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Clinical and immunological analysis of patients with X-linked agammaglobulinemia: single center experience

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Abstract

The retrospective analysis of a immunological and clinical survey in 33 boys with definitive diagnosis of X-linked agammaglobulinemia, based on ESID criteria, is presented. Recurrent lower respiratory tract infections were the most common presentation with the onset at the age of 1 year 3 months, when maternal antibodies disappeared. The clinical diagnosis was usually delayed by 2 years and 6 months. The mean IgG level at diagnosis was 1.03 g/l (range 0-4.0 g/l). Mean IgA was 0.039 g/l (range 0-0.38 g/l) and IgM – 0.189 g/l (range 0.021-3.51 g/l). The total number of B cells was decreased and varied between 0-391 cells/μl, with the mean value of 26 cells/μl and 0.65%. The replacement therapy with intravenous immunoglobulin (IVIG) preparation was also delayed, and introduced at the mean age of 3 years 5 months. In some patients it was followed with subcutaneous immunoglobulin (SCIG). The overall prognosis in XLA patients is good if diagnosis and immunoglobulin replacement therapy are done early before the onset of chronic complications. Diagnosis was established through molecular analysis.

Key words: *agammaglobulinemia, primary immunodeficiencies, immunoglobulin replacement therapy, Btk gene.*

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Introduction

The first description of X-linked agammaglobulinemia (XLA) comes from 1952 when Dr O. Bruton described a boy with recurrent infection treated successfully with immunoglobulins [1]. It is a rare genetic disorder presenting a B cell differentiation arrest. XLA is caused by mutations in a gene encoding a cytoplasmic tyrosine kinase (*Bruton's tyrosine kinase – Btk*), localised at Xq21.3-Xq22 chromosome and encompasses 37.5 kb containing 19 exons. *Btk* is regarded as required for the proliferation and differentiation of B lymphocytes [2]. About 50% of affected boys have a family history of the disease [3]. Mutations in *Btk* are highly variable but no clear genotype-phenotype correlation has been found to date. The disorder is maintained in the population by new mutations [2, 4, 5].

Patients with XLA present a marked reduction in B cells in the peripheral circulation, while T cell subsets are usually normal or relatively increased. Laboratory studies generally show very low but detectable serum immunoglobulin levels of all isotopes, and lack of specific antibody production. On physical examination individuals with XLA have no, or barely detectable, tonsils and cervical lymph nodes [3, 4].

XLA is characterized by an increased susceptibility to infections, particularly of the lower respiratory tract as well as gastrointestinal tract. Patients with XLA may present sepsis, meningitis, osteomyelitis, or skin infections. To prevent them, life-long immunoglobulin replacement therapy is indicated.

The onset of infections occurs before the end of the first year of life, when maternal antibodies disappear. Few individuals present later onset of infections and almost normal

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or even normal immunoglobulin concentration, which usually declines with age [6]. The diagnosis is delayed due to less severe or even atypical courses of infection. Some authors report the diagnosis of XLA in adult patients [7, 8].

There are a few reports on long-term observation of agammaglobulinemic patients [5, 6, 9]. In this paper we present clinical analysis of 33 XLA patients from the biggest pediatric referral center for primary immune deficiencies (PID) in Poland.

Material and methods

Almost fifteen hundred primary immunodeficiency (PID) patients were diagnosed at the Department of Immunology, Children's Memorial Health Institute (CMHI) in 1980-2012. The most common were primary antibody deficiencies (PAD), recognized in 56% of patients. Among them IgA deficiencies (IgAD) or IgG subclass deficiencies were of highest prevalence. In 66 boys with recurrent infections, a diminished immunoglobulin level, and low B cells number, diagnosis of Bruton's agammaglobulinemia was made according to ESID definitive and probable diagnostic criteria [10]. The definitive diagnostic criteria with Btk sequence analysis were met by 33 boys. The retrospective analysis of clinical and laboratory data, involving personal data, family history, onset of clinical manifestation, levels of main immunoglobulin classes, number and percentage of T and B cells, date of diagnosis, and immunoglobulin replacement therapy introduction was done on that group.

Serum immunoglobulin levels of three main classes IgG, IgA and IgM were analysed at the time of diagnosis and during replacement therapy, approximately every 3-4 months, using the nephelometry technique. The number and percentages of circulating B and T lymphocytes were evaluated by FACS analysis using anti-CD3, anti-CD19 or anti-CD20 monoclonal antibodies at least twice.

Table 1. Demographic and clinical characteristics of 33 males with XLA

Feature	Mean value	Range
Family history	33%	
Onset of symptoms (years)	1.3	0.2-7
Clinical diagnosis (years)	3.9	0.11-12
IgG (g/l)	1.03	0-4.0
IgA (g/l)	0.039	0-0.38
IgM (g/l)	0.189	0.021-3.51
B cells (%), [cells/μl]	0.65 [26]	0-9 [0-391]
IVIG – age of introduction (years)	3.5	0.11-12
Genetic confirmation (years)	7.9	1- 25
Deaths	1	

BTK mutation analysis was performed in the Department of Immunology, Erasmus MC in Rotterdam, the Netherlands, courtesy of prof. J.J. van Dongen.

Results

Thirty-three boys meeting ESID definitive criteria for diagnosis of XLA were included in the study. Demographic and main laboratory data are shown in Table 1. First symptoms of the disease occurred at mean age of 15 months (range 2 months-7 years). In two boys, recurrent infections occur relatively late, at the age of 7 years. Clinical diagnosis of XLA was delayed by 2 years 6 months (range 11 months-12 years). The vast majority of patients presented very low levels of all three main immunoglobulin isotypes. The mean IgG level at diagnosis before immunoglobulin replacement therapy was 1.03 g/l (range 0-4.0 g/l). One patient presented an IgG level of 4.0 g/l, which diminished over time. Other immunoglobulin classes were also below 2SD in all but one boy with a high IgM concentration (3.51 g/l). Mean IgA was 0.039 g/l (range 0-0.38 g/l) and IgM – 0.189 g/l (range 0.021-3.51 g/l).

All patients presented a normal number and percentage of CD3+ cells, as well as CD4+ and CD8+ cells (data not shown in this article). The total number of B cells was decreased and varied between 0-391 cells/μl, with the mean value of 26 cells/μl and 0.65%. However, in two boys B lymphocytes reached 6% (391 cells/μl) and 9% (375 cells/μl), accompanied by very low IgG (1.34 g/l and 0.5 g/l), IgM (0.29 and 0.08 g/l) and no detectable IgA. In the subsequent analysis the values were below the lower range border. Diagnosis was established through molecular analysis.

The clinical diagnosis based on clinical and laboratory symptoms was made approximately 2 years and 6 months after the onset of infections. Genetic confirmation of XLA was performed at mean age of 7 years and 9 months due to availability of molecular analyses of the *Btk* gene.

The immunoglobulin infusions were administered sporadically in a few individuals with very serious infections as a supportive therapy prior to clinical diagnosis. Replacement immunoglobulin therapy was introduced at the mean age of 3 years and 5 months and was generally delayed in comparison to the disease onset. All discussed patients were treated with intravenous immunoglobulin (IVIG), with a mean dose of 0.4-0.6 g/kg, given every 3 or 4 weeks. In 2001, treatment with subcutaneous immunoglobulin (SCIG) was initiated in a group of 15 patients with PAD, among them in 9 with XLA. An equivalent monthly dose, divided into 4 weekly infusions, was given i.e. approximately 0.1-0.15 g/kg/week. Patients and their families generally preferred SCIG than IVIG due to the possibility of self-infusions at home, less frequent visits to the hospital, and a more comfortable lifestyle.

The family history for XLA was positive in 27% of patients, including two brothers, two cousins and relevant history of severe recurrent infections and deaths of very young boys on the maternal side.

Molecular analysis of the *Btk* gene, confirming diagnosis, was performed on all of them at Erasmus MC in Rotterdam.

The frequency and type of infections are shown in Table 2. The lower respiratory tract infections were of the highest frequency: 78% of patients suffered from bronchitis, over half of them – from pneumonia (51%). Upper respiratory tract infections (URTI) were reported in 30% of the analysed group. Almost one third (30%) presented otitis, and 21% sinusitis. Other clinical manifestations, mostly of infectious origin, such as meningitis, skin infections and abscesses were observed less commonly. Four boys had a history of sepsis, in two of them with pneumococcal origin, as documented.

Gastrointestinal problems were observed in 12 boys. One boy suffered from abdominal pain, and weight loss several years after diagnosis of XLA. He also presented consistently diminishing IgG through levels in spite of proper replacement immunoglobulin therapy (either IVIG and SCIG). Following the clinical course, laboratory disorders and endoscopic evaluation (gastroscopy and colonoscopy) with several biopsies and histological analysis, the diagnosis of inflammatory bowel disease was made. Treatment with sulfasalazine, steroids and azathioprine was successfully introduced. Eleven boys complained of diarrhea, which was resolved shortly after immunoglobulin therapy introduction.

In one boy with established diagnosis of hemophilia A referred to the center due to recurrent infections – XLA was recognized.

Almost all patients received vaccines according to the national immunization program until severe infections started or diagnosis was made. No serious adverse events following live vaccines, i.e. vaccine-associated paralytic poliomyelitis, or BCG-tis were noticed in vaccinated boys. Eight out of nineteen boys had no antibodies to HbsAg. In eight out of ten tested boys there were no anti-diphtheria antibodies, while in four out of ten – against tetanus. Other patients were not tested due to earlier infusions of immunoglobulins or incomplete vaccination program or presented a satisfactory response. In thirteen out of 15 boys low isohemagglutinin titers were shown.

Neutropenia was noticed in 2 boys and resolved during IVIG therapy.

Four boys presented chronic lung disease (CLD). In two of them bronchiectasis was recognized , before the diagnosis of XLA and substitution immunoglobulin therapy was established. In one patient, lung cirrhosis was also diagnosed beforehand. The other one developed bronchiectasis during follow-up, in spite of immunoglobulin therapy and IgG trough level of 7-8 g/l. Chronic bronchitis was recognized in another one.

One boy died at the age of 1 year due to neurological sequelae of severe meningoencephalitis. The infection appeared just before diagnosis and was the only symptom of disease. Two older brothers died at the age of 1 year and 2.5, respectively, because of pulmonary infections.

Table 2. Clinical manifestation of X-linked agammaglobulinemia

Clinical manifestation	Number of patients	% of patients
pneumonia	17	51
bronchitis	26	78
otitis	10	30
sinusitis	7	21
sepsis	4	12
meningitis/encephalitis	4	12
arthritis	6	18
abscesses	4	12
URTI	16	48
diarrhea	11	33
conjunctivitis	6	18
skin allergy	5	15
neutropenia	2	6
urinary tract infections	2	6
stomatitis	4	12
fever of unknown origin	3	9
inflammatory bowel disease	1	3
bronchiectasis	3	9
lung cirrhosis	1	3

Discussion

There are some reports on clinical presentation and long-term observation on a large group of patients with XLA [5, 6, 9]. It is the first clinical report on such a large group of children from one center in Poland. With this aim, the charts of 33 boys fulfilling definitive ESID criteria of X-linked agammaglobulinemia were analysed. In one of them, with no family history and almost normal immunoglobulin level, and in two with quite a high percentage and number of B cells, and low immunoglobulin concentration, diagnosis was established by molecular analysis.

The mean onset of symptoms occurred at 15 months of age, while clinical diagnosis of XLA usually was made 30 months later. It is compatible with Conley *et al.*'s report, where 48.3% of patients had onset of symptoms between 13 and 40 m.o., and 31.7% – after 40 m.o. [3]. Two of our patients demonstrated the first symptoms relatively late – at the age of 7 y.o. Some authors indicated later onset of

symptoms in few cases – over 7 years of age [3]. Usuiu *et al.* described a 27-year-old male with mild course of the disease and recurrent pneumonias since the age of 25 [7]. One of our patients had quite high IgG levels, reaching 0.4 g/l, in four – B lymphocytes reached 2% and more. The mean value of all immunoglobulin levels was diminished below 2 SD, with IgG < 0.1 g/l. B cells percentage varied between 0 up to 6% (0-391 cells/μl), with mean value of 0.65% (28 cells/μl). All patients presented a normal number and percentage of CD3+ cells, as well as CD4+ and CD8+ cells.

Our observations confirm the thesis that the immunological spectrum is broader than originally thought [3, 4, 6]. A number of "leaky" phenotypes as well as atypical phenotypes have been reported [8, 11].

Relatively late molecular confirmation of XLA at the mean age of almost 8 years was connected with availability of molecular diagnostic methods.

Respiratory tract infections were reported as the most common feature, with a predominance of lower respiratory tract infection (LRTI). Respiratory infections (RI) were still observed during replacement immunoglobulin therapy, but less frequently. The analyses of frequency of infections and days of antibiotics were published in another report [12, 13]. The observations are in concordance with others [14, 15].

Delayed or suboptimal replacement therapy may result in chronic problems, such as chronic pulmonary disease or bronchiectasis [3, 6]. Inappropriate immunoglobulin substitution therapies (i.e. plasma infusion, intramuscular immunoglobulin treatment given in the 1970s and 1980s, low-dose IVIG) amplify the risk of chronic lung disease. Our patients were treated either with IVIG or SCIG therapy, with regular control of IgG through levels. CLD occurred in the majority of them before diagnosis and proper treatment. One patient developed bronchiectasis in spite of regular infusion, correction of dose according to weight, infections and IgG levels. In the rest of the analyzed population, infections were controlled.

The main goal of IgG replacement treatment is to reduce the frequency and severity of infections. In spite of the above, the quality of patients' life must be considered when the route of administration is chosen. There are several reports on safety and efficacy of SCIG therapy both in children and adults [14-17]. They revealed efficacy, good tolerability and safety of subcutaneous gamma globulin treatment, with a very low risk of adverse events and possibility of self-infusion at home. A group of 9 agammaglobulinemic boys together with six patients with other primary antibody deficiencies (autosomal recessive agammaglobulinemia, common variable immunodeficiency, Nijmegen breakage syndrome) were with subcutaneous immunoglobulin. The comparison of effectiveness of both types of therapy was done and published in an earlier paper [12]. The study revealed slightly higher IgG trough levels during subcutaneous treatment and reduction in the number and severity of infections. As observed in other clinical trials, both patients and their families report better quality of life when treated with subcutaneous immunoglobulins [18]. Regular subcutaneous infusions keep the IgG level more stable. It leads to less infectious complications on the one hand, and to lower costs of treatment on the other hand [16, 18].

Teahon *et al.* pay attention to the effect of gamma globulins on the reduction in frequency of gastrointestinal infections, caused particularly by Giardia lamblia in hypogammaglobulinemia patients [19]. In the studied group, no Giardia lamblia infections were reported, and it is hard to say either if there were any or if immunoglobulin therapy could have reduced this. But for sure, immunoglobulin therapy stopped recurrent diarrhea. In one boy, diagnostic procedures of the gastrointestinal tract were performed to explain weight loss and abdominal pain. Finally, inflammatory bowel disease (IBD) was recognized. There are sporadic reports on IBD in XLA patients, different from common variable immunodeficiency.

No cancers were observed in our XLA patients, although there were reports on GI cancers in literature [20].

Early diagnosis together with replacement immunoglobulin therapy allows a decrease in the number of infectious complications, antibiotics therapy and serious lung complications. Proper treatment warrants better quality of life. The overall prognosis in XLA is not bad until diagnosis and treatment are established before onset of chronic complications.

The authors declare no conflict of interests.

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