adequately manage their disease may have led to XLA being a 'forgotten PID' in place of new PIDs being discovered with the advent of next-generation sequencing and novel therapies for other PID such as small molecule therapy, gene therapy and improved outcomes for HSCT.

The overarching question for clinicians, researchers and patients alike is, 'is current therapy for XLA satisfactory?'. Most health care professionals and patients would agree satisfactory

is defined as minimum infections, no end-organ damage, minimal impact on daily activities and a normal quality of life. This study shows that XLA patients suffer from high rates of bronchiectasis as well as other complications and a subsequent significant impact on their quality of life.

While some may argue that some of these poorer outcomes relate to older patients from older suboptimal therapy, this study clearly shows patients that have never been on intramuscular therapy and those born in more recent times (benefiting from greater awareness and improve age a diagnosis) still suffer from these complications. Furthermore, this study suggests that there are no obvious amenable methods to current treatment strategies to improve this. While improving age at diagnosis may play a role as suggested as others, this cohort suggests we have a reached a plateau in out improvements in reducing the age at diagnosis. This study was not able to ascertain as to whether asymptomatic screening (e.g. with a family history) can improve outcomes, as a proxy for newborn screening. Although, these data suggested that early diagnosis (<2 years) was associated with a decreased risk of developing bronchiectasis and therefore even earlier diagnosis, at birth, may reduce this risk even further. However, this question could only be answered by a large, multination prospective study. The time this would take would mean any changes that are suggested could take many years to reach fruition.

This study argues that current outcomes are so poor; a more considerable effort should be made for alternative therapies now. Realistically, this means cure with the only current feasible option being gene therapy. However, there remains hesitancy to develop research into these areas further due to a lack of any convincing evidence that current therapy is significantly ineffective. Hopefully, these data will fil this gap and demonstrate that further research into gene therapy for XLA is now warranted. Gene therapy for PID is now reaching fruition with numerous clinical trials, and even Strimvellis being approved by NICE for the treatment of ADA-SCID, demonstrating both a clinical and business case success.

Most importantly, these data, after confirming previous XLA cohort studies, give patients and their families a realistic and more accurate idea of prognosis. The risk of bronchiectasis and other end-organ damage should hopefully provide a further emphasis for both patients and clinicians to make sure IGRT is closely followed and monitored, and breakthrough infections treated early. These data on longer-term outcomes also provide essential information for

patients and their relatives regarding family planning and the implications for daughters and grandsons.

With IVIG only being made available since the 1980s and long-term survival available, longer-term outcomes data (i.e. 50 years plus) is still a relative unknown. However, again, these data would suggest tightly controlled, and monitored therapy can either delay the onset of bronchiectasis or at least significantly reduce its severity and onset if it does occur.

9.15.1 Potential impact for other PID patients

XLA is just one disease of a much wider group of primary antibody deficiency. The largest group within this being CVID, which has been extensively studied before, and the difficulties of lung disease (both bronchiectasis and granulomatous-lymphocytic interstitial lung disease) are well known. A great deal of work is underway to better define disease groups within CVID, especially those with proven monogenic defects. There are groups of PAD patients for whom, like XLA, IGRT is the mainstay of their treatment. Many are rarer than XLA making studies of them potentially unfeasible. While not sharing the same defect, they likely share the same limitations in treatment, with the same risks of recurrent infections and end-organ damage, notably bronchiectasis. These data could prove useful for clinicians caring for PADs other than XLA.

A significant number of patients who undergo HSCT for PID do not achieve B-lymphocyte reconstitution. In essence, they become primary antibody deficiency patients and require lifelong IGRT. This can have a significant effect on QoL compared to patients who achieve full reconstitution (483). While these patients do not share the underlying BTK defect, they are at risk of many of the complications XLA patients are due to the demonstrated limits in IGRT in preventing infections and end-organ damage. This work strengthens to the argument that B cell reconstitution in HSCT for PID should be a significant outcome measure.

9.15.2 Potential impacts for patients in developing countries

Thanks to the efforts of the Jeffrey Modell Foundation and others, there is now increased awareness of PID worldwide with increasing numbers of diagnosis being made in developing countries and the development of specialised immunology centres (253). This is reflected by the vast body of research output generated by these counties (191,248,484). As discussed in Chapter 2, outcomes in these countries are invariably worse than developed nations with significantly higher mortality rates. A significant reason for this is likely to be the limited

access to regular health care review and to replacement immunoglobulin therapy these patients are reliant on. This is likely to be due to several reasons; logistics of trying to reach a small number of immunology specialists within an extensive geographical area and an inability to afford regular IGRT both from a patient and healthcare provider perspective. It may be possible that for these nations, novel therapies such as a single administration of gene therapy may be more cost-effective than current mainstays of treatments consisting of lifelong clinical reviews and IGRT.

9.16 A role for adjunct and novel therapies

9.16.1 Role of HSCT

HSCT is not standard care for patients with XLA in developed health care systems. Very occasionally, XLA patients may require HSCT for treatment of a malignancy, which co-incidentally, cures their XLA (485), so there certainly is the possibility of cure through HSCT. However, it has always been felt that whatever the limitations of current therapy, the benefits of potential cure through HSCT do not outweigh the risks. This remains true even when considering the ongoing improvements in outcomes following HSCT for PID.

While this study demonstrates that recurrent infections and bronchiectasis remain a major burden for this cohort, the severity of these complications does not warrant HSCT becoming a standard of care for XLA. It should be noted that the median age of this cohort 26.87 years is relatively young and it would be interesting to continue to monitor disease severity in XLA which may swing the risk-benefit ratio in favour of HSCT.

However, as reported in this thesis, during the course of this study, two patients were referred for consideration of HSCT as a result of significant nutritional compromise from chronic norovirus infection. One patient is now 18 months post HSCT, with good immune reconstitution and has satisfactory vaccine responses off IGRT (Figure 9-4). It should be noted that clearance of the Norovirus was associated with T-cell reconstitution, occurring some time before reconstitution of humoral immunity. This highlights that the defects in XLA must have an impact on T cell function, with complications occurring due to these defects not being amenable to current therapies. Currently, the main modality of curing T cell defects is through HSCT.

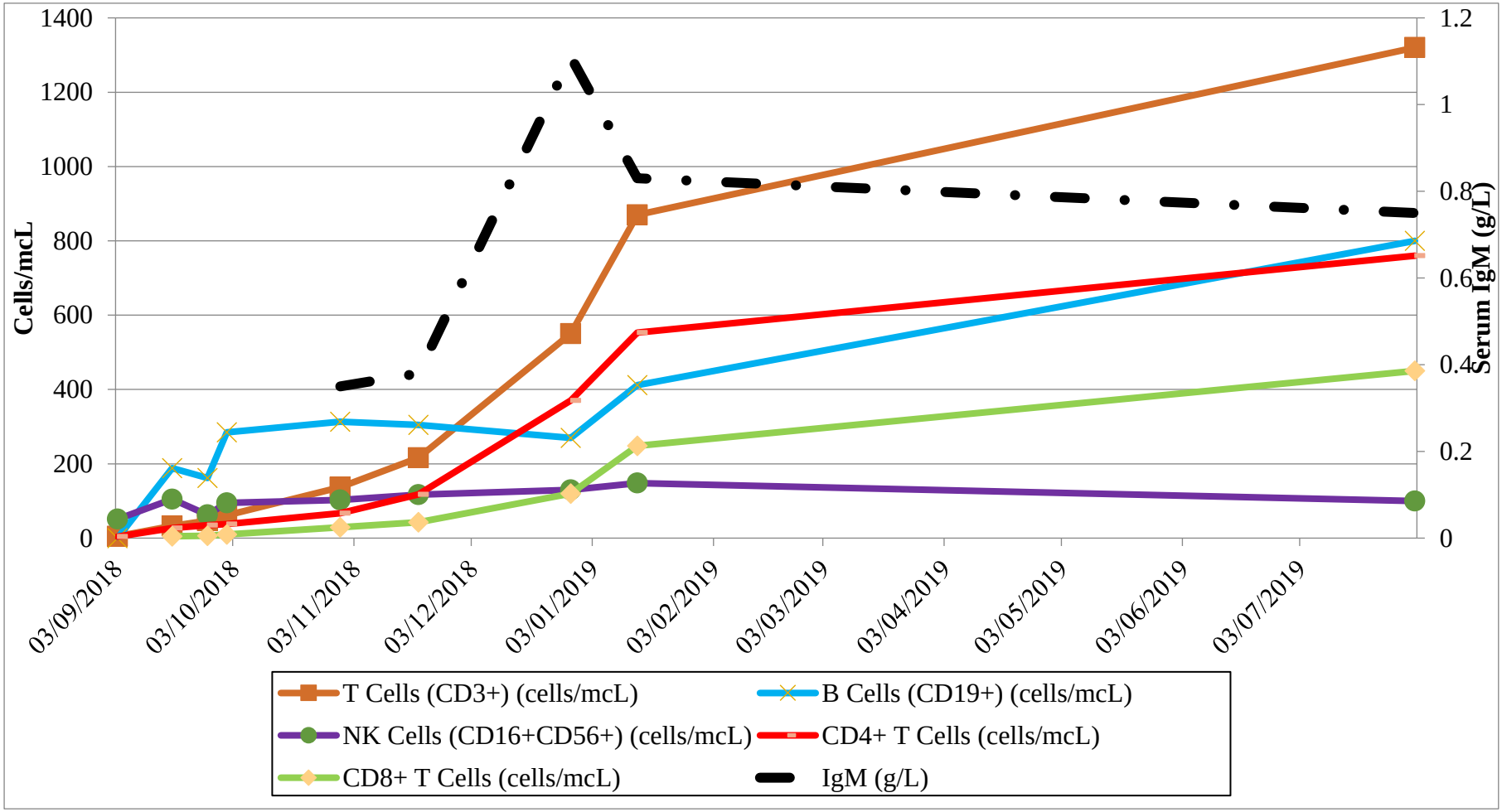
This example clearly shows that HSCT for XLA plays a role for patients with complications resistant to standard therapy. As the safety profile and outcomes for HSCT improve the

threshold for deciding who and when to offer HSCT may decrease with time, but unlikely to reach a stage where HSCT would ever be offered as standard care for XLA. Even so, this example offering HSCT solely to cure XLA is a major paradigm shift for developed health care settings. As far as our unit is aware, the patient in this cohort is the first XLA patient in Europe and America to be transplanted solely to cure their HSCT. As described previously, XLA patients have been transplanted before but in the context of other diseases where HSCT is standard of care, most notably leukaemia. I am aware through personal communication, of one other XLA patient in Europe who has successfully undergone HSCT. In this case, the patient had Sickle Cell disease and several complications related to this. In this case, the joint cure of XLA and Sickle Cell, it was felt the risk-benefit ratio swung in favour of HSCT. The Japanese have recently reported the successful HSCT of an adult patient (233). The decision here was made to progress due to recurrent infections and the ever-increasing risk of end-organ damage (233). Still, the role HSCT in XLA is controversial, and the experience of our group is due to be presented at international meetings soon, and the role of HSCT in XLA debated.

There have been other PIDs described where, historically, HSCT was not routinely performed. However, with improving HSCT outcomes and evidence that current therapies are suboptimal, HSCT is now offered for these patients. One example would be CGD where recent data demonstrated that outcomes were poor with supportive therapy and now HSCT is offered as standard of care in many centres (486). HSCT outcomes continue to improve with efforts made to tailor or reduce conditioning therapy and to improve HLA-matching with new graft manipulation strategies such as TCR αβ depletion of haploidentical or mismatched donors (487,488). A promising future therapy is the use of antibody-drug-conjugate targeting the CD117 (c-kit) antigen (489). This potentially offers the promise of excellent HSC depletion with minimal toxicity. As these developments progress and HSCT becomes safer, it is possible the risk benefit ratio swings in the favour of HSCT for XLA.

As this study demonstrates, there are other significant complications other than bronchiectasis, which can affect this cohort. Therefore, on a case by case basis, consideration of HSCT for XLA should be given, even in developed health care systems.

Figure 9-4 Immune reconstitution post HSCT for XLA



There may also be paediatric patients who develop significant lung disease or bronchiectasis early on life. As demonstrated by these data, the degree of lung disease will not improve with time and would, if anything, likely deteriorate over time. Therefore, there may be a potential argument to offer HSCT to paediatric XLA patients who have developed significant lung disease early on life, in a bid to offer to cure their XLA and prevent any further deterioration.

Some countries, most notably India, the benefits of HSCT do outweigh the risks as cost and barriers to regular IGRT mean that many patients in developing healthcare systems receive suboptimal care with prolonged periods of no IGRT or monitoring (250).

9.16.2 The role of gene therapy

These data demonstrate that XLA is not a benign disease, and complication rates remain high despite the best current therapies. It is possible through closer monitoring and more aggressive targeting of IgG trough levels and antibiotic use that outcomes for these patients may improve with time. However, the existing limitations (e.g. lack of IgA/IgM and BTK function) will always remain.

The only current prospect of dramatically improving clinical outcomes would be to offer cure via gene therapy. As discussed above, while HSCT could offer the best outcome for a select number of patients, it is highly unlikely ever to become the standard of care. Gene therapy has the potential to offer a better safety profile than HSCT while matching its effectiveness. The two major potential criticisms of gene therapy are regarding the efficacy and its safety profile. As newer gene therapy products are established, it would appear efficacy is improving with recent ADA-SCID trials demonstrating 96% event-free survival (event-free defined as the need for rescue enzyme therapy or HSCT) (490). While initial gene therapy trials were marred by insertions of the viral vectors near oncogenes, and the subsequent development of malignancy, newer designed viral vectors have a much greater safety profile (239).

Gene therapy has already become established enough to warrant inclusion into NICE guidance where guideline recommend gene therapy should be offered for patients with ADA-SCID where a matched sibling donor is not available (242). Also, there are several human clinical trials examining the use of gene therapy for other PIDs, and these data will help improve techniques and establish good quality safety data. However, there still remain no

plans for human clinical trials for XLA despite several murine models (212,404,405). The justification for XLA remains difficult. Currently, diseases under review for gene therapy are ones where HSCT would be the standard of care, e.g. ADA-SCID, Wiskott-Aldrich Syndrome (WAS) and CGD. In these cases, it is clear that any other method of reducing complications by reducing conditioning load and negating the risk of GvHD would be desirable. The problem with XLA is that current therapy (i.e., IGRT) is relatively safe. What these PhD data confirm, however, is the limitations of these therapy resulting in real-life effects with recurrent infections and end-organ damage. The debate now will entail whether these complications warrant gene therapy.

Further work into gene therapy would necessitate significant time and investment. While existing therapies could be used as a basis for XLA gene therapy, new basic science and then clinical trials specifically for XLA would need to be set up. Recruiting enough patients for such a rare disease would require a multi-centre international trial. These trials would likely require significant commercial investment, and it is only fair that these companies should expect a realistic time frame for their return of investment (ROI). Again, with such a rare disease (approximately 2-3 new cases per year in the UK) the time to ROI may be quite significant. However, the recent establishment of gene therapy for ADA-SCID with Strimvelis® and the ongoing commercial support for gene therapy trials strongly suggest that pharmaceutical companies see this area as a significant and profitable area to pursue.

9.16.3 The role of newborn screening

Although these data failed to show that very early or newborn diagnosis significantly improves outcomes, they do strongly suggest that much of the damage leading to bronchiectasis occurs early in life (potentially pre XLA diagnosis), and that diagnosis before the age of 2 may help reduce this risk. Newborn screening for XLA, via KREC analysis, may, therefore, still be a promising method for improving outcomes for these patients.

Current KREC analysis has a high false-positive rate due to a lack of consensus regarding an accurate cut off level for PAD patients. However, with further studies, these cut-off values could likely be refined, improving the false positive rate and improving the feasibility of introducing a KREC screening program. The UK is shortly due to start a pilot TREC screening program. If successful and rolled out across the UK, adding a KREC screening program would be relatively cheap (approximately £30, 000 for the laboratory costs) and

providing a more accurate cut-off level could be defined to reduce the burden of false positives (395).

9.16.4 Prophylactic Antibiotics

The effectiveness of prophylactic antibiotics in non-CF bronchiectasis is well established (275). All XLA patients with bronchiectasis should be prophylactic antibiotics. This should be based upon their microbiology results. With *Haemophilus influenzae* being the predominant organism found in these sputum samples appropriate prophylaxis could be co-trimoxazole, azithromycin or amoxicillin.

There are currently no data in the literature or guidelines examining the use of prophylactic antibiotics in XLA. However, a recent RCT examining 89 patients with PAD and bronchiectasis randomised patients to receiving prophylactic azithromycin or placebo (319). These data showed those patients who received prophylactic azithromycin reported a significant reduction in the number of exacerbations (HR 0.5, 95% 0.3 – 0.9) and hospitalisations (HR 0.5 95% CI 0.2-1.1) compared to the placebo group (319). These data, and those in non-CF bronchiectasis, therefore, clearly show prophylactic antibiotics can play a useful role for XLA patients with bronchiectasis.

What is potentially more controversial is whether prophylactic antibiotics play a role for those patients who do not have bronchiectasis, as a small number of patients did so within this study. The risk of antimicrobial resistance has to be borne in mind before deciding whether to prescribe long term antibiotics. However, the clear burden of bronchiectasis in this cohort demonstrates that more must be done to reduce the risk of developing of bronchiectasis and therefore serious consideration just be given as to whether all patients with XLA should be on prophylactic antibiotics, be that all year round or just in the winter months. Better quantification of the risk of antimicrobial resistance may soon be available through a UK RCT examining the use of prophylactic flucloxacillin in newly diagnosed infants with cystic fibrosis (ISRCTN 18130649).

9.16.5 *Cost effectiveness*

There have been no analyses of the cost effectiveness for the above strategies in XLA, but we can draw some conclusions from the available data for other PIDs, most notably SCID.

In regard to the costs of current therapy, this will consist largely of the product and staff costs for immunoglobulin therapy. As noted above, the current annual mean cost in this cohort for

2018 is £24, 171 per patient. The overall cost is likely to be influenced by the place of therapy and whether it is self-administered. Beaute et al., calculated for the French PAD cohort the costs were €19, 484 for home based IVIG and €25, 583 for hospital based IVIG. The costs for home based SCIG were €24, 952 per year (491).

It would also be worth noting the cost impacts of complications, particularly bronchiectasis. Spanish work has calculated annual costs at between €3892 and €7520 per year for patients with bronchiectasis (492). Similar US data calculated annual costs for bronchiectasis management of $5681, increasing to as much as $56, 499 for those with *P. aeruginosa* infections and exacerbations requiring hospital (493).

There is no work examining the clinical or cost effectiveness of newborn screening for XLA. The argument for newborn screening for SCID is well established, with a cost effectiveness of £20, 000 per QALY (494). Further work would need to be done to accurately calculate the costs effectiveness of a newborn screening program for XLA but it is promising that adding a KREC screening program onto an existing TREC panel would only accumulate a further £30, 000 for the laboratory costs per year for the UK (395).

Although HSCT would offer the chance of complete cure, and little in the way of long term follow up costs, there are significant costs associated with the transplant and the follow up shortly after. The total cost depends on a number of factors, namely age at transplant and clinical condition (a proxy for expected complications). Swedish data has estimated a mean cost of €301, 832 for those who were less 6 months old, and €423, 642 for those who were older than 6 months at the time of HSCT. These costs include the transplant itself, admissions and investigations pre HSCT and up to 5 years of follow up and necessary investigations for monitoring the transplant (495) . UK data would estimate the costs of HSCT for a child diagnosed with SCID at between £128, 363 and £231, 186 depending on complications encountered (494). Follow up of children with SCID post HSCT is estimated to between £251 and £1005 a year depending on complications (494).

Costing for gene therapy is difficult to analyse with only one product (Strimvellis) being licensed for use. However, we can use these costings to draw some preliminary conclusions regarding costings of any future gene therapy program for XLA. The cost of the Strimvellis therapy is £509, 027 (494). The cost of hospital admission, administration and treatment of

any associated complications is between £527, 829 and £585, 994 (494). Follow up costs are similar to those post allogenic HSCT for SCID (494).

Whilst novel or more intensive therapies may have a high initial upfront cost, they are likely to be cost effective given their potential for reducing the risk of long-term complications. Further work is needed to analyse the cost-effectiveness of novel therapies against the costs of current treatments and associated complications.

9.17 Future research

Studies like this one, studying rare diseases are always hampered by small numbers even on a national scale, which is why multi-centre, multinational studies are needed.

The first piece of work leading on from this project will be to extended recruitment across the UK, which is already underway. I have also been granted access to mortality data from the UKPID registry which will be included within these dataset. This work will aim to be the most detail population-based study of XLA patients in the literature.

There could also be potential to recruit across Europe, with the aid of the ESID and national registries. It is necessary to help garner interest and funding to do this as part of a more extensive study of primary antibody patients or patients on IGRT. This information would also be useful, as many of these patient group may also face the same limitations in therapy and complications as XLA patients do.

Incorporating regular lung function recording in a prospective manner, e.g. the UKPID registry may give a more accurate picture of progression of lung disease. In addition, it would be useful to know if there are any changes in HRQoL over time, including associations with respiratory symptoms. The exact microbiology of the respiratory tract and of infections, including the impact of viruses, is poorly understood in this group. Potential future work could include the regular collection of samples from the upper respiratory tract to describe the organisms isolated in XLA patients and those associated with infections. This work could also analyse the impact of viruses for these patients. These samples would be easy to collect and could be timed with patient's regular visits to hospital appointments for clinics and immunoglobulin infusions.

It would be hoped that this work and the planned UK wide work will prompt further research into gene therapy, be it viral vector or gene-editing techniques.

The setup of a newborn screening programme of SCID is still underway in the UK. Potentially, when this is established, this work could form the basis for initial thoughts about adding KRECs to the newborn screening.

Chapter 10 Conclusion

This study has demonstrated that the majority of XLA patients suffer at least one chronic complication of their XLA. The most common co-morbidity is bronchiectasis, with 44% developing bronchiectasis. The development of bronchiectasis is not affected by IgG trough levels, IGRT dose or infection incidence. Furthermore, these data suggest that significant lung damage has already occurred before the start of IGRT, and the path to bronchiectasis has already begun by the time the patient is diagnosed with XLA. While it may be that an early diagnosis can mitigate these risks, any further improvements in age at diagnosis would only likely be achievable via newborn screening.

There is potential for improvement with stricter monitoring of lung disease and with more aggressive increases in IgG doses to eliminate all infections and symptoms of chronic sinusitis and infection. There may be a role for prophylactic antibiotics for all XLA patients, even without bronchiectasis, and this should be looked into further.

The limitations of current therapy remain, namely the lack of IgA and IgM and the development of bronchiectasis may be inevitable to many patients with XLA. These data demonstrated that, in the absence of bronchiectasis, XLA patients could expect a normal quality of life and therefore, future work should concentrate on improvements in current therapy or novel therapies to reduce or eliminate the risk of bronchiectasis. These data and findings provide a strong argument that further research into gene therapy for XLA is now warranted. Furthermore, these data have demonstrated that, in select cases, attempting cure with HSCT may also be warranted.

References

1. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 1993 Jan 29;72(2):279–90.

2. Vetrie D, Vorechovský I, Sideras P, Holland J, Davies A, Flinter F, et al. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein-tyrosine kinases. Nature. 1993;361(6409):226–33.

3. Bruton OC. Agammaglobulinemia. Pediatrics. 1952;9(6):722–8.

4. Conley ME, Notarangelo LD, Etzioni A. Diagnostic Criteria for Primary Immunodeficiencies. Clin Immunol. 1999 Dec;93(3):190–7.

5. Edgar JDM, Buckland M, Guzman D, Conlon NP, Knerr V, Bangs C, et al. The United Kingdom Primary Immune Deficiency (UKPID) Registry: report of the first 4 years' activity 2008-2012. Clin Exp Immunol. 2014 Jan;175(1):68–78.

6. Tiselius A, Kabat EA. An Electrophoretic Study of Immune Sera and Purified Antibody Preperations. J Exp Med. 1939 Jan 1;69(1):119–31.

7. Tiselius A. A new apparatus for electrophoretic analysis of colloidal mixtures. Trans Faraday Soc. 1937;33(0):524–31.

8. Bruton OC. A decade with agammaglobulinemia. J Pediatr. 1962 May;60:672–6.

9. Siegal FP, Pernis B, Kunkel HG. Lymphocytes in human immunodeficiency states: a study of membrane-associated immunoglobulins. Eur J Immunol. 1971 Jan 1;1(6):482–6.

10. Cooper MD, Lawton AR. Circulating B-cells in patients with immunodeficiency. Am J Pathol. 1972 Dec;69(3):513–28.

11. Geha RS, Rosen FS, Merler E. Identification and Characterization of Subpopulations of Lymphocytes in Human Peripheral Blood after Fractionation on Discontinuous Gradients of Albumin: The Cellular Defect in X-Linked Agammaglobulinaemia. J Clin Invest. 1973 Jul 1;52(7):1726–34.

12. Preud'Homme JL, Griscelli C, Seligmann M. Immunoglobulins on the surface of

lymphocytes in fifty patients with primary immunodeficiency diseases. Clin Immunol Immunopathol. 1973 Jan;1(2):241–56.

13. Pearl ER, Vogler LB, Okos AJ, Crist WM, Lawton AR, Cooper MD. B Lymphocyte Precursors in Human Bone Marrow: An Analysis of Normal Individuals and Patients with Antibody-Deficiency States. J Immunol. 1978 Apr;120(4):1169–75.

14. Kwan SP, Kunkel L, Bruns G, Wedgwood RJ, Latt S, Rosen FS. Mapping of the X-linked agammaglobulinemia locus by use of restriction fragment-length polymorphism. J Clin Invest. 1986 Feb 1;77(2):649–52.

15. Hagemann TL, Chen Y, Rosen FS, Kwan SP. Genomic organization of the Btk gene and exon scanning for mutations in patients with x-linked agammaglobulinemia. Hum Mol Genet. 1994 Oct;3(10):1743–9.

16. Ohta Y, Haire RN, Litman RT, Fu SM, Nelson RP, Kratz J, et al. Genomic organization and structure of Bruton agammaglobulinemia tyrosine kinase: localization of mutations associated with varied clinical presentations and course in X chromosome-linked agammaglobulinemia. Proc Natl Acad Sci U S A. 1994 Sep 13;91(19):9062–6.

17. Rohrer J, Parolini O, Conley ME, Belmont JW. The genomic structure of human BTK, the defective gene in X-linked agammaglobulinemia. Immunogenetics. 1994;40(5):319–24.

18. Sideras P, Müller S, Shiels H, Jin H, Khan WN, Nilsson L, et al. Genomic organization of mouse and human Bruton's agammaglobulinemia tyrosine kinase (Btk) loci. J Immunol. 1994 Dec 15;153(12):5607–17.

19. Takada H, Kanegane H, Nomura A, Yamamoto K, Ihara K, Takahashi Y, et al. Female agammaglobulinemia due to the Bruton tyrosine kinase deficiency caused by extremely skewed X-chromosome inactivation. Blood. 2004;103(1):185–7.

20. Conley ME, Broides A, Hernandez-Trujillo V, Howard V, Kanegane H, Miyawaki T, et al. Genetic analysis of patients with defects in early B-cell development. Immunol Rev. 2005 Feb;203(1):216–34.

21. Mohamed AJ, Yu L, Bäckesjö CM, Vargas L, Faryal R, Aints A, et al. Bruton's tyrosine kinase (Btk): Function, regulation, and transformation with special emphasis

on the PH domain. Immunol Rev. 2009 Mar;228(1):58–73.

22. Kornfeld SJ, Haire RN, Strong SJ, Tang H, Sung SS, Fu SM, et al. A novel mutation (Cys145-->Stop) in Bruton's tyrosine kinase is associated with newly diagnosed X-linked agammaglobulinemia in a 51-year-old male. Mol Med. 1996 Sep;2(5):619–23.

23. Conley ME, Fitch-hilgenberg ME, Cleveland JL, Parolini O, Rohrer J. Screening of genomic DNA to identify mutations in the gene for bruton's tyrosine kinase. Hum Mol Genet. 1994 Oct;3(10):1751–6.

24. Bykowsky MJ, Haire RN, Ohta Y, Tang H, Sung SS, Veksler ES, et al. Discordant phenotype in siblings with X-linked agammaglobulinemia. Am J Hum Genet. 1996 Mar;58(3):477–83.

25. Gathmann B, Grimbacher B, Beauté J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: Results 2006-2008. Clin Exp Immunol. 2009 Sep;157(SUPPL. 1):3–11.

26. Conley ME. Genetics of hypogammaglobulinemia: what do we really know? Curr Opin Immunol. 2009;21(5):466–71.

27. Silva P, Justicia A, Regueiro A, Fariña S, Couselo JM, Loidi L. Autosomal recessive agammaglobulinemia due to defect in μ heavy chain caused by a novel mutation in the IGHM gene. Genes Immun. 2017 Aug 3;18(3):197–9.

28. Hendriks RW, Bredius RG, Pike-Overzet K, Staal FJ. Biology and novel treatment options for XLA, the most common monogenetic immunodeficiency in man. Expert Opin Ther Targets. 2011 Aug 2;8222(December 2015):9–18.

29. Tangye SG, Al-Herz W, Bousfiha A, Chatila T, Cunningham-Rundles C, Etzioni A, et al. Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee. J Clin Immunol. 2020;40(1):24–64.

30. LeBien TW, Tedder TF. B lymphocytes : how they develop and function. Am Soc Hematol. 2008 Sep 1;112(5):1570–80.

31. Cooper MD, Alder MN. The Evolution of Adaptive Immune Systems. Cell. 2006 Feb

24;124(4):815–22.

32. Tiselius A, Kabat EA. Electrophoresis of Immune Serum. Science (80-). 1938 May 6;87(2262):416–7.

33. Fagraeus A. Plasma Cellular Reaction and its Relation to the Formation of Antibodies in vitro. Nature. 1947 Apr 12;159(4041):499–499.

34. Mitchell GF, Miller JF. Cell to cell interaction in the immune response. II. The source of hemolysin-forming cells in irradiated mice given bone marrow and thymus or thoracic duct lymphocytes. J Exp Med. 1968 Oct 1;128(4):821–37.

35. Miller JF, Mitchell GF. Cell to cell interaction in the immune response. I. Hemolysin-forming cells in neonatally thymectomized mice reconstituted with thymus or thoracic duct lymphocytes. J Exp Med. 1968 Oct 1;128(4):801–20.

36. Coombs RR, Feinstein A, Wilson AB. Immunoglobulin determinants on the surface of human lymphocytes. Lancet (London, England). 1969 Nov 29;2(7631):1157–60.

37. Fröland S, Natvig JB, Berdal P. Surface-bound immunoglobulin as a marker of B lymphocytes in man. Nat New Biol. 1971 Dec 22;234(51):251–2.

38. Schroeder HW, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010 Feb;125(2):S41–52.

39. Langereis JD, van der Flier M, de Jonge MI. Limited Innovations After More Than 65 Years of Immunoglobulin Replacement Therapy: Potential of IgA- and IgM-Enriched Formulations to Prevent Bacterial Respiratory Tract Infections. Front Immunol. 2018 Aug 23;9:1925.

40. Davis AC, Roux KH, Shulman MJ. On the structure of polymeric IgM. Eur J Immunol. 1988 Jul 1;18(7):1001–8.

41. Crago SS, Kulhavy R, Prince SJ, Mestecky J. Secretory component of epithelial cells is a surface receptor for polymeric immunoglobulins. J Exp Med. 1978 Jun 1;147(6):1832–7.

42. Mostov KE, Blobel G. A transmembrane precursor of secretory component. The receptor for transcellular transport of polymeric immunoglobulins. J Biol Chem. 1982

Oct 10;257(19):11816–21.

43. Mostov KE, Kraehenbuhl JP, Blobel G. Receptor-mediated transcellular transport of immunoglobulin: synthesis of secretory component as multiple and larger transmembrane forms. Proc Natl Acad Sci U S A. 1980 Dec;77(12):7257–61.

44. Waldmann TA, Strober W. Metabolism of immunoglobulins. Prog Allergy. 1969;13(7):1–110.

45. Barth WF, Wochner RD, Waldmann TA, Fahey JL. Metabolism of Human Gamma Macroglobulins. J Clin Invest. 1964 Jun 1;43(6):1036–48.

46. Chen K, Cerutti A. The function and regulation of immunoglobulin D. Curr Opin Immunol. 2011 Jun;23(3):345–52.

47. Bindon CI, Hale G, Brüggemann M, Waldmann H. Human monoclonal IgG isotypes differ in complement activating function at the level of C4 as well as C1q. J Exp Med. 1988 Jul 1;168(1):127–42.

48. Tao MH, Smith RI, Morrison SL. Structural features of human immunoglobulin G that determine isotype-specific differences in complement activation. J Exp Med. 1993 Aug 1;178(2):661–7.

49. Mankarious S, Lee M, Fischer S, Pyun KH, Ochs HD, Oxelius VA, et al. The half-lives of IgG subclasses and specific antibodies in patients with primary immunodeficiency who are receiving intravenously administered immunoglobulin. J Lab Clin Med. 1988 Nov;112(5):634–40.

50. Frangione B, Wolfenstein-Todel C. Partial duplication in the “hinge” region of IgA 1 myeloma proteins. Proc Natl Acad Sci U S A. 1972 Dec;69(12):3673–6.

51. Pilette C, Ouadrhiri Y, Godding V, Vaerman JP, Sibille Y. Lung mucosal immunity: Immunoglobulin-A revisited. Eur Respir J. 2001;18(3):571–88.

52. Woof JM, Mestecky J. Mucosal immunoglobulins. Immunol Rev. 2005 Aug;206(1):64–82.

53. Johnson S, Sypura WD, Gerding DN, Ewing SL, Janoff EN. Selective neutralization of a bacterial enterotoxin by serum immunoglobulin A in response to mucosal disease.

Infect Immun. 1995 Aug;63(8):3166–73.

54. Delacroix DL, Dive C, Rambaud JC, Vaerman JP. IgA subclasses in various secretions and in serum. Immunology. 1982 Oct;47(2):383–5.

55. Pier G, Lyczak J, Wetzler L. Immunology, Infection, and Immunity. 1st ed. ASM Press; 2004.

56. Ron Y, De Baetselier P, Tzehoval E, Gordon J, Feldman M, Segal S. Defective induction of antigen-reactive proliferating T cells in B cell-deprived mice II. Anti-μ treatment affects the initiation and recruitment of T cells. Eur J Immunol. 1983;13(2):167–71.

57. Meyers G, Ng Y, Bannock JM, Lavoie A, Walter JE, Notarangelo LD. Activation-induced cytidine deaminase (AID) is required for B-cell tolerance in humans. 2011;108(28).

58. Li R, Rezk A, Li H, Gommerman JL, Prat A, Bar-Or A, et al. Antibody-Independent Function of Human B Cells Contributes to Antifungal T Cell Responses. J Immunol. 2017 Apr 15;198(8):1601572.

59. Stiehm ER, Chin TW, Haas A, Peerless AG. Infectious complications of the primary immunodeficiencies. Clin Immunol Immunopathol. 1986 Jul;40(1):69–86.

60. Yamano T, Steinert M, Klein L. Thymic B Cells and Central T Cell Tolerance. Front Immunol. 2015 Jul 22;6:376.

61. Genevier HC, Hinshelwood S, Gaspar HB, Rigley KP, Brown D, Saeland S, et al. Expression of Bruton's tyrosine kinase protein within the B cell lineage. Eur J Immunol. 1994 Dec;24(12):3100–5.

62. Smith CI, Baskin B, Humire-Greiff P, Zhou JN, Olsson PG, Maniar HS, et al. Expression of Bruton's agammaglobulinemia tyrosine kinase gene, BTK, is selectively down-regulated in T lymphocytes and plasma cells. J Immunol. 1994 Jan 15;152(2):557–65.

63. Conley ME, Rohrer J, Minegishi Y. X-linked agammaglobulinemia. Clin Rev Allergy Immunol. 2000;19(2):183–204.

64. Conley ME, Cooper MD. Genetic basis of abnormal B cell development. Curr Opin Immunol. 1998 Aug;10(4):399–406.

65. Corneth OBJ, Klein Wolterink RGJ, Hendriks RW. BTK Signaling in B Cell Differentiation and Autoimmunity. In 2015. p. 67–105.

66. Andreotti AH, Bunnell SC, Feng S, Berg LJ, Schreiber SL. Regulatory intramolecular association in a tyrosine kinase of the tec family. Nature. 1997 Jan 2;385(6611):93–7.

67. Ellmeier W, Jung S, Sunshine MJ, Hatam F, Xu Y, Baltimore D, et al. Severe B Cell Deficiency in Mice Lacking the Tec Kinase Family Members Tec and Btk. J Exp Med. 2000 Dec 4;121300(11):1611–23.

68. Smith CIE, Islam KB, Vořechovský I, Olerup O, Wallin E, Rabbani H, et al. X-Linked Agammaglobulinemia and Other Immunoglobulin Deficiencies. Immunol Rev. 1994 Apr;138(1):159–83.

69. Vihinen M, Nilsson L, Smith CIE. Tec homology (TH) adjacent to the PH domain. FEBS Lett. 1994 Aug 22;350(2–3):263–5.

70. Vihinen M, Nore BF, Mattsson PT, Bäckesjö CM, Nars M, Koutaniemi S, et al. Missense mutations affecting a conserved cysteine pair in the TH domain of Btk. FEBS Lett. 1997 Aug 18;413(2):205–10.

71. Hyvönen M, Saraste M. Stucture of the PH domain and Btk motif from Bruton's tyrosine kinase: Molecular explanations for X-linked agammaglobulinaemia. EMBO J. 1997 Jun 15;16(12):3396–404.

72. Wahl MI, Fluckiger AC, Kato RM, Park H, Witte ON, Rawlings DJ. Phosphorylation of two regulatory tyrosine residues in the activation of Bruton's tyrosine kinase via alternative receptors. Proc Natl Acad Sci U S A. 1997 Oct 14;94(October):11526–33.

73. Honda F, Kano H, Kanegane H, Nonoyama S, Kim E, Lee S-K, et al. The kinase Btk negatively regulates the production of reactive oxygen species and stimulation-induced apoptosis in human neutrophils. Nat Immunol. 2012 Feb 26;13(4):369–78.

74. Bao Y, Zheng J, Han C, Jin J, Han H, Liu Y, et al. Tyrosine kinase Btk is required for NK cell activation. J Biol Chem. 2012 Jul 6;287(28):23769–78.

75. Koprulu AD, Ellmeier W. The role of Tec family kinases in mononuclear phagocytes. Crit Rev Immunol. 2009;29(4):317–33.

76. Lee SH, Kim T, Jeong D, Kim N, Choi Y. The Tec family tyrosine kinase Btk regulates RANKL-induced osteoclast maturation. J Biol Chem. 2008 Apr 25;283(17):11526–34.

77. Shinohara M, Koga T, Okamoto K, Sakaguchi S, Arai K, Yasuda H, et al. Tyrosine Kinases Btk and Tec Regulate Osteoclast Differentiation by Linking RANK and ITAM Signals. Cell. 2008 Mar 7;132(5):794–806.

78. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. Saunders/Elsevier; 2017. 545 p.

79. Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med. 2015 Aug 20;21(8):922–6.

80. Melchers F, Ten Boekel E, Seidl T, Kong XC, Yamagami T, Onishi K, et al. Repertoire selection by pre-B-cell receptors, and B-cell receptors, and genetic control of B-cell development from immature to mature B cells. Immunol Rev. 2000 Jun;175:33–46.

81. Hendriks RW, Middendorp S. The pre-BCR checkpoint as a cell-autonomous proliferation switch. Trends Immunol. 2004 May 1;25(5):249–56.

82. Herzog S, Reth M, Jumaa H. Regulation of B-cell proliferation and differentiation by pre-B-cell receptor signalling. Nat Rev Immunol. 2009 Mar;9(3):195–205.

83. Brack C, Hirama M, Lenhard-Schuller R, Tonegawa S. A complete immunoglobulin gene is created by somatic recombination. Cell. 1978 Sep;15(1):1–14.

84. Schatz DG, Oettinger MA, Baltimore D. The V(D)J recombination activating gene, RAG-1. Cell. 1989 Dec 22;59(6):1035–48.

85. Raff MC, Megson M, Owen JJT, Cooper MD. Early production of intracellular IgM by B-lymphocyte precursors in mouse. Nature. 1976 Jan 22;259(5540):224–6.

86. Lam KP, Kühn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell. 1997 Sep

19;90(6):1073–83.

87. Nomura K, Kanegane H, Karasuyama H, Tsukada S, Agematsu K, Murakami G, et al. Genetic defect in human X-linked agammaglobulinemia impedes a maturational evolution of pro-B cells into a later stage of pre-B cells in the B-cell differentiation pathway. Blood. 2000 Jul 15;96(2):610–7.

88. Campana D, Farrant J, Inamdar N, Webster AD, Janossy G. Phenotypic features and proliferative activity of B cell progenitors in X-linked agammaglobulinemia. J Immunol. 1990 Sep 15;145(6):1675–80.

89. Minegishi Y, Conley ME. Negative selection at the pre-BCR checkpoint elicited by human mu heavy chains with unusual CDR3 regions. Immunity. 2001 May;14(5):631–41.

90. Minegishi Y, Rohrer J, Conley ME. Recent progress in the diagnosis and treatment of patients with defects in early B-cell development. Curr Opin Pediatr. 1999 Dec;11(6):528–32.

91. Conley ME, Brown P, Pickard AR, Buckley RH, Miller DS, Raskind WH, et al. Expression of the Gene Defect in X-Linked Agammaglobulinemia. N Engl J Med. 1986 Aug 28;315(9):564–7.

92. Fearon ER, Winkelstein JA, Civin CI, Pardoll DM, Vogelstein B. Carrier detection in X-linked agammaglobulinemia by analysis of X-chromosome inactivation. N Engl J Med. 1987 Feb 19;316(316):427–31.

93. Conley ME, Parolini O, Rohrer J, Campana D. X-linked agammaglobulinemia: new approaches to old questions based on the identification of the defective gene. Immunol Rev. 1994 Apr;138(138):5–21.

94. Guo B, Kato RM, Garcia-Lloret M, Wahl MI, Rawlings DJ. Engagement of the human pre-B cell receptor generates a lipid raft-dependent calcium signaling complex. Immunity. 2000 Aug;13(2):243–53.

95. Saito K, Scharenberg AM, Kinet J-P. Interaction between the Btk PH Domain and Phosphatidylinositol-3,4,5-trisphosphate Directly Regulates Btk. J Biol Chem. 2001 May 11;276(19):16201–6.

96. Mahajan S, Fargnoli J, Burkhardt AL, Kut SA, Saouaf SJ, Bolen JB. Src family protein tyrosine kinases induce autoactivation of Bruton's tyrosine kinase. Mol Cell Biol. 1995 Oct;15(10):5304–11.

97. Rawlings DJ, Scharenberg AM, Park H, Wahl MI, Lin S, Kato RM, et al. Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. Science. 1996 Feb 9;271(5250):822–5.

98. Hendriks RW, Yuvaraj S, Kil LP. Targeting Bruton's tyrosine kinase in B cell malignancies. Nat Rev Cancer. 2014 Apr 24;14(4):219–32.

99. Wang J, Sohn H, Sun G, Milner JD, Pierce SK. The autoinhibitory C-terminal SH2 domain of phospholipase C-γ2 stabilizes B cell receptor signalosome assembly. Sci Signal. 2014 Sep 16;7(343):ra89–ra89.

100. Takata M, Kurosaki T. A role for Bruton's tyrosine kinase in B cell antigen receptor-mediated activation of phospholipase C-gamma 2. J Exp Med. 1996 Jul 1;184(1):31–40.

101. Fluckiger AC, Li Z, Kato RM, Wahl MI, Ochs HD, Longnecker R, et al. Btk/Tec kinases regulate sustained increases in intracellular Ca2+ following B-cell receptor activation. EMBO J. 1998 Apr 1;17(7):1973–85.

102. Kim YJ, Sekiya F, Poulin B, Bae YS, Rhee SG. Mechanism of B-cell receptor-induced phosphorylation and activation of phospholipase C-gamma2. Mol Cell Biol. 2004 Nov;24(22):9986–99.

103. Hogan PG, Lewis RS, Rao A. Molecular Basis of Calcium Signaling in Lymphocytes: STIM and ORAI. Annu Rev Immunol. 2010;28(1):491–533.

104. Hashimoto A, Okada H, Jiang A, Kurosaki M, Greenberg S, Clark EA, et al. Involvement of guanosine triphosphatases and phospholipase C-gamma2 in extracellular signal-regulated kinase, c-Jun NH2-terminal kinase, and p38 mitogen-activated protein kinase activation by the B cell antigen receptor. J Exp Med. 1998 Oct 5;188(7):1287–95.

105. Shinohara H, Maeda S, Watarai H, Kurosaki T. IkappaB kinase beta-induced phosphorylation of CARMA1 contributes to CARMA1 Bcl10 MALT1 complex formation in B cells. J Exp Med. 2007 Dec 24;204(13):3285–93.

106. Kang SW, Wahl MI, Chu J, Kitaura J, Kawakami Y, Kato RM, et al. PKCβ modulates antigen receptor signaling via regulation of Btk membrane localization. EMBO J. 2001 Oct 15;20(20):5692–702.

107. Anderson JS, Teutsch M, Dong Z, Wortis HH. An essential role for Bruton's tyrosine kinase in the regulation of B-cell apoptosis. Proc Natl Acad Sci U S A. 1996 Oct 1;93(20):10966–71.

108. Solvason N, Wu WW, Kabra N, Lund-Johansen F, Roncarolo MG, Behrens TW, et al. Transgene expression of bcl-xL permits anti-immunoglobulin (Ig)-induced proliferation in xid B cells. J Exp Med. 1998 Apr 6;187(7):1081–91.

109. Glassford J, Soeiro I, Skarell SM, Banerji L, Holman M, Klaus GGB, et al. BCR targets cyclin D2 via Btk and the p85α subunit of PI3-K to induce cell cycle progression in primary mouse B cells. Oncogene. 2003 Apr 17;22(15):2248–59.

110. Spaargaren M, Beuling EA, Rurup ML, Meijer HP, Klok MD, Middendorp S, et al. The B Cell Antigen Receptor Controls Integrin Activity through Btk and PLCγ2. J Exp Med. 2003 Nov 17;198(10):1539–50.

111. Kouro T, Nagata K, Takaki S, Nisitani S, Hirano M, Wahl MI, et al. Bruton's tyrosine kinase is required for signaling the CD79b-mediated pro-B to pre-B cell transition. Int Immunol. 2001 Apr;13(4):485–93.

112. Aoki Y, Isselbacher KJ, Pillai S. Bruton tyrosine kinase is tyrosine phosphorylated and activated in pre-B lymphocytes and receptor-ligated B cells. Proc Natl Acad Sci U S A. 1994 Oct 25;91(22):10606–9.

113. Saouaf SJ, Mahajan S, Rowley RB, Kut SA, Fargnoli J, Burkhardt AL, et al. Temporal differences in the activation of three classes of non-transmembrane protein tyrosine kinases following B-cell antigen receptor surface engagement. Proc Natl Acad Sci U S A. 1994 Sep 27;91(20):9524–8.

114. de Weers M, Brounsnj GS, Hinshelwoodll S, Kinnonll C, Schuurmans RKB, Hendrikst RW, et al. B-cell Antigen Receptor Stimulation Activates the Human Bruton's Tyrosine Kinase, Which Is Deficient in X-linked Agammaglobulinemia*. J Biol Chem. 1994 Sep 30;269(39):23657–60.

115. de Gorter DJJ, Beuling EA, Kersseboom R, Middendorp S, van Gils JM, Hendriks

RW, et al. Bruton's Tyrosine Kinase and Phospholipase Cγ2 Mediate Chemokine-Controlled B Cell Migration and Homing. Immunity. 2007 Jan;26(1):93–104.

116. Santos-argumedo L, Lund FE, Heath AW, Solvason N, Wu WW, Grimaldi JC, et al. CD38 unresponsiveness of xid B cells implicates Bruton's tyrosine kinase (btk) as a regulator of CD38 induced signal transduction. Int Immunol. 1995 Feb;7(2):163–70.

117. Schmidt U, van den Akker E, Parren-van Amelsvoort M, Litos G, de Bruijn M, Gutiérrez L, et al. Btk Is Required for an Efficient Response to Erythropoietin and for SCF-controlled Protection against TRAIL in Erythroid Progenitors. J Exp Med. 2004 Mar 15;199(6):785–95.

118. Kawakami Y, Yao L, Miura T, Tsukada S, Witte ON, Kawakami T. Tyrosine phosphorylation and activation of Bruton tyrosine kinase upon Fc epsilon RI cross-linking. Mol Cell Biol. 1994 Aug;14(8):5108–13.

119. Launay P, Lehuen A, Kawakami T, Blank U, Monteiro RC. IgA Fc receptor (CD89) activation enables coupling to syk and Btk tyrosine kinase pathways: differential signaling after IFN-gamma or phorbol ester stimulation. J Leukoc Biol. 1998 May;63(5):636–42.

120. Jongstra-Bilen J, Puig Cano A, Hasija M, Xiao H, Smith CIE, Cybulsky MI. Dual Functions of Bruton's Tyrosine Kinase and Tec Kinase during Fc Receptor-Induced Signaling and Phagocytosis. J Immunol. 2008 Jul 1;181(1):288–98.

121. Quek LSS, Bolen J, Watson SPP. A role for Bruton's tyrosine kinase (Btk) in platelet activation by collagen. Curr Biol. 1998 Oct 8;8(20):1137–40.

122. Sato S, Katagiri T, Takaki S, Kikuchi Y, Hitoshi Y, Yonehara S, et al. IL-5 receptor-mediated tyrosine phosphorylation of SH2/SH3-containing proteins and activation of Bruton's tyrosine and Janus 2 kinases. J Exp Med. 1994 Dec 1;180(6):2101–11.

123. Matsuda T, Takahashi-Tezuka M, Fukada T, Okuyama Y, Fujitani Y, Tsukada S, et al. Association and activation of Btk and Tec tyrosine kinases by gp130, a signal transducer of the interleukin-6 family of cytokines. Blood. 1995 Feb 1;85(3):627–33.

124. Horwood NJ, Page TH, McDaid JP, Palmer CD, Campbell J, Mahon T, et al. Bruton's tyrosine kinase is required for TLR2 and TLR4-induced TNF, but not IL-6, production. J Immunol. 2006 Mar 15;176(6):3635–41.

125. Jefferies CA, Doyle S, Brunner C, Dunne A, Brin E, Wietek C, et al. Bruton's tyrosine kinase is a Toll/interleukin-1 receptor domain-binding protein that participates in nuclear factor ??B activation by toll-like receptor 4. J Biol Chem. 2003 Jul 11;278(28):26258–64.

126. Khan WN, Alt FW, Gerstein RM, Malynn BA, Larsson I, Rathbun G, et al. Defective B cell development and function in Btk-deficient mice. Immunity. 1995 Sep;3(3):283–99.

127. Doyle SL, Jefferies CA, Feighery C, O'Neill LAJ. Signaling by toll-like receptors 8 and 9 requires Bruton's tyrosine kinase. J Biol Chem. 2007 Dec 21;282(51):36953–60.

128. Taneichi H, Kanegane H, Mohamed Sira M, Futatani T, Agematsu K, Sako M, et al. Toll-like receptor signaling is impaired in dendritic cells from patients with X-linked agammaglobulinemia. Clin Immunol. 2008 Feb;126(2):148–54.

129. Halcomb KE, Musuka S, Gutierrez T, Wright HL, Satterthwaite AB. Btk regulates localization, in vivo activation, and class switching of anti-DNA B cells. Mol Immunol. 2008 Dec;46(2):233–41.

130. Tsukamoto Y, Nagai Y, Kariyone A, Shibata T, Kaisho T, Akira S, et al. Toll-like receptor 7 cooperates with IL-4 in activated B cells through antigen receptor or CD38 and induces class switch recombination and IgG1 production. Mol Immunol. 2009 Apr;46(7):1278–88.

131. Lee KG, Xu S, Wong ET, Tergaonkar V, Lam KP. Bruton's tyrosine kinase separately regulates NFκB p65RelA activation and cytokine interleukin (IL)-10/IL-12 production in TLR9-stimulated B cells. J Biol Chem. 2008 Apr 25;283(17):11189–98.

132. Röck J, Schneider E, Grün JR, Grützkau A, Küppers R, Schmitz J, et al. CD303 (BDCA-2) signals in plasmacytoid dendritic cells via a BCR-like signalosome involving Syk, Slp65 and PLCγ2. Eur J Immunol. 2007 Dec;37(12):3564–75.

133. Weber ANR, Bittner Z, Liu X, Dang T-M, Radsak MP, Brunner C. Bruton's Tyrosine Kinase: An Emerging Key Player in Innate Immunity. Front Immunol. 2017 Nov 8;8:1454.

134. Mirsafian H, Ripen AM, Leong W-MM, Chear CT, Bin Mohamad S, Merican AF. Transcriptome profiling of monocytes from XLA patients revealed the innate immune

function dysregulation due to the BTK gene expression deficiency. Sci Rep. 2017 Dec 28;7(1):1–13.

135. Braga Amoras AL, Kanegane H, Miyawaki T, Dos Santos Vilela MM. Defective Fc-, CR1 - and CR3-Mediated Monocyte Phagocytosis and Chemotaxis in Common Variable Immunodeficiency and X-linked Agammaglobulinemia Patients. J Investig Allergol Clin Immunol. 2003;13(3):181–8.

136. Cavaliere FM, Prezzo A, Bilotta C, Iacobini M, Quinti I. The lack of BTK does not impair monocytes and polymorphonuclear cells functions in X-linked agammaglobulinemia under treatment with intravenous immunoglobulin replacement. Boissonnas A, editor. PLoS One. 2017 Apr 19;12(4):1–21.

137. Nagai Y, Garrett KP, Ohta S, Bahrun U, Kouro T, Akira S, et al. Toll-like Receptors on Hematopoietic Progenitor Cells Stimulate Innate Immune System Replenishment. Immunity. 2006 Jun;24(6):801–12.

138. Melcher M, Unger B, Schmidt U, Rajantie IA, Alitalo K, Ellmeier W. Essential roles for the Tec family kinases Tec and Btk in M-CSF receptor signaling pathways that regulate macrophage survival. J Immunol. 2008 Jun 15;180(12):8048–56.

139. Fiedler K, Sindrilaru A, Terszowski G, Kokai E, Feyerabend TB, Bullinger L, et al. Neutrophil development and function critically depend on Bruton tyrosine kinase in a mouse model of X-linked agammaglobulinemia. Blood. 2011 Jan 27;117(4):1329–39.

140. Kozlowski C, Evans DI. Neutropenia associated with X-linked agammaglobulinaemia. J Clin Pathol. 1991 May 1;44(5):388–90.

141. Farrar JE, Rohrer J, Conley ME. Neutropenia in X-linked agammaglobulinemia. Clin Immunol Immunopathol. 1996 Dec;81(3):271–6.

142. Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, Burks AW, et al. X-linked agammaglobulinemia: report on a United States registry of 201 patients. Medicine (Baltimore). 2006 Jul;85(4):193–202.

143. Marron TU, Rohr K, Martinez-Gallo M, Yu J, Cunningham-Rundles C. TLR signaling and effector functions are intact in XLA neutrophils. Clin Immunol. 2010 Oct;137(1):74–80.

144. Kawakami Y, Inagaki N, Salek-Ardakani S, Kitaura J, Tanaka H, Nagao K, et al. Regulation of dendritic cell maturation and function by Bruton's tyrosine kinase via IL-10 and Stat3. Proc Natl Acad Sci U S A. 2006 Jan 3;103(1):153–8.

145. Köprülü AD, Kastner R, Wienerroither S, Lassnig C, Putz EM, Majer O, et al. The Tyrosine Kinase Btk Regulates the Macrophage Response to Listeria monocytogenes Infection. Lenz LL, editor. PLoS One. 2013 Mar 27;8(3):e60476.

146. Liu X, Pichulik T, Wolz O-O, Dang T-M, Stutz A, Dillen C, et al. Human NACHT, LRR, and PYD domain–containing protein 3 (NLRP3) inflammasome activity is regulated by and potentially targetable through Bruton tyrosine kinase. J Allergy Clin Immunol. 2017 Oct 1;140(4):1054-1067.e10.

147. Lee K-G, Xu S, Kang Z-H, Huo J, Huang M, Liu D, et al. Bruton's tyrosine kinase phosphorylates Toll-like receptor 3 to initiate antiviral response. Proc Natl Acad Sci. 2012 Apr 10;109(15):5791–6.

148. Herbst S, Shah A, Mazon Moya M, Marzola V, Jensen B, Reed A, et al. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to Aspergillus fumigatus. EMBO Mol Med. 2015 Mar 1;7(3):e201404556.

149. Sochorova K, Horvath R, Rozkova D, Litzman J, Bartunkova J, Sediva A, et al. Impaired Toll-like receptor 8–mediated IL-6 and TNF-α production in antigen-presenting cells from patients with X-linked agammaglobulinemia. Blood. 2007 Mar 15;109(6):2553–6.

150. Li YF, Lee KG, Ou X, Lam KP. Bruton's tyrosine kinase and protein kinase C μ are required for TLR7/9-induced IKKα and IRF-1 activation and interferon-β production in conventional dendritic cells. Zhang L, editor. PLoS One. 2014 Aug 29;9(8):e105420.

151. Lougaris V, Baronio M, Vitali M, Tampella G, Cattalini M, Tassone L, et al. Bruton tyrosine kinase mediates TLR9-dependent human dendritic cell activation. J Allergy Clin Immunol. 2014 Jun;133(6):1644-1650.e4.

152. Mukhopadhyay S, Mohanty M, Mangla A, George A, Bal V, Rath S, et al. Macrophage effector functions controlled by Bruton's tyrosine kinase are more crucial than the cytokine balance of T cell responses for microfilarial clearance. J Immunol. 2002 Mar 15;168(6):2914–21.

153. Schmidt NW, Thieu VT, Mann BA, Ahyi A-NN, Kaplan MH. Bruton's tyrosine kinase is required for TLR-induced IL-10 production. J Immunol. 2006 Nov 15;177(10):7203–10.

154. Hasan M, Lopez-Herrera G, Blomberg KEM, Lindvall JM, Berglöf A, Smith CIE, et al. Defective Toll-like receptor 9-mediated cytokine production in B cells from Bruton's tyrosine kinase-deficient mice. Immunology. 2008 Aug 28;123(2):239–49.

155. Broderick L, De Nardo D, Franklin BS, Hoffman HM, Latz E. The Inflammasomes and Autoinflammatory Syndromes. Annu Rev Pathol Mech Dis. 2015 Jan 24;10(1):395–424.

156. Dubois H, Wullaert A, Lamkanfi M. General strategies in inflammasome biology. In: Inflammasome Signaling and Bacterial Infections. Springer, Cham; 2016. p. 1–22.

157. de Jesus AA, Canna SW, Liu Y, Goldbach-Mansky R. Molecular Mechanisms in Genetically Defined Autoinflammatory Diseases: Disorders of Amplified Danger Signaling. Annu Rev Immunol. 2015 Mar 21;33(1):823–74.

158. Ito M, Shichita T, Okada M, Komine R, Noguchi Y, Yoshimura A, et al. Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. Nat Commun. 2015 Dec 10;6(1):7360.

159. Yang Y, Wang H, Kouadir M, Song H, Shi F. Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis. 2019 Feb 12;10(2):128.

160. Bousfiha AA, Jeddane L, Ailal F, Al Herz W, Conley ME, Cunningham-Rundles C, et al. A phenotypic approach for IUIS PID classification and diagnosis: Guidelines for clinicians at the bedside. J Clin Immunol. 2013;33(6):1078–87.

161. Ng Y-S, Wardemann H, Chelnis J, Cunningham-Rundles C, Meffre E. Bruton's Tyrosine Kinase Is Essential for Human B Cell Tolerance. J Exp Med. 2004 Oct 4;200(7):927–34.

162. Wang J, Lau K-Y, Jung J, Ravindran P, Barrat FJ. Bruton's tyrosine kinase regulates TLR9 but not TLR7 signaling in human plasmacytoid dendritic cells. Eur J Immunol. 2014 Apr;44(4):1130–6.

163. Jansson L, Holmdahl R. Genes on the X chromosome affect development of collagen-induced arthritis in mice. Clin Exp Immunol. 1993 Dec;94(3):459–65.

164. Crofford LJ, Nyhoff LE, Sheehan JH, Kendall PL. The role of Bruton's tyrosine kinase in autoimmunity and implications for therapy. Expert Rev Clin Immunol. 2016 Jul 2;12(7):763–73.

165. Conley ME, Howard VC. X-Linked Agammaglobulinemia. GeneReviews(®). 2001. 1–13 p.

166. Ochs H, Winkelstein J. Disorders of the B-cell system. In: Stiehm E, editor. Immunoligic disorders in infants and children. 4th ed. Philadelphia: The W. B. Saunders Co.; 1996. p. 296–338.

167. Lederman HM, Winkelstein J a. X-linked agammaglobulinemia: an analysis of 96 patients. Medicine (Baltimore). 1985;64(3):145–56.

168. Hermaszewski R, Webster A. Primary hypogammaglobulinaemia: a survey of clinical manifestations and complications. Q J Med. 1993;86(1):31–42.

169. LoGalbo PR, Sampson HA, Buckley RH. Symptomatic giardiasis in three patients with X-linked agammaglobulinemia. J Pediatr. 1982 Jul;101(1):78–80.

170. Roifman CM, Rao CP, Lederman HM, Lavi S, Quinn P, Gelfand EW. Increased susceptibility to Mycoplasma infection in patients with hypogammaglobulinemia. Am J Med. 1986 Apr;80(4):590–4.

171. Furr PM, Taylor-Robinson D, Webster AD. Mycoplasmas and ureaplasmas in patients with hypogammaglobulinaemia and their role in arthritis: microbiological observations over twenty years. Ann Rheum Dis. 1994 Mar;53(3):183–7.

172. Linnemann Jr. CC, May DB, Schubert WK, Caraway CT, Schiff GM. Fatal viral encephalitis in children with X-linked hypogammaglobulinemia. Am J Dis Child. 1973 Jul;126(1):100–3.

173. Wyatt H V. Poliomyelitis in hypogammaglobulinemics. J Infect Dis. 1973 Dec;128(6):802–6.

174. Wilfert CM, Buckley RH, Mohanakumar T, Griffith JF, Katz SL, Whisnant JK, et al.

Persistent and Fatal Central-Nervous-System ECHOvirus Infections in Patients with Agammaglobulinemia. N Engl J Med. 1977 Jun 30;296(26):1485–9.

175. Bardelas JA, Winkelstein JA, Seto DSY, Tsai T, Rogol AD. Fatal ECHO 24 infection in a patient with hypogammaglobulinemia: Relationship to dermatomyositis-like syndrome. J Pediatr. 1977 Mar;90(3):396–9.

176. McKinney RE, Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. Rev Infect Dis. 1987;9(2):334–56.

177. Bearden D, Collett M, Quan PL, Costa-Carvalho BT, Sullivan KE. Enteroviruses in X-Linked Agammaglobulinemia: Update on Epidemiology and Therapy. J Allergy Clin Immunol Pract. 2016 Nov 1;4(6):1059–65.

178. Rahmani F, Aghamohammadi A, Ochs HD, Rezaei N. Agammaglobulinemia: comorbidities and long-term therapeutic risks. Expert Opin Orphan Drugs. 2017 Jul 3;5(7):559–74.

179. Halliday E, Winkelstein J, Webster ADB. Enteroviral infections in primary immunodeficiency (PID): A survey of morbidity and mortality. J Infect. 2003 Jan;46(1):1–8.

180. Ziegler JB, Penny R. Fatal Echo 30 virus infection and amyloidosis in X-linked hypogammaglobulinemia. Clin Immunol Immunopathol. 1975 Jan;3(3):347–52.

181. Lucas M, Lee M, Lortan J, Lopez-Granados E, Misbah S, Chapel H. Infection outcomes in patients with common variable immunodeficiency disorders: relationship to immunoglobulin therapy over 22 years. J Allergy Clin Immunol. 2010;125(6):1354-1360.e4.

182. Galama JMD, Vogels MTE, Jansen GH, Gielen M, Heesen FWA. Antibodies against enteroviruses in intravenous Ig preparations: Great variation in titres and poor correlation with the incidence of circulating serotypes. J Med Virol. 1997 Nov;53(3):273–6.

183. Galama JMD, Gielen M, Weemaes CMR. Enterovirus antibody titers after IVIG replacement in agammaglobulinemic children. Clin Microbiol Infect. 2000 Nov;6(11):630–2.

184. Kainulainen L, Vuorinen T, Rantakokko-Jalava K, Österback R, Ruuskanen O, Osterback R. Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia. J Allergy Clin Immunol. 2010 Jul 1;126(1):120–6.

185. Kempf B, Edgar JD, Mc Caughey C, Devlin LA. Nitazoxanide Is an Ineffective Treatment of Chronic Norovirus in Patients With X-Linked Agammaglobulinemia and May Yield False-Negative Polymerase Chain Reaction Findings in Stool Specimens. J Infect Dis. 2017 Feb 1;215(3):486–7.

186. Plebani A, Soresina A, Rondelli R, Amato GM, Azzari C, Cardinale F, et al. Clinical, immunological, and molecular analysis in a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. Clin Immunol. 2002;104(3):221–30.

187. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. J Pediatr. 2002 Oct;141(4):566–71.

188. Kanegane H, Taneichi H, Nomura K, Futatani T, Miyawaki T. Severe neutropenia in Japanese patients with X-linked agammaglobulinemia. J Clin Immunol. 2005;25(5):491–5.

189. Aghamohammadi A, Cheraghi T, Rezaei N, Kanegane H, Abdollahzede S, Talaei-Khoei M, et al. Neutropenia Associated with X-Linked Agammaglobulinemia in an Iranian Referral Center. Iran J Allergy, Asthma Immunol. 2009 Mar;8(March):43–7.

190. Muñoz-Miguelsanz M, Álvarez Morales T, Martín García J, Martínez Gallo M, Santos Pérez J. Pseudomonas aeruginosa Liver Abscess as the First Manifestation of X-Linked Agammaglobulinemia With a Novel Mutation. J Investig Allergol Clin Immunol. 2017 Apr 10;27(2):129–31.

191. Abolhassani H, Hirbod-Mobarakeh A, Shahinpour S, Panahi M, Mohammadinejad P, Mirminachi B, et al. Mortality and morbidity in patients with X-linked agammaglobulinaemia. Allergol Immunopathol (Madr). 2015;43(1):62–6.

192. Mamishi S, Shahmahmoudi S, Tabatabaie H, Teimourian S, Pourakbari B, Gheisari Y, et al. Novel BTK mutation presenting with vaccine-associated paralytic poliomyelitis. Eur J Pediatr. 2008 Nov 4;167(11):1335–8.

193. Gofshteyn J, Cárdenas AM, Bearden D. Treatment of chronic enterovirus encephalitis

with fluoxetine in a patient with X-linked agammaglobulinemia. Pediatr Neurol. 2016;64:94–8.

194. Thomas J, Sideras P, Smith C, Vorechovsky I, Chapman V, Paul W. Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. Science (80-). 1993 Jul 16;261(5119):355–8.

195. Vihinen M, Brandau O, Brandén LJ, Kwan SP, Lappalainen I, Lester T, et al. BTKbase, mutation database for X-linked agammaglobulinemia (XLA). Nucleic Acids Res. 1998 Jan 1;26(1):242–7.

196. Hendriks RW, de Bruijn MF, Maas A, Dingjan GM, Karis A, Grosveld F. Inactivation of Btk by insertion of lacZ reveals defects in B cell development only past the pre-B cell stage. EMBO J. 1996 Sep 16;15(18):4862–72.

197. Cohn EJ, Oncley JL, Strong LE, Hughes WL, Armstrong SH. Chemical, Clinical, and Immunological Studies on the Products of Human Plasma Fractionation. I. The Characterization of the Protein Fractions of Human Plasma. J Clin Invest. 1944 Jul 1;23(4):417–32.

198. Barandun S, Kistler P, Jeunet F, Isliker H. Intravenous administration of human gamma-globulin. Vox Sang. 1962;7:157–74.

199. Abolhassani H, Asgardoon MH, Rezaei N, Hammarstrom L, Aghamohammadi A. Different brands of intravenous immunoglobulin for primary immunodeficiencies: how to choose the best option for the patient? Expert Rev Clin Immunol. 2015 Nov 2;11(11):1229–43.

200. Cunningham-Rundles C. Immunoglobulin Replacement Therapy. In: Immunodeficiency and Disease. Dordrecht: Springer Netherlands; 1988. p. 43–60.

201. Pirofsky B, Campbell SM, Montanaro A. Individual patient variations in the kinetics of intravenous immune globulin administration. J Clin Immunol. 1982 Apr;2(2 Suppl):7S-14S.

202. Garbett ND, Currie DC, Cole PJ. Comparison of the clinical efficacy and safety of an intramuscular and an intravenous immunoglobulin preparation for replacement therapy in idiopathic adult onset panhypogammaglobulinaemia. Clin Exp Immunol. 1989 Apr;76(1):1–7.

203. Bjoro K, Froland SS, Yun Z, Samdal HH, Haaland T. Hepatitis C Infection in Patients with Primary Hypogammaglobulinemia after Treatment with Contaminated Immune Globulin. N Engl J Med. 1994 Dec 15;331(24):1607–11.

204. Sriaroon P, Ballow M. Immunoglobulin Replacement Therapy for Primary Immunodeficiency. Immunol Allergy Clin North Am. 2015 Nov;35(4):713–30.

205. Healey C, Chapel H. Intravenous immunoglobulin and hepatitis C virus: the British episode. Clin Ther. 2016 Feb 8;18:93–5.

206. Biesert L. Virus validation studies of immunoglobulin preparations. Clin Exp Rheumatol. 1996;14 Suppl 1:S47-52.

207. Kerr J, Quinti I, Eibl M, Chapel H, Späth PJ, Sewell WAC, et al. Is dosing of therapeutic immunoglobulins optimal? A review of a three-decade long debate in Europe. Front Immunol. 2014 Dec 12;5:629.

208. Smith GN, Griffiths B, Mollison D, Mollison PL. Uptake of IgG after intramuscular and subcutaneous injection. Lancet (London, England). 1972 Jun 3;1(7762):1208–12.

209. Gardulf A, Hammarström L, Smith CI. Home treatment of hypogammaglobulinaemia with subcutaneous gammaglobulin by rapid infusion. Lancet (London, England). 1991 Jul 20;338(8760):162–6.

210. Ochs HD, Fischer SH, Lee ML, Delson ES, Kingdon HS, Wedgwood RJ. Intravenous immunoglobulin home treatment for patients with primary immunodeficiency diseases. Lancet (London, England). 1986 Mar 15;1(8481):610–1.

211. Jolles S. Hyaluronidase facilitated subcutaneous immunoglobulin in primary immunodeficiency. ImmunoTargets Ther. 2013 Sep;2:125.

212. Quartier P, Debré M, De Blic J, de Sauverzac R, Sayegh N, Jabado N, et al. Early and prolonged intravenous immunoglobulin replacement therapy in childhood agammaglobulinemia: a retrospective survey of 31 patients. J Pediatr. 1999;134(5):589–96.

213. de Gracia J, Vendrell M, Alvarez A, Pallisa E, Rodrigo M-J, de la Rosa D, et al. Immunoglobulin therapy to control lung damage in patients with common variable immunodeficiency. Int Immunopharmacol. 2004;4(6):745–53.

214. Eijkhout HW, van Der Meer JW, Kallenberg CG, Weening RS, van Dissel JT, Sanders LA, et al. The effect of two different dosages of intravenous immunoglobulin on the incidence of recurrent infections in patients with primary hypogammaglobulinemia. A randomized, double-blind, multicenter crossover trial. Ann Intern Med. 2001 Aug 7;135(3):165–74.

215. Chen Y, Stirling RG, Paul E, Hore-Lacy F, Thompson BR, Douglass J a. Longitudinal decline in lung function in patients with primary immunoglobulin deficiencies. J Allergy Clin Immunol. 2011;127(6):1414–7.

216. Ghurye R, Hodkinson J, Longhurst H. The Use Of Intravenous Pentaglobin In A Subset Of Patients With Severe Mucosal Complications Related To Primary Immune Deficiency. In: UKPIN 2015 Meeting. Belfast; 2015.

217. Kiani-Alikhan S, Yong PFK, Grosse-Kreul D, Elston C, Ibrahim MAA. Immunoglobulin replacement therapy: Is there a role for IgA and IgM? J Allergy Clin Immunol. 2012 Aug;130(2):553–4.

218. Maarschalk-Ellerbroek LJ, Hoepelman IM, Ellerbroek PM. Immunoglobulin treatment in primary antibody deficiency. Vol. 37, International Journal of Antimicrobial Agents. Elsevier B.V.; 2011. p. 396–404.

219. Kerr M a. The structure and function of human IgA. Hum cell Off J Hum Cell Res Soc. 1990;271(2):285–96.

220. Baumann U, Miescher S, Vonarburg C. Immunoglobulin replacement therapy in antibody deficiency syndromes: are we really doing enough? Clin Exp Immunol. 2014;178:83–5.

221. Quinti I, Soresina A, Spadaro G, Martino S, Donnanno S, Agostini C, et al. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. J Clin Immunol. 2007 May 17;27(3):308–16.

222. Quinti I, Soresina A, Guerra A, Rondelli R, Spadaro G, Agostini C, et al. Effectiveness of Immunoglobulin Replacement Therapy on Clinical Outcome in Patients with Primary Antibody Deficiencies: Results from a Multicenter Prospective Cohort Study. J Clin Immunol. 2011 Jun 2;31(3):315–22.

223. Razvi S, Schneider L, Jonas MM, Cunningham-Rundles C. Outcome of intravenous

immunoglobulin-transmitted hepatitis C virus infection in primary immunodeficiency. Clin Immunol. 2001 Dec;101(3):284–8.

224. Pappenheim K. UK inquiry should establish why contaminated blood products were given to people with haemophilia. BMJ. 1999 Jul 3;319(7201):52.

225. The National CJD Research & Surveillance Unit. Creutzfeldt-Jakob Disease Surveillance in the UK - 25th Annual Report. 2016.

226. Carne E, Ponsford M, El-Shanawany T, Jolles S. Skin Necrosis Following Subcutaneous Immunoglobulin (SCIg). J Clin Immunol. 2017;37(1):27–8.

227. Takeguchi M, Korematsu S, Miyahara H, Kuga S, Izumi T. IVIG-triggered tubulointerstitial nephritis in X-linked agammaglobulinemia. Pediatr Int. 2017 Aug 1;59(8):945–6.

228. Yoshino A, Honda M, Kanegane H, Obata K, Matsukura H, Sakazume S, et al. Membranoproliferative glomerulonephritis in a patient with X-linked agammaglobulinemia. Pediatr Nephrol. 2006 Jan 11;21(1):36–8.

229. Endo LM, Giannobile J V, Dobbs AK, Foote JB, Szymanska E, Warnock DG, et al. Membranous glomerulopathy in an adult patient with X-linked agammaglobulinemia receiving intravenous gammaglobulin. J Investig Allergol Clin Immunol. 2011;21(5):405–9.

230. Sugimoto K, Nishi H, Miyazawa T, Wada N, Izu A, Enya T, et al. Tubulointerstitial nephritis complicating IVIG therapy for X-linked agammaglobulinemia. BMC Nephrol. 2014 Jul 8;15(1):109.

231. Howard V, Myers L a., Williams D a., Wheeler G, Turner EV, Cunningham JM, et al. Stem cell transplants for patients with X-linked agammaglobulinemia. Clin Immunol. 2003;107(2):98–102.

232. Abu-Arja RF, Chernin LR, Abusin G, Auletta J, Cabral L, Egler R, et al. Successful Hematopoietic Cell Transplantation in a Patient With X-Linked Agammaglobulinemia and Acute Myeloid Leukemia. Pediatr Blood Cancer. 2015 Sep;62(9):1674–6.

233. Ikegame K, Imai K, Yamashita M, Hoshino A, Kanegane H, Morio T, et al. Allogeneic stem cell transplantation for X-linked agammaglobulinemia using reduced intensity

conditioning as a model of the reconstitution of humoral immunity. J Hematol Oncol. 2016 Feb 13;9(1):9.

234. Gennery AR, Slatter M a, Grandin L, Taupin P, Cant AJ, Veys P, et al. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? J Allergy Clin Immunol. 2010;126(3):602-610.e1-e11.

235. Borte S, von Dobeln U, Fasth A, Wang N, Janzi M, Winiarski J, et al. Neonatal screening for severe primary immunodeficiency diseases using high-throughput triplex real-time PCR. Blood. 2012 Mar 15;119(11):2552–5.

236. Chan K, Puck JM. Development of population-based newborn screening for severe combined immunodeficiency. J Allergy Clin Immunol. 2005 Feb;115(2):391–8.

237. Siminovitch KA, Bakhshi A, Goldman P, Korsmeyer SJ. A uniform deleting element mediates the loss of kappa genes in human B cells. Nature. 1985 Jul;316(6025):260–2.

238. Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K, et al. Quantification of k-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects. J Allergy Clin Immunol. 2011 Dec 15;128(1):223–5.

239. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest. 2008 Sep 2;118(9):3132–42.

240. Howe SJ, Mansour MR, Schwarzwaelder K, Hubank M, Kempski H, Brugman MH, et al. Insertional mutagenesis in combination with acquired somatic mutations leads to leukemogenesis following gene therapy of SCID-X1. J Clin. 2008;118(9):3143–50.

241. Ferrua F, Brigida I, Aiuti A. Update on gene therapy for adenosine deaminase-deficient severe combined immunodeficiency. Curr Opin Allergy Clin Immunol. 2010 Dec;10(6):551–6.

242. NICE. Strimvelis for treating adenosine deaminase deficiency–severe combined immunodeficiency [Internet]. NICE; 2018 [cited 2018 Oct 27]. Available from: https://www.nice.org.uk/guidance/hst7

243. Rivat C, Santilli G, Gaspar HB, Thrasher AJ. Gene therapy for primary immunodeficiencies. Hum Gene Ther. 2012;23(7):668–75.

244. De Ravin SS, Li L, Wu X, Choi U, Allen C, Koontz S, et al. CRISPR-Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease. Sci Transl Med. 2017;9(372).

245. Chang CW, Lai YS, Westin E, Khodadadi-Jamayran A, Pawlik KM, Lamb LS, et al. Modeling Human Severe Combined Immunodeficiency and Correction by CRISPR/Cas9-Enhanced Gene Targeting. Cell Rep. 2015;12(10):1668–77.

246. Hill LE. Hypogammaglobulinaemia in the United Kingdom. 3. Clinical features of hypogammaglobulinaemia. Spec Rep Ser Med Res Counc (G B). 1971;310:9–34.

247. Shillitoe B, Bangs C, Guzman D, Gennery ARR, Longhurst HJJ, Slatter M, et al. The United Kingdom Primary Immune Deficiency (UKPID) registry 2012 to 2017. Clin Exp Immunol. 2018 Jun;192(3):284–91.

248. Basile N, Danielian S, Oleastro M, Rosenzweig S, Prieto E, Rossi J, et al. Clinical and molecular analysis of 49 patients with X-linked agammaglobulinemia from a single center in Argentina. J Clin Immunol. 2009;29(1):123–9.

249. Zaidi SK, Qureshi S, Qamar FN. X-linked agammaglobulinemia — first case with Bruton tyrosine kinase mutation from Pakistan. J Pak Med Assoc. 2017;67(3):471–3.

250. Singh S, Rawat A, Suri D, Gupta A, Garg R, Saikia B, et al. X-linked agammaglobulinemia: Twenty years of single-center experience from North West India. Ann Allergy, Asthma Immunol. 2016 Oct;117(4):405–11.

251. Moin M, Aghamohammadi A, Farhoudi A, Pourpak Z, Rezaei N, Movahedi M, et al. X-linked agammaglobulinemia: a survey of 33 Iranian patients. Immunol Invest. 2004;33(1):81–93.

252. Jeffrey Modell Foundation. 10 Warning Signs - JMF [Internet]. [cited 2020 Jan 26]. Available from: http://www.info4pi.org/library/educational-materials/10-warning-signs

253. Arkwright PD, Gennery AR. Ten warning signs of primary immunodeficiency: A new paradigm is needed for the 21st century. Ann N Y Acad Sci. 2011;1238(1):7–14.

254. Monto AS, Napier JA, Metzner HL. The Tecumseh study of respiratiry illness. Am J Epidemiol. 1971 Sep 1;94(3):269–79.

255. Fleming DW, Cochi SL, Hightower AW, Broome C V. Childhood upper respiratory tract infections: to what degree is incidence affected by day-care attendance? Pediatrics. 1987 Jan;79(1):55–60.

256. Chan H-Y, Yang Y-H, Yu H-H, Chien Y-H, Chiang L-L, Chiang B-L. Clinical characteristics and outcomes of primary antibody deficiency: A 20-year follow-up study. J Formos Med Assoc. 2012;113(6):340–8.

257. Mohammadinejad P, Pourhamdi S, Abolhassani H, Mirminachi B, Havaei A, Masoom SN, et al. Primary antibody deficiency in a tertiary referral hospital: A 30-year experiment. J Investig Allergol Clin Immunol. 2015;25(6):416–25.

258. Howard V, Greene JM, Pahwa S, Winkelstein J a., Boyle JM, Kocak M, et al. The health status and quality of life of adults with X-linked agammaglobulinemia. Clin Immunol. 2006;118(2–3):201–8.

259. Aghamohammadi A, Moin M, Farhoudi A, Rezaei N, Pourpak Z, Movahedi M, et al. Efficacy of intravenous immunoglobulin on the prevention of pneumonia in patients with agammaglobulinemia. FEMS Immunol Med Microbiol. 2004;40(2):113–8.

260. Stubbs A, Bangs C, Shillitoe B, Edgar JD, Burns SO, Thomas M, et al. Bronchiectasis and deteriorating lung function in agammaglobulinaemia despite immunoglobulin replacement therapy. Clin Exp Immunol. 2018 Feb 3;191(2):212–9.

261. Pac MM, Bernatowska EA, Kierkuś J, Ryżko JP, Cielecka-Kuszyk J, Jackowska T, et al. Gastrointestinal disorders next to respiratory infections as leading symptoms of X-linked agammaglobulinemia in children – 34-year experience of a single center. Arch Med Sci. 2017;2:412–7.

262. Winkelstein J a., Conley ME, James C, Howard V, Boyle JM. Status of Adults with X-Linked Agammaglobulinemia. Med. 2010;9(2):1–14.

263. Kim JY, Park SY, Lee JM, Kim YK, Kim SY. Intracranial abscess as a complication of X-linked agammaglobulinemia. Child's Nerv Syst. 2016;32(11):2049–51.

264. Buehring I, Friedrich B, Schaaf J, Schmidt H, Ahrens P, Zielen S. Chronic sinusitis

refractory to standard management in patients with humoral immunodeficiencies. Clin Exp Immunol. 1997;109(3):468–72.

265. Mazza JM, Lin SY. Primary immunodeficiency and recalcitrant chronic sinusitis: a systematic review. Int Forum Allergy Rhinol. 2016 Oct 1;6(10):1029–33.

266. Guilemany JM, Angrill J, Alobid I, Centellas S, Pujols L, Bartra J, et al. United airways again: High prevalence of rhinosinusitis and nasal polyps in bronchiectasis. Allergy Eur J Allergy Clin Immunol. 2009 May;64(5):790–7.

267. Rusconi F, Panisi C, Dellepiane RM, Cardinale F, Chini L, Martire B, et al. Pulmonary and sinus diseases in primary humoral immunodeficiencies with chronic productive cough. Arch Dis Child. 2003;88(12):1101–5.

268. Bryan BA, Battersby A, Shillitoe BMJ, Barge D, Bourne H, Flood T, et al. Respiratory Health and Related Quality of Life in Patients with Congenital Agammaglobulinemia in the Northern Region of the UK. J Clin Immunol. 2016 Jul 18;36(5):472–9.

269. Sperlich JM, Grimbacher B, Workman S, Haque T, Seneviratne SL, Burns SO, et al. Respiratory Infections and Antibiotic Usage in Common Variable Immunodeficiency. J Allergy Clin Immunol Pract. 2018 Jan 1;6(1):159-168.e3.

270. Hurst JR, Workman S, Garcha DS, Seneviratne SL, Haddock J a., Grimbacher B. Activity, severity and impact of respiratory disease in primary antibody deficiency syndromes. J Clin Immunol. 2014;34(1):68–75.

271. Sweinberg SK, Wodell RA, Grodofsky MP, Greene JM, Conley ME. Retrospective analysis of the incidence of pulmonary disease in hypogammaglobulinemia. J Allergy Clin Immunol. 1991 Jul;88(1):96–104.

272. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012 Feb 16;119(7):1650–7.

273. Morales P, Hernández D, Vicente R, Solé A, Moreno I, Torres J., et al. Lung transplantation in patients with x-linked agammaglobulinemia. Transplant Proc. 2003;35(5):1942–3.

274. Barnes S, Kotecha S, Douglass JA, Paul E, Hore-Lacey F, Stirling R, et al. Evolving

practice: X-linked agammaglobulinemia and lung transplantation. Am J Transplant. 2015 Apr 1;15(4):1110–3.

275. Pasteur MC, Bilton D, Hill a. T. British Thoracic Society guideline for non-CF bronchiectasis. Thorax. 2010 Jul 1;65(Suppl 1):i1–58.

276. King PT, Holdsworth SR, Freezer NJ, Villanueva E, Gallagher M, Holmes PW. Outcome in adult bronchiectasis. COPD. 2005 Mar;2(1):27–34.

277. Martínez-García MA, Soler-Cataluña J-J, Perpiñá-Tordera M, Román-Sánchez P, Soriano J. Factors associated with lung function decline in adult patients with stable non-cystic fibrosis bronchiectasis. Chest. 2007;132(5):1565–72.

278. Goeminne PC, Scheers H, Decraene A, Seys S, Dupont LJ. Risk factors for morbidity and death in non-cystic fibrosis bronchiectasis: a retrospective cross-sectional analysis of CT diagnosed bronchiectatic patients. Respir Res. 2012;13(1):21.

279. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A Self-complete Measure of Health Status for Chronic Airflow Limitation: The St. George's Respiratory Questionnaire. Am Rev Respir Dis. 1992 Jun;145(6):1321–7.

280. Masekela R, Green RJ. The Role of Macrolides in Childhood Non-Cystic Fibrosis-Related Bronchiectasis. Mediators Inflamm. 2012 Apr 18;2012:1–7.

281. Chalmers JD, Aliberti S, Blasi F. Management of bronchiectasis in adults. Eur Respir J. 2015 May;45(5):1446–62.

282. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92(1):34–48.

283. Jolles S. The variable in common variable immunodeficiency: A disease of complex phenotypes. J Allergy Clin Immunol Pract. 2013 Nov;1(6):545–56.

284. Jolles S. Subclinical infection and dosing in primary immunodeficiencies. Clin Exp Immunol. 2014 Dec;178(S1):67–9.

285. Reynolds H. Immunoglobulin G and Its Function in the Human Respiratory Tract. Mayo Clin Proc. 1988 Feb 1;63(2):161–74.

286. Stubbs A, Bangs C, Shillitoe B, Edgar JD, Burns SO, Thomas M, et al. Bronchiectasis

and deteriorating lung function in agammaglobulinaemia despite immunoglobulin replacement therapy. Clin Exp Immunol. 2017 Nov 3;

287. Wood P. Primary antibody deficiencies: Recognition, clinical diagnosis and referral of patients. Clin Med (Northfield Il). 2009;9(6):595–9.

288. Stanojevic S, Quanjer P, Miller MR, Stocks J. The Global Lung Function Initiative: dispelling some myths of lung function test interpretation. Breathe. 2013 Dec;9(6):462–74.

289. Kainulainen L, Nikoskelainen J, Vuorinen T, Tevola K, Liippo K, Ruuskanen O. Viruses and bacteria in bronchial samples from patients with primary hypogammaglobulinemia. Am J Respir Crit Care Med. 1999 Apr;159(4 I):1199–204.

290. Kainulainen L, Suonpää J, Nikoskelainen J, Svedström E, Vuorinen T, Meurman O, et al. Bacteria and viruses in maxillary sinuses of patients with primary hypogammaglobulinemia. Arch Otolaryngol Head Neck Surg. 2007 Jun 1;133(6):597–602.

291. Duraisingham SS, Manson A, Grigoriadou S, Buckland M, Tong CYW, Longhurst HJ. Immune deficiency: Changing spectrum of pathogens. Clin Exp Immunol. 2015 Aug;181(2):267–74.

292. Janssen WJ, Mohamed Hoesein F, Van de Ven AA, Maarschalk J, van Royen F, de Jong PA, et al. IgG trough levels and progression of pulmonary disease in pediatric and adult common variable immunodeficiency disorder patients. J Allergy Clin Immunol. 2017 Jan;140(1):303-306.e4.

293. Journy NMY, Lee C, Harbron RW, McHugh K, Pearce MS, Berrington de González A. Projected cancer risks potentially related to past, current, and future practices in paediatric CT in the United Kingdom, 1990-2020. Br J Cancer. 2017;116(1):109–16.

294. Serra G, Milito C, Mitrevski M, Granata G, Martini H, Pesce AM, et al. Lung MRI as a possible alternative to CT scan for patients with primary immune deficiencies and increased radiosensitivity. Chest. 2011;140(6):1581–9.

295. Guillerman RP. Imaging of Childhood Interstitial Lung Disease. Pediatr Allergy Immunol Pulmonol. 2010 Mar 5;23(1):43–68.

296. Odegard KC, DiNardo JA, Tsai-Goodman B, Powell AJ, Geva T, Laussen PC. Anaesthesia considerations for cardiac MRI in infants and small children. Pediatr Anesth. 2004 Jun;14(6):471–6.

297. Verma N, Grimbacher B, Hurst JR. Review Lung disease in primary antibody deficiency. Lancet Respir. 2015;3(8):651–60.

298. Seed L, Wilson D, Coates AL. Children Should Not Be Treated Like Little Adults in the PFT Lab. Respir Care. 2012 Jan 1;57(1):61–74.

299. Jolles S, Sánchez-Ramón S, Quinti I, Soler-Palacín P, Agostini C, Florkin B, et al. Screening protocols to monitor respiratory status in primary immunodeficiency disease: findings from a European survey and subclinical infection working group. Clin Exp Immunol. 2017 Nov 14;190(2):226–34.

300. Cunningham-Rundles C. How I treat common variable immune deficiency. Blood. 2010 Jul 8;116(1):7–15.

301. Maarschalk-Ellerbroek LJ, de Jong PA, van Montfrans JM, Lammers JWJ, Bloem AC, Hoepelman AIM, et al. CT Screening for Pulmonary Pathology in Common Variable Immunodeficiency Disorders and the Correlation with Clinical and Immunological Parameters. J Clin Immunol. 2014 Aug 21;34(6):642–54.

302. Kuo W, Ciet P, Tiddens HAWM, Zhang W, Guillerman RP, van Straten M. Monitoring Cystic Fibrosis Lung Disease by Computed Tomography. Radiation Risk in Perspective. Am J Respir Crit Care Med. 2014 Jun 1;189(11):1328–36.

303. Jesenak M, Banovcin P, Jesenakova B, Babusikova E. Pulmonary manifestations of primary immunodeficiency disorders in children. Front Pediatr. 2014 Jul 25;2(July):77.

304. Brusselle GG, Van Braeckel E. Sputum Neutrophil Elastase as a Biomarker for Disease Activity in Bronchiectasis. Am J Respir Crit Care Med. 2017 May 15;195(10):1289–91.

305. Olveira G, Olveira C, Dorado A, García-Fuentes E, Rubio E, Tinahones F, et al. Cellular and plasma oxidative stress biomarkers are raised in adults with bronchiectasis. Clin Nutr. 2013 Feb 1;32(1):112–7.

306. Quinti I, Pulvirenti F, Giannantoni P, Hajjar J, Canter DL, Milito C, et al. Development

and Initial Validation of a Questionnaire to Measure Health-Related Quality of Life of Adults with Common Variable Immune Deficiency: The CVID_QoL Questionnaire. J Allergy Clin Immunol Pract. 2016 Nov;4(6):1169-1179.e4.

307. Andersen JB, Midttun K, Feragen KJB. Measuring quality of life of primary antibody deficiency patients using a disease-specific health-related quality of life questionnaire for common variable immunodeficiency (CVID_QoL). J Patient-Reported Outcomes. 2019 Dec 26;3(1):15.

308. Jones PW, Quirk FH, Baveystock CM. The St George's Respiratory Questionnaire. Respir Med. 1991 Sep;85:25–31.

309. Chalmers JD, Goeminne P, Aliberti S, McDonnell MJ, Lonni S, Davidson J, et al. The bronchiectasis severity index an international derivation and validation study. Am J Respir Crit Care Med. 2014 Mar 1;189(5):576–85.

310. Gregersen S, Aaløkken TM, Mynarek G, Fevang B, Holm AM, Ueland T, et al. Development of pulmonary abnormalities in patients with common variable immunodeficiency: associations with clinical and immunologic factors. Ann Allergy, Asthma Immunol. 2010 Jun;104(6):503–10.

311. Rich AL, Le Jeune IR, McDermott L, Kinnear WJM. Serial lung function tests in primary immune deficiency. Clin Exp Immunol. 2008;151(1):110–3.

312. Bonilla FA, Bernstein IL, Khan DA, Ballas ZK, Chinen J, Frank MM, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. Ann allergy, asthma Immunol. 2005 May;94(5 Suppl 1):S1-63.

313. Welsh EJ, Evans DJ, Fowler SJ, Spencer S. Interventions for bronchiectasis: an overview of Cochrane systematic reviews. Cochrane Database Syst Rev. 2015;(7).

314. Altenburg J, de Graaff CS, Stienstra Y, Sloos JH, van Haren EHJ, Koppers RJH, et al. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. Jama. 2013;309(12):1251–9.

315. O'Donnell AE. Bronchiectasis. Chest. 2008;134(4):815–23.

316. Garrod R, Lasserson T. Role of physiotherapy in the management of chronic lung

diseases: An overview of systematic reviews. Respir Med. 2007;101(12):2429–36.

317. Wilkinson M, Sugumar K, Milan SJ, Hart A, Crockett A, Crossingham I. Mucolytics for bronchiectasis. Cochrane Database Syst Rev. 2014 May 2;2014(5).

318. Bott J, Blumenthal S, Buxton M, Ellum S, Falconer C, Garrod R, et al. Guidelines for the physiotherapy management of the adult, medical, spontaneously breathing patient. Thorax. 2009;64(Suppl 1):i1–52.

319. Milito C, Pulvirenti F, Cinetto F, Lougaris V, Soresina A, Pecoraro A, et al. Double-blind, placebo-controlled, randomized trial on low-dose azithromycin prophylaxis in patients with primary antibody deficiencies. J Allergy Clin Immunol. 2019 Aug;144(2):584-593.e7.

320. Fan L-C, Lu H-W, Wei P, Ji X-B, Liang S, Xu J-F. Effects of long-term use of macrolides in patients with non-cystic fibrosis bronchiectasis: a meta-analysis of randomized controlled trials. BMC Infect Dis. 2015 Dec 27;15(1):160.

321. Kainulainen L, Varpula M, Liippo K, Svedström E, Nikoskelainen J, Ruuskanen O. Pulmonary abnormalities in patients with primary hypogammaglobulinemia. J Allergy Clin Immunol. 1999;104(5):1031–6.

322. Oksenhendler E, Gérard L, Fieschi C, Malphettes M, Mouillot G, Jaussaud R, et al. Infections in 252 Patients with Common Variable Immunodeficiency. Clin Infect Dis. 2008 May 15;46(10):1547–54.

323. Arkwright PD. Autoimmunity in human primary immunodeficiency diseases. Blood. 2002 Apr 15;99(8):2694–702.

324. Hernandez-Trujillo VP, Scalchunes C, Cunningham-Rundles C, Ochs HD, Bonilla F a., Paris K, et al. Autoimmunity and Inflammation in X-linked Agammaglobulinemia. J Clin Immunol. 2014;34(6):627–32.

325. Ramírez AS, Rosas A, Hernández-Beriain JA, Orengo JC, Saavedra P, de la Fe C, et al. Relationship between rheumatoid arthritis and Mycoplasma pneumoniae: A case-control study. Rheumatology. 2005 Jul 1;44(7):912–4.

326. Barmettler S, Otani IM, Minhas J, Abraham RS, Chang Y, Dorsey MJ, et al. Gastrointestinal Manifestations in X-linked Agammaglobulinemia. J Clin Immunol.

2017 Apr 24;37(3):287–94.

327. Shah S, Terdiman J, Gundling K, Mahadevan U. Immunoglobulin therapy for refractory Crohn's disease. Therap Adv Gastroenterol. 2013 Mar;7(2):99–102.

328. Rogosnitzky M, Danks R, Holt D. Intravenous immunoglobulin for the treatment of Crohn's disease. Autoimmun Rev. 2012 Dec;12(2):275–80.

329. Glassner KL, Abraham BP, Quigley EMM. The microbiome and inflammatory bowel disease. J Allergy Clin Immunol. 2020 Jan 1;145(1):16–27.

330. Lavilla P, Gil A, Rodríguez MC, Dupla ML, Pintado V, Fontán G. X-linked agammaglobulinemia and gastric adenocarcinoma. Cancer. 1993 Sep 1;72(5):1528–31.

331. van der Meer JW, Weening RS, Schellekens PT, van Munster IP, Nagengast FM. Colorectal cancer in patients with X-linked agammaglobulinaemia. Lancet (London, England). 1993 Jun 5;341(8858):1439–40.

332. Mueller B, Pizzo P. Cancer in children with primary or secondary immunodeficiencies. J Pediatr. 1995;126(January):1–10.

333. Hoshino A, Okuno Y, Migita M, Ban H, Yang X, Kiyokawa N, et al. X-Linked Agammaglobulinemia Associated with B-Precursor Acute Lymphoblastic Leukemia. J Clin Immunol. 2015 Feb 16;35(2):108–11.

334. Staines Boone AT, Torres Martínez MG, López Herrera G, de Leija Portilla JO, Espinosa Padilla SE, Espinosa Rosales FJ, et al. Gastric Adenocarcinoma in the Context of X-linked Agammaglobulinemia : Case Report and Review of the Literature. J Clin Immunol. 2013;10–3.

335. Hajjar J, Hasan S, Forbes LR, Hemmige V, Orange JS. Gastric Adenocarcinoma in a Patient with X-Linked Agammaglobulinemia and HIV: Case Report and Review of the Literature. Front Pediatr. 2016 Sep 23;4(September):6–8.

336. Richter D, Conley ME, Rohrer J, Myers LA, Zahradka K, Kelecic J, et al. A contiguous deletion syndrome of X-linked agammaglobulinemia and sensorineural deafness. Pediatr Allergy Immunol. 2001 Apr;12(2):107–11.

337. Šedivá A, Smith CIE, Asplund AC, Hadač J, Janda A, Zeman J, et al. Contiguous X-

chromosome deletion syndrome encompassing the BTK, TIMM8A, TAF7L, and DRP2 genes. J Clin Immunol. 2007 Nov 12;27(6):640–6.

338. Brown L-AKAK, Clark I, Brown JR, Breuer J, David |, Lowe M, et al. Norovirus infection in primary immune deficiency. Rev Med Virol. 2017 May 1;27(3):e1926.

339. Dellepiane RM, Dell'Era L, Beilis LV, Pavesi P, Raimondi M, Soresina A, et al. Nutritional Status in Agammaglobulinemia: An Italian Multicenter Study. J Clin Immunol. 2015;3–5.

340. Similuk MN, Wang A, Lenardo MJ, Erby LH. Life with a Primary Immune Deficiency: a Systematic Synthesis of the Literature and Proposed Research Agenda. J Clin Immunol. 2016;

341. Gardulf A, Bjorvell H, Gustafson R, Hammarstrom L, Smith CI. The life situations of patients with primary antibody deficiency untreated or treated with subcutaneous gammaglobulin infusions. Clin Exp Immunol. 1993 May;92(2):200–4.

342. Gardulf A, Nicolay U. Replacement IgG therapy and self-therapy at home improve the health-related quality of life in patients with primary antibody deficiencies. Curr Opin Allergy Clin Immunol. 2006 Dec;6(6):434–42.

343. Nicolay U, Haag S, Eichmann F, Herget S, Spruck D, Gardulf a. Measuring treatment satisfaction in patients with primary immunodeficiency diseases receiving lifelong immunoglobulin replacement therapy. Qual Life Res. 2005 Sep;14(7):1683–91.

344. Ballow M, Conaway MR, Sriaroon P, Rachid RA, Seeborg FO, Duff CM, et al. Construction and Validation of a Novel Disease-Specific Quality of Life Instrument for Patients with Primary Antibody Deficiency Disease (PADQOL -16). J Allergy Clin Immunol. 2017;(2017).

345. Quinti I, Pulvirenti F. Health-Related Quality of Life and Patients' Empowerment in the Health Care of Primary Immune Deficiencies. J Clin Immunol. 2017 Oct 17;37(7):615–6.

346. Tabolli S, Giannantoni P, Pulvirenti F, La Marra F, Granata G, Milito C, et al. Longitudinal Study on Health-Related Quality of Life in a Cohort of 96 Patients with Common Variable Immune Deficiencies. Front Immunol. 2014 Nov 26;5:605.

347. Titman P, Allwood Z, Gilmour C, Malcolmson C, Duran-Persson C, Cale C, et al. Quality of Life in Children with Primary Antibody Deficiency. J Clin Immunol. 2014;34(7):844–52.

348. Soresina A, Nacinovich R, Bomba M, Cassani M, Molinaro A, Sciotto A, et al. The quality of life of children and adolescents with X-linked agammaglobulinemia. J Clin Immunol. 2009;29(4):501–7.

349. Sultan S, Rondeau É, Levasseur M-CC, Dicaire R, Decaluwe H, Haddad É. Quality of Life, Treatment Beliefs, and Treatment Satisfaction in Children Treated for Primary Immunodeficiency with SCIg. J Clin Immunol. 2017 Jul 8;37(5):496–504.

350. Cohen J. A power primer. Psychol Bull. 1992 Jul;112(1):155–9.

351. Gannoni AF, Shute RH. Parental and child perspectives on adaptation to childhood chronic illness: a qualitative study. Clin Child Psychol Psychiatry. 2010 Jan;15(1):39–53.

352. de Ridder D, Geenen R, Kuijer R, van Middendorp H. Psychological adjustment to chronic disease. Lancet (London, England). 2008 Jul 19;372(9634):246–55.

353. Lavigne J V, Faier-Routman J. Psychological adjustment to pediatric physical disorders: a meta-analytic review. J Pediatr Psychol. 1992 Apr;17(2):133–57.

354. Cadman D, Boyle M, Szatmari P, Offord DR. Chronic illness, disability, and mental and social well-being: findings of the Ontario Child Health Study. Pediatrics. 1987 May;79(5):805–13.

355. Conley ME, Sweinberg SK. Females with a disorder phenotypically identical to X-linked agammaglobulinemia. J Clin Immunol. 1992 Mar;12(2):139–43.

356. Yel L, Minegishi Y, Coustan-Smith E, Buckley RH, Trübel H, Pachman LM, et al. Mutations in the mu heavy-chain gene in patients with agammaglobulinemia. N Engl J Med. 1996 Nov 14;335(20):1486–93.

357. Minegishi Y, Rohrer J, Coustan-smith E, Lederman HM, Pappu R, Campana D, et al. An Essential Role for BLNK in Human B Cell Development. Science. 1999 Dec 3;286(December):1954–8.

358. Minegishi Y, Coustan-Smith E, Wang Y-H, Cooper MD, Campana D, Conley ME. Mutations in the Human 5/14.1 Gene Result in B Cell Deficiency and Agammaglobulinemia. J Exp Med. 1998 Jan 5;187(1):71–7.

359. Sawada A, Takihara Y, Kim JY, Matsuda-Hashii Y, Tokimasa S, Fujisaki H, et al. A congenital mutation of the novel gene LRRC8 causes agammaglobulinemia in humans. J Clin Invest. 2003 Dec 1;112(11):1707–13.

360. Minegishi Y, Coustan-Smith E, Rapalus L, Ersoy F, Campana D, Conley ME. Mutations in Igα (CD79a) result in a complete block in B-cell development. J Clin Invest. 1999 Oct 15;104(8):1115–21.

361. Khalili A, Plebani A, Vitali M, Abolhassani H, Lougaris V, Mirminachi B, et al. Autosomal Recessive Agammaglobulinemia: A Novel Non-sense Mutation in CD79a. J Clin Immunol. 2014 Feb 1;34(2):138–41.

362. Wang Y, Kanegane H, Sanal O, Tezcan I, Ersoy F, Futatani T, et al. Novel Igα (CD79a) gene mutation in a Turkish patient with B cell-deficient agammaglobulinemia. Am J Med Genet. 2002 Apr 1;108(4):333–6.

363. Ferrari S, Lougaris V, Caraffi S, Zuntini R, Yang J, Soresina A, et al. Mutations of the Igβ gene cause agammaglobulinemia in man. J Exp Med. 2007 Sep 3;204(9):2047–51.

364. Dobbs AK, Yang T, Farmer D, Kager L, Parolini O, Conley ME. Cutting Edge: A Hypomorphic Mutation in Ig (CD79b) in a Patient with Immunodeficiency and a Leaky Defect in B Cell Development. J Immunol. 2007 Aug 15;179(4):2055–9.

365. Conley ME, Dobbs a. K, Quintana AM, Bosompem A, Wang Y-DY-DY-DY-D, Coustan-Smith E, et al. Agammaglobulinemia and absent B lineage cells in a patient lacking the p85α subunit of PI3K. J Exp Med. 2012 Mar 12;209(3):463–70.

366. Abolhassani H, Vitali M, Lougaris V, Giliani S, Parvaneh N, Parvaneh L, et al. Cohort of Iranian Patients with Congenital Agammaglobulinemia: Mutation Analysis and Novel Gene Defects. Expert Rev Clin Immunol. 2016 Apr 2;12(4):479–86.

367. Morwood K, Bourne H, Philpot R, Gold M, Gillis D, Benson EM. Phenotypic variability: clinical presentation between the 6th year and the 60th year in a family with X-linked agammaglobulinemia. J Allergy Clin Immunol. 2004 Apr;113(4):783–5.

368. Broides A, Yang W, Conley ME. Genotype/phenotype correlations in X-linked agammaglobulinemia. Clin Immunol. 2006;118(2–3):195–200.

369. Conley ME. B cells in patients with X-linked agammaglobulinemia. J Immunol. 1985 May;134(5):3070–4.

370. Gaspar HB, Lester T, Levinsky RJ, Kinnon C. Bruton's tyrosine kinase expression and activity in X-linked agammaglobulinaemia (XLA): The use of protein analysis as a diagnostic indicator of XLA. Clin Exp Immunol. 1998 Feb;111(2):334–8.

371. Futatani BT, Miyawaki T, Tsukada S, Hashimoto S, Kunikata T, Arai S, et al. Deficient Expression of Bruton's Tyrosine Kinase in Monocytes From X-Linked Agammaglobulinemia as Evaluated by a Flow Cytometric Analysis and Its Clinical Application to Carrier Detection. Blood1. 1998 Jan 15;91(2):595–602.

372. Futatani T, Watanabe C, Baba Y, Tsukada S, Ochs HD. Bruton's tyrosine kinase is present in normal platelets and its absence identifies patients with X-linked agammaglobulinaemia and carrier females. Br J Haematol. 2001 Jul;114(1):141–9.

373. Saffran DC, Parolini O, Fitch-Hilgenberg ME, Rawlings DJ, Afar D, Witte ON, et al. A Point Mutation in the SH2 Domain of Bruton's Tyrosine Kinase in Atypical X-Linked Agammaglobulinemia. N Engl J Med. 1994 May 26;330(21):1488–91.

374. Stewart DM, Tian L, Nelson DL. A case of X-linked agammaglobulinemia diagnosed in adulthood. Clin Immunol. 2001 Apr;99(1):94–9.

375. Noordzij JG, De Bruin-Versteeg S, Hartwig NG, Weemaes CMR, Gerritsen EJA, Bernatowska E, et al. XLA patients with BTK splice-site mutations produce low levels of wild-type BTK transcripts. J Clin Immunol. 2002 Sep;22(5):306–18.

376. López-Granados E, Pérez de Diego R, Ferreira Cerdán A, Fontán Casariego G, García Rodríguez MC. A genotype-phenotype correlation study in a group of 54 patients with X-linked agammaglobulinemia. J Allergy Clin Immunol. 2005 Sep;116(3):690–7.

377. Gaspar HB, Bradley LAD, Katz F, Lovering RC, Roifman CM, Morgan G, et al. Mutation analysis in bruton's tyrosine kinase, the X-linked agammaglobulinaemia gene, including identification of an insertional hotspot. Vol. 4, Human Molecular Genetics. Oxford University Press; 1995. p. 755–7.

378. Jin H, Webster ADB, Vihinen M, Sideras P, Vorechovsky L, Hammarström L, et al. Identification of Btk mutations in 20 unrelated patients with x-iinked agammaglobulinaemia (XLA). Hum Mol Genet. 1995 Apr 1;4(4):693–700.

379. Holinski-Feder E, Weiss M, Brandau O, Jedele KB, Nore B, Backesjo CM, et al. Mutation Screening of the BTK Gene in 56 Families With X-Linked Agammaglobulinemia (XLA): 47 Unique Mutations Without Correlation to Clinical Course. Pediatrics. 1998 Feb;101(2):276–84.

380. Kobayashi S, Iwata T, Saito M, Iwasaki R, Matsumoto H, Naritaka S, et al. Mutations of theBtk gene in 12 unrelated families with X-linked agammaglobulinemia in Japan. Hum Genet. 1996 Apr;97(4):424–30.

381. Wang Y, Kanegane H, Wang X, Han X, Zhang Q, Zhao S, et al. Mutation of the BTK Gene and Clinical Feature of X-Linked Agammaglobulinemia in Mainland China. J Clin Immunol. 2009 May 28;29(3):352–6.

382. Orange JS, Grossman WJ, Navickis RJ, Wilkes MM. Impact of trough IgG on pneumonia incidence in primary immunodeficiency: A meta-analysis of clinical studies. Clin Immunol. 2010;137(1):21–30.

383. Tarzi MD, Grigoriadou S, Carr SB, Kuitert LM, Longhurst HJ. Clinical immunology review series: An approach to the management of pulmonary disease in primary antibody deficiency. Clin Exp Immunol. 2009;155(2):147–55.

384. Lucas M, Lee M, Oksenhendler E, Chapel H. The ratio of mean daily IgG increment/mean daily dose in immunoglobulin replacement therapy in primary antibody deficiencies. J Allergy Clin Immunol Pract. 2015 Dec 14;3(6):998-1000.e2.

385. Gouilleux-Gruart V, Chapel H, Chevret S, Lucas M, Malphettes M, Fieschi C, et al. Efficiency of immunoglobulin G replacement therapy in common variable immunodeficiency: Correlations with clinical phenotype and polymorphism of the neonatal Fc receptor. Clin Exp Immunol. 2013;171(2):186–94.

386. Litzman J. Influence of FCRN expression on lung decline and intravenous immunoglobulin catabolism in common variable immunodeficiency patients. Clin Exp Immunol. 2014;178(S1):103–4.

387. Wasserman RL, Melamed I, Stein MR, Gupta S, Puck J, Engl W, et al. Recombinant

human hyaluronidase-facilitated subcutaneous infusion of human immunoglobulins for primary immunodeficiency. J Allergy Clin Immunol. 2012 Oct;130(4):951-957.e11.

388. Bienvenu B, Cozon G, Hoarau C, Pasquet M, Cherin P, Clerson P, et al. Does the route of immunoglobin replacement therapy impact quality of life and satisfaction in patients with primary immunodeficiency? Insights from the French cohort “Visages”. Orphanet J Rare Dis. 2016;11(1):83.

389. Hodkinson JP, Bangs C, Wartenberg-Demand A, Bauhofer A, Langohr P, Buckland MS, et al. Low IgA and IgM Is Associated with a Higher Prevalence of Bronchiectasis in Primary Antibody Deficiency. J Clin Immunol. 2017 May 14;37(4):329–31.

390. Micol R, Kayal S, Mahlaoui N, Beauté J, Brosselin P, Dudoit Y, et al. Protective effect of IgM against colonization of the respiratory tract by nontypeable Haemophilus influenzae in patients with hypogammaglobulinemia. J Allergy Clin Immunol. 2012;129(3):770–7.

391. Ludvigsson JF, Neovius M, Hammarström L. Risk of Infections Among 2100 Individuals with IgA Deficiency: a Nationwide Cohort Study. J Clin Immunol. 2016;134–40.

392. Kuehn BM. After 50 Years, Newborn Screening Continues to Yield Public Health Gains. JAMA. 2013 Mar 27;309(12):1215.

393. Gaspar HB, Hammarström L, Mahlaoui N, Borte M, Borte S. The case for mandatory newborn screening for severe combined immunodeficiency (SCID). J Clin Immunol. 2014;34(4):393–7.

394. King J, Ludvigsson J, Hammarström L. Newborn Screening for Primary Immunodeficiency Diseases: The Past, the Present and the Future. Int J Neonatal Screen. 2017 Aug 3;3(3):19.

395. Barbaro M, Ohlsson A, Borte S, Jonsson S, Zetterström RH, King J, et al. Newborn Screening for Severe Primary Immunodeficiency Diseases in Sweden—a 2-Year Pilot TREC and KREC Screening Study. J Clin Immunol. 2017 Jan 21;37(1):51–60.

396. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn Screening for Severe Combined Immunodeficiency in 11 Screening Programs in the United States. JAMA. 2014 Aug 20;312(7):729.

397. Somech R, Lev A, Simon AJ, Korn D, Garty BZ, Amariglio N, et al. Newborn screening for severe T and B cell immunodeficiency in Israel: A pilot study. Isr Med Assoc J. 2013;15(8):404–9.

398. Chien Y-H, Chiang S-C, Chang K-L, Yu H-H, Lee W-I, Tsai L-P, et al. Incidence of severe combined immunodeficiency through newborn screening in a Chinese population. J Formos Med Assoc. 2015;114(1):12–6.

399. Wilson JM, Jungner G. The principles and practice of mass screening for disease. World Health Organisation. 1968. 163 p.

400. UK National Screening Committe. Criteria for appraising the viability, effectiveness and appropriateness of screening programme [Internet]. 2015 [cited 2018 Mar 29]. p. 1–2. Available from: https://www.gov.uk/government/publications/evidence-review-criteria-national-screening-programmes/criteria-for-appraising-the-viability-effectiveness-and-appropriateness-of-a-screening-programme

401. Bestas B, Turunen JJ, Blomberg KEM, Wang Q, Månsson R, EL Andaloussi S, et al. Splice-Correction Strategies for Treatment of X-Linked Agammaglobulinemia. Curr Allergy Asthma Rep. 2015;15(3):4.

402. Bestas B, Moreno PMD, Blomberg KEM, Mohammad DK, Saleh AF, Sutlu T, et al. Splice-correcting oligonucleotides restore BTK function in X-linked agammaglobulinemia model. J Clin Invest. 2014;124(9):4067–81.

403. Yamamoto H, Ishimura M, Ochiai M, Takada H, Kusuhara K, Nakatsu Y, et al. BTK gene targeting by homologous recombination using a helper-dependent adenovirus/adeno-associated virus hybrid vector. Vol. 23, Gene Therapy. Nature Publishing Group; 2016. 205–213 p.

404. Moreau T, Barlogis V, Bardin F, Nunes J a, Calmels B, Chabannon C, et al. Development of an enhanced B-specific lentiviral vector expressing BTK: a tool for gene therapy of XLA. Gene Ther. 2008;15(12):942–52.

405. Yu PW, Tabuchi RS, Kato RM, Astrakhan A, Humblet-Baron S, Kipp K, et al. Sustained correction of B-cell development and function in a murine model of X-linked agammaglobulinemia (XLA) using retroviral-mediated gene transfer. Blood. 2004;104(5):1281–90.

406. Kerns HM, Ryu BY, Stirling B V, Sather BD, Astrakhan A, Humblet-Baron S, et al. B cell-specific lentiviral gene therapy leads to sustained B-cell functional recovery in a murine model of X-linked agammaglobulinemia. Blood. 2010;115(11):2146–55.

407. Aghamohammadi A, Allahverdi A, Abolhassani H, Moazzami K, Alizadeh H, Gharagozlou M, et al. Comparison of pulmonary diseases in common variable immunodeficiency and X-linked agammaglobulinaemia. Respirology. 2010 Feb;15(2):289–95.

408. Carlos Poston WS, Foreyt JP. Body Mass Index. Strength Cond J. 2002 Aug 3;24(4):15–7.

409. Royal College of Paediatrics & Child Health. Body mass index (BMI) chart | RCPCH [Internet]. [cited 2018 Dec 3]. Available from: https://www.rcpch.ac.uk/resources/body-mass-index-bmi-chart

410. Newton EHJ, Cox NJ, Baum C, College B, Bellocco R, Institutet K, et al. Standardizing anthropometric measures in children and adolescents with new functions for egen. Stata J.

411. Cole TJ, Freeman J V, Preece MA. British 1990 growth reference centiles for weight, height, body mass index and head circumference fitted by maximum penalized likelihood. Stat Med. 1998 Feb 28;17(4):407–29.

412. Bates D, Christie D. Respiratory Function in Disease. Philadelphia and London: Saunders; 1964. 91 p.

413. Lum S, Bonner R, Kirkby J, Sonnappa S, Stocks J. S33 Validation of the GLI-2012 Multi-Ethnic Spirometry Reference Equations in London School Children. Thorax. 2012 Dec 19;67(Suppl 2):A18.2-A18.

414. Hall GL, Thompson BR, Stanojevic S, Abramson MJ, Beasley R, Coates A, et al. The Global Lung Initiative 2012 reference values reflect contemporary Australasian spirometry. Respirology. 2012 Oct;17(7):1150–1.

415. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: The global lung function 2012 equations. Eur Respir J. 2012 Dec;40(6):1324–43.

416. Martinez-Garcia MA, de Gracia J, Vendrell Relat M, Giron R-M, Maiz Carro L, de la Rosa Carrillo D, et al. Multidimensional approach to non-cystic fibrosis bronchiectasis: the FACED score. Eur Respir J. 2014 May 1;43(5):1357–67.

417. Ellis HC, Cowman S, Fernandes M, Wilson R, Loebinger MR. Predicting mortality in bronchiectasis using bronchiectasis severity index and FACED scores: a 19-year cohort study. Eur Respir J. 2016 Feb 1;47(2):482–9.

418. Fletcher CM. Standardized Questionaries on Respiratory Symptoms. BMJ. 1960 Dec 3;2(5213):1665–1665.

419. Genome Reference Consortium. Genome Reference Consortium Human Build 37 (GRCh37) [Internet]. 2009 [cited 2018 Jun 14]. Available from: https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.13/

420. BTK gene homepage - Global Variome shared LOVD [Internet]. [cited 2018 Dec 3]. Available from: https://databases.lovd.nl/shared/genes/BTK

421. Ng PC. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003 Jul 1;31(13):3812–4.

422. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Vol. 7, Nature Methods. 2010. p. 248–9.

423. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. 2015 Aug 15;31(16):2745–7.

424. MutationTaster [Internet]. [cited 2018 Jun 14]. Available from: http://www.mutationtaster.org/

425. FATHMM - Functional Analysis through Hidden Markov Models [Internet]. [cited 2018 Jun 14]. Available from: http://fathmm.biocompute.org.uk/

426. Jian X, Boerwinkle E, Liu X. In silico prediction of splice-altering single nucleotide variants in the human genome. Nucleic Acids Res. 2014;42(22):13534–44.

427. Mesbah-Uddin M. Prediction of deleterious nonsynonymous SNPs by integrating multiple classifiers – An application to neurodegenerative diseases. 2015 Apr 21;

428. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May 8;17(5):405–24.

429. Quang D, Chen Y, Xie X. DANN: a deep learning approach for annotating the pathogenicity of genetic variants. Bioinformatics. 2015 Mar 1;31(5):761–3.

430. WHO | The world health report 2001 - Mental Health: New Understanding, New Hope. WHO. 2013;

431. Jenkinson C, Stewart-Brown S, Petersen S, Paice C. Assessment of the SF-36 version 2 in the United Kingdom. J Epidemiol Community Health. 1999;53(1):46–50.

432. Gee L, Abbott J, Conway SP, Etherington C, Webb AK. Validation of the SF-36 for the assessment of quality of life in adolescents and adults with cystic fibrosis. J Cyst Fibros. 2002 Sep 1;1(3):137–45.

433. Upton P, Eiser C, Cheung I, Hutchings H a, Jenney M, Maddocks A, 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;3:22.

434. Thomas C, Mitchell P, O'Rourke P, Wainwright C. Quality-of-life in children and adolescents with cystic fibrosis managed in both regional outreach and cystic fibrosis center settings in queensland. J Pediatr. 2006 Apr 1;148(4):508-516.e1.

435. Jones PW. SGRQ Manual June 2009. 2009;44(June):0–16.

436. Padilla A, Olveira G, Olveira C, Dorado A, Plata AJ, Gaspar I, et al. Validity and reliability of the St. George Respiratory Questionnaire in the adult population with cystic fibrosi. Arch Bronconeumol. 2007 Apr 1;43(4):205–11.

437. Breeman S, Cotton S, Fielding S, Jones GT, Gareth •, Jones T. Normative data for the Hospital Anxiety and Depression Scale. Qual Life Res. 2015 Feb 27;24(2):391–8.

438. Duff AJA, Abbott J, Cowperthwaite C, Sumner C, Hurley MA, Quittner A. Depression and anxiety in adolescents and adults with cystic fibrosis in the UK: A cross-sectional study. J Cyst Fibros. 2014 Dec;13(6):745–53.

439. Bagley C, Mallick K. Normative Data and Mental Health Construct Validity for the Rosenberg Self-Esteem Scale in British Adolescents. Int J Adolesc Youth. 2001;9(2–3):117–26.

440. Platten MJ, Newman E, Quayle E. Self-Esteem and Its Relationship to Mental Health and Quality of Life in Adults with Cystic Fibrosis. J Clin Psychol Med Settings. 2013 Sep 21;20(3):392–9.

441. Lincoln R. User's manual for the SF36v2 Health Survey. 3rd ed. Maruish ME, editor. QualityMetric Incorporated; 2011.

442. Varni JW, Seid M, Kurtin PS. 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):800–12.

443. Wilson CB, Jones PW, O'Leary CJ, Cole PJ, Wilson R. Validation of the St. George's respiratory questionnaire in bronchiectasis. Am J Respir Crit Care Med. 1997;156(2 I):536–41.

444. Loebinger MR, Wells AU, Hansell DM, Chinyanganya N, Devaraj A, Meister M, et al. Mortality in bronchiectasis: A long-term study assessing the factors influencing survival. Eur Respir J. 2009;34(4):843–9.

445. Muris P, Meesters C, van den Berg F. The Strengths and Difficulties Questionnaire (SDQ). Eur Child Adolesc Psychiatry. 2003;12(1):1–8.

446. Ding T, Hall A, Jacobs K, David J. Psychological functioning of children and adolescents with juvenile idiopathic arthritis is related to physical disability but not to disease status. Rheumatology. 2008 Jan 29;47(5):660–4.

447. Bruce S, Rodgers J, Firth M, Freeston M. Mum knows best? Psychological status in an oncology sample. Child Care Health Dev. 2005 Nov;31(6):643–8.

448. Youth in Mind. SDQ: Generating scores in STATA [Internet]. [cited 2018 Jun 11]. Available from: http://www.sdqinfo.org/c3.html

449. Meltzer H, Gatward R, Goodman R, Ford T. Mental health of children and adolescents in Great Britain. Int Rev Psychiatry. 2003 Jan 11;15(1–2):185–7.

450. White, D., Leach, C., Sims, R., & Cottrell D. Validation of the Hospital Anxiety and Depression Scale for use with adolescents. Br J Psychiatry. 1999;175(5):452–4.

451. Snaith RP. The Hospital Anxiety And Depression Scale. Heal Qual Life Outcomes. 2003;1:29.

452. Rosenberg M. Society and the adolescent self-image. Princeton: Princeton University Press; 1965. 326 p.

453. Baumeister RF, Campbell JD, Krueger JI, Vohs KD. Rosenberg Self-Esteem Scale. 2003;

454. Joint Formulary Committee. BNF: British National Formulary - NICE. NICE; 2017.

455. Cole T, Pearce MS, Cant AJ, Cale CM, Goldblatt D, Gennery AR. Clinical outcome in children with chronic granulomatous disease managed conservatively or with hematopoietic stem cell transplantation. J Allergy Clin Immunol. 2013;132(5):1150–5.

456. Battersby a. C, Cale CM, Goldblatt D, Gennery a. R. Clinical manifestations of disease in X-linked carriers of chronic granulomatous disease. J Clin Immunol. 2013;33(8):1276–84.

457. Hilliard ME, Lawrence JM, Modi AC, Anderson A, Crume T, Dolan LM, et al. Identification of Minimal Clinically Important Difference Scores of the PedsQL in Children, Adolescents, and Young Adults With Type 1 and Type 2 Diabetes. Diabetes Care. 2013 Jul 1;36(7):1891–7.

458. Office for National Statistics. ONS Population Estimates [Internet]. [cited 2017 Mar 8]. Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates

459. Mahlaoui N, Warnatz K, Jones A, Workman S, Cant A. Advances in the Care of Primary Immunodeficiencies (PIDs): from Birth to Adulthood. J Clin Immunol. 2017 Jul 18;37(5):452–60.

460. Hammer J, Eber E. Paediatric pulmonary function testing. Karger; 2005. 288 p.

461. Heather JM, Chain B. The sequence of sequencers: The history of sequencing DNA.

Genomics. 2016 Jan;107(1):1–8.

462. van Schouwenburg PA, Davenport EE, Kienzler A-K, Marwah I, Wright B, Lucas M, et al. Application of whole genome and RNA sequencing to investigate the genomic landscape of common variable immunodeficiency disorders. Clin Immunol. 2015 Oct;160(2):301–14.

463. NHS England. NHS England » NHS Long Term Plan to reduce toll of 'hidden killer' sepsis [Internet]. [cited 2020 Jan 3]. Available from: https://www.england.nhs.uk/2019/03/nhs-long-term-plan-to-reduce-toll-of-hidden-killer-sepsis/

464. Hay AD, Heron J, Ness A, ALSPAC study team. The prevalence of symptoms and consultations in pre-school children in the Avon Longitudinal Study of Parents and Children (ALSPAC): a prospective cohort study. Fam Pract. 2005 Aug 1;22(4):367–74.

465. Ponsford MJ, Price C, Farewell D, Greene G, Moore C, Perry M, et al. Increased Respiratory Viral Detection and Symptom Burden Among Patients with Primary Antibody Deficiency: Results from the BIPAD Study. J Allergy Clin Immunol Pract. 2020;

466. Littlewood JM, Bevan A, Connett G, Conway S, Govan J, Hodson M. Antibiotic treatment for cystic fibrosis: report of the UK Cystic Fibrosis Trust Antibiotic Group. 2009;(May 2009).

467. Shillitoe B, Hollingsworth R, Foster M, Garcez T, Guzman D, Edgar JD, et al. Immunoglobulin use in immune deficiency in the UK: A report of the UKPID and National Immunoglobulin Databases. Clin Med J R Coll Physicians London. 2018 Oct 4;18(5):364–70.

468. Stiehm ER. Plasma therapy: an alternative to gamma globulin injections in immunodeficiency. Birth Defects Orig Artic Ser. 1975;11(1):343–6.

469. Buckley RH. Plasma therapy in immunodeficiency diseases. Am J Dis Child. 1972 Sep 1;124(3):376–81.

470. Kerstens PJSM, Endtz HP, Meis JFGM, Oyen WJG, Koopman RJI, van den Broek PJ, et al. Erysipelas-like skin lesions associated with Campylobacter jejuni septicemia in patients with hypogammaglobulinemia. Eur J Clin Microbiol Infect Dis. 1992

Sep;11(9):842–7.

471. Stephan W, Dichtelmüller H, Schedel I. [Properties and efficacy of a human immunoglobulin M preparation for intravenous administration]. Arzneimittelforschung. 1985;35(6):933–6.

472. Garbett ND, Munro CS, Cole PJ. Opsonic activity of a new intravenous immunoglobulin preparation: Pentaglobin compared with sandoglobulin. Clin Exp Immunol. 1989 Apr;76(1):8–12.

473. Rossmann FS, Kropec A, Laverde D, Saaverda FR, Wobser D, Huebner J. In vitro and in vivo activity of hyperimmune globulin preparations against multiresistant nosocomial pathogens. Infection. 2015 Apr 27;43(2):169–75.

474. Welte T, Dellinger RP, Ebelt H, Ferrer M, Opal SM, Singer M, et al. Efficacy and safety of trimodulin, a novel polyclonal antibody preparation, in patients with severe community-acquired pneumonia: a randomized, placebo-controlled, double-blind, multicenter, phase II trial (CIGMA study). Intensive Care Med. 2018 Apr 1;44(4):438–48.

475. Eibl MM, Wolf HM, Fürnkranz H, Rosenkranz A. Prevention of Necrotizing Enterocolitis in Low-Birth-Weight Infants by IgA–IgG Feeding. N Engl J Med. 1988 Jul 7;319(1):1–7.

476. Casswall T, Hammarström L, Veress B, Nord C, Bogstedt A, Brockstedt U, et al. Oral IgA-IgG treatment of chronic non-specific diarrhoea in infants and children. Acta Paediatr. 1996 Sep;85(9):1126–8.

477. Delacroix DL, Elkom KB, Geubel AP, Hodgson HF, Dive C, Vaerman JP. Changes in size, subclass, and metabolic properties of serum immunoglobulin A in liver diseases and in other diseases with high serum immunoglobulin A. J Clin Invest. 1983 Feb;71(2):358–67.

478. Longet S, Miled S, Lötscher M, Miescher SM, Zuercher AW, Corthésy B. Human plasma-derived polymeric IgA and IgM antibodies associate with secretory component to yield biologically active secretory-like antibodies. J Biol Chem. 2013 Feb 8;288(6):4085–94.

479. Vonarburg C, Loetscher M, Spycher MO, Kropf A, Illi M, Salmon S, et al. Topical

application of nebulized human IgG, IgA and IgAM in the lungs of rats and non-human primates. Respir Res. 2019 May 22;20(1).

480. Monteiro RC. Immunoglobulin A as an anti-inflammatory agent. Clin Exp Immunol. 2014 Dec 1;178(S1):108–10.

481. Li Y, Jin L, Chen T, Pirozzi CJ. The Effects of Secretory IgA in the Mucosal Immune System. Vol. 2020, BioMed Research International. Hindawi Limited; 2020.

482. Conley ME. Are Patients with X-Linked Agammaglobulinemia at Increased Risk of Developing Acute Lymphoblastic Leukemia? J Clin Immunol. 2015;35(2):98–9.

483. Abd Hamid IJ, Slatter MA, McKendrick F, Pearce MS, Gennery AR. Long-Term Health Outcome and Quality of Life Post-HSCT for IL7Rα-, Artemis-, RAG1- and RAG2-Deficient Severe Combined Immunodeficiency: a Single Center Report. J Clin Immunol. 2018 Aug 1;38(6):727–32.

484. Chen X-F, Wang W-F, Zhang Y-D, Zhao W, Wu J, Chen T-X. Clinical characteristics and genetic profiles of 174 patients with X-linked agammaglobulinemia. Medicine (Baltimore). 2016 Aug;95(32):e4544.

485. Pumar M, Fong C, Aui P, van Zelm M, Bosco J. Acute Pre-B Lymphoblastic Leukaemia in a patient with X-Linked agammaglobulinaemia. Intern Med J. 2017 Sep 1;47(S5):39–39.

486. Lum SH, Flood T, Hambleton S, McNaughton P, Watson H, Abinun M, et al. Two decades of excellent transplant survival for chronic granulomatous disease: A supraregional immunology transplant center report. Blood. 2019 Jun 6;133(23):2546–9.

487. Lum SH, Sobh A, Carruthers K, Nademi Z, Watson H, McNaughton P, et al. Improved survival and graft function in ex vivo T-cell depleted haploidentical hematopoietic cell transplantation for primary immunodeficiency. Bone Marrow Transplantation. Springer Nature; 2020.

488. Elfeky R, Shah RM, Unni MNM, Ottaviano G, Rao K, Chiesa R, et al. New graft manipulation strategies improve the outcome of mismatched stem cell transplantation in children with primary immunodeficiencies. J Allergy Clin Immunol. 2019 Jul 1;144(1):280–93.

489. Czechowicz A, Palchaudhuri R, Scheck A, Hu Y, Hoggatt J, Saez B, et al. Selective hematopoietic stem cell ablation using CD117-antibody-drug-conjugates enables safe and effective transplantation with immunity preservation. Nat Commun. 2019 Dec 1;10(1).

490. Cicalese MP, Ferrua F, Castagnaro L, Pajno R, Barzaghi F, Giannelli S, et al. Update on the safety and efficacy of retroviral gene therapy for immunodeficiency due to adenosine deaminase deficiency. Blood. 2016;(April).

491. Beauté J, Levy P, Millet V, Debré M, Dudoit Y, Le Mignot L, et al. Economic evaluation of immunoglobulin replacement in patients with primary antibody deficiencies. Clin Exp Immunol. 2010 May;160(2):240–5.

492. Sánchez-Muñoz G, López de Andrés A, Jiménez-García R, Carrasco-Garrido P, Hernández-Barrera V, Pedraza-Serrano F, et al. Time Trends in Hospital Admissions for Bronchiectasis: Analysis of the Spanish National Hospital Discharge Data (2004 to 2013). Waterer G, editor. PLoS One. 2016 Sep 13;11(9):e0162282.

493. Blanchette C, Noone J, Stone G, Zacherle E, Patel R, Howden R, et al. Healthcare Cost and Utilization before and after Diagnosis of Pseudomonas aeruginosa among Patients with Non-Cystic Fibrosis Bronchiectasis in the U.S. Med Sci. 2017 Sep 23;5(4):20.

494. Bessey A, Chilcott J, Leaviss J, de la Cruz C, Wong R. A Cost-Effectiveness Analysis of Newborn Screening for Severe Combined Immunodeficiency in the UK. Int J Neonatal Screen. 2019 Aug 30;5(3):28.

495. Gardulf AR, Winiarski J, Thorin M, Heibert Arnlind MPH MR, von Döbeln U, Hammarström L. Costs associated with treatment of severe combined immunodeficiency-rationale for newborn screening in Sweden. 2017;

Appendices

Appendix A - Consent forms and patient information leaflets

Adult information sheet – version 5 – 25/06/2018
IRAS ID 212528

Investigation into the General Health and Quality of Life of Patients with Congenital Agammaglobulinemia

Information Sheet for Patients
We would like to invite you to take part in a research study, which is being undertaken as part of a PhD qualification. Before you decide you need to understand why the research is being done and what it involves. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask if anything is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1 tells you the purpose of this study and what will happen if you take part.
Part 2 gives you more detailed information about the conduct of the study.

Part 1:
What is the purpose of this study?
The purpose of this study is to look at the health of patients with congenital agammaglobulinemia (antibody deficiency). We know that despite treatment with replacement antibody, sometimes patients can experience repeated chest, sinus, throat and ear infections. We don't know how often this happens or if there are other symptoms that patients might experience.

From looking after people with other immune problems, we know that immunodeficiency that requires ongoing treatment can have an impact on how a person feels and their quality of life. This has not been studied in patients with antibody deficiency before. We would therefore like to investigate this in more detail.

Why have I been invited?
You have been invited because your doctor has identified that you have congenital agammaglobulinemia and receive regular antibody treatment (immunoglobulins).

Do I have to take part?
No, it is up to you to decide. We will give you time to read and consider this information sheet. If you would like to take part, we ask that you to sign the included consent form to show you have agreed to take part and post it back in the supplied envelope. If you prefer to do so, you can wait until your next clinic appointment to discuss this study further and sign the consent form at this point if you wish to do so.

What will happen to me if I take part?
To gather information about your health and treatment we will look at your hospital notes. This information will be kept for 5 years in a secure fashion. Your data will only be accessed by members of the research team and will be destroyed after 5 years. It will not be removed before this unless you decide not to continue being involved in this study. We will ask you to complete a questionnaire about your health.

We may want to take a blood sample from you to look at how your antibody deficiency affects an important protein. This will involve a small extra blood sample taken at the same time as the rest of your routine blood tests. If we would like a blood sample from you, we will consent for this separately. There will be no genetic testing as part of this study. We will also ask your permission to store this sample at Newcastle University for possible future research. These samples will be anonymized and will not be identifiable to the research team at Newcastle University. This future research would be carried out by researchers at Newcastle University examining your disease, and diseases similar to it. You do not have to consent to this blood sample, and not doing so will not affect your participation in the rest of the study.

We will ask you to complete questionnaires about your emotions and quality of life. This will last approximately 90 minutes. If you agree to take part you will be given a full explanation of the process, along with the results and what they mean for you. We will do this when you are attending a routine clinic appointment so you do not have to make an extra journey.

What are the disadvantages to taking part?
We know it may be difficult to talk about your feelings and how they are affected by having antibody

deficiency. If you think it would be helpful to see a trained psychologist we will arrange this.

What are the benefits to taking part?
We cannot promise the study will help you immediately, but the information from this study will help us understand about the health of patients with antibody deficiency and may affect what treatments they, and you, are offered in the future.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practices. All information about you will be handled in confidence. The details are included in Part 2.

This completes part 1. If the information has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2:
What will happen if I don't want to carry on with the study?
You can withdraw from the study at any time and it will not have an impact on your normal care or on the care of your relative. You do not have to give a reason for why you want to withdraw.

What happens if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure.

How will my taking part be kept confidential?
All information which is collected about you during the course of the research will be kept strictly confidential. Information will be stored in an anonymous fashion with a code so nothing can be related back to the individual. With your consent, we will write to your GP to let them know you are involved in this study.

What will happen to the results of the study?
The results of this study will be published in medical journals and the findings will be communicated to patients and families. Anonymous data only will be used and no individual will be identifiable.

Who is funding this research?
This research is funded by a grant from the Bubble Foundation, a charity for children with immunodeficiency (http://www.bubblefoundation.org.uk/). No-one receives a payment for your inclusion in the study.

Contact details

Researcher: Dr Ben Shillitoe
Clinical Research Associate/Paediatric Registrar
c/o Roz Gale
Paediatric Immunology
Floor 4, Block 2
Clinical Resource Building
Royal Victoria Infirmary
NE1 4LP
0191 282 5234
Email: b.shillitoe2@newcastle.ac.uk

Chief Investigator: Dr Andrew Gennery
Reader/ Consultant in Paediatric Immunology
Paediatric Immunology
Floor 4, Block 2
Clinical Resource Building
Royal Victoria Infirmary
NE1 4LP
0191 282 5234
Email: andrew.gennery@ncl.ac.uk

Investigation into the General Health and Quality of Life of Patients with Congenital Agammaglobulinemia

Information Sheet for parents
We would like to invite you and your child to take part in a research study, which is being undertaken as part of a PhD qualification. Before you decide you need to understand why the research is being done and what it involves. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask if anything is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1 tells you the purpose of this study and what will happen if you take part.
Part 2 gives you more detailed information about the conduct of the study.

Part 1:
What is the purpose of this study?
The purpose of this study is to look at the health of patients with congenital agammaglobulinemia (antibody deficiency). We know that despite treatment with replacement antibody (immunoglobulin), sometimes patients can experience repeated chest, sinus, throat and ear infections. We don't know how often this happens or if there are other symptoms that patients might experience.

From looking after people with other immune problems, we know that immunodeficiency that requires ongoing treatment can have an impact on how a person feels and their quality of life. This has not been studied in patients with antibody deficiency before. We would therefore like to investigate this in more detail.

Why has my child been invited?
Your child has been invited because their doctor has identified that they have antibody deficiency and receive regular antibody treatment.

Does my child have to take part?
No, it is up to you and your child to decide. We will give you time to read and consider this information sheet. If you would like to take part, we ask that you to sign the included consent form to show you have agreed for your child to take part and post it back in the supplied envelope. If you prefer to do so, you can wait until your child's next clinic appointment to discuss this study further and sign the consent form at this point if you wish to do so.

What will happen to my child if we take part?
To gather information about your child's health and treatment we will look at their hospital notes. This information will be kept for 5 years in a secure fashion. Their data will only be accessed by members of the research team and will be destroyed after 5 years. It will not be removed before this unless you decide not to continue being involved in this study.

We may want to take a blood sample from your child to look at how your child's antibody deficiency affects an important protein. This will involve a small extra blood sample taken at the same time as the rest of his/her routine blood tests. If we would like a blood sample from your child we will consent for this separately. There will be no genetic testing as part of this study. We will also ask your permission to store this sample at Newcastle University for possible future research. These samples will be anonymized and will not be identifiable to the research team at Newcastle University. This future research would be carried out by researchers at Newcastle University examining your child's disease, and diseases similar to it. You do not have to consent to this blood sample, and not doing so will not affect your child's participation in the rest of the study.

We will ask you or your child to complete a questionnaire about their health.

We will ask you or your child to complete questionnaires about your emotions and quality of life. This will last approximately 90 minutes. If you agree to take part you and your child will be given a full explanation of the process, along with the results and what they mean for you. We will do this when you are attending a routine clinic appointment so you do not have to make an extra journey.

What are the disadvantages to taking part?
We know it may be difficult to talk about your feelings and how they are affected by having antibody

deficiency. If you think it would be helpful to see a trained psychologist we will arrange this.

What are the benefits to taking part?
We cannot promise the study will help you and your child immediately, but the information from this study will help us understand about the health of patients with antibody deficiency and may affect what treatments they, and your child, are offered in the future.

Will my taking part in the study be kept confidential?
Yes. We will follow ethical and legal practices. All information about you will be handled in confidence. The details are included in Part 2.

This completes part 1. If the information has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2:
What will happen if I don't want to carry on with the study?
You can withdraw from the study at any time and it will not have an impact on your normal care or on the care of your relative. You do not have to give a reason for why you want to withdraw.

What happens if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure.

How will my taking part be kept confidential?
All information which is collected about your child during the course of the research will be kept strictly confidential. Information will be stored in an anonymous fashion with a code so nothing can be related back to the individual. With your consent, we will write to your child's GP to let them know you are involved in this study.

What will happen to the results of the study?
The results of this study will be published in medical journals and the findings will be communicated to patients and families. Anonymous data only will be used and no individual will be identifiable.

Who is funding this research?
This research is funded by a grant from the Bubble Foundation, a charity for children with immunodeficiency (http://www.bubblefoundation.org.uk/). No-one receives a payment for your inclusion in the study.

Contact details

Researcher: Dr Ben Shillitoe
Clinical Research Associate/Paediatric Registrar
c/o Roz Gale
Paediatric Immunology
Floor 4, Block 2
Clinical Resource Building
Royal Victoria Infirmary
NE1 4LP
0191 282 5234
Email: b.shillitoe2@newcastle.ac.uk

Chief Investigator: Dr Andrew Gennery
Reader/ Consultant in Paediatric Immunology
Paediatric Immunology
Floor 4, Block 2
Clinical Resource Building
Royal Victoria Infirmary
NE1 4LP
0191 282 5234
Email: andrew.gennery@ncl.ac.uk

Newcastle University

4th Floor, Block 2
Clinical Resource Building
Royal Victoria Infirmary
Newcastle NE1 4LP

Name:

Consultant:

Hospital:

Study ID:

Investigation into the General Health and Quality of Life of Patients with Congenital Agammaglobulinemia

	Please initial box
1. I confirm I have read and understood the information sheet (version 5 - 25/06/18) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.	☐
3. I agree to participate in the quality of life component of the project	☐
4. I agree to my GP being informed that I am involved in this study.	☐
5. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from regulatory authorities or from the NHS trust, where it is relevant to my taking park in this research. I give permission for these individuals to have access to my records.	☐

Participant:
Print name:
Signature:

Date:

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

4th Floor, Block 2
Clinical Resource Building
Royal Victoria Infirmary
Newcastle NE1 4LP

Name of child:

Consultant:

Hospital:

Study ID:

Investigation into the General Health and Quality of Life of Patients with Congenital Agammaglobulinemia

	Please initial box
1. I confirm I have read and understood the information sheet (version 5 -25/06/2018) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2. I understand that my child's participation is voluntary and that I am free to withdraw them at any time without giving any reason, without their medical care or legal rights being affected.	☐
3. I agree to my child's participation in quality of life component of the project.	☐
4. I agree to my child's GP being informed that they are involved in this study.	☐
5. I understand that relevant sections of my child's medical notes and data collected during the study, may be looked at by individuals from regulatory authorities or from the NHS trust, where it is relevant to my taking park in this research. I give permission for these individuals to have access to my child's records.	☐

Parent:
Print name:

Signature:

Date:

Patient: (Optional)
Print name:

Signature:

Date:

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

Appendix B - Data collection proforma

Newcastle University
Institute of Cellular Medicine

Clinical Data Pro forma

Investigation into the Health Status and Quality of Life of Patients with Congenital Agammaglobulinaemia

Patient Study Number ☐☐☐ Local Study Number..... ☐☐☐

Enrolment Date..... Date of data collection.....

Demographics

Date of Birth...../...../..... Sex Male ☐ Female☐
Hospital.....Consultant.....
Unemployed ☐ Self Employed ☐ Employed ☐
Occupation.....
Caucasian ☐ African American ☐ North East Asian ☐ South East Asian ☐
Other/mixed ☐

Diagnosis

Diagnosis XLA ☐ Autosomal Recessive ☐ Details.....

Genetic Defect Yes ☐ Detail..... None found ☐
Not tested ☐

BTK Expression Normal ☐ Absent ☐ Reduced ☐ Not tested ☐
Flow Cytometry ☐ Western Blot ☐
Age at diagnosis.....Date of Diagnosis.....
Immunoglobulins at Diagnosis (g/dL): IgA.....IgG.....IgM.....
Subsets CD3_____CD4_____
CD8_____CD19/20_____
CD56/16_____
B Cell Markers CD27 –ve_____
CD27+ve IgM/IgD +ve_____CD27+ve IgM/IgD -ve_____
Proliferative Responses PHA_____Control_____
Vaccine Responses Tetanus (IU/ml)_____Hib (mcg/ml)_____
Pneumococcal Titre_____
Family History? Yes ☐ No ☐
Details.....

Clinical Presentation

Screened pre-clinical presentation (if applicable) Yes ☐ No ☐ Details.....
ITU/HDU Admission Pre Diagnosis Yes ☐ No ☐
Details.....

Number of infections (pre – diagnosis)

	In Patient	Outpatient	Total
Respiratory (Total)			
URTI			
LRTI			
Penumonia Proven			
Sinus			
Otitis			
Skin			
Eyes			
GI			
CNS			
Bone/Joint			
Sepsis			
TB			
Other/Misc			

Is the above data complete? Yes ☐ No ☐

Details……

……

Current Clinical Status

Current Immunoglobulins (g/dL) Date……
IgA…… IgM……IgG……
Subsets (Date……)
CD3_____CD4_____
CD8_____CD19/20_____
CD56/16_____

ITU/HDU Admission Yes ☐ No ☐
Details……
……

FBC Date……
Hb……WCC……Neuts……Lypmh……Plts……
Medications……
……

	2018		2017		2016		2015		2014		2013		2012		2011		2010		2009		2008	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
URTI																						
LRTI																						
Penumonia																						
Sinus																						
Otitis																						
Skin																						
Eyes																						
GI																						
CNS																						
Bone/Joint																						
Sepsis																						
GU																						
Other/Misc																						

	2007		2006		2005		2004		2003		2002		2001		2000		1999		1998		1997	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
URTI																						
LRTI																						
Penumonia																						
Sinus																						
Otitis																						
Skin																						
Eyes																						
GI																						
CNS																						
Bone/Joint																						
Sepsis																						
GU																						
Other/Misc																						

	1996		1995		1994		1993		1992		1991		1990		1989		1988		1987		1986	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
URTI																						
LRTI																						
Penumonia																						
Sinus																						
Otitis																						
Skin																						
Eyes																						
GI																						
CNS																						
Bone/Joint																						
Sepsis																						
GU																						
Other/Misc																						

	1985		1984		1983		1982		1981		1980		1979		1978		1977		1976		1975	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
URTI																						
LRTI																						
Penumonia																						
Sinus																						
Otitis																						
Skin																						
Eyes																						
GI																						
CNS																						
Bone/Joint																						
Sepsis																						
GU																						
Other/Misc																						

From what year does the above data represent?.....

Comorbidities/Complications

.....

.....

BMI

Date	Height	Weight	BMI	Date	Height	Weight	BMI

Immunoglobulin Therapy

Current Ig Route SC ☐ IV ☐
Ever IM? Yes ☐ No ☐
Current Dose and Interval.....
Current Brand.....
Home therapy Yes ☐ No ☐
Side effects Yes ☐ No ☐
Date of last infusion.....
Compliance Issues?.....

Date	Dose	Interval	Dose/Kg/Month	Route	Brand

Trough Levels

Date	Level	Date	Level	Date	Level	Date	Level

Gastrointestinal Complications

Any? Yes ☐ No ☐

Diagnosis?.....

Enteropathy Yes ☐ No ☐

IDB 'Like' Disease Yes ☐ No ☐

Histology.....
.....
.....

Surgery.....
.....
.....

Psychological Health

Any Psychological Health Disorders?

Yes ☐ No ☐ Resolved ☐

Details.....
.....

Pulmonary Health

Respiratory Diagnosis Yes ☐ No ☐
Details……
Diagnosed How?……Diagnosed When?……
Prophylactic Antibiotics Yes ☐ No ☐
Details……
Other interventions e.g.
Physio/Surgery……
Non Smoker ☐ Ex Smoker ☐ Current Smoker ☐

Last Sputum Sample

Date	Organism	Sensitives	Resistance

HRCT

Date	Findings

Lung Function

Date	Height	FEV1	FVC	Ratio	TLC	RV/TLC	KCO

Appendix C – Quality of life questionnaires

PedsQl 4.0

ID# _____

Date:_____

PedsQL™

Paediatric Quality of Life Inventory

Version 4.0 – UK English

TEENAGER REPORT (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **PAST MONTH** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the **PAST MONTH**, how much of a **problem** has this been for you …*

ABOUT MY HEALTH AND ACTIVITIES *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than a couple of streets (about 100 metres)	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activities or exercise	0	1	2	3	4
4. It is hard for me to lift heavy things	0	1	2	3	4
5. It is hard for me to have a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I have aches and pains	0	1	2	3	4
8. I feel tired	0	1	2	3	4

ABOUT MY FEELINGS *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ON WITH OTHERS *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. I have trouble getting on with other teenagers	0	1	2	3	4
2. Other teenagers do not want to be my friend	0	1	2	3	4
3. Other teenagers tease me	0	1	2	3	4
4. I cannot do things that other teenagers my age can do	0	1	2	3	4
5. It is hard to keep up with other teenagers my age	0	1	2	3	4

ABOUT SCHOOL / COLLEGE *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my school / college work	0	1	2	3	4
4. I miss school / college because of not feeling well	0	1	2	3	4
5. I miss school / college to go to the doctor or hospital	0	1	2	3	4

ID# _____

Date:_____

PedsQL™

Paediatric Quality of Life Inventory

Version 4.0 – UK English

CHILD REPORT (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **PAST MONTH** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the **PAST MONTH**, how much of a **problem** has this been for you …*

ABOUT MY HEALTH AND ACTIVITIES *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to walk more than a couple of streets (about 100 metres)	0	1	2	3	4
2. It is hard for me to run	0	1	2	3	4
3. It is hard for me to do sports activities or exercise	0	1	2	3	4
4. It is hard for me to lift heavy things	0	1	2	3	4
5. It is hard for me to have a bath or shower by myself	0	1	2	3	4
6. It is hard for me to do chores around the house	0	1	2	3	4
7. I have aches and pains	0	1	2	3	4
8. I feel tired	0	1	2	3	4

ABOUT MY FEELINGS *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel afraid or scared	0	1	2	3	4
2. I feel sad	0	1	2	3	4
3. I feel angry	0	1	2	3	4
4. I have trouble sleeping	0	1	2	3	4
5. I worry about what will happen to me	0	1	2	3	4

HOW I GET ON WITH OTHERS *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. I have trouble getting on with other children	0	1	2	3	4
2. Other children do not want to be my friend	0	1	2	3	4
3. Other children tease me	0	1	2	3	4
4. I cannot do things that other children my age can do	0	1	2	3	4
5. It is hard to keep up when I play with other children	0	1	2	3	4

ABOUT SCHOOL *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard to pay attention in class	0	1	2	3	4
2. I forget things	0	1	2	3	4
3. I have trouble keeping up with my schoolwork	0	1	2	3	4
4. I miss school because of not feeling well	0	1	2	3	4
5. I miss school to go to the doctor or hospital	0	1	2	3	4

ID#_____

Date:_____

PedsQL™

Pediatric Quality of Life Inventory (UK)

Version 4.0

PARENT REPORT for **TEENAGERS** (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your teenager.** Please tell us **how much of a problem** each one has been for **your teenager** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has your teenager had with …*

PHYSICAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking down the road a little bit	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports or running games	0	1	2	3	4
4. Lifting heavy things	0	1	2	3	4
5. Having a bath or shower by him or herself	0	1	2	3	4
6. Tidying up around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Feeling very tired	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or unhappy	0	1	2	3	4
3. Feeling angry or cross	0	1	2	3	4
4. Trouble sleeping at night	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting on with other teenagers	0	1	2	3	4
2. Other teenagers not wanting to be his or her friend	0	1	2	3	4
3. Getting bullied by other teenagers	0	1	2	3	4
4. Not able to do things that other teenagers his or her age can do	0	1	2	3	4
5. Keeping up with other teenagers during activities	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Having days off school because of not feeling well	0	1	2	3	4
5. Having days off school to go to the doctor or hospital	0	1	2	3	4

ID#
_____

Date:_____
_

PedsQL™

Pediatric Quality of Life Inventory (UK)

Version 4.0

PARENT REPORT for **CHILDREN** (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month,** how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking down the road a little bit	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports or running games	0	1	2	3	4
4. Lifting heavy things	0	1	2	3	4
5. Having a bath or shower by him or herself	0	1	2	3	4
6. Tidying up around the house	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Feeling very tired	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or unhappy	0	1	2	3	4
3. Feeling angry or cross	0	1	2	3	4
4. Trouble sleeping at night	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting on with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting bullied by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with schoolwork	0	1	2	3	4
4. Having days off school because of not feeling well	0	1	2	3	4
5. Having days off school to go to the doctor or hospital	0	1	2	3	4

ID#_____

Date:_____

PedsQL™

Paediatric Quality of Life Inventory

Version 4.0 – UK English

PARENT REPORT for **TODDLERS** (ages **2-4**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **PAST MONTH** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the **PAST MONTH**, how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Walking	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in active play and exercise	0	1	2	3	4
4. Lifting heavy things	0	1	2	3	4
5. Bathing	0	1	2	3	4
6. Helping to pick up his or her toys	0	1	2	3	4
7. Having aches or pains	0	1	2	3	4
8. Feeling tired	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad	0	1	2	3	4
3. Feeling angry	0	1	2	3	4
4. Having trouble sleeping	0	1	2	3	4
5. Worrying	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Playing with other children	0	1	2	3	4
2. Other children not wanting to play with him or her	0	1	2	3	4
3. Getting teased by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

***Please complete this section if your child attends nursery or day care**

NURSERY/DAY CARE FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Doing the same nursery/day care activities as peers	0	1	2	3	4
2. Missing nursery/day care because of not feeling well	0	1	2	3	4
3. Missing nursery/day care to go to the doctor or hospital	0	1	2	3	4

ID#
_____

Date:_____
_

PedsQL™

Pediatric Quality of Life Inventory (UK)

Version 4.0

PARENT REPORT for **YOUNG CHILDREN** (ages **5-7**)

DIRECTIONS

On the following page is a list of things that might be a problem for **your child**. Please tell us **how much of a problem** each one has been for **your child** during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **ONE month**, how much of a **problem** has your child had with …*

PHYSICAL FUNCTIONING (PROBLEMS WITH…)	Never	Almost Never	Some-times	Often	Almost Always
1. Walking down the road a little bit	0	1	2	3	4
2. Running	0	1	2	3	4
3. Participating in sports or running games	0	1	2	3	4
4. Lifting heavy things	0	1	2	3	4
5. Having a bath or shower by him or herself	0	1	2	3	4
6. Helping to pick up his or her toys	0	1	2	3	4
7. Having hurts or aches	0	1	2	3	4
8. Feeling very tired	0	1	2	3	4

EMOTIONAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Feeling afraid or scared	0	1	2	3	4
2. Feeling sad or unhappy	0	1	2	3	4
3. Feeling angry or cross	0	1	2	3	4
4. Trouble sleeping at night	0	1	2	3	4
5. Worrying about what will happen to him or her	0	1	2	3	4

SOCIAL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Getting on with other children	0	1	2	3	4
2. Other kids not wanting to be his or her friend	0	1	2	3	4
3. Getting bullied by other children	0	1	2	3	4
4. Not able to do things that other children his or her age can do	0	1	2	3	4
5. Keeping up when playing with other children	0	1	2	3	4

SCHOOL FUNCTIONING *(problems with…)*	Never	Almost Never	Some-times	Often	Almost Always
1. Paying attention in class	0	1	2	3	4
2. Forgetting things	0	1	2	3	4
3. Keeping up with school activities	0	1	2	3	4
4. Having days off school because of not feeling well	0	1	2	3	4
5. Having days off school to go to the doctor or hospital	0	1	2	3	4

ID# _____

Date:_____

PedsQL™

Paediatric Quality of Life Inventory

Version 4.0 – UK English

YOUNG CHILD REPORT (ages **5-7**)

Instructions for interviewer:

I am going to ask you some questions about things that might be a problem for some children. I want to know how much of a problem any of these things might be for you.

Show the child the template and point to the responses as you read.

If it is <u>not at all</u> a problem for you, point to the smiling face.

If it is <u>sometimes</u> a problem for you, point to the middle face.

If it is a problem for you <u>a lot</u>, point to the frowning face.

I will read each question. Point to the pictures to show me how much of a problem it is for you. Let's try a practice one first.

	Not at all	Sometimes	A lot
Is it hard for you to click your fingers?	☺	😐	☹

Ask the child to demonstrate clicking his or her fingers to determine whether or not the question was answered correctly. Repeat the question if the child demonstrates a response that is different from his or her action.

Think about how you have been doing for the last few weeks. Please listen carefully to each sentence and tell me how much of a problem this is for you.

After reading the item, gesture to the template. If the child hesitates or does not seem to understand how to answer, read the response options while pointing at the faces.

PHYSICAL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Is it hard for you to walk?	0	2	4
2. Is it hard for you to run?	0	2	4
3. Is it hard for you to play sports or exercise?	0	2	4
4. Is it hard for you to lift big things?	0	2	4
5. Is it hard for you to have a bath or shower?	0	2	4
6. Is it hard for you to help in the home (like picking up your toys)?	0	2	4
7. Do you have aches and pains? (***Where?***_____)	0	2	4
8. Do you ever feel too tired to play?	0	2	4

Remember, tell me how much of a problem this has been for you for the last few weeks.

EMOTIONAL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Do you feel scared?	0	2	4
2. Do you feel sad?	0	2	4
3. Do you feel angry?	0	2	4
4. Do you have trouble sleeping?	0	2	4
5. Do you worry about what will happen to you?	0	2	4

SOCIAL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Do you have trouble getting on with other children?	0	2	4
2. Do other children say they do not want to play with you?	0	2	4
3. Do other children tease you?	0	2	4
4. Can other children do things you cannot do?	0	2	4
5. Is it hard for you to keep up when you play with other children?	0	2	4

SCHOOL FUNCTIONING *(problems with…)*	Not at all	Some-times	A lot
1. Is it hard for you to pay attention in school?	0	2	4
2. Do you forget things?	0	2	4
3. Do you have trouble keeping up with schoolwork?	0	2	4
4. Do you miss school because of not feeling well?	0	2	4
5. Do you miss school to go to the doctor or hospital?	0	2	4

Strength and Difficulties Questionnaire

Strengths and Difficulties Questionnaire P 2-4

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of the child's behaviour over the last six months.

Child's Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often argumentative with adults	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Can stop and think things out before acting	☐	☐	☐
Can be spiteful to others	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Do you have any other comments or concerns?

Please turn over - there are a few more questions on the other side

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes- minor difficulties	Yes- definite difficulties	Yes- severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

- How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

- Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

- Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

- Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Mother/Father/Other (please specify:)

Thank you very much for your help

Strengths and Difficulties Questionnaire P 4-17

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of the child's behaviour over the last six months.

Child's Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children (treats, toys, pencils etc.)	☐	☐	☐
Often has temper tantrums or hot tempers	☐	☐	☐
Rather solitary, tends to play alone	☐	☐	☐
Generally obedient, usually does what adults request	☐	☐	☐
Many worries, often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, down-hearted or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Thinks things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets on better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Sees tasks through to the end, good attention span	☐	☐	☐

Do you have any other comments or concerns?

Please turn over - there are a few more questions on the other side

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes- minor difficulties	Yes- definite difficulties	Yes- severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

- How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

- Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

- Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

- Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Signature ..... Date .....

Mother/Father/Other (please specify:)

Thank you very much for your help

Strengths and Difficulties Questionnaire S 11-17

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain or the item seems daft! Please give your answers on the basis of how things have been for you over the last six months.

Your Name ..... Male/Female

Date of Birth.....

	Not True	Somewhat True	Certainly True
I try to be nice to other people. I care about their feelings	☐	☐	☐
I am restless, I cannot stay still for long	☐	☐	☐
I get a lot of headaches, stomach-aches or sickness	☐	☐	☐
I usually share with others (food, games, pens etc.)	☐	☐	☐
I get very angry and often lose my temper	☐	☐	☐
I am usually on my own. I generally play alone or keep to myself	☐	☐	☐
I usually do as I am told	☐	☐	☐
I worry a lot	☐	☐	☐
I am helpful if someone is hurt, upset or feeling ill	☐	☐	☐
I am constantly fidgeting or squirming	☐	☐	☐
I have one good friend or more	☐	☐	☐
I fight a lot. I can make other people do what I want	☐	☐	☐
I am often unhappy, down-hearted or tearful	☐	☐	☐
Other people my age generally like me	☐	☐	☐
I am easily distracted, I find it difficult to concentrate	☐	☐	☐
I am nervous in new situations. I easily lose confidence	☐	☐	☐
I am kind to younger children	☐	☐	☐
I am often accused of lying or cheating	☐	☐	☐
Other children or young people pick on me or bully me	☐	☐	☐
I often volunteer to help others (parents, teachers, children)	☐	☐	☐
I think before I do things	☐	☐	☐
I take things that are not mine from home, school or elsewhere	☐	☐	☐
I get on better with adults than with people my own age	☐	☐	☐
I have many fears, I am easily scared	☐	☐	☐
I finish the work I'm doing. My attention is good	☐	☐	☐

Do you have any other comments or concerns?

Please turn over - there are a few more questions on the other side

Overall, do you think that you have difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get on with other people?

No	Yes-minor difficulties	Yes-definite difficulties	Yes-severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

- How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

- Do the difficulties upset or distress you?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

- Do the difficulties interfere with your everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

- Do the difficulties make it harder for those around you (family, friends, teachers, etc.)?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Your Signature .....

Today's Date .....

Thank you very much for your help

Strength and Difficulties STATA do file for scoring responses

```
recode pobeys (0=2) (1=1) (2=0) (else=.), gen(qobeys)
recode preflect (0=2) (1=1) (2=0) (else=.) , gen(qreflect)
recode pattends (0=2) (1=1) (2=0) (else=.), gen(qattends)
recode pfriend (0=2) (1=1) (2=0) (else=.), gen(qfriend)
recode ppopular (0=2) (1=1) (2=0) (else=.), gen(qpopular)

recode pdistres (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqdistres)
recode pimphome (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimphome)
recode pimpfrie (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimpfrie)
recode pimpclas (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimpclas)
recode pimpleis (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimpleis)

egen nemotion=robs(psomatic pworries punhappy pclingy pafraid)
egen pemotion=rmean(psomatic pworries punhappy pclingy pafraid) if nemotion>2
replace pemotion=round(pemotion*5)

egen nconduct=robs(ptantrum qobeys pfights plies psteals)
egen pconduct=rmean(ptantrum qobeys pfights plies psteals) if nconduct>2
replace pconduct=round(pconduct*5)

egen nhyper=robs(prestles pfidgety pdistrac qreflect qattends)
egen phyper=rmean(prestles pfidgety pdistrac qreflect qattends) if nhyper>2
replace phyper=round(phyper*5)

egen npeer=robs(ploner qfriend qpopular pbullied poldbest)
egen ppeer=rmean(ploner qfriend qpopular pbullied poldbest) if npeer>2
replace ppeer=round(ppeer*5)

egen nprosoc=robs(pconsid pshares pcaring pkind phelpout)
egen pprosoc=rmean(pconsid pshares pcaring pkind phelpout) if nprosoc>2
replace pprosoc=round(pprosoc*5)

egen nimpact=robs(pdistres pimphome pimpfrie pimpclas pimpleis)
gen pimpact=qqdistres+qqimphome+qqimpfrie+qqimpclas+qqimpleis if (nimpact!=0)
replace pimpact=0 if pebddiff==0

drop qobeys qreflect qattends qfriend qpopular qqdistres qqimphome qqimpfrie qqimpclas
qqimpleis nemotion nconduct nhyper npeer nprosoc nimpact

gen pebdtot=pemotion+pconduct+phyper+ppeer
label variable pemotion "Parent SDQ Emotion Score"
label variable pconduct "Parent SDQ Conduct Score"
label variable phyper "Parent SDQ Hyperactivity Score"
label variable ppeer "Parent SDQ Peer Score"
label variable pprosoc "Parent SDQ ProScoial Score"
label variable pimpact "Parent SDQ Impact Score"
label variable pebdtot "Parent SDQ Total Score"

*** Recoding variables and then scoring the parent SDQ scores 2-4 year olds
recode pobeys (0=2) (1=1) (2=0) (else=.), gen(qobeys)
```

```
recode preflect (0=2) (1=1) (2=0) (else=.) , gen(qreflect)
recode pattends (0=2) (1=1) (2=0) (else=.), gen(qattends)
recode pfriend (0=2) (1=1) (2=0) (else=.), gen(qfriend)
recode ppopular (0=2) (1=1) (2=0) (else=.), gen(qpopular)

recode pdistres (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqdistres)
recode pimphome (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimphome)
recode pimpfrie (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimpfrie)
recode pimpclas (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimpclas)
recode pimpleis (0=0) (1=0) (2=1) (3=2) (.=0), gen(qqimpleis)

egen nemotion=robs(psomatic pworries punhappy pclingy pafraid)
egen pemotion=rmean(psomatic pworries punhappy pclingy pafraid) if nemotion>2
replace pemotion=round(pemotion*5)

egen nconduct=robs(ptantrum qobeys pfights pargues pspite)
egen pconduct=rmean(ptantrum qobeys pfights pargues pspite) if nconduct>2
replace pconduct=round(pconduct*5)
```

Rosenberg Self Esteem Scale

NICHD SECCYD—Wisconsin

ROSENBERG SELF-ESTEEM SCALE

The next questions ask about your current feelings about yourself. For each of the following, please circle the number that corresponds with the answer that best describes how strongly you agree or disagree with the statement about yourself now.

	Strongly agree	Somewhat agree	Somewhat disagree	Strongly disagree
1. I feel that I am a person of worth, or at least on an equal plane with others.	1	2	3	4
2. I feel that I have a number of good qualities.	1	2	3	4
3. All in all, I'm inclined to feel that I am a failure.	1	2	3	4
4. I am able to do things as well as most other people.	1	2	3	4
5. I feel I do not have much to be proud of.	1	2	3	4
6. I take a positive attitude toward myself.	1	2	3	4
7. On the whole, I am satisfied with myself.	1	2	3	4
8. I certainly feel useless at times.	1	2	3	4
9. I wish I could have more respect for myself.	1	2	3	4
10. At times, I think I am no good at all.	1	2	3	4

Hospital and Anxiety Depression Screen

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:
Total score: Depression (D) _____ Anxiety (A) _____
0-7 = Normal
8-10 = Borderline abnormal (borderline case)
11-21 = Abnormal (case)

Short Form 36 version 2

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. *Thank you for completing this survey!*

For each of the following questions, please tick the one box that best describes your answer.

1. **In general, would you say your health is:**

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. **<u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?**

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. <u>Vigorous activities</u>, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b. <u>Moderate activities</u>, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c. Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d. Climbing <u>several</u> flights of stairs	☐ 1	☐ 2	☐ 3
e. Climbing <u>one</u> flight of stairs	☐ 1	☐ 2	☐ 3
f. Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g. Walking <u>more than a mile</u>	☐ 1	☐ 2	☐ 3
h. Walking <u>several hundred yards</u>	☐ 1	☐ 2	☐ 3
i. Walking <u>one hundred yards</u>	☐ 1	☐ 2	☐ 3
j. Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

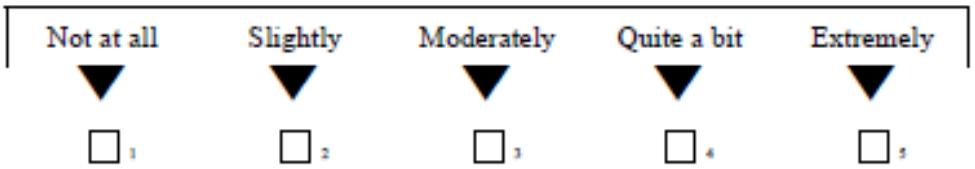
4. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Had difficulty performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

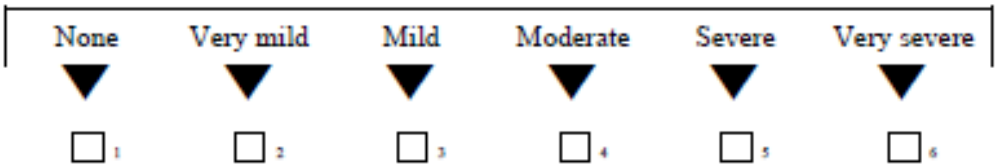
5. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

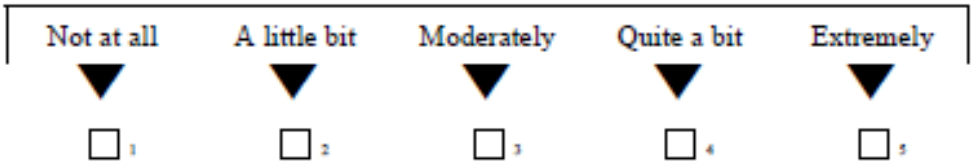
6. **During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?**



7. **How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?**



8. **During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?**



9. **These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Have you been very nervous?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. Have you felt downhearted and low?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g. Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h. Have you been happy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
i. Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

0. **During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?**

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a. I seem to get ill more easily than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

St George's Respiratory Questionnaire

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE ORIGINAL ENGLISH VERSION

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE (SGRQ)

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.

Before completing the rest of the questionnaire:

Please tick in one box to show how you describe your current health:

Very good	Good	Fair	Poor	Very poor
☐	☐	☐	☐	☐

P.W. Jones, PhD FRCP
Professor of Respiratory Medicine,
St. George's University of London,
Jenner Wing,
Cranmer Terrace,
London SW17 ORE, UK.

Tel. +44 (0) 20 8725 5371
Fax +44 (0) 20 8725 5955

continued…

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (✓) *one box for each question*:

	most days a week	several days a week	a few days a month	only with chest infections	not at all
1. Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2. Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3. Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4. Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) *one*:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick (✓) *one*:

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) *one*:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) *one*:

- No ☐
- Yes ☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please tick (✓) *one*:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes no problem ☐

If you have ever had paid employment.

Please tick (✓) *one*:

- My chest trouble made me stop work altogether ☐
- My chest trouble interferes with my work or made me change my work ☐
- My chest trouble does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

Please tick (✓) in *each box* that applies to you *these days*:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness <u>these days</u>.

	Please tick (✓) in *each box* that applies to you *these days*: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you <u>these days</u>.

	Please tick (✓) in *each box* that applies to you *these days*: True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

	Please tick (✓) in *each box* that applies to you *these days*: True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick (✓) in *each box* that applies to you *because of your breathing*:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest <u>usually</u> affects your daily life.

Please tick (✓) in *each box* that applies to you *because of your chest trouble*:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

continued…

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church, pub, club or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

.....

.....

.....

.....

Now would you tick in the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐

It stops me doing one or two things I would like to do ☐

It stops me doing most of the things I would like to do ☐

It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.

Appendix D – Presentations and publications pertaining to this thesis

Published abstracts

1. Bryan BA, Battersby A, Shillitoe BMJ, Barge D, Bourne H, Flood T, et al. Respiratory Health and Related Quality of Life in Patients with Congenital Agammaglobulinemia in the Northern Region of the UK. J Clin Immunol. 2016 Jul 18;36(5):472–9.
2. Shillitoe B, Gennery A. X-Linked Agammaglobulinaemia: Outcomes in the modern era. Clin Immunol. 2017 Oct;183:54–62.
3. Shillitoe BMJ, Gennery AR. An update on X-Linked agammaglobulinaemia. Curr Opin Allergy Clin Immunol. 2019 Dec;19(6):571–7.

Presentations

Oral – International

1. European Society for Immunodeficiencies meeting, Edinburgh, 11-14th September 2017. *The clinical health of XLA patients in northern England*
2. Inborn Errors Working Party (European Society for Blood and Marrow Transplantation) Meeting, London 11th-13th October 2019. *HSCT in XLA: Who benefits?*
3. European Society for Immunodeficiencies, Lisbon, 24-27th October 2019. *UK Outcomes in XLA*

Oral – Regional and national

1. Institute of Health and Society, Newcastle University, Applied epidemiology study day, Newcastle, 20th September 2017. *XLA Outcomes in Northern England.*
2. UCL Primary Immunodeficiency Winter School, Windsor, 19th-21st February 2018. *A case of enteropathy in XLA*

Poster -international

1. European Society for Immunodeficiencies, Lisbon, 24-27th October 2019. *Health related quality of life for UK XLA patients*

Poster – Regional and national

1. UK Primary Immunodeficiency Network Annual Meeting, Brighton, 7th-8th December. The clinical health of XLA patients in Northern England

Prizes

1. Best oral poster presentation. *XLA Outcomes in Northern England.* Institute of Health and Society, Newcastle University, applied epidemiology study day, September 2017
2. Best oral poster presentation. *The clinical health of XLA patients in northern England.* European Society for Immunodeficiencies, Edinburgh, September 2017