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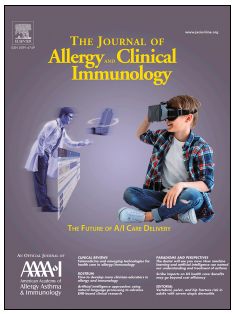

Long term follow-up of 168 patients with X-linked agammaglobulinemia reveals increased morbidity and mortality

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Title page

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Abstract

Background: X-linked agammaglobulinemia (XLA) is the prototype of primary humoral immunodeficiencies. Long-term follow-up studies regarding disease-related complications and outcome are scarse.

Objective: To describe the natural history of X-linked agammaglobulinemia.

Methods: A nationwide multicenter study based on the IPINet registry was established in 2000 in Italy. Affected patients were enrolled by documenting centers and patients' laboratory, clinical and imaging data were recorded on an annual base.

Results: Patients' data (N=168) derived from a cumulative follow-up of 1370 patient years with a mean follow-up of 8.35 years per patient. Mean age at diagnosis decreased upon the establishment of the IPINet registry (84 months before versus 23 months after). Respiratory, skin and gastrointestinal manifestations were the most frequent clinical symptoms at diagnosis and during long-term follow-up. Regular immunoglobulin replacement treatment reduced the incidence of invasive infections. Affected patients developed chronic lung disease over time (47% after 40 years of follow-up) in the presence of chronic sinusitis (84%). Malignancies were documented in a minority of cases (3.7%). Overall survival for affected patients was significantly reduced when compared to the healthy male Italian population, and further deteriorated in the presence of chronic lung disease.

Conclusions. This is the first detailed long-term follow-up study for XLA patients revealing that while immunoglobulin replacement treatment reduces the incidence of invasive infections, it does not appear to influence the development of chronic lung disease. Overall survival of affected patients is reduced. Further studies are warranted in order to improve patients' clinical management and increase awareness among physicians.

Key messages

- Patients affected with X-linked agammaglobulinemia suffer from respiratory, cutaneous and gastrointestinal complications
- Despite regular immunoglobulin replacement treatment, affected patients tend to develop chronic lung disease
- Overall survival of affected patients is significantly reduced when compared to healthy male subjects

Capsule summary: This is the first real-life study involving patients with X-linked agammaglobulinemia revealing important co-morbidities and significantly reduced overall survival during long-term follow-up, suggesting that patients' current clinical management may necessitate of additional measures.

Keywords: X-linked agammaglobulinemia; Bruton's tyrosin kinase; chronic lung disease

Abbreviations

XLA: X-linked agammaglobulinemia

BTK: Bruton's tyrosine kinase

IPINet: Italian Primary Immunodeficiency Network

CLD: Chronic Lung Disease

Introduction

X-linked agammaglobulinemia (XLA; Bruton type agammaglobulinemia) (OMIM 300755) is a rare form of primary immunodeficiency with an X-recessive pattern of transmission that affects males and is characterized by severe reduction of peripheral B cells (less than 2%), severely reduced immunoglobulin serum levels of all classes and lack of recall humoral response to antigens [1]. Ogden Carr Bruton, a pediatrician, described the first case of XLA in 1952 [2]. In 1993, two separate groups identified the genetic cause of XLA in monoallelic mutations in BTK (Bruton's tyrosine kinase) which encodes for a kinase essential for early B cell development [3, 4]. Since then, more than 500 different mutations in BTK have been identified (http://structure.bmc.lu.se/idbase/) [5]. In recent decades, the prevalence of XLA is estimated to be approximately 1 case per 250.000 live births. [6-10] One third of cases are familial, while the remaining two thirds of cases are believed to arise from new mutations. Clinical onset of affected patients is typically in early childhood, frequently in the first year of life, when maternal antibodies wane and the patients fail to produce their own immunoglobulins [7,11-18]. The spectrum of infectious manifestations is quite wide and may include respiratory infections of the upper and lower tract, gastrointestinal infections, as well as invasive infections such as sepsis and meningitis. Pathogens involved are mainly encapsulated bacteria, but viruses and parasites may complicate the clinical course of the disease as well [7,11-18]. Once diagnosis is made, immunoglobulin replacement treatment has been shown to be effective, at least in part, in reducing the incidence and frequency of the infectious episodes in affected patients [7,11-19]. Nonetheless, one of the major complications for patients with XLA is the high risk of developing bronchiectasis and chronic lung disease [7,11-13,19].

Although XLA is known for more than 6 decades, data in the literature regarding long term follow-up of affected patients are lacking, rendering thus the natural history of this disease still not well characterized. In this study, we collected data on the clinical presentation, treatment, and follow-up

of a large cohort of patients with XLA in order to better define the natural history, the real-life overall survival and long-term associated co-morbidities.

Methods

Patients' data

Patients' data were collected from the online database of the IPINet (Italian primary immunodeficiency network) registry (https://www.aipeop.org/web/). Sixty medical centers, members of the IPINet, documented data for XLA patients followed at their clinic at diagnosis and at least once for every year of follow-up. Enrollment data included the patient's personal data, family pedigree, date of diagnosis, immunologic data, clinical manifestations and treatment at diagnosis and during follow-up. A questionnaire including relevant clinical and immunological features was compiled annually upon enrollment. This collective effort was initiated in 2000. Data included in this study were collected for the period 2000-2017. Cumulative follow-up data for 1370 patient-years were collected with a mean follow-up of 8.35 years per patient.

Flow cytometric evaluation of peripheral T and B lymphocytes was performed at diagnosis by using monoclonal antibodies (anti-CD3, anti-CD4, anti-CD8 and anti-CD19 or anti-CD20). Patients with a suspected diagnosis of XLA were analyzed for BTK mutations by Sanger sequencing or next generation sequencing (NGS). Evaluation of BTK expression was performed in a limited number of patients.

Statistical analysis

Comparisons between patients and healthy subjects were performed using Student's t-test. Significance threshold was set at $p<0.05$. The Prism GraphPad software (version 8) was used for statistical analysis (GraphPad Software Inc., La Jolla, CA, USA) (* $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$).

Results

Patient cohort

One hundred and sixty eight male patients affected with XLA were included in the study. Mean age at diagnosis was 66 months (median 36 months; range 1-592 months) with an evident descending trend in recent years (Figure 1A). In particular, while the mean age at diagnosis for patients born before 2000 (118/168) was 84 months, the mean age at diagnosis for patients born after the year 2000 (50/168) resulted significantly lower: 23 months, underlining the role of the IPINet registry and related activities in increasing awareness for XLA among physicians and specialists. Positive family history for XLA was identified in 66/168 patients (39.3%), while the remaining 102/168 patients (60.7%) were sporadic cases. No significant differences were observed for mean age at diagnosis between the two groups (familial: 64 months versus sporadic: 67 months).

Immunoglobulin serum levels at diagnosis

Immunoglobulin serum levels were low for all classes with the following mean values: IgG: 191.4 mg/dl; IgA: 10.2 mg/dl; IgM: 15.2 mg/dl (Figure 1B, 1C and 1D respectively). A small percentage of patients (21.4%) (36/168 cases) presented with at least one Ig class within normal range for age (Figure 1B, 1C, 1D and Online Repository Table E1). Seventeen out of these thirty six patients showed normal IgG serum values for age. Of these, eight had an age at diagnosis below or equal to 12 months, suggesting that the IgGs were most likely of maternal origin. Seven out of these eight patients also had a positive family history for XLA. The single patient without a positive family history under the age of 12 months presented with undetectable IgA, IgM and peripheral B cells (0%) (Online Repository Table E1). The remaining nine patients with IgG serum levels within normal range for age showed a positive family history for XLA in 4/9 cases (44.4%) and low peripheral B cells (≤2%) in 7/9 cases (77.8%) (Online Repository Table E1). One of these nine patients had normal IgG and peripheral B cells (9%), but low IgA and IgM serum levels for age with a positive family history for XLA (Online Repository Table E1). The remaining nineteen

patients showed low IgG for age with either IgA or IgM within normal range (Online Repository Table E1). All nineteen patients showed low peripheral B cells (≤2%); seven out of nineteen patients had a positive family history for XLA (Online Repository Table E1).

Lymphocyte subsets at presentation

Peripheral lymphocyte subset evaluation was performed at diagnosis and showed normal $CD3^+$ T cell percentages in all patients ($CD3^+$ cells: 84.9%, mean value), with conserved $CD8^+$ and $CD4^+$ T cell distribution ($CD8^+$: 30.54%; $CD4^+$: 52.1%, mean values) (Figure 1E). Peripheral B cells were below 2% in the majority of cases (147/168 cases; 87.5%) ($CD19^+$ B cells: 0.58%; mean value)**.** Twenty one patients presented with ≥ 2% of peripheral B cells (Figure 1E and Online Repository Table E2). Eleven out of these twenty one patients had a positive family history for XLA. Thirteen out of these twenty one patients showed reduction of all three Ig classes, while seven showed reduction of one out of three Ig classes; one patient with two out of three Ig classes within normal range for age displayed a positive family history for XLA (Online Repository Table E2). Thirteen out of twenty one patients presented with 2% of peripheral B cells, which is the classical cut-off for XLA suspicion, with a positive family history for XLA in six cases; eight out of thirteen patients had all Ig classes below normal range for age. The remaining 8 patients with >2% of peripheral B cells exhibited a positive family history in five out of eight cases; only three out of these eight patients had one out of three Ig classes within normal range for age (Online Repository Table E2). Collectively, our data on patients' immunological presentation at diagnosis suggest that the combination of peripheral B cell percentages, Ig serum levels and family history allow for identification of XLA patients even in the minority of cases where one of these parameters may not fully satisfy the classical diagnostic criteria for this disease.

BTK mutation analysis

Among the patients, 157 belonging to 125 families were genetically characterized while the remaining ones had reduced BTK expression. Mutations were reported using RefSeq NM_000061 under the HVSG recommendations. BTK sequencing revealed 104 different mutations, among which 20 are recurrent and 18 are novel (Table 1 and Online repository Table E3).

More in detail, the most frequent types of mutations were missense (49%), followed by indels (18%), non-sense (17%) and mutations affecting splice-sites (12%), with a minor incidence of large deletions (4%). Among the 18 novel mutations, missense and non-sense were the most frequent ones (8 and 7 respectively), followed by mutations affecting splice sites, indels and large deletions (1 for each category) (Table 1).

Clinical features at diagnosis

The prevalence and type of clinical manifestations before diagnosis are shown in Figure 2A. Respiratory infections were the most frequent clinical features, with pneumonias recorded in 39.9% (67/168) of patients, otitis media in 32.7% (55/168) of patients and sinusitis in 17.9% (30/168). Of note, 13.1% (22/168) of patients were affected with chronic lung disease (CLD) at diagnosis based on lung CT scan (presence of bronchiectasis, peribronchial wall thickening, atelectasias) [11]. Besides respiratory infections, affected patients presented frequently skin infections (20.8%; 35/168). The gastrointestinal tract was also involved with a positive history for gastroenteritis in 19% (32/168) of cases, while chronic diarrhea was reported in 8.3% (14/168). Invasive infections were also reported at diagnosis: sepsis in 7.7% (13/168) of patients, meningitis in 4.8% (8/168) and encephalitis in 0.6% (1/168). Post vaccination poliomyelitis related to the oral attenuated Sabin vaccine was reported in 2.4% (4/168) of cases. Finally, other manifestations such as arthritis and hepatitis were documented in 9.5% (16/168) and 3.6% (6/168) of affected subjects, respectively.

Comparison of clinical presentation between familial and sporadic cases showed that respiratory, gastrointestinal and skin infections were slightly more frequent among sporadic ones. (Online

Repository Figure E1A). Encephalitis and sepsis were mostly seen among the sporadic cases (Online Repository Figure E1A).

Patients' presentation resulted particularly different when the year of birth was taken into consideration setting the limit at year 2000 (Online Repository Figure E1B), year of establishment of the IPINet registry for XLA. Patients born before 2000 showed a more severe clinical history with an increased prevalence of recurrent respiratory infections of the upper and lower tract when compared to the patients born after 2000 (Online Repository Figure E1B). Of note, all patients with CLD at diagnosis (13.1%, 22/168) were born before 2000. Invasive infections such as meningitis and sepsis were mainly observed in patients born before 2000, while encephalitis and post-vaccination poliomyelitis were exclusively reported in patients born before 2000. An increased prevalence of gastrointestinal and skin involvement was also observed in patients born before 2000 (Online Repository Figure E1B).

Clinical features during follow-up

During a cumulative follow-up of 1370 patient-years, one hundred and sixty four XLA patients receiving regular Ig replacement treatment were followed for a mean follow-up of 8.35 years per patient (range 1-18). The most common clinical manifestations registered during follow-up were respiratory infections (Figure 2B). More in detail, 34.1% (56/164) of patients suffered from pneumonias with a mean number of 0.08 episodes/patient-year (Table 2); 33.5% (55/164) of cases presented otitis media with a mean number of 0.095 episodes/patient-year (Table 2); 56.7% (93/164) of subjects experienced sinusitis with a mean number of 0.28 episodes/patient-year (Table 2).

While chronic lung disease (CLD) was present at diagnosis in 13.1% (22/168) of patients, during follow-up and under regular immunoglobulin replacement treatment, another 38,4% (63/164) developed CLD, reaching a combined 51.8% (85/164) patients with XLA affected with CLD.

Diagnosis of CLD was made after an overall mean of follow-up of 12.78 years (range: 1-39 years) at an overall mean age of 19.67 years (range: 2-43 years). Development of CLD was not associated with the IgG dose administered for immunoglobulin replacement treatment (Online Repository Figure E2). A small percentage (15.8%) of XLA patients was under antibiotic prophylaxis before the development of CLD; this percentage increased upon diagnosis of CLD (40.3%). The long term follow-up of our cohort of XLA patients allowed us to calculate the real life cumulative risk for developing CLD among patients with XLA which resulted to be 47% after 40 years of follow-up (Figure 2C). The cumulative risk for developing CLD at 50 years of age was 47% (Figure 2D).

Chronic sinusitis based on pathologic CT scans and clinical criteria was present in 56.7% (93/164) of patients during the last follow-up. Mean age at diagnosis of chronic sinusitis was 16.29 years (range: 4-41 years) with a mean follow-up of 13.56 years (range: 1-30 years). Of note, during the last follow-up, 53/164 XLA patients showed both chronic sinusitis and CLD.

Gastrointestinal involvement was identified in 52.4% (86/164) of patients with a mean number of 0.14 episodes/patient-year (Table 2). Skin infections were reported in 30.5% (50/164) of cases with a mean number of 0.08 episodes/patient-year (Table 2). Arthritis was recorded in 10,4% (26/164) of patients with a mean number of 0.02 episodes/patient-year of (Table 2). Finally, invasive infections such as sepsis, meningitis and encephalitis were registered in 2.4% (4/164), 0.6% (1/164) and 0% of subjects respectively (Table 2). While the majority of clinical complications during follow-up showed a similar prevalence between sporadic and familial cases, invasive infections of the central nervous system were only reported in the sporadic ones.

Pathogen isolation

As previously reported [7,11], identifying the cause of infectious complications in XLA is not always feasible. Considering the long term follow-up and the size of our XLA cohort, we analyzed the pathogens isolated during the most frequent infectious complications involving lungs, gastrointestinal tract and skin. A total of 107 pneumonia episodes were recorded. Pathogen isolation

from sputum cultures were available in 25% of cases. *Haemophilus influenzae* and *Streptococcus pneumoniae* were the most frequent pathogens isolated (53.58% and 17.86% respectively), followed by *Pseudomonas spp* (10.71%), *Staphylococcus spp* (7.14%), *Klebsiella pneumoniae* (3.57%), *Branhamella catharralis* (3.57%) and *Pneumocystis jiroveci* (3.57%). (Online Repository Figure E3A).

During follow-up, a total of 200 episodes of gastrointestinal manifestations were registered. Pathogens were isolated only in 18.5% of cases. *Giardia lamblia* was the most frequently isolated pathogen (48.65%), followed by *Salmonella* (24.32%), *Campylobacter* (18.92%), *Escherichia coli* (5.41%) and *Blastocistis hominis* (2.70%) (Online Repository Figure E3B).

Finally, a total of 115 episodes of skin infections were reported. Pathogens were isolated in 28.7% of cases. The most frequent one was *Staphylococcus aureus* (75.75%), followed by *Herpes virus* (21.21%) and *Candida spp* (3.03%) (Online Repository Figure E3C).

Genotype-phenotype correlation in XLA

Considering the size of our cohort and the long term follow-up, we investigated whether a genotype-phenotype correlation could be established in XLA, both at diagnosis and during follow-up. Mutations were divided in 5 separate groups (Missense, Non Sense, InDels, Splicing and Deletions) and symptoms were evaluated for patients belonging to each group. Our data did not reveal a significant genotype-phenotype correlation in XLA, neither at diagnosis or during follow-up (Online Repository Figure E4 and E5).

Malignancy

During follow-up, a minority of XLA patients (3.7%; 6/164 cases) were diagnosed with a malignancy (Table 3). Four out of six malignancies were localized in the gastrointestinal tract: colon n=2; stomach n=1; liver n=1.The remaining two malignancies involved the thyroid gland and the central nervous system (Table 3). Three out of these six patients (50%) died due to the

malignancy at the last follow-up. No lymphomas were reported in this cohort. The incidence of the above mentioned malignancies in XLA patients resulted higher when compared to the one reported for healthy male Italians of the same age-group (source: www.gco.iarc.fr) (Table 3).

Survival

Long-term follow-up data regarding survival of patients with XLA are limited. Analysis of our cohort's data shows that the overall survival at 43 years of age is 92.7%, significantly lower when compared to age-matched healthy controls (98%) (source: www.istat.it) (Figure 3). Subdivision of XLA patients based on the presence or absence of CLD (CLD^+ and CLD^- respectively) revealed that CLD^+ patients showed an even lower survival at 43 years of age (90.5%), which resulted significantly lower when compared to age-matched healthy controls (Figure 3). Of note, although the absence of CLD in XLA ameliorates survival at 43 years of age (97.4%), their survival still remains significantly lower when compared to healthy age-matched controls (Figure 3). Causes of death for 13 XLA patients are reported in Table 4. All deceased patients were born before the year 2000.

Immunoglobulin replacement treatment

During follow-up, all patients were under regular immunoglobulin (Ig) replacement treatment. At the last follow-up, the endovenous (IVIG) and the subcutaneous route of administration were almost equally represented: 51% versus 49% respectively (Figure 4). Regarding the subcutaneous route, 41% of patients were treated with conventional products (SCIG), while 8% of patients with the facilitated (fSCIG) ones. Almost all patients under SCIG treatment had been previously treated with IVIG (96.7%). 58,3% of patients under fSCIG treatment had been previously mainly treated with SCIG (Figure 4).

Discussion

In a nationwide longitudinal collaborative effort co-ordinated by IPINet, in order to better define the natural history of XLA, 168 affected patients with documented XLA were regularly evaluated for a cumulative follow-up for 1370 patient-years, the longest reported to date.

Overall mean age at diagnosis was 66 months. The introduction of the national XLA registry in 2000 determined a reduction of the diagnostic delay from 84 months (before 2000) to 23 months (after 2000), suggesting that the establishment of a nationwide registry for primary immunodeficiencies increases awareness among physicians and leads to early diagnosis for affected patients. In fact, in a recent cohort study of 174 agammaglobulinemic patients that did not utilize a nationwide registry [14], average age at diagnosis was 7.09+/-3.98 years, further underlining the potential role of a national registry for primary immunodeficiencies in increasing awareness among physicians, thus leading to earlier diagnosis for affected patients.

The immunological presentation of affected patients at onset satisfied the classical diagnostic criteria in the majority of cases. For the remaining cases, diagnosis of XLA was achieved by integrating the Ig class levels of all classes with the percentages of peripheral B cells and the family history for XLA, underlining how the combined use of these three parameters allows for diagnosis of XLA even in the minority of cases without complete satisfaction of the classical diagnostic criteria for this disease.

Mean age at diagnosis was unexpectedly similar between sporadic and familial cases (67 months versus 64 months respectively). A similar diagnostic delay for familial cases was observed in another cohort study, where only one third of patients with positive family history were diagnosed before becoming symptomatic [7], further underscoring that physicians should pay attention to positive family history in order to achieve early diagnosis of XLA.

Infections of the respiratory tract, mainly otitis media, pneumonia and sinusitis, were the most common clinical presentations at onset, in accordance with previously reported data [7,11-19]. Of note,

13.1% of affected patients was affected with CLD at diagnosis. The occurrence of this complication already at diagnosis may be partially explained by various factors such as advanced age, delayed diagnosis and initial treatment with intramuscular Ig known to be considerably less effective than endovenous Ig in reducing the incidence of respiratory infections. Gastrointestinal infections were reported in 19% of patients, confirming that this is a frequent co-morbidity as previously reported [7,11-20]. The incidence of post-vaccination poliomyelitis (PPV) due to the oral attenuated Sabin vaccine was 2.4%, quite higher than previous reports (1%) [7]. Both PPV and encephalitis were exclusively documented for patients born before the establishment of the IPINet registry in 2000. Regarding PPV, this is also related to the fact that, after 2002, the national vaccine schedule includes only the inactivated Salk vaccine.

During follow-up, and under regular Ig replacement treatment, infections of the respiratory tract, such as otitis media, pneumonia and sinusitis, remained one of the major clinical burdens for XLA patients, suggesting that the lack of mucosal IgA cannot be adequately compensated by polyspecific IgG replacement treatment. A recent meta-analysis [21] demonstrated that increasing the administered dose of IgG in patients affected with PIDs, may reduce the incidence of pneumonias and thus the risk of developing CLD. The high prevalence of CLD in our cohort suggest that regular IgG supplementation does not prevent the development of this complication. The long-term follow-up of our cohort allowed us to calculate the cumulative risk of CLD which is equal to 47% after 40 years of follow-up and equal to 47% at 50 years of age, findings that have not been reported before. In our previous study [11], the prevalence of CLD resulted higher, but this was dependent on the composition of the cohort, including the mean age at diagnosis and the type of treatment received. In any case, these findings underline that the development of CLD is a real-life complication in XLA and may interest almost half of affected patients by their 40s-50s. Considering the impact of CLD in everyday life [22] and especially in long-term outcome, physicians should pay more attention to lung morbidity in XLA and consider, as early as possible, a personalized respiratory physiotherapy program and/or antibiotic prophylaxis [23] regimen for affected patients.

Gastrointestinal and skin infections were recorded in a significant number of patients, consistent with previous XLA case series [7,11-13]. Previous studies have suggested that the gastrointestinal tract is frequently involved in tumor transformation in XLA patients [24-38]. In our cohort, four XLA patients developed tumor of the gastrointestinal tract during follow-up, one gastric adenocarcinoma, one liver carcinoma and two colon adenocarcinoma.

Finally, overall survival for XLA patients was 92.7% at 43 years of age, and CLD was the major factor found to increase the mortality of XLA patients in agreement with an international survey reporting that CLD was the leading cause of death among XLA patients [19]. This is the first real life definition of long-term survival in XLA patients, since previously published data for patients' registries had a limited follow up period (4.5 years) [7].

In conclusion, our data describe in a detailed and substantial manner the natural history of XLA during the longest follow-up described to date and underline that Ig replacement treatment is not sufficient to control all co-morbidities that arise over the years. Considering that the life expectancy of affected patients is reduced when compared to age-matched healthy controls, clinical management should focus on the prevention and prompt treatment of associated complications, mainly CLD, in order to improve patients' quality of life and overall survival.

Acknowledgments

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References

1. Picard C, Bobby Gaspar H, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, et al. International Union of Immunological Societies: 2017 Primary Immunodeficiency Diseases Committee Report on Inborn Errors of Immunity. J Clin Immunol 2018 Jan;38(1):96-128.

2. Bruton OC. Agammaglobulinemia. Pediatrics 1952 Jun; 9(6): 722-8

3. Vetrie D, Vorechovský I, Sideras P, Holland J, Davies A, Flinter F, et al. The gene involved in X-linked agammmaglobulinemia is a member of the src family of protein-tyrosine kinases. Nature 1993 Jan 21;361(6409):226-33.

4. 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.

5. Väliaho J, Smith CI, Vihinen M. BTKbase: the mutation database for X-Linked agammaglobulinemia. Hum Mutat 2006 Sep 12;27(12):1209-1217

6. Ryser O, Morell A, Hiztig WH. Primary immunodeficiencies in Switzerland: first report of the national registry in adults and children. J Clin Immunol 1988;8(6):479–485.

7. 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

8. Matamoros Florí N, Mila Llambi J, Español Boren T, Raga Borja S, Fontan Casariego G. Primary immunodeficiency syndrome in Spain: first report of the national registry in children and adults. J Clin Immunol 1997;17(4):333–339.

9. Stray Pedersen A, Abrahamsen TG, Froland SS. Primary immunodeficiency diseases in Norway. J Clin Immunol 2000;20(6):477–485.

10. 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;12(4):479-86.

11. Plebani A, Soresina A, Rondelli R, Amato GM, Azzari C, Cardinale F, et al. Clinical, immunological and molecular analysis of a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. Clin Immunol 2002 Sep;104(3):221-30.

12. 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 Feb;33(1):81-93

13. Pac M, 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 Mar 1;13(2):412-417.

14. Chen XF, Wang WF, Zhang YD, Zhao W, Wu J, Chen TX. Clinical characteristics and genetic profiles of 174 patients with X-linked agammaglobulinemia: Report from Shanghai, China (2000-2015). Medicine (Baltimore) 2016 Aug;95(32): e4544.

15. Esenboga S, Cagdas D, Ozgur TT, Gur Cetinkaya P, Turkdemir LM, Sanal O, et al. Clinical and genetic features of the patients with X-Linked agammaglobulinemia from Turkey: Single-centre experience. Scand J Immunol 2018 Mar;87(3).

16. 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):405-411.

17. García-García E, Staines-Boone AT, Vargas-Hernández A, González-Serrano ME, Carrillo-Tapia E, Mogica-Martínez D, et al. Clinical and mutational features of X-linked agammaglobulinemia in Mexico. Clin Immunol 2016 Apr;165:38-44.

18. Aadam Z, Kechout N, Barakat A, Chan KW, Ben-Ali M, Ben-Mustapha I, et al. X-Linked Agammagobulinemia in a Large Series of North African Patients: Frequency, Clinical Features and Novel BTK Mutations. J Clin Immunol 2016 Apr;36(3):187-94.

19. El-Sayed ZA, Abramova I, Aldave JC, Al-Herz W, Bezrodnik L, Boukari R, et al. X-linked agammaglobulinemia (XLA): Phenotype, diagnosis, and therapeutic challenges around the world. World Allergy Organ J 2019 Mar 22;12(3):100018.

20. 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;37(3):287-294

21. 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 Oct;137(1):21-30.

22. Bryan BA, Battersby A, Shillitoe BM, 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;36(5):472-9.

23. 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.

24. Van der Hilst JC, Smits BW, van der Meer JW. Hypogammaglobulinaemia: cumulative experience in 49 patients in a tertiary care institution. Neth J Med 2002;60(3):140–7.

25. Kinlen LJ, Webster AD, Bird AG, Haile R, Peto J, Soothill JF, et al. Prospective study of cancer in patients with hypogammaglobulinaemia. Lancet 1985;1(8423):263–6.

26. Zenone T, Souillet G. Cancer and primary humoral immunodeficiency. Bull Cancer 1997;84(8):813–21.

27. Vajdic C. M, Mao L, van Leeuwen MT, Kirkpatrick P, Grulich AE, Riminton S. Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency? Blood2010;116(8):1228–34.

599 28. Lederman HM. Cancer in children with primary or secondary immunodeficiencies. J Pediatr
600 1995;127(2):335.
601
602 29. Bachmeyer C, Monge M, Cazier A, Le Deist F, de Saint Basile G, Durandy A, et al. Gastric
603 adenocarcinoma in a patient with X-linked agammaglobulinaemia. Eur J Gastroenterol Hepatol
604 2000;12(9):1033–5.
605
606 30. Lackmann GM, Poremba C, Wahn V, Niehues T. X-gebundene Agammaglobulinämie,
607 chronisch-atrophische Gastritis und Adenokarzinom des Magens bei einem 15-jährigen Jungen.
608 Monatsschr Kinderheilkd 2007;155(1):S6–9.
609
610 31. Lavilla P, Gil A, Rodriguez MC, Dupla ML, Pintado V, Fontan G. X-linked
611 agammaglobulinemia and gastric adenocarcinoma. Cancer 1993;72(5):1528–31.
612
613 32. Brosens LA, Tytgat KM, Morsink FH, Sinke RJ, Ten Berge IJ, Giardiello F. M, et al. Multiple
614 colorectal neoplasms in X-linked agammaglobulinemia. Clin Gastroenterol Hepatol 2008;6(1):115–
615 9.
616
617 33. Chisuwa H, Mori T, Fujimori K, Shigeno T, Maejima T. Colorectal cancer in a young adult with
618 X-linked agammaglobulinemia (XLA). Report of a case. Nihon Shokakibyo Gakkai Zasshi
619 1999;96(12):1392–5.
620
621 34. van der Meer JW, Weening RS, Schellekens PT, van Munster IP, Nagengast FM. Colorectal
622 cancer in patients with X-linked agammaglobulinaemia. Lancet 1993;341(8858):1439–40.
623

624 35. Adachi Y, Mori M, Kido A, Shimono R, Suehiro T, Sugimachi K. Multiple colorectal neoplasms
625 in a young adult with hypogammaglobulinemia. Report of a case. Dis Colon Rectum
626 1992;35(2):197–200.
627
628 36. Sasi OA, Sathiapalan R, Rifai A, Tulbah A, M, Al-Mehaidib A, Kofide A, et al. Colonic
629 neuroendocrine carcinoma in a child. Pediatr Radiol 2005;35(3):339–43.
630
631 37. Wang Y, Kanegane H, Wang X, Han X, Zhang Q, Zhao S, et al. Mutation of the BTK gene and
632 clinical feature of X-linked agammaglobulinemia in mainland China. J Clin Immunol
633 2009;29(3):352–6.
634
635 38. Lackmann G. M, Wahn
636 V, Poremba C, Niehues TA teenager with X-linked agammaglobulinemia and vitamin B12
637 deficiency anemia. J Pediatr Gastroenterol Nutr 2005;41(3):360–2.
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Figure legends

Figure 1. Age and immunological presentation of 168 XLA patients at diagnosis. A. Age at diagnosis (shown in months) in correlation with the year of birth. **B.** Immunoglobulin IgG serum levels at diagnosis (grey area depict normal values for age). **C.** Immunoglobulin IgA serum levels at diagnosis (grey area depict normal values for age). **D.** Immunoglobulin IgM serum levels at diagnosis (grey area depict normal values for age). **E.** Peripheral lymphocyte subsets (CD3, CD8, CD4, CD19) at diagnosis (dotted-light grey area depicts ≥2% of peripheral B cells).

Figure 2. Clinical manifestations of XLA patients at diagnosis and during follow-up. A. Clinical symptoms at diagnosis. **B.** Clinical complications during follow-up. **C.** Cumulative risk for the development of chronic lung disease (CLD) based on the years of follow-up for affected patients. **D.** Cumulative risk for the development of chronic lung disease (CLD) based on the age of affected patients.

Figure 3. Survival of XLA Patients during long term follow-up. Survival curve of affected patients (total: black line; without chronic lung disease: dotted line; with chronic lung disease: dashed line) compared to age matched Italian males (red line) (source: www.istat.it). The numbers indicate patients censored at each time point (total: black; without chronic lung disease: green; with chronic lung disease: blue). Comparisons between patients and general population were performed using Student's t-test. Significance threshold was set at $p<0.05$. Statistical analysis was performed using Prism GraphPad software, version 8 (GraphPad Software Inc., La Jolla, CA, USA) (* $p<0.05$; ** $p<0.01$; *** $p<0.001$; $p<0.0001$).

Figure 4. Immunoglobulin replacement treatment in Italian XLA patients. Immunoglobulin replacement treatment (IVIG: black bar; SCIG: dark grey bar; fSCIG: light grey bar) at last follow-

675 up (right panel); previous route of Ig administration (left panel) for patients under IVIG (upper left
676 panel), SCIG (mid left panel) and fSCIG (lower left panel) replacement treatment.

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Table 1: 18 novel mutations of BTK in 157 XLA patients

#	BTK_EXON	BTK_MUT	BTK_EFF	Polyphen	MutationTaster	CADD
1	2	c.40T>C	p.Ser14Pro	1/D	1/D	27.4
2	2	c.40T>C	p.Ser14Pro	1/D	1/D	27.4
3	2	c.A52del	p.Lys18Argfs*6	-	1/D	-
4	3	c.221delC	p.Pro74Leufs*47	-	1/D	-
5 **§**	4	c.307C>T	p.Gln103*	-	1/D	29.3
6 **§**	4	c.307C>T	p.Gln103*	-	1/D	29.3
7	6	c.493T>C	p.Cys165Arg	1/D	1/D	25.9
8	8	c.592_596delinsCTAACTACATA	Lys199Thrfs*3	-	1/D	-
9	IVS10	c.895-2A>G	splicing defect	-	-	-
10 **§**	12	c.1032T>G	p.Tyr344*	-	1/D	18.73
11 **§**	12	c.1032T>G	p.Tyr344*	-	1/D	18.73
12 **§**	12	c.1032T>G	p.Tyr344*	-	1/D	18.73
13	14	c.1228delA	Thr410Leufs*6	-	1/D	-
14	15	c.1375C>T	p.Gln459*	-	1/D	26
15	15	c.1399C>T	p.Gln467*	-	1/D	38
16	15	c.1507A>T	p.Lys503*	-	1/D	26.9
17 **§**	15	c.1541C>A	p.Ser514*	-	1/D	28.5
18 **§**	15	c.1541C>A	p.Ser514*	-	1/D	28.5
19	IVS15	c.1567-1G>A	splicing defect	-	-	-
20	16	c.1579T>G	p.Cys527Arg	0.4/B	1/D	32
21	IVS16	c.1632-1G>A	splicing defect	-	-	-
22	17	c.1691C>A	p.Ser564Tyr	1/D	1/D	22.7
23	17	c.1702_1704delGTC	p.Val568del	-	1/D	-

§ = familiar cases

B = Benign

D = Damaging

Table 2. Incidence of infectious episodes among XLA patients during follow-up

	number of patients (%)	number of episodes	number of episodes/patient-year
Pneumonias	56 (34,1%)	107	0,08
Otitis	55 (33,5%)	130	0,095
Sinusitis	93 (56,7%)	382	0,28
Sepsis	4 (2,4%)	4	0,003
Meningitis	1 (0,6%)	1	0,0007
Encephalitis	0 (0%)	0	0
Arthritis	17 (10,4%)	26	0,02
Gastroenteritis	86 (52,4%)	198	0,14
Skin Infections	50 (30,5%)	113	0,08

Table 3. Malignancies diagnosed in XLA patients during follow-up

#	Year of Birth	Age at Diagnosis of Malignancies	Type of Malignancies	Status	% of Malignancies in XLA	% of Malignancies in Healthy Italian Males*
8	1999	11	ependymal astrocytoma variant giant cells	alive	0,61	0,0022
30	1972	33	colon adenocarcinoma	alive	1,20	0,0041
74 §	1969	38	liver carcinoma HCV^+	dead	0,61	0,0033
77	1980	36	thyroid papillar carcinoma follicular type	alive	0,61	0,0109
78	1980	34	colon adenocarcinoma	dead	1,20	0,0041
88	1967	37	gastric carcinoma	dead	0,61	0,0012

§ = familial case

HCV^+ = Hepatitis C virus positive

*** = data source: https://gco.iarc.fr**

Table 4. Deceased XLA patients during long term follow-up

#	Year of Birth	Age at Diagnosis	Age at Death	Cause of Death
10 §	1974	5	29	Infectious BPN
35	1966	40	42	Septic Shock
41 §	1979	1	37	Neuroacanthosis
43	1999	3	3	Pseudomonas Sepsis
74 §	1969	8	38	Cardiac Arrest/Liver Disease
78	1980	11	38	Colon adenocarcinoma
86	1981	12	19	Car accident
88	1967	7	37	Gastric Carcinoma
96	1972	6	21	Cardio-respiratory Failure
104	1969	13	35	Cardio-respiratory Failure
145	1969	4	34	CLD/Cardiac Arrest
146	1986	1	29	Cerebral Hemorrhage/Thrombocytopenia/Liver Cirrhosis
149 §	1968	12	32	Pneumocistis jirovecii infection/Cardiac Arrest

§= familial cases

Figure 1

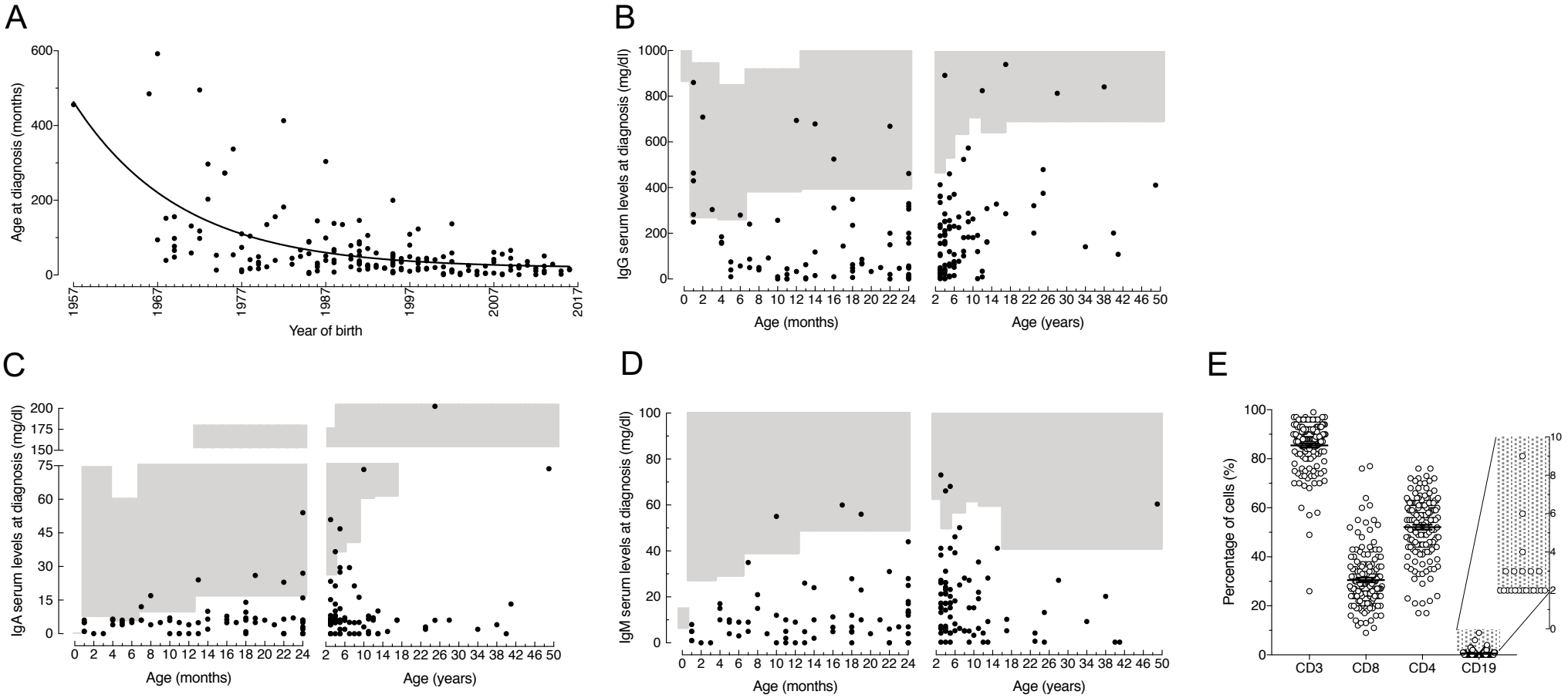

A
Age at diagnosis (months)
Year of birth
B
IgG serum levels at diagnosis (mg/dl)
Age (months)
Age (years)
C
IgA serum levels at diagnosis (mg/dl)
Age (months)
Age (years)
D
IgM serum levels at diagnosis (mg/dl)
Age (months)
Age (years)
E
Percentage of cells (%)
CD3
CD8
CD4
CD19

Figure 2

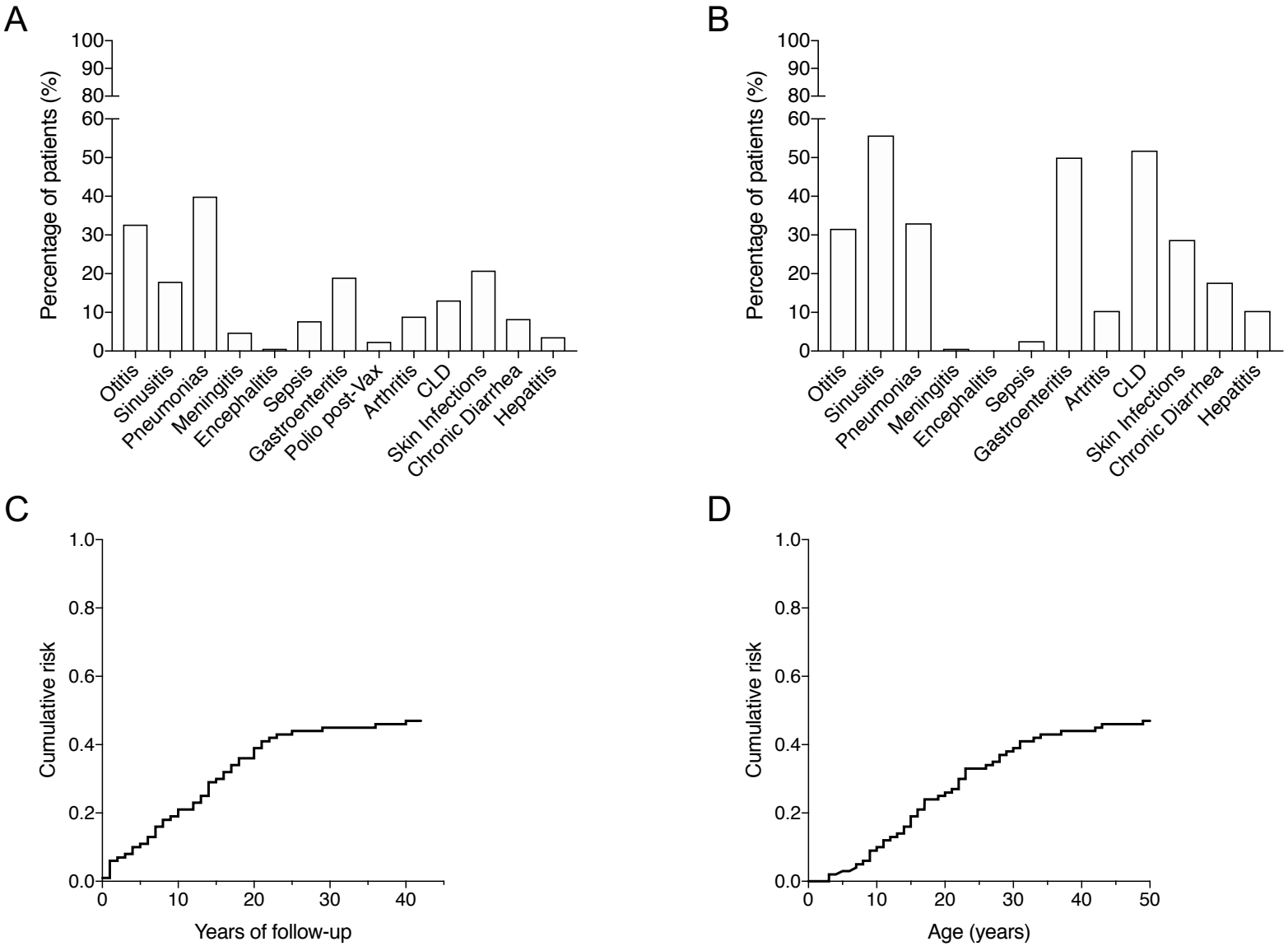
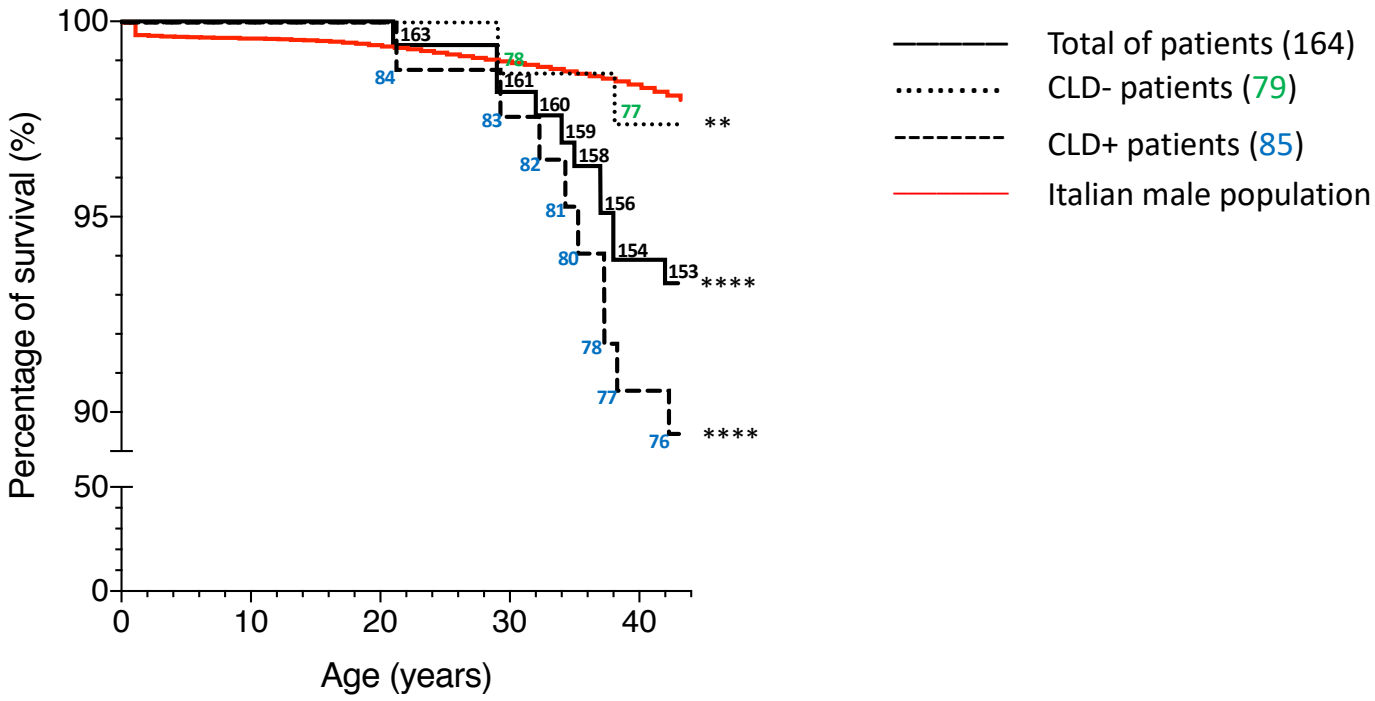

A
Percentage of patients (%)
100
90
80
60
50
40
30
20
10
0
Otitis
Sinusitis
Pneumonias
Meningitis
Encephalitis
Sepsis
Gastroenteritis
Polio post-Vax
Arthritis
CLD
Skin Infections
Chronic Diarrhea
Hepatitis
B
Percentage of patients (%)
100
90
80
60
50
40
30
20
10
0
Otitis
Sinusitis
Pneumonias
Meningitis
Encephalitis
Sepsis
Gastroenteritis
Artritis
CLD
Skin Infections
Chronic Diarrhea
Hepatitis
C
Cumulative risk
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
Years of follow-up
D
Cumulative risk
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
Age (years)

Figure 3


100
95
90
50
0
Percentage of survival (%)
0
10
20
30
40
Age (years)
163
161
160
159
158
156
154
153
84
83
82
81
80
78
77
76
78
77
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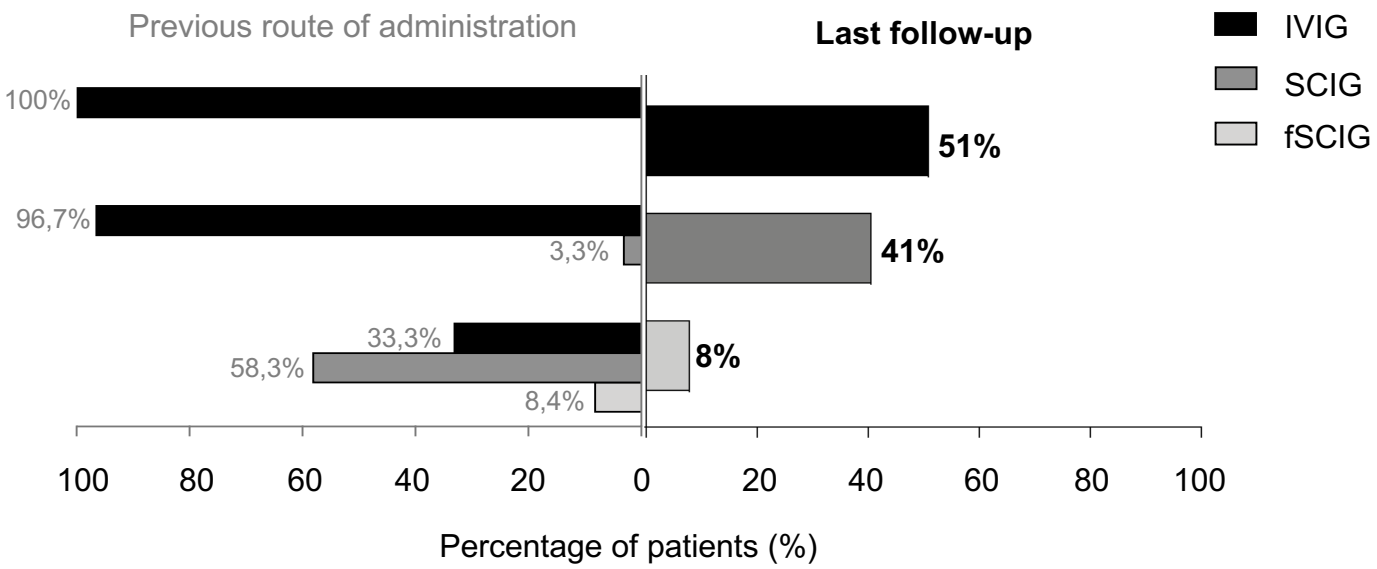
****
Total of patients (164)
CLD- patients (79)
CLD+ patients (85)
Italian male population

Figure 4


Previous route of administration
Last follow-up
IVIG
SCIG
fSCIG
100%
51%
96,7%
3,3%
41%
33,3%
58,3%
8,4%
8%
100
80
60
40
20
0
20
40
60
80
100
Percentage of patients (%)