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The Recovery of Immune System Parameters in Children Following Lymphoblastic Leukemia Therapy – Preliminary Report

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Acute lymphoblastic leukemia (ALL) is the most common pediatric neoplasm. Long-term survival is achieved in approximately 80% of patients. One of the more common complications of ALL treatment is immunosuppression.

Objectives. The aim of the study is to assess the reconstruction rate of the most important immune system parameters in children after ALL treatment.

Material and Methods. The study included 47 children (22 boys, 25 girls) diagnosed and treated for ALL in Department of Pediatric Hematology and Oncology at Wroclaw Medical University. The study used peripheral blood collected at the time treatment was completed and in the first, second, third and sixth months following treatment, then at yearly intervals up to 10 years after treatment. In order to determine the immune status of the tested samples the following parameters were assessed: white blood cell count, absolute lymphocyte count, the proportions of individual subpopulations of lymphocytes – T (CD3 +), Th (CD4 +), Ts (CD8 +), B (CD19 +), NK (CD16 + 56 +), the concentration of immunoglobulins A, G and M, interleukin 10 activity, as well as the expression of ICAM-1 adhesion molecules.

Results. At the end of anti-neoplastic therapy a reduction in both the absolute number leukocytes and various subpopulations of lymphocytes was observed. The lower limits of normal range were achieved about two years after the end of treatment. The concentrations of immunoglobulin IgA, IgG and IgM at the end of treatment were within low normal limits. Normal concentrations of immunoglobulin levels and stability were observed about two years after the end of treatment. There was a slow, steady increase in the production of interleukin-10 and the expression of ICAM-1 adhesion molecules. These results confirm previous observations that after ALL treatment children are in an immunocompromised state for up to 12 months, in terms of both humoral and cellular immunity.

Conclusions. Knowing the average growth trends for the main immune system parameters after ALL treatment can be important in clinical practice for children in whom immune reconstruction proceeds slowly. Predicting the expected time required to restore immune function could be of help, for example in combating infections and planning vaccinations (**Adv Clin Exp Med 2014, 23, 1, 97–102**).

Key words: acute lymphoblastic leukemia, immune system, reconstruction.

Acute lymphoblastic leukemia (ALL) is the most common pediatric neoplasm, representing approximately 75% of all leukemias, which in turn constitute a third of all cancers, with an average incidence of 5/100,000 children from 0 to 14 years of age [1]. As a result of the progress made in recent years in the treatment of ALL, long-term survival is achieved in approximately 80% of children. These results in an increasing population of survivors, and thus the problem of dealing with this group and monitoring the early and late sequelae of antineoplastic treatment is of increasing importance in clinical practice, both specialized and primary. One of the more common complications of ALL treatment is immunosuppression, stemming from the nature of the disease and the side effects

of chemotherapeutic drugs. A reduction in cellular and humoral immunity during the treatment of ALL [2–4] and after treatment [3, 5, 6, 15, 17] has been observed. As a result, there is a greater risk of developing serious infections (including systemic infections) and even septic shock. This also applies to pathogens against which the child had been vaccinated before the start of ALL treatment. For example, Ek et al. showed reduced immunity against diphtheria, tetanus and *Hemophilus influenza B* in children after ALL treatment [7, 9]. Another study [8] showed a decreased level of antibodies against pneumococcus specific antigens for up to 9 months after the treatment of ALL – below the threshold providing immunity, but also below the average value observed in unvaccinated healthy children. Vaccinations against measles and rubella given to children before the onset of ALL also did not provide protective antibody levels after treatment [14]. Hence the need to monitor the immune status of treated children as early as during remission maintenance therapy and after its completion. In some children re-vaccination against certain diseases is recommended.

The duration of full immune system reconstruction depends on various factors, including the age of the child, and more importantly the intensity of the chemotherapy; there are high risk groups (HR) and standard risk groups (SR) [4, 5]. Each of the components of the immune system is renewed at a different rate, but in the first 12 months after the completion of treatment, there are irregularities in total lymphocyte count, its percentage composition and the content of lymphocyte subsets and in immunoglobulin concentrations [10, 11].

The increased risk of systemic infection affects how fever is dealt with in children in immunosupression, whether or not they present with signs of infection. In this group, the indications for starting antibiotic therapy with a spectrum encompassing both Gram positive and Gram negative bacteria are much broader than among healthy children. This applies especially to patients with persistent leucopenia and granulocytopenia [2].

The inadequate production of cytokines and/or impaired expression of their receptors causes a cell maturation block and proliferation at different levels of oncogenetic development, initiating the neoplastic process. Interleukin-10 is produced by Th1 lymphocyte suppression and thereby inhibits the production of IL-2 and IFN-gamma [19, 20].

The role of the immune system in the initiation of the neoplastic process, its pathogenesis and clinical course is not fully understood. The proper cellular function of this system depends on cytokines that act in a genetically programmed network as signal substances stimulating cells by, among other things, uncovering adhesion molecules on their surface. The discovery of adhesion molecules has shed new light on the medical profession's understanding of some pathophysiological processes. These molecules play an important role in morphogenesis, cell migration, blood clotting, inflammation and metastasis formation [20, 21].

Knowledge of the basic parameters of the immune system and their estimated growth trends could facilitate therapeutic decision-making without the repetition of numerous laboratory studies.

The primary objective of this study was to assess the average recovery rate of the main parameters of the immune system (levels of lymphocytes and their subpopulations, and IgG, IgA, IgM concentrations) in children following treatment for acute lymphoblastic leukemia. The expression of ICAM-1 adhesion molecules on the surface of immune cells was also analyzed, and their role in infection and the occurrence of metastases was assessed. The prognostic value of cytokine IL-10 and IL-12 production in children after acute lymphoblastic leukemia treatment was also assessed. Plans were also made for investigating the relationship between severe systemic infections and the risk of a relapse of leukemia.

Material and Methods

The study involved 47 children who had been treated for ALL: 22 boys and 25 girls between the ages of 4 years and 5 months and 15 years and 8 months (with a median age of 8 years and 4 months) at the end of their treatment. All the patients had been diagnosed and treated at the Department of Pediatric Hematology and Oncology in Wroclaw, Poland, according to therapeutic protocols adopted by the Polish Pediatric Leukemia/Lymphoma Study Group in the study period. None of the cases involved allogeneic stem cell transplantation. There were no cases of relapse or any other adverse events during the entire 10-year observation period.

The study used peripheral blood collected at the time of the completion of ALL treatment, in the first, second, third and sixth months following the completion of treatment, then at yearly intervals up to 10 years after treatment. In order to determine the immune status of the tested samples, the following parameters were determined:

1. White blood cell (WBC) count.
2. Absolute lymphocyte count.
3. The proportions of individual subpopulations of lymphocytes – T (CD3 +), Th (CD4 +), Ts (CD8 +), B (CD19 +), NK (CD16 + 56).

4. The concentration of immunoglobulins A, G and M.

5. Interleukin-10 activity, as well as the expression of ICAM-1 adhesion molecules.

The levels of leukocytes and lymphocytes in the peripheral blood were determined using routine methods (both automatic and manual). The phenotype of lymphocyte subsets was determined using two-and three-color flow cytometry (Coulter EPICS XL flow cytometer, USA; Coulter/Immunotech monoclonal antibody sets, France, Becton Dickinson, USA, DAKO, Denmark). Immunoglobulin IgA, IgG and IgM concentrations were determined by turbidimetry using Turbiqant apparatus (Dade Behring Marburg GmbH, Germany).

Interleukin-10 activity as well as the expression of ICAM-1 adhesion molecules in serum and in supernatants of a 24-h culture of peripheral blood mononuclear leucocytes were tested by the ELISA method, interleukins using a Quantikine HS test set (R&D Systems, USA) and s-ICAM-1 using a kit from Bender MedSystems (Vienna, Austria).

The results were analyzed in the following groups depending on the period in which the study was performed: group 0: results from the studies performed after the completion of cytostatic therapy; group 2: results from the studies performed in the second year following treatment; group 5: results from the studies performed in the fifth year after treatment; group 10: results from the studies performed after the fifth year after treatment.

Due to the statistically small number of studies performed in each group, the analysis of the results was based on polynomial mean value trends after confirmation of normal distribution. The method of least squares based on the polynomial $y = b + c_1x+c_2x^2 + c_3x^3 +... + c_nx^n$ was used, aiming to best fit the polynomial curve. The resulting trend curve and the mean value curves for maximum and minimum values of the examined parameter are presented in the form of graphs.

Results

At the time of the completion of antineoplastic therapy, completion a reduction in the absolute numbers of both total leukocytes and various subpopulations of lymphocytes was observed. The lowest total leukocyte count was 2100 cells/mL. In subsequent years, an increasing WBC trend was observed, up to normal levels 4–5 years after treatment (Fig. 1). The total number of lymphocytes was initially reduced to 1100 cells/mL, and it normalized approximately two years after the completion of therapy. T cells (CD3 +), on average, achieve the lower normal ranges after the second

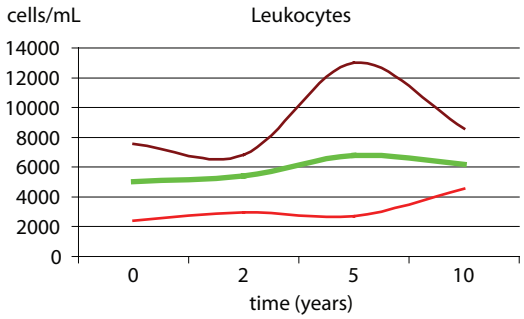

Fig. 1. Leucocyte values after cessation of therapy

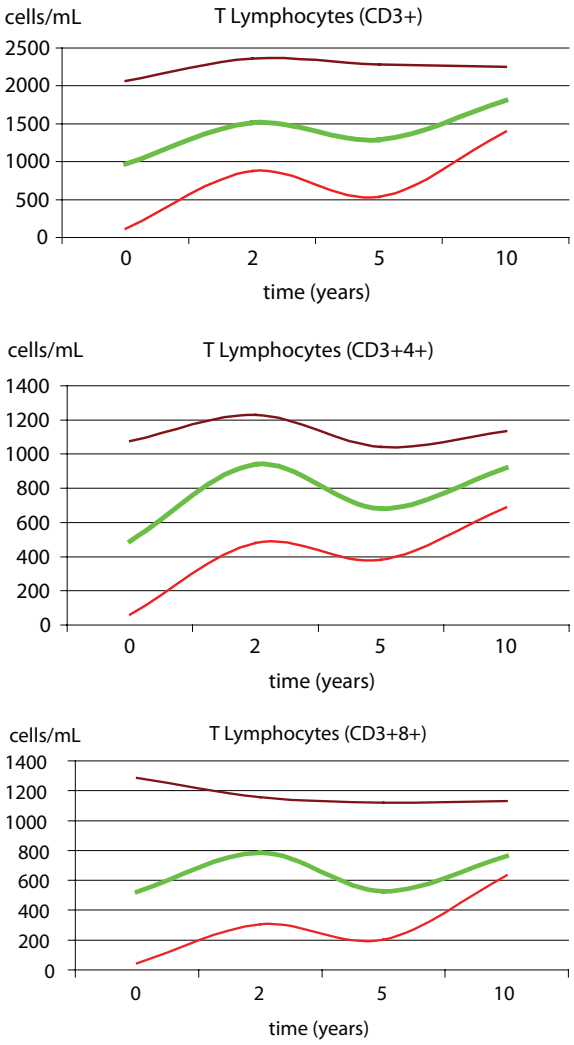

Fig. 2. T lymphocyte (helper and suppressor) values after cessation of therapy

year. Reduced levels of *T* helper lymphocytes (CD3 4 +) (< 85 cells/mL) and suppressor *T* cells (CD3 +8 +) (to a minimum value of 36 cells/mL) at the end of treatment are notable. The lowest values returned to normal about 2 years after the end of treatment (Fig. 2).

The number of NK cells (CD16 +3– 56) after the completion of treatment did not differ from the average values in healthy children (Fig. 3).

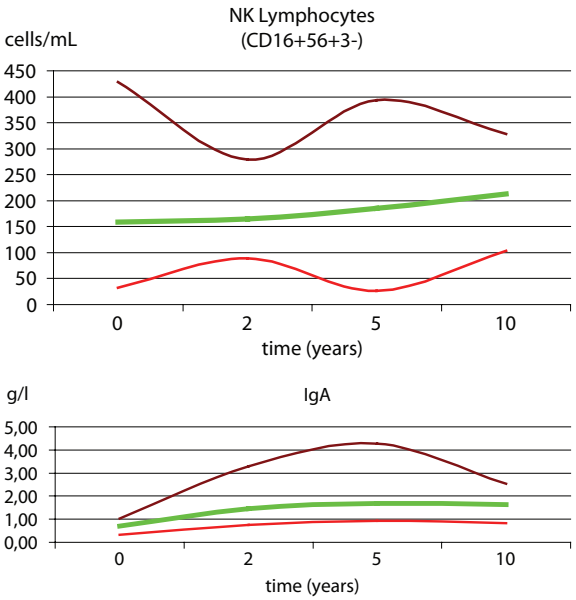

Fig. 3. NK (natural killer) lymphocyte values after cessation of therapy

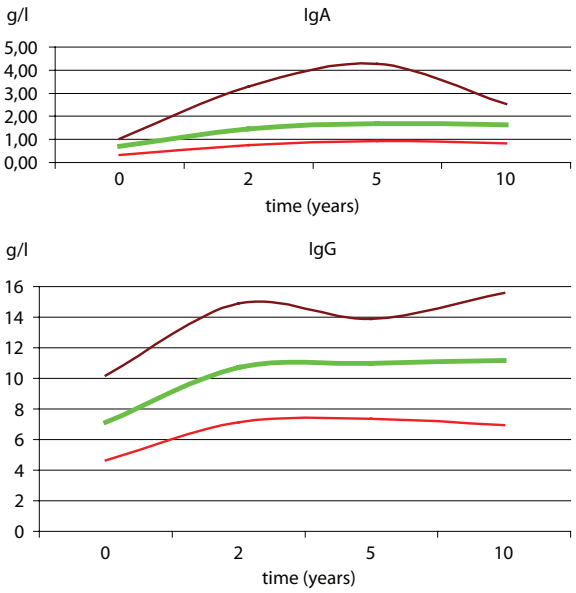

Fig. 4. *B* lymphocyte values after cessation of therapy

The number of *B* cells (CD19 +3–) was slightly reduced just after treatment, and the lower limit of normal range is achieved within the first year (Fig. 4).

The concentrations of immunoglobulin IgA, IgG and IgM at the end of treatment were within low-normal limits. The lowest values were, respectively, for IgA 0.5 g/L, IgG 5.3 g/L for IgM 0.46 g/L. Normal concentrations of immunoglobulin levels and stability were observed about 2 years after the end of treatment (Fig. 5).

There was a slow, steady increase in the production of interleukin-10 and the expression of ICAM-1 adhesion molecules (Fig. 6).

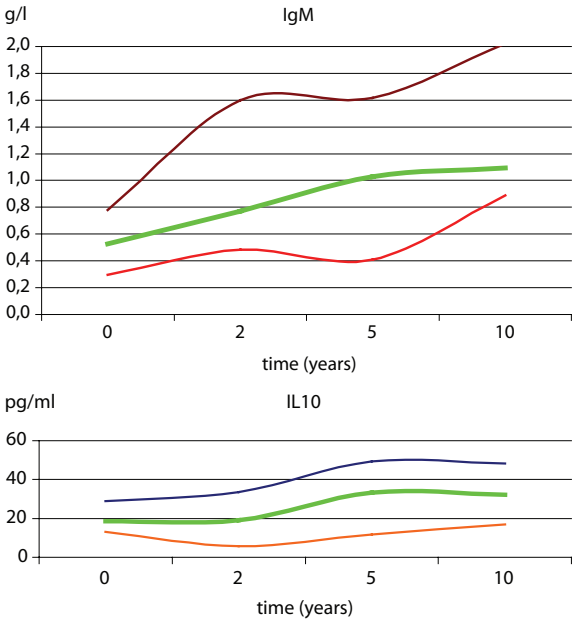

Fig. 5. Concentrations of immunoglobulins A, G & M after cessation of therapy

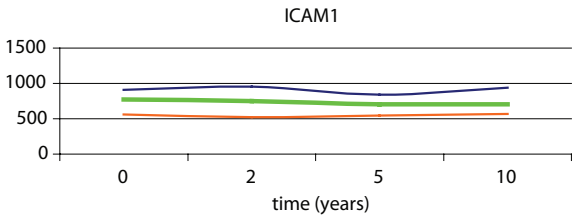

Fig. 6. Interleukin-10 production and expression of ICAM-1 after cessation of therapy

Discussion

The results obtained in the current study confirm the observations of other authors [3, 5, 6, 11, 13, 17, 19] that after ALL treatment children are in an immunocompromised state for up to 12 months. This concerns both cellular and humoral immunity. Despite this, during the 10-year observation period no serious systemic infections or significant increases in the number of infectious incidents were observed in the study group as compared to the population of healthy children of corresponding age. This corresponds to the observations of Kovacs et al. [11], among others.

There is a noticeable difference in the mean of values obtained in the initial period after treatment and those obtained after 5 and 10 years. It should also be noted that the differences between the maximum and minimum values are in most cases similar to the standard deviation of the given group of parameters. There was no significant correlation between the parameters studied.

Knowing the average growth trends for the main parameters of the immune system – leukocyte

and lymphocyte counts divided into subpopulations (T, B, NK) and primary immunoglobulin (IgA, IgM, IgG) levels – after treatment for ALL can be important in clinical practice. After testing a larger population of children it could be possible to develop specific standards in the form of "centile charts" for these parameters. With their help, patients in whom immune system recovery is slower than average, and who are thus at greater risk for complications associated with immunosuppression, could be identified. These children require more careful observation, frequent repetition of laboratory studies, more aggressive antibiotic therapy in case of infection, the continuation of prophylaxis against *Pneumocystis carini* or longer isolation from their peer group (for example home schooling).

Predicting the restoration of immune function could help in the planning of vaccinations. Vaccinating children with reduced immune system responsiveness may be inefficient or even dangerous, in the case of live vaccines. Therefore, knowing the average growth trends could be helpful in deciding whether to administer missing vaccinations according to the standard schedule of successive vaccinations.

In the next stage of the study the correlation between the rate of immune recovery and the incidence of relapse will be examined. The immune system plays an important role in fighting cancer cells, especially in their early stages. This phenomenon is used in allogeneic stem cell transplantation as a graft-versus-leukemia (GVL) effect. GVL allows residual disease to be controlled and destroyed after megachemotherapy in a large proportion of cases. It has also been shown that the absolute lymphocyte count during the induction phase of lymphoblastic leukemia and myeloid leukemia treatment in children is an independent risk factor, and that children with an absolute lymphocyte count (ALC) < 350/μL on day 15th of treatment had a significantly worse 5-year survival rate in comparison to children with an ALC > 350/mL [14]. Conducting a prospective analysis will answer the question of whether a deeper and more prolonged immunosuppression after treatment is associated with an increased risk of leukemia relapse and a worse overall survival rate. In these preliminary studies there seems to be no relation between the immune status and the risk of relapse or the overall 5-year survival rate, but the small sample size does not allow definitive conclusions.

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