COVID-19 and X-linked agammaglobulinemia (XLA) – insights from a monogenic antibody deficiency

Authors:

Mark J Ponsford [† 1,2]

Ben MJ Shillitoe [† 3]

Ian R Humphreys [2,4]

Andrew R Gennery [χ 3,5]

Stephen Jolles [*,χ 1]

Affiliations

[1] Immunodeficiency Centre for Wales, University Hospital for Wales, Heath Park, Cardiff, UK

[2] Henry Wellcome Building, Division of Infection & Immunity, School of Medicine, Cardiff University, UK.

[3] Paediatric Immunology, Great North Children's Hospital, Newcastle upon Tyne, UK

[4] Systems Immunity Research Institute, School of Medicine, Cardiff University, Cardiff, UK.

[5] Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK

[†] are joint first authors

[χ] are joint senior authors

[*] **Corresponding author**: jollessr@cardiff.ac.uk

Keywords – X-linked agammaglobulinemia (XLA), Bruton's Tyrosine Kinase (BTK), Convalescent Plasma Therapy (CPT), Secondary Antibody Deficiency (SAD), Immunoglobulin Replacement Therapy (IgRT)

FOR SUBMISSION PURPOSES	ORCID ID	Email
Mark J Ponsford † [1,2]	0000-0002-0236-1059	Ponsfordm@Cardiff.ac.uk
Ben MJ Shillitoe † [3]	0000-0003-4407-5998	Benjamin.shillitoe@nhs.net
Ian R Humphreys [2,4]	0000-0002-9512-5337	HumphreysIR@cardiff.ac.uk
Andrew R Gennery * [3,5]	0000-0002-6218-1324	Andrew.gennery@ncl.ac.uk
Stephen Jolles * [1]	0000-0002-7394-6804	jollessr@cardiff.ac.uk

ABSTRACT

Purpose of review

The clinical outcomes from COVID-19 in monogenic causes of predominant antibody deficiency have pivotal implications for our understanding of the antiviral contribution of humoral immunity to protection from severe disease, persistent infection, and vaccine responses. This has wider implications in secondary antibody deficiency. This review summarizes outcomes from COVID-19 infection in X-linked agammaglobulinemia (XLA) due to genetic defects in Bruton's tyrosine kinase (BTK) and therapeutic inhibition of BTK alongside other B-cell ablative agents.

Recent findings

We summarise outcomes for 28 XLA patients with confirmed SARS-CoV-2 infection, with a crude mortality rate of 4%. Mean duration of SARS-CoV-2 detection was 38.3 days (95% CI: 20.3 to 56.2 days), consistent with the protracted infection course observed in secondary antibody-deficient populations. Individualised approaches have included convalescent plasma and monoclonal antibody therapy. Early experience with mRNA vaccinations suggests individuals with XLA can mount a viral-specific T-cell response, however the clinical significance remains uncertain.

Summary

XLA patients remain susceptible to severe disease or death. Persistent infection is likely to favour the emergence of novel viral variants. COVID-19 infection in antibody-deficient groups due to genetic, therapeutic or disease therefore warrants prompt recognition and specific interventions for both patient and societal benefit.

Introduction

Since its emergence in 2019, the novel pandemic coronavirus (SARS-CoV-2) causing COVID-19 has placed enormous pressure on health-care systems worldwide. Individuals with primary immunodeficiency (PID) appear at particularly elevated risk of mortality (1,2). The diagnosis of PID encompasses a heterogenous patient group however, with over 430 distinct monogenic disorders recognised to date, accompanied by a spectrum of immunological and clinical phenotypes (3). Analysis of young individuals hospitalised with COVID-19 has revealed inborn- and acquired- dysregulation of the innate type I interferon signalling pathway is highly enriched within those with risk of severe disease (4,5). However less is known about the impact of monogenic defects affecting other immune compartments.

XLA is a congenital antibody deficiency caused by mutations in Bruton's Tyrosine Kinase (BTK). Treatment consists of lifelong immunoglobulin replacement therapy (IgRT). It should be noted this treatment does not replace IgA or IgM, nor can current therapies compensate for the other lost roles of BTK outside of B-cell development. These limitations in therapy are being increasingly recognised, particularly in relation to ongoing risk of recurrent respiratory tract infections and the frequent development of end organ damage such as bronchiectasis.

Initial experience during the pandemic suggested that individuals with congenital forms of agammaglobulinaemia appeared to experience only asymptomatic or milder disease, relative to those with common variable immunodeficiency (CVID), but considered only 2 congenital agammaglobulinemia cases and 7 patients overall (6). We and others have recently described the heavy burden associated with persistent and symptomatic respiratory and gastro-intestinal tract infections in individuals with both XLA and CVID (7–10), suggesting a re-evaluation of this assessment as more reports became available. Here, we conduct a rapid review of the peer-reviewed and pre-print literature for the outcomes from COVID-19 in cases of congenital agammaglobulinaemia in relation to disease severity and persistence, and consider biological and epidemiological factors which may contribute to these findings. PubMed, MedRxiv, Medline and EMBASE were interrogated for articles published until June 30th 2021 with the search terms; "COVID-19", "SARS-CoV-2", "XLA", "Agammaglobulinaemia", "BTK" and "Bruton". Finally, given continued occurrence of new viral variants with enhanced transmissibility, we assess the evidence for T-cell responses to vaccination in this patient group. Together, the findings emphasise the unique balance between pathogen clearance and immunopathology with specific immunological defects, and highlight a need for greater focus on individualised approaches to protect and treat immunocompromised patients.

Viral infections in XLA

Whilst the predisposition to bacterial infection is well recognised in XLA there is in addition both consistent historical evidence of viral susceptibility before and after the advent of IgRT able to maintain stable levels of IgG well into the normal range. This includes enteroviral infection leading to chronic and potentially fatal enteroviral infections (chronic meningoencephalitis in agammaglobulinaemia (CEMA)), from Echovirus type 11 and Coxsackievirus B5 (11,12). The exact mechanism underlying this susceptibility in XLA is unknown, although it has been suggested the loss of BTK in TLR signalling may play a role (13). Severe herpesvirus infections are described prior to optimal IgRT and vaccine-associated paralytic poliomyelitis is reported (14). Live vaccinations are therefore contraindicated in XLA. Respiratory viruses occur more frequently in antibody deficiency and are prolonged with rhinoviral persistence described for 4 months in XLA (10). Chronic type 2 norovirus related gut failure necessitating parenteral nutrition and ultimately HSCT to cure the infection and the antibody deficiency has been recently reported (8). Taken together the findings suggest an important role for antibody in viral defence with the caveat regarding the other potential functions of BTK.

A role for therapeutic antibodies and BTK inhibition in COVID-19 infection?

Antibody responses are active in the early immune response to COVID-19, as demonstrated by detectable SARS-CoV-2 specific antibody responses within the first days to weeks of infection (15). Neutralising antibodies to viral spike protein are an important correlate of protective immunity following vaccination (16). Studies of antibody therapy in COVID-19 support the importance of early intervention for convalescent plasma (17), and whilst initial results from the 5795 patients allocated to convalescent plasma within the RECOVERY trial suggested this to be ineffective, subsequent reanalysis strongly suggests benefit in sero-negative individuals (18). In addition, 2 monoclonal antibodies targeting the receptor-binding domain of the SARS-CoV-2 spike protein reduce viral load (19), and offer clear benefit in reducing 28-day inpatient mortality amongst hospitalised sero-negative adults with COVID-19 (20). Importantly, this benefit was not seen when administered to patients with antibodies to SARS-CoV-2 (20). The results support a dose dependent reduction in viral load with clinical benefit only when given early and also benefit in seronegative over seropositive individuals (18).

B-cell aplasia and pan-hypogammaglobulinaemia are hallmarks of X-linked and autosomal recessive antibody deficiency (3) which mean that these patients will neither mount an antibody response to COVID-19 and nor are they likely to have anti-interferon antibodies - in contrast to autoimmune polyglandular syndrome type 1 (APS-1), a rare autoimmune disease that results from autosomal recessive mutations of the autoimmune regulatory (AIRE) gene. Instances of severe COVID-19 in individuals with APS-1 coinciding with pre-existing neutralising antibodies type I interferons stimulated the search and discovery of similar auto-antibodies in up to 10% of individuals with severe COVID-19 pneumonia (5).

As noted in Figure 1, BTK has wide ranging roles beyond B-cell development, and is expressed across haematopoietic lineages apart from T-cells, where it participates in Toll-like receptor (TLR) and NLRP3 inflammasome signalling (including thrombo-inflammation in platelets) (**Figure 1**) (21–23). It has been suggested that BTK deficiency may protect from the cytokine storm seen in severe disease based on the role of BTK in macrophage activation and IL-6 production (6,24–26), although murine and human studies have shown conflicting results (27). Recent studies of blood monocytes from general adult patients with severe COVID-19 showed consistent increases in BTK activation and production of interleukin-6 (28). Blockade of IL-6 using Tocilizumab has been shown also to improve survival in severe disease (29), and BTK inhibitors (such as ibrutinib and acalabrutinib) are now being studied for the treatment of severe COVID-19 following promise in case series (28,30). It is therefore plausible that agammaglobulinaemia or genetic BTK deficiency may confer protection from severe COVID-19.

Outcomes from COVID-19 in XLA patients

The clinical outcomes 28 XLA patients with SARS-CoV-2 infection identified from peer-reviewed and pre-print literature are summarised in **Table 1**. Age was reported in 23/28 individuals, with a median age at presentation of COVID-19 infection of 30.5 years (range: 5 to 55 years). Clinical presentations were available in 24 patients, with asymptomatic presentations uncommon (2/24, 8%). Fever was present in 83%, cough in 50%, and shortness of breath in 38%; a background of bronchiectasis was documented in 11 patients. Twenty-two (79%) were admitted to hospital, including 2 children (1,31). Supplemental oxygen was required in the majority of hospitalised patients (8/11, 73%), with evidence of elevated inflammatory markers. The median length of stay was 22 days (range: 11-73 days) with three patients (11%) admitted to an intensive case setting. A single death was reported, a 55 year old male (32), equating to an overall mortality rate of 4% (1/28). Therapies were defined in 17/21 hospitalised individuals, and included hydroxychloroquine (n=10), remdesivir (n=4), and corticosteroids (n=3). In contrast to the initial experience of Quinti et al (6), tociluzimab use was reported in 2 individuals with protracted hospital stays (39-56 days). Convalescent plasma therapy (CPT) was the most frequently reported therapeutic agent, employed in 11 patients (52%). Individual groups reported rapid recovery after CPT (31,33,34), including Buckland et al, where CPT was successful in achieving viral clearance after two independent courses of remdesivir had failed. Interestingly, remdesivir achieved symptomatic response and suppression of viral load, but its cessation was followed by recrudescence of symptoms and rise in viral load. Genomic sequencing indicated persistent infection rather than re-infection. Detailed longitudinal investigation of this patient confirmed the absence of viral-specific antibodies produced by the patient and within replacement immunoglobulin therapy. Additionally, complement activation similar to critically-ill COVID-19 admissions was demonstrated (34,35). Polyfunctional CD8 T-cell responses were also observed, comparable to those seen in healthcare workers, and these increased in magnitude during the course of COVID-19 infection (34). This case illustrates the power of a personalised experimental approach, and suggests that SARS-CoV-2 specific antibodies provide a non-redundant contribution to reduction in viral load and eventual viral clearance. The patient continued regular immunoglobulin replacement therapy (IgRT) throughout their admission, suggesting that clearance was not due to correction of defects of dendritic cell maturation arising in the absence of IgRT (34).

Overall, the small sample size in the XLA patient group reported to date, possible reporting bias, and high mortality in this age-range leaves us underpowered to address the hypothesis that individuals with XLA have a different inpatient mortality rate to the general population (**Table 2**). Mortality appears comparable to modelling of inpatient mortality for COVID-19 in the UK population of a similar age-band (36). Time until viral clearance was reported in 8/23 patients (35%) (**Figure 2).** Mean duration was 38.3 days (95% confidence interval: 20.3 to 56.2 days) and ranged from “6 to 14” days (handled as 10 days) to 64 days. By comparison, a recent meta-analysis estimated the mean duration of SARS-

CoV-2 shedding from the upper respiratory tract to be 17.0 days (95% CI: 15.5 to 18.6 days) and 17.2 days (95% CI: 14.4 to 20.1 days) from the lower respiratory tract, up to a maximum of 83 days (37). Similar observations of prolonged SARS-COV-2 infection in patients who have received Rituximab help triangulate the critical defect in XLA patients to the B-cell compartment (38,39). A case series of 17 B-cell–depleted patients with protracted COVID-19 were treated with CP at a median of 56 days from onset of symptoms. Prior to CPT, SARS-CoV-2 specific IFN-gamma producing T-cells were evident in all patients studied, accompanied by profound hypogammaglobulinaemia with absent neutralising antibody responses to SARS-CoV-2 (38). Similar to the XLA patient described by Buckland et al (34), symptomatic and inflammatory marker improvement occurred in all but 1 within 48 hours, followed by falling viral load and reduction in oxygen requirements (38,39). Together, this provides evidence for viral persistence of SARS-CoV-2 in patients with XLA and iatrogenic B-cell depletion and suggests a role for antibodies in reduction in viral load and contribution to viral clearance. The United States Food and Drug Administration reissued emergency use authorisation for high-titre COVID-19 CPT in hospitalized patients with impaired humoral immunity (39) in March 2021, however, a potential concern in immunosuppressed individuals was raised by Kemp et al (40). Performing phylogenetic analysis on ultra-deep whole genome sequential sequencing on samples from a chronically infected immunosuppressed individual with SARS-CoV-2, they showed CPT was accompanied by viral evolution and reduced sensitivity to neutralizing antibodies (40). Recent analysis of a second immunocompromised adult with hypogammaglobulinaemia and SARS-CoV-2 infection for over 290 days has subsequently demonstrated similar viral escape mutations can also arise independently of CPT (41). In this instance, following failure of remdesivir, treatment with the combination of casirivimab and imdevimab at day 265 achieved progressive resolution of all symptoms over the next 8 weeks, and clearance by RT-PCR was achieved by day 311 (41).

Vaccine responses to SARS-CoV-2 in XLA

We identified 2 reports assessing vaccine response following 2 doses of Pfizer-BioNTech in 10 individuals with genetically-confirmed XLA (42,43). As expected, these individuals mounted no detectable serological response, however robust production of IFNy was evident following stimulation with spike peptide using an *ex vivo* ELISpot approach in 9/10 individuals (and IL-2 production in 5/5). Stimulation with peptides representing SARS-CoV-2 membrane protein induced no cytokine responses, consistent with vaccine-induced cellular immunity over post-infection responses (42). The relevance of such T-cell assessments, as a reflection of nature and durability of cellular immunity and risk of future severe COVID-19, remain uncertain. The presence of polyfunctional CD8+ T-cells during prolonged infection, described by Buckland et al (34), does not exclude protection following vaccination. It is relevant to note both Hagin and Salinas et al identified a higher rate of vaccine-induced T-cell non-responders within genetically-undefined common variable immunodeficiency (CVID) patients, compared to XLA (42,43). Although patients were not age or gender matched, this difference in T-cell response mirrors differences in reported mortality between XLA and CVID (1,6), and suggests assessment of T-cell responses maybe relevant to ongoing risk stratification, **Figure 3**. The efficacy of alternative vaccination strategies, such as subunit or adenoviral vectors has yet to be examined. However, previous assessment of a trivalent inactivated influenza vaccine in 12 XLA patients showed induction of influenza virus-specific CD4 and CD8 T-cell responses comparable to healthy controls when assessed up to 6 months post-vaccination (44).

Conclusion

In this review we draw together the international experience of COVID-19 in XLA, representing the largest description of outcomes for this rare patient group to date, highlighting the vulnerability of such individuals to chronic SARS-CoV-2 infection. In contrast to reports made early in the pandemic, we show these patients remain susceptible to severe disease despite any immunomodulation mediated by their underlying selective immunodeficiency. Although low, the crude case fatality rate of 4% associated with acute COVID-19 in XLA should be viewed in the context of the young age of the cohort described, likelihood of reporting bias, and the high level of background lung disease (i.e. bronchiectasis). Protracted SARS-CoV-2 infection appeared common, with fever and respiratory symptoms in over 80% of cases at presentation, often accompanied by systemic inflammation and respiratory impairment. This is closely analogous to the marked symptom burden and impaired quality of life associated with persistent viral infections of the respiratory tract, gastro-intestinal, and central nervous system reported in this patient group (7,8,11,12,14). The psychosocial impact of protracted COVID-19 infection on these individuals and their families is likely multiplied given requirements to self-isolate accompanied by experiencing progressive debilitation due to declining respiratory function (41). We also highlight the far-reaching societal and clinical implications of chronic viral infection within primary and the expanding field of secondary predominant antibody deficiency disorders (45). Whilst viral reversion of attenuated vaccine strains during chronic infection in XLA can result in vaccine-associated paralytic poliomyelitis for the individual (14), evolution of novel highly-transmissible SARS-CoV-2 variants in hypogammaglobulinemic individuals poses a continued threat to vaccine-induced herd immunity (40,41). The significance of increasing titres of anti-SARS-CoV-2 antibody within pooled purified immunoglobulin replacement therapy (IgRT) products over time warrants further investigation, as they may complement T-cell induced vaccine responses. Caution is necessary however, given immune selection pressure from CPT or future IgRT may accelerate generation of escape variants (40). This suggests virological surveillance with immunological risk stratification should go hand in hand in order to recognise infection in this subset of vulnerable individuals and support a rapid individualised response.

The paradigm that XLA is simply an antibody deficiency is slowly being challenged. In particular, susceptibility to both severe and persistence viral infections is also being recognised. Beyond the lack of ongoing of IgA/IgM in IgRT, the wider roles of BTK that cannot be compensated for in XLA are likely to play a major role in the control of viral infections.

Summary

- **Individuals with XLA remain susceptible to severe disease or death following COVID-19.**
- **Protracted SARS-CoV-2 infection appears common in B-cell ablated and antibody-deficient individuals, and may favour emergence of novel viral variants.**
- **COVID-19 infection in antibody deficient groups due to genetic, therapeutic or disease therefore warrant specific interventions for both patient and societal benefit.**
- **Individualised therapeutic approaches include monoclonal anti-SARS-CoV-2 antibody cocktails.**
- **COVID-19 mRNA vaccination successfully elicit T-cell responses in XLA patients however the degree of clinical protection remains uncertain.**

Conflicts of Interest

SJ has participated in advisory boards, trials, projects, and has been a speaker with Baxalta, CSL Behring, Shire, Thermofisher, Swedish Orphan Biovitrum, Biotest, Binding Site, Grifols, BPL, Octapharma, LFB, GSK, Weatherden, Zarodex, Sanofi, and UCB Pharma. The remaining authors have no conflicts of interest to declare.

Author Contributions

SJ and AG conceived the study. All authors contributed to the literature review. MJP and BS wrote the first draft. Figure 1 was created by BS; MJP created Figures 2 and 3. All authors critically reviewed and approved the final manuscript

Financial Support and Sponsorship

MJP is supported by the Welsh Clinical Academic Training (WCAT) programme and a Career Development Award from the Association of Clinical Pathologists and is a participant in the NIH Graduate Partnership Program. IH is a Wellcome Trust Senior Research Fellow in Basic Biomedical Sciences. BJMS received funding from the Jeffrey Model Foundation and Bubble Foundation, UK. The funding sources did not have any role in designing the study, performing analysis or communicating findings.

Ethical approval

The data used in this work obtained relevant participant consent and ethical approval, therefore no additional approvals were required.

Acknowledgements

None

References

1. Meyts I, Bucciol G, Quinti I, Neven B, Fischer A, Seoane E, et al. Coronavirus Disease 2019 in patients with inborn errors of immunity: an international study. The Journal of allergy and clinical immunology. 2020;(September).

** International experience of the outcomes of 94 patients with IEI, demonstrating 30% with mild coronavirus disease 2019 (COVID-19) and relevance of risk factors predisposing to severe disease/mortality including within young patients.

2. Shields AM, Burns SO, Savic S, Richter AG, Anantharachagan A, Arumugakani G, et al. COVID-19 in patients with primary and secondary immunodeficiency: The United Kingdom experience. Journal of Allergy and Clinical Immunology. 2021;147(3):870-875.e1.

** UK experience of the outcomes of 67 individuals with primary and 33 with symptomatic secondary immunodeficiency. In comparison to the general population, adult patients with PID and symptomatic SID display greater morbidity and mortality from COVID-19.

3. Bousfiha A, Jeddane L, Picard C, Al-Herz W, Ailal F, Chatila T, et al. Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. Journal of Clinical Immunology. 2020 Jan 1;40(1):66–81.

4. Zhang, Qian, Bastard P, Liu Z, Le Pen J, Moncada-Velez M, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020;370(6515). Available from: https://science.sciencemag.org/content/370/6515/eabd4570

** Landmark study exploring genetic susceptibility to severe COVID-19 based on exome or genome sequencing from 1193 patients. At least 3.5% had genetic defects in loci involved in the TLR3- and IRF7-dependent induction and amplification of type I IFNs.

5. Bastard P, Rosen LB, Zhang Q, Michailidis E, Hoffmann HH, Zhang Y, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. Science. 2020 Oct 23;370(6515).

** Landmark study complementing Zhang et al, identifying individuals with high titres of neutralizing autoantibodies against type I IFN-α2 and IFN-ω in about 10% of patients with severe COVID-19 pneumonia, triggering subsequent use of plasma exchange as a rescue therapy.

6. Quinti I, Lougaris V, Milito C, Cinetto F, Pecoraro A, Mezzaroma I, et al. A possible role for B cells in COVID-19? Lesson from patients with agammaglobulinemia. Journal of Allergy and Clinical Immunology. 2020 Jul 1;146(1):211-213.e4.

* Early insights from COVID-19 outcomes in a case series of 4 CVID, 1 XLA, and 1 autosomal recessive agammaglobulinaemic individuals, suggesting the hypothesis that Btk/B-cell deficiency may confer protection from severe disease.

7. Ponsford M, 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. The Journal of Allergy and Clinical Immunology: In Practice. 2020 Aug 22;

* Prospective observation study following 44 patients with primary antibody deficiency (PAD) and 42 healthy controls (including 34 household pairs) over 12 months. Participants returned over 22,500 days of symptom scores and 1,496 nasal swabs to reveal the burden of viral infections (typically rhinovirus) and symptoms in PAD.

8. Shillitoe BMJ, Ponsford M, Slatter MA, Evans J, Struik S, Cosgrove M, et al. Haematopoietic Stem Cell Transplant for Norovirus-Induced Intestinal Failure in X-linked Agammaglobulinemia. Journal of Clinical Immunology. 2021 Jun 23; Available from: https://doi.org/10.1007/s10875-021-01088-2

* Describes cure of persistent severe chronic norovirus-associated enteropathy with intestinal failure in XLA, contributing to the growing evidence base that HSCT can correct XLA.

9. Sperlich JM, Grimbacher B, Workman S, Haque T, Seneviratne SL, Burns SO, et al. Respiratory Infections and Antibiotic Usage in Common Variable Immunodeficiency. The Journal of Allergy and Clinical Immunology: In Practice. 2018 Jan 1;6(1):159-168.e3.

10. Kainulainen L, Vuorinen T, Rantakokko-Jalava K, Österback R, Ruuskanen O. Recurrent and persistent respiratory tract viral infections in patients with primary hypogammaglobulinemia. Journal of Allergy and Clinical Immunology. 2010 Jul 1;126(1):120–6.

* Seminal description of prolonged respiratory-tract viral infections in 12 patients (2 XLA, 10 CVID), with notable demonstration of recurrent and persistent human rhinovirus infection.

11. Linnemann CC Jr, May DB, Schubert WK, Caraway CT, Schiff GM. Fatal Viral Encephalitis in Children With X-Linked Hypogammaglobulinemia. American Journal of Diseases of Children. 1973 Jul 1;126(1):100–3.

12. 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.

13. Sochorová K, Horváth R, Rozková D, Litzman J, Bartunková J, Sedivá A, et al. Impaired Toll-like receptor 8-mediated IL-6 and TNF-alpha production in antigen-presenting cells from patients with X-linked agammaglobulinemia. Blood. 2007 Mar 15;109(6):2553–6.

14. Shahmahmoodi S, Mamishi S, Aghamohammadi A, Aghazadeh N, Tabatabaie H, Gooya M. Vaccine-associated Paralytic Poliomyelitis in Immunodeficient Children, Iran, 1995–2008. Emerg Infect Dis. 2010;16(7):1133–6.

15. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: Current State of the Science. Immunity. 2020 Jun 16;52(6):910–41.

16. Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science. 2020 Jul 3;369(6499):77.

17. Libster R, Pérez Marc G, Wappner D, Coviello S, Bianchi A, Braem V, et al. Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. New England Journal of Medicine. 2021

* Early randomized, double-blind, placebo-controlled trial support for the utility of convalescent plasma therapy in older adult patients within 72 hours after the onset of mild Covid-19 symptoms.

18. Hamilton F, Lee T, Arnold D, Lilford R, Hemming K. Is convalescent plasma futile in COVID-19? A Bayesian re-analysis of the RECOVERY randomised controlled trial. International Journal of Infectious Diseases. 2021 [cited 2021 Jul 11]; Available from: https://doi.org/10.1016/j.ijid.2021.06.034

19. Weinreich DM, Sivapalasingam S, Norton T, Ali S, Gao H, Bhore R, et al. REGN-COV2, a neutralizing antibody cocktail, in outpatients with covid-19. New England Journal of Medicine. 2021;384(3):238–51.

20. Horby, W P, Mafham M, Peto L, Campbell M, Pessoa-Amorim G, et al. Casirivimab and imdevimab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. medRxiv pre-print server. 2021; Available from: https://www.medrxiv.org/content/early/2021/06/16/2021.06.15.21258542

* Randomised controlled trial evidence that REGENERON (**REGN-COV2)** monoclonal antibody cocktail reduces mortality when administered to seronegative individuals hospitalised with acute COVID-19.

21. Brunner C, Müller B, Wirth T. Bruton's Tyrosine Kinase is involved in innate and adaptive immunity. Histol Histopathol. 2005 Jul;20(3):945–55.

22. 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.

23. Murthy P, Durco F, Miller-Ocuin JL, Takedai T, Shankar S, Liang X, et al. The NLRP3 inflammasome and bruton's tyrosine kinase in platelets co-regulate platelet activation, aggregation, and in vitro thrombus formation. Biochem Biophys Res Commun. 2017 Jan 29;483(1):230–6.

24. Soresina A, Moratto D, Chiarini M, Paolillo C, Baresi G, Focà E, et al. Two X-linked agammaglobulinemia patients develop pneumonia as COVID-19 manifestation but recover. Pediatric Allergy and Immunology. 2020;31(5):565–9.

25. Cunningham-Rundles C. Human B cell defects in perspective. Vol. 54, Immunologic Research. Immunol Res; 2012. p. 227–32.

26. Lougaris V, Baronio M, Vitali M, Tampella G, Cattalini M, Tassone L, et al. Bruton tyrosine kinase mediates TLR9-dependent human dendritic cell activation. Journal of Allergy and Clinical Immunology. 2014 Jun;133(6):1644-1650.e4.

27. Marron TU, Martinez-Gallo M, Yu JE, Cunningham-Rundles C, York N, Spain B. Toll-like receptor 4-, 7-, and 8-activated myeloid cells from patients with X-linked agammaglobulinemia produce enhanced inflammatory cytokines. Journal of Allergy and Clinical Immunology. 2012;129:184-190.e4.

28. Roschewski M, Lionakis MS, Sharman JP, Roswarski J, Goy A, Monticelli MA, et al. Inhibition of Bruton tyrosine kinase in patients with severe COVID-19. Science Immunology. 2020;5(48):eabd0110.

** Monocytes from patients with severe COVID-19 show BTK activation and enhanced IL-6, relative to healthy controls. Non-randomised BTK-inhibition with acalabrutinib in 19 patients showed improvement of systemic inflammation, prompting larger randomised trial evaluation of this approach.

29. Abani O, Abbas A, Abbas F, Abbas M, Abbasi S, Abbass H, et al. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. The Lancet. 2021 May 1;397(10285):1637–45.

30. Stack M, Sacco K, Castagnoli R, Livinski AA, Notarangelo LD, Lionakis MS. BTK inhibitors for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): A Systematic Review. Res Sq. 2021 Mar 22;rs.3.rs-319342.

* Systematic review of observational studies reporting outcomes of off-label Btk-inhibitor use in COVID-19 to date.

31. Jin H, Reed JC, Liu STH, Ho H en, Lopes JP, Ramsey NB, et al. Three patients with X-linked agammaglobulinemia hospitalized for COVID-19 improved with convalescent plasma. Journal of Allergy and Clinical Immunology: In Practice. 2020 Nov 1;8(10):3594-3596.e3.

32. Loh SY, Bassett J, Hoodless EJ, Walshaw M. Possible COVID-19 reinfection in a patient with X-linked agammaglobulinaemia. BMJ Case Reports. 2021 Mar 4;14(3):240765.

33. Iaboni A, Wong N, Betschel SD. A Patient with X-Linked Agammaglobulinemia and COVID-19 Infection Treated with Remdesivir and Convalescent Plasma. Journal of Clinical Immunology. 2021;(day 5):8–10.

34. Buckland MS, Galloway JB, Fhogartaigh CN, Meredith L, Provine NM, Bloor S, et al. Treatment of COVID-19 with remdesivir in the absence of humoral immunity: a case report. Nature Communications. 2020;11(1).

** Deep phenotyping in protracted SARS-CoV-2 infection in a case of XLA demonstrates complement and T-cell activation independent of virus-specific antibody response. Following 2 independent trials of the antiviral remdesivir, convalescent plasma therapy is followed by viral clearance.

35. Zelek WM, Cole J, Ponsford MJ, Harrison RA, Schroeder BE, Webb N, et al. Complement Inhibition with the C5 Blocker LFG316 in Severe COVID-19. Am J Respir Crit Care Med. 2020 Sep 8;

36. Verity R, Okell LC, Dorigatti I, Winskill P, Whittaker C, Imai N, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. The Lancet Infectious Diseases. 2020;20(6):669–77.

37. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. The Lancet Microbe. 2021 Jan 1;2(1):e13–22.

38. Hueso T, Pouderoux C, Péré H, Beaumont A-L, Raillon L-A, Ader F, et al. Convalescent plasma therapy for B-cell–depleted patients with protracted COVID-19. Blood. 2020 Nov 12;136(20):2290–5.

* Observational study suggesting convalescent plasma therapy appears of clinical benefit in selected individuals with B-cell depletion and variable hypogammaglobulinaemia post-Rituximab.

39. Aviv R, Weber A, Anzum T, Federbush M, Horowitz D, Singas E. Prolonged Coronavirus Disease 2019 in a Patient With Rheumatoid Arthritis on Rituximab Therapy. The Journal of Infectious Diseases [Internet]. 2021 May 8 [cited 2021 Jul 7];(jiab248). Available from: https://doi.org/10.1093/infdis/jiab248

40. Kemp SA, Collier DA, Datir RP, Ferreira IATM, Gayed S, Jahun A, et al. SARS-CoV-2 evolution during treatment of chronic infection. Nature. 2021 Apr 1;592(7853):277–82.

** Case report of prolonged SARS-CoV-2 infection up to 101 days in individual with combined immunodeficiency following past therapy for haematological malignancy. Treatment with convalescent plasma failed to clear infection and was associated with large shifts in the viral populating including emergence of a spike deletion mutant with reduced susceptibility to neutralising antibodies.

41. Williamson MK, Hamilton F, Hutchings S, Pymont HM, Hackett M, Arnold D, et al. Chronic SARS-CoV-2 infection and viral evolution in a hypogammaglobulinaemic individual. medRxiv. 2021 Jan 1;2021.05.31.21257591.

****** Case report of prolonged SARS-CoV-2 infection up to 311 days, with evidence of shifts in the viral populating including emergence of a spike deletion mutant in the absence of convalescent plasma therapy. REGENERON (**REGN-COV2)** monoclonal antibody cocktail administration preceded viral clearance.

42. Hagin D, Freund T, Navon M, Halperin T, Adir D, Marom R, et al. Immunogenicity of pfizer-biontech COVID-19 vaccine in patients with inborn errors of immunity. The Journal of allergy and clinical immunology. 2021 Jun; Available from: https://europepmc.org/articles/PMC8168345

** Early experience of immune correlates of SARS-COV-2 mRNA vaccination in 26 immunodeficient individuals, including 4 XLA cases.

43. Salinas AF, Mortari EP, Terreri S, Quintarelli C, Pulvirenti F, Cecca SD, et al. SARS-CoV-2 vaccine induced atypical immune responses in antibody defects: everybody does their best. medRxiv; 2021. Available from: https://doi.org/10.1101/2021.06.24.21259130

* Rapid assessment of humoral and ELISpot T-cell responses 1 week following 2nd dose mRNA vaccination in 41 CVID, 6 XLA, and 28 healthy age-matched controls.

44. Liu Y, Yuet W, Tu W, Lau Y-L. Dendritic cell function and T cell responses to influenza viruses in patients with X-linked agammaglobulinemia (106.12). J Immunol. 2011 Apr 1;186(1 Supplement):106.12.

45. Patel SY, Carbone J, Jolles S. The expanding field of secondary antibody deficiency: Causes, diagnosis, and management. Frontiers in Immunology. 2019;10:33.

46. Devassikutty FM, Jain A, Edavazhippurath A, Joseph MC, Peedikayil MMT, Scaria V, et al. X-linked agammaglobulinemia and COVID-19: Two case reports and review of literature.

47. Milošević I, Jovanović J, Stevanović O. Atypical course of COVID-19 in patient with Bruton agammaglobulinemia. Journal of Infection in Developing Countries. 2020;14(11):1248–51.

48. Marcus N, Frizinsky S, Hagin D, Ovadia A, Hanna S, Farkash M, et al. Minor Clinical Impact of COVID-19 Pandemic on Patients With Primary Immunodeficiency in Israel. Frontiers in Immunology. 2021;11(January):1–9.

49. Guetl K, Moazedi-Fuerst F, Rosskopf K, Brodmann M, Krause R, Eller P, et al. SARS-CoV-2 positive virus culture 7 weeks after onset of COVID-19 in an immunocompromised patient suffering from X chromosome-linked agammaglobulinemia. Journal of Infection. 2021 Mar;82(3):414–51.

50. Mira E, Yarce OA, Ortega C, Fernández S, Pascual NM, Gómez C, et al. Rapid recovery of a SARS-CoV-2–infected X-linked agammaglobulinemia patient after infusion of COVID-19 convalescent plasma. Journal of Allergy and Clinical Immunology: In Practice. 2020;8(8):2793–5.

51. Hovey JG, Tolbert D, Howell D. Burton's Agammaglobulinemia and COVID-19. Cureus. 2020 Nov 25;12(11).

52. Almontasheri A, Al-Husayni F, Alsuraihi AK, Binyahib H, Albanna AS. The clinical course of COVID-19 pneumonia in a 19-year-old man on intravenous immunoglobulin replacement therapy for X-linked agammaglobulinemia. American Journal of Case Reports. 2021;22(1):1–5.

53. Weber ANR, Bittner Z, Liu X, Dang T-M, Radsak MP, Brunner C. Bruton's tyrosine kinase: An emerging key player in innate immunity. Frontiers in Immunology. 2017;8:1454.

Table 1*:* A summary of the reported cases of SARS-CoV-2 infection in XLA patients.

NA = Data not available. *Admitted for infection control measures only.

Paper	Age (years)	Bronchiectasis	Presentation	Peak CRP (mg/L)	Peak Ferritin (ng/mL)	Hospital admission	Oxygen	ICU	Therapies	Length of stay (days)	Virus detection duration (days)
Devassikutty et al., (46)	18	Yes	Fever, myalgia,loss of taste/smell	11.9	NA	Yes*	No	No	None	17*	17
	19	Yes	Fever, cough	NA	NA	Yes	Yes	Yes	None	22	NA
Milosevic et al., (47)	34	Yes	Fever	205.8	493	Yes	Yes	No	IVIG, chloroquine, nadroparin, tocilizumab, convalescent plasma, steroids	56	52
Meyts et al., (1)	3-12	No	Fever, cough, shortness of breath, chest pain	NA	NA	Yes	Yes	No	Remdesivir, convalescent plasma, enoxaparin	NA	NA
	19-24	Yes	Fever, cough, shortness of breath	NA	NA	Yes	No	No	Chloroquine, convalescent plasma, enoxaparin	NA	NA
	25-34	No	Cough, fever	NA	NA	Yes	No	No	Steroids, Immunoglobulin, chloroquine	NA	64
	35-44	No	Cough, fever	NA	NA	Yes	No	No	Chloroquine, lopinavir, ritonavir	NA	6-14

Paper	Age (years)	Bronchiectasis	Presentation	Peak CRP (mg/L)	Peak Ferritin (ng/mL)	Hospital admission	Oxygen	ICU	Therapies	Length of stay (days)	Virus detection duration (days)
	45-54	Yes	Asymptomatic	NA	NA	No	No	No	None	0	NA
	45-54	Yes	Fever	NA	NA	Yes	No	No	None	NA	NA
Shields et al., (2)	Median age 30.5	NA	NA	NA	NA	Yes	NA	NA	NA	NA	NA
		NA	NA	NA	NA	No	No	No	NA	0	NA
		NA	NA	NA	NA	No	No	No	NA	0	NA
		NA	NA	NA	NA	No	No	No	NA	0	NA
Quinti et al., (6)	34	NA	Fever	NA	NA	Yes	No	No	Hydroxychloroquine, lopinavir, ritonavir	NA	NA
Marcus et al., (48)	6	No	Fever	NA	NA	No	No	No	None	0	NA
	5	No	Asymptomatic	NA	NA	No	No	No	None	0	NA
Geutl et al., (49)		Yes	Fever, fatigue	NA	NA	Yes	Yes	Yes	Hydroxychloroquine, lopinavir, ritonavir, IVIG, convalescent plasma, tocilizumab	39	49-70 days
Jin et al., (31)	10	No	Fever, cough, chest pain	22.4	642	Yes	Yes	No	Remdesivir, IVIG, Convalescent plasma,	29	25
	24	No	Fever, cough, shortness of beath	64	185	Yes	Yes	No	Convalescent plasma	19	NA

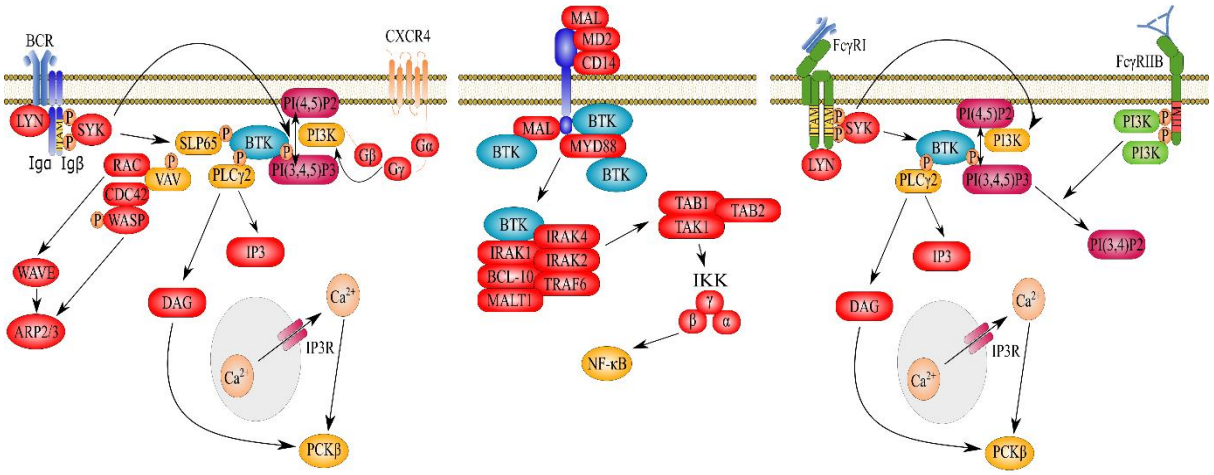
Paper	Age (years)	Bronchiectasis	Presentation	Peak CRP (mg/L)	Peak Ferritin (ng/mL)	Hospital admission	Oxygen	ICU	Therapies	Length of stay (days)	Virus detection duration (days)
	40	Yes	Fatigue, fever, cough, respiratory distress	16.4	967	Yes	Yes	No	Convalescent plasma	45	NA
Soresina et al., (24)	34	No	Fever, cough	78	269	Yes	No	No	Hydroxychloroquine, lopinavir, ritonavir	22	NA
	26	No	Vomiting, asthenia, fever	3.6	774	Yes	No	No	Hydroxychloroquine		
Mira et al., (50)	39	No	Cough, respiratory distress, fever	NA	NA	Yes	No	No	Hydroxychloroquine, IVIG, Convalescent plasma	30	25
Hovey et al., (51)	26	No	Fever, respiratory distress, diarrhoea	12.86	1324.3	Yes	No	No	IVIG, convalescent plasma	14	NA
Loh et al., (32)	55	Yes	Fever, respiratory distress	258	NA	Yes	Yes	No	Steroids, IVIG	Died	NA
Iaboni et al., (33)	28	No	Respiratory distress	127	>1500	Yes	Yes	Yes	Remdesivir, convalescent plasma	13	NA
Buckland et al., (34)	31	Yes	Fever, cough, diarrhoea, vomiting	NA	1063.5	Yes	Yes	No	Hydroxychloroquine, Remdesivir, convalescent plasma	73	64

Paper	Age (years)	Bronchiectasis	Presentation	Peak CRP (mg/L)	Peak Ferritin (ng/mL)	Hospital admission	Oxygen	ICU	Therapies	Length of stay (days)	Virus detection duration (days)
Almontasheri et al., (52)	19	Yes	Fever, respiratory distress, diarrhoea	47.6	58	Yes	No	No	IVIG	11	NA

Table 2: Age stratified mortality for individuals with Primary Immunodeficiency, X-linked Agammaglobulinemia, and the general UK population.
Table adapted from Shields et al, (2). IFR, infection fatality rate- proportion of mortality in all suspected or proven infected SARS-CoV-2 infections; CFR, case fatality rate- proportion of deaths from all confirmed infections.

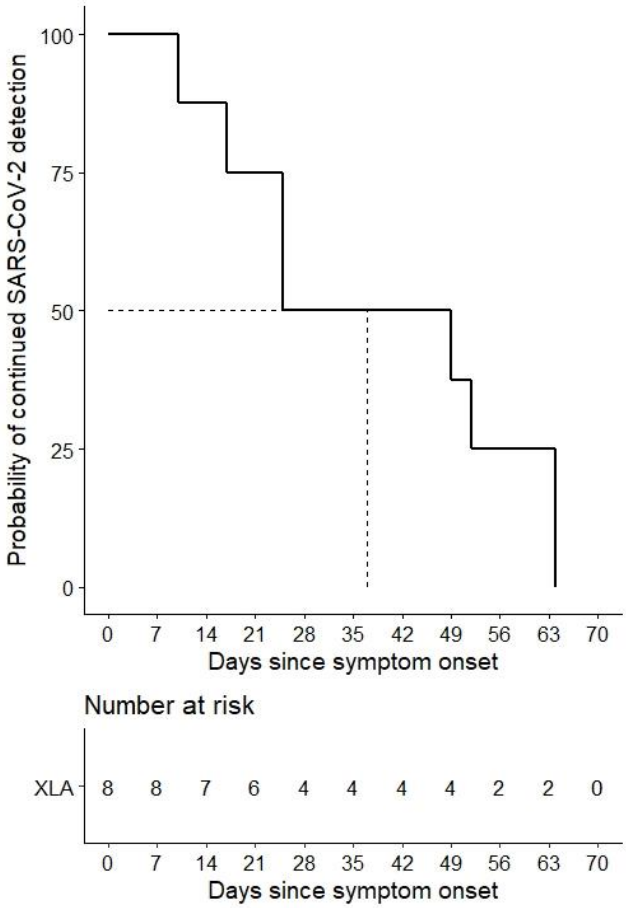
	Primary Immunodeficiency, all diagnoses (United Kingdom, UK (2)**)**								**X-linked agammaglobulinaemic (International)**					**General Population, (United Kingdom,** (36)**)**	
Age group (years)	**Patients, n**	**PCR$^+$, n**	**Hospitalized, n (%)**	**Deaths, n**	**%**	**IFR (%)**	**CFR (%)**	**Inpatient mortality (%)**	**XLA, n**	**Hospitalised, n (%)**	**Deaths, n**	**CFR (%)**	**Inpatient mortality (%)**	**UK IFR (general population), %**	**UK inpatient mortality (general population), %**
0-9	2	2	1 (50%)	0	0	0	0	0	3	1 (33%)	0	0	0	0.001	0.7
10-19	1	0	0 (0%)	0	0	0	0	NA	4	4 (100%)	0	0	0	0.007	1.9
20-29	12	5	3 (25%)	1	8%	8%	20	33.3	7	7 (100%)	0	0	0	0.03	4.3
30-39	12	7	6 (50%)	0	0	0	0	0	9	6 (67%)	0	0	0	0.08	4.2
40-49	9	5	4 (44%)	1	8%	11.1	20	25	3	2 (67%)	0	0	0	0.16	6.3
50-59	11	7	7 (64%)	4	33%	36.4	57.1	57.1	1	1 (100%)	1	100.0	100.0	0.6	10.8
60-69	3	2	2 (67%)	1	8.3	33.3	50	50	0	-	-	-	-	1.93	20.2
70-79	6	6	5 (83%)	2	16.7	16.7	16.7	40	0	-	-	-	-	4.28	34.1
>80	4	4	4 (100%)	3	25	75	75	75	0	-	-	-	-	7.8	41.7

Figure 1: The wider roles of BTK in downstream signalling



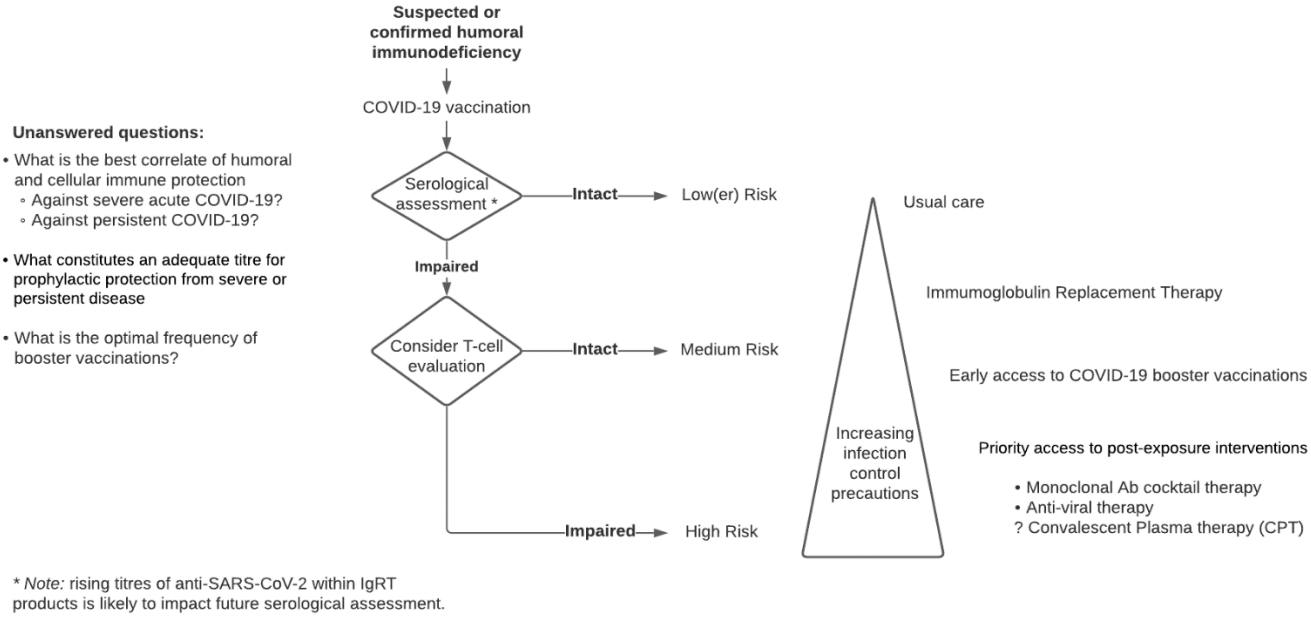
BTK is widely expressed including in neutrophils, natural killer cells, dendritic cells, macrophages, and monocytes of myeloid origin. The wider functions of BTK are shown with involvement in downstream signalling of not only the B cell receptor (BCR) in B-lymphocytes but also the i) chemokine receptor (CXCR4) activating PI3K and subsequently BTK, AKT and MAP-K dependent pathways in B cells, ii) Toll Like receptors (TLRs) recruiting TIR, MYD88, IRAK1, TIRAP/MAL activating NF-κB, in macrophages, basophils and dendritic cells and iii) the activating Fcγ receptor (FcγR1), activating Src-kinases, SYK, PI3K-γ and BTK in mast cells and basophils. There is increasing evidence for involvement of BTK in roles beyond B cell signalling in phagocytosis, the NLRP3 inflammasome (including thrombo-inflammation in platelets, (23)), microbe recognition via TLRs, nucleic acid recognition, maturation, differentiation signals and chemotaxis (53).

Figure 2: SARS-CoV-2 persistence in XLA patients.



Kaplan Maier plot showing time until SARS-CoV-2 no longer detectable by respiratory tract RT-PCR sampling in 8 XLA patients. Created using R version 4.0.2 in RStudio (version 1.3.959, R Foundation, Vienna, Austria) using the survival and survminer packages.

Figure 3: Putative role of vaccine response profiling in risk stratification to individualise approaches to SARS-CoV-2 in individuals with suspected humoral immunodeficiency



* *Note:* rising titres of anti-SARS-CoV-2 within IgRT products is likely to impact future serological assessment.