A prolonged progressive lymphopenia with hypogammaglobulinemia after FCR treatment of chronic lymphocytic leukemia: case report and literature review

EWELINA GRYWALSKA[1], AGATA SURDACKA[1], MONIKA PIECZYKOLAN[1], ELŻBIETA STAROSŁAWSKA[2], KAROLINA OLSZEWSKA-BOŻEK[1], JACEK ROLIŃSKI[1]

[1]Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Poland
[2]Lublin Oncology Center, Poland

Abstract

The combination of two chemotherapeutic drugs, fludarabine and cyclophosphamide (FC), with the rituximab (RTX) – FCR – in the treatment of chronic lymphocytic leukemia (CLL) is associated with a high complete remission rate. In recent years FCR has become the first-line choice for CLL patients under 65 year of age. Delayed leucopenia has been described in rituximab-treated CLL patients. However among reported cases, the late onset and progressive lymphopenia was not noticed in individuals with CLL.

The present study reports the case of a 53-year-old Caucasian man who developed prolonged progressive lymphopenia after 18 weeks RTX treatment-free period. Irrespective of no drug intake, in 13-week follow up, the amount of B lymphocytes as well as the serum levels of IgG decreased (93.22% and 20% reduction, respectively). Progressive neutropenia with hypogammaglobulinemia has constantly intensified leading to the necessity of gamma globulin supplementation and administration of granulocyte colony-stimulating factor (G-CSF). The present findings suggests that FCR therapy in some individuals may lead to the induction of persistent alteration of the cellular and humoral immunity.

Key words: chronic lymphocytic leukemia, hypogammaglobulinemia, lymphopenia, neutropenia, rituximab.

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Introduction

In recent years, chemoimmunotherapy combining rituximab (RTX) with fludarabine and cyclophosphamide (FCR) has become the first-line choice for persons suffering from chronic lymphocytic leukemia (CLL) [1]. The FCR regimen in treatment-naive CLL patients is associated with better outcome, especially in younger persons.

In the present study, we report a first case of a prolonged progressive lymphopenia with hypogammaglobulinemia after the end of CLL treatment with the use of FCR. The patient had no family history of immunodeficiency; before, during and in first six months after FCR treatment did not notice any infection or fever of unknown origin. During 7-month observation after the end of the treatment, the absolute number of neutrophils and B lymphocytes, and in consequence of which, immunoglobulin levels gradually decreased leading to the septic arthritis and necessity of gamma-globulin substitution and G-CSF admission.

Case presentation

The patient at the time of CLL treatment admission (October 2010) was a 52-year-old Caucasian. The laboratory tests were as follows: WBC, $117.9 \times 10^3/\text{mm}^3$ ($106.3 \times 10^3/\text{mm}^3$ lymphocytes); Hg – 12.9 g/dl, hematocrit – 38%, platelet count – $276 \times 10^3/\mu\text{l}$, C-reactive protein –

Correspondence: Ewelina Grywalska, Department of Clinical Immunology and Immunotherapy, Medical University of Lublin, Chodzki 4a, 20-093 Lublin, Poland, tel. +48 81 756 48 40, e-mail: ewelina.grywalska@gmail.com
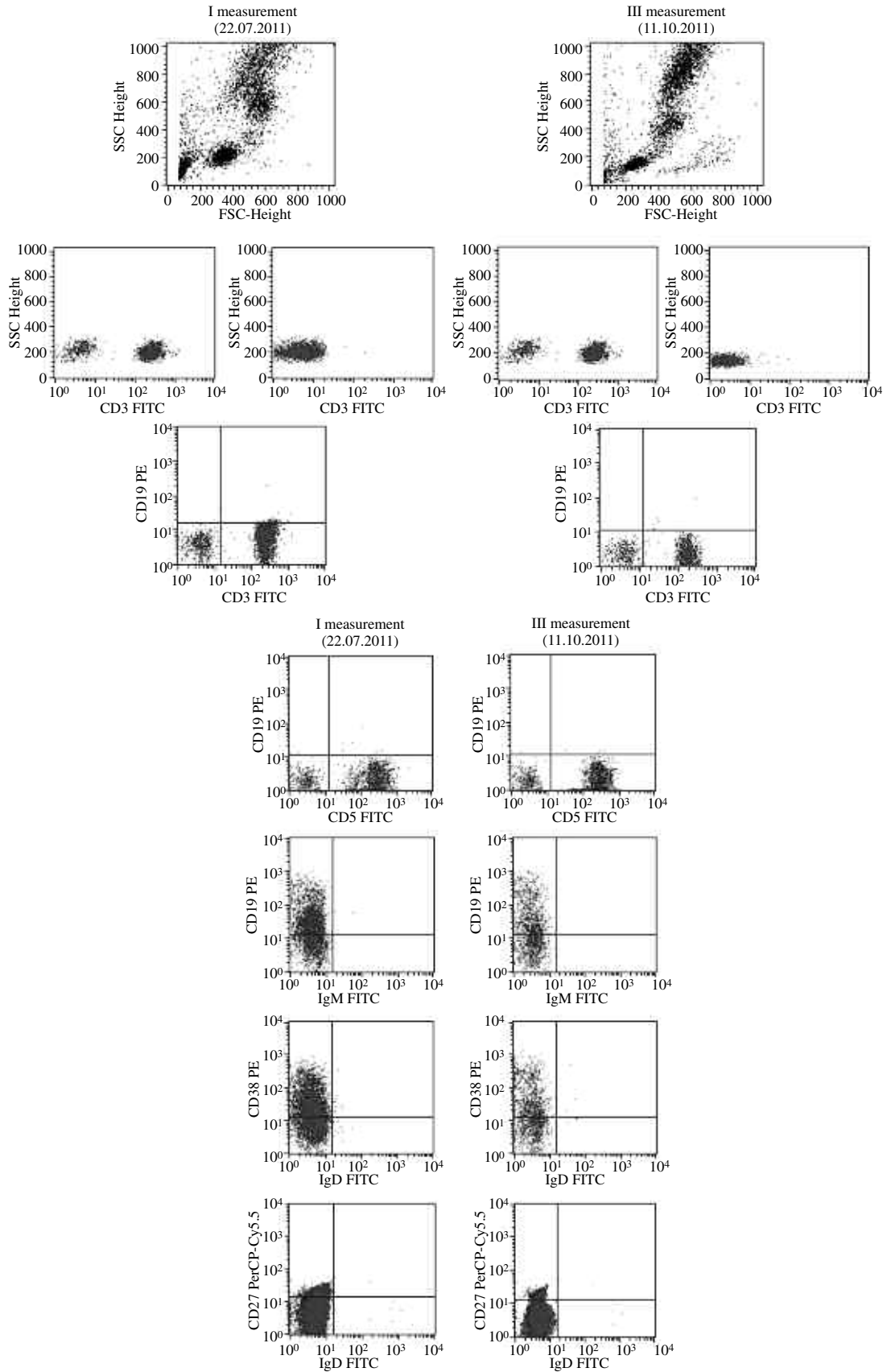
Fig. 1. Phenotypes of peripheral B cells of the patient in the first and third measurement. PBMCs were stained for the expression of CD3, CD5, CD19, CD27, CD38, IgM, and IgD and analyzed by flow cytometer. Analysis was performed by gating on CD19+cells, and dead cells were excluded by forward/side scatter gating

4.3 mg/l. The staging results were Rai stage 2 and Binet stage A and the patient was ZAP-70 and CD38 negative.

The patient from October 2010 to March 2011 received 6 cycles of FCR chemotherapy with positive improvement. FCR regimen was chosen as an initial treatment strategy due to its remarkably higher potential of preventing disease progression in younger, healthier, treatment-naive CLL patients [2]. After the 6th cycle, the patient's WBC was $4.34 \times 10^3/mm^3$ with lymphocytes count $1.48 \times 10^3/mm^3$ and PMNs count $2.02 \times 10^3/mm^3$. From that time, his complete blood count was checked monthly, but despite progressive lymphopenia, the globulin levels were not checked.

The described man enrolled at Immunological Outpatient Clinic in July 2011 in order to assess his immunological status and receive preventive vaccination against selected pathogens after the treatment of CLL (18 weeks treatment-free period). Patient did not report any signs or symptoms suggesting the occurrence of the immunological disturbances during and after the end of chemotherapy. At the time of patient's submission, main laboratory test findings included leukopenia – $3.42 \times 10^3/mm^3$ (lymphopenia – $0.8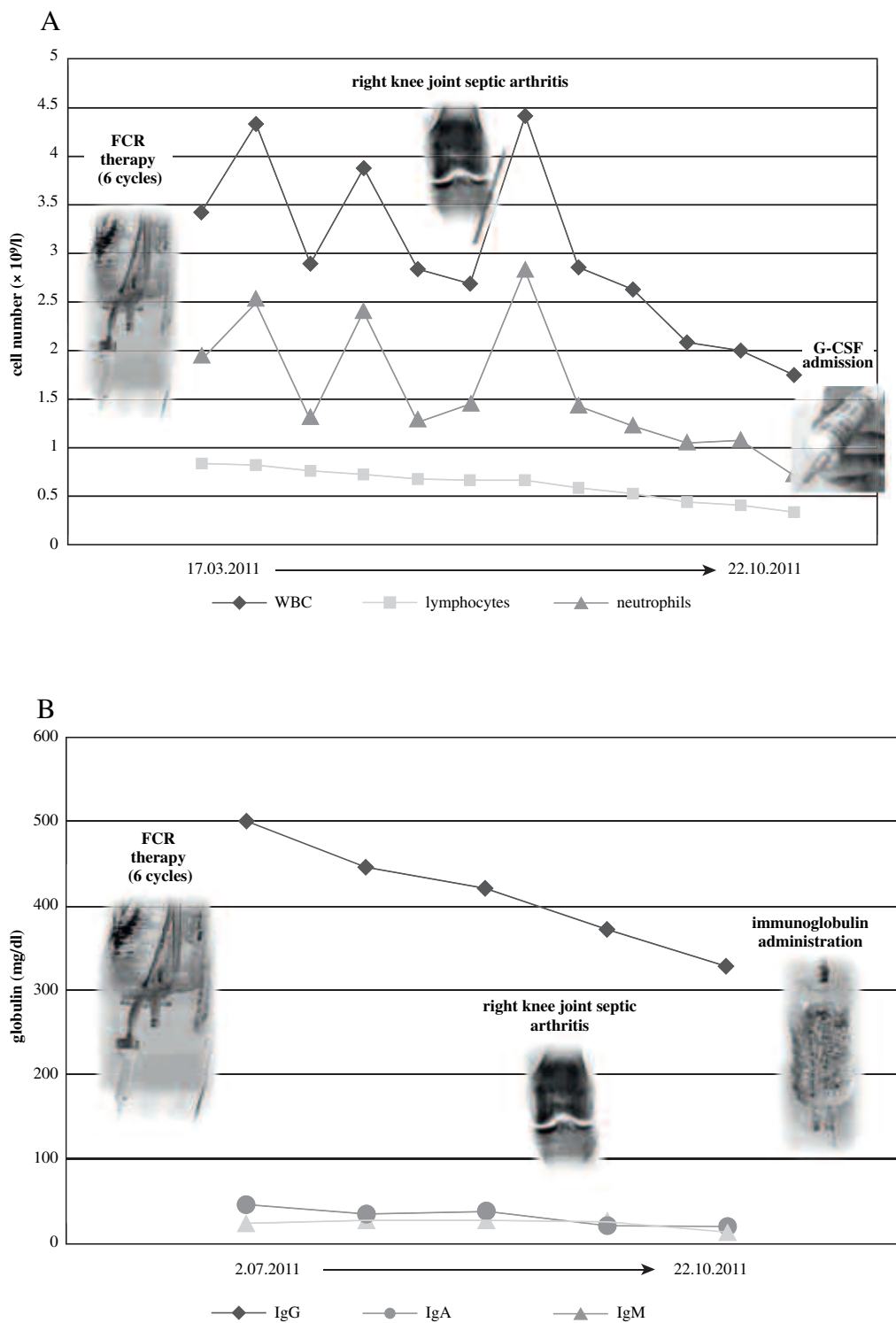3 \times 10^3/mm^3$ and neutropenia $1.86 \times 10^3/mm^3$) and low serum levels of γ-globulin (IgG – 477 mg/dl, IgA – 48 mg/dl, IgM – 36 mg/dl) with red blood cells and platelets parameters in normal ranges (RBC – $5.07 \times 10^6/mm^3$, hemoglobin – 15.1 g/dl, hematocrit – 41.8%, platelet count – $265 \times 10^3/mm^3$).

Flow cytometric analyses (FACSCalibur cytometer with Cell-Quest Software; Becton Dickinson and relevant monoclonal antibodies; Pharmingen) presented gradually lowering levels of B lymphocytes (Fig. 1) and alteration in other cells subsets (Table 1), irrespective of no drug intake.

At the end of September 2011, the patient developed knee arthritis without any injury or purulent changes on the skin and was treated with the use of oral amoxicillin in the dose of 1 g/24 h and clindamycin in a dose of 1.2 g/24 h with no results. Therefore patient received treatment with the use of intravenous ceftriaxone 1 g/12 h for the period of 10 days. A progressive neutropenia with lymphopenia and hypogammaglobulinemia persisted, leading to the necessity of gamma globulin supplementation and administration of granulocyte colony-stimulating factor (Fig. 2).

Table 1. Percentages and absolute numbers of lymphocyte subsets over 11 weeks of follow-up

Lymphocyte subset	I measurement (22.07.2011)		II measurement (6.09.2011)		III measurement (11.10.2011)	
	% of all lymphocytes	absolute count (/µl)	% of all lymphocytes	absolute count (/µl)	% of all lymphocytes	absolute count (/µl)
CD19+	0.59	5	0.34	3	0.04	0
CD5+CD19+	0.22	2	0.12	1	0.01	0
CD19+CD25+	0.12	1	0.1	1	0.02	0
CD19+CD69+	0.15	1	0.09	1	0.03	0
CD3+	78.58	652	76.09	632	79.87	535
CD4+CD3+	39.61	329	32.44	269	39.51	265
CD8+CD3+	39.99	332	45.07	374	39.98	268
CD4/CD8 T lymphocytes ratio	0.99	–	0.72	–	0.99	–
CD3+CD25+	17.91	149	12.36	103	17.19	115
CD3+CD69+	1.05	9	4.88	41	7.54	51
CD3+HLA-DR+	35.22	292	33.41	277	35.57	238
CD4+CD25+	17.37	144	11.05	92	17.88	120
CD4+CD25+high	3.61	30	0.87	7	2.51	17
CD45RA+CD4+CD3+	12.3	102	13.87	115	19.27	129
CD45RO+CD4+CD3+	88.2	732	90.61	752	85.82	575
CD3+CD16+CD56+	3.57	30	5.86	49	4.72	32
CD3−CD16+CD56+	12.16	101	14.07	117	13.56	91

Fig. 2. A – the levels of WBC, lymphocytes and neutrophils in peripheral blood of the patient from the time of the end of the treatment to the G-CSF admission moment. B – serum immunoglobulin concentrations from the time of patient's admission to the Immunological Outpatient Clinic to the beginning of intravenous immunoglobulin (IVIG) supplementation

Discussion

Rituximab – mechanisms of acting

Rituximab (RTX) is a human immunoglobulin G1-κ antibody conjugated with mouse variable regions isolated from a murine anti-CD20 monoclonal Ab. It is the first monoclonal antibody successfully developed for treatment of malignant hematologic diseases [3]. The drug seems to act mainly by causing antibody dependent cell-mediated cytotoxicity (ADCC) [4] ultimately resulting in the lysis of target B cells [5]. Complement activation leading to the cytotoxicity is another mechanism of action of RTX [6]. The last important mechanism is binding of rituximab to CD20, which can induce intracellular signals resulting in programmed cell death [7, 8]. The expression of CD20 begins at the pre-B cell stage and is lost prior to differentiation into immunoglobulin-secreting plasma cells [9, 10]. Thus, RTX should not affect hematopoietic progenitor cells and mature, antibody-secreting plasma cells. Despite the B-cell depleting, the T-cell compartment may also be modulated by RTX [11].

Rituximab related B-cell depletion – a common finding?

Transient peripheral B cell depletion is common after the end of RTX therapy and appears during the treatment, but immunoglobulin levels are generally not affected due to the fact that CD20 is expressed on pre-B and mature B lymphocytes but not on stem cells or plasma cells [9-11]. However our observations are in line with the results of Nishio *et al.*, who described 14 patients treated with rituximab as an adjuvant therapy [12]. Six persons developed hypogammaglobulinemia. A phenotypical analysis revealed a severe delay in the recovery of CD27$^+$ memory B cells, especially in the IgD$^-$/CD27$^+$ switched populations and these data suggested that RTX can affect the B-cell quantities as well as B-cell repertoires [12]. Irie *et al.* [11] similarly to us found out that after RTX therapy may cause a reduction of class-switched CD27$^+$IgM$^-$IgD$^-$ memory B cells. Keating *et al.* [13] reported that two-thirds of patients evaluated with flow cytometry had less than 1% CD5$^+$/CD19$^+$ cells in bone marrow after FCR therapy which provided that the progression-free survival was significantly longer in the FCR group than in the FC group. Recovery of normal B cells usually occurs more than 6 months after therapy [14].

In our study, in observation period (July-October) we noticed 20% reduction of IgG level together with over than 90% B-lymphocyte count decrease. This finding is similar to the results obtained by Diwarak *et al.* [15], who found out in two adult patients with pre-existing primary antibody deficiency treated with various immunosuppressive agents due to idiopathic thrombocytopenic purpura without any notable infective problems but a few weeks after treatment with RTX, these patients presented with clinically significant

immunodeficiency requiring intravenous immunoglobulin replacement therapy. CLL causing secondary immunodeficiency seems to induce the same problem.

Single separate cases of other transient hematologic toxicity

Lymphopenia may also be induced by fludarabine. Purine analogs are associated with lymphocytopenia, especially in absolute CD4 cell counts. Mentioned defect may persist for several years, while other mononuclear cell populations, such as B cells, recover more rapidly [16]. Fludarabine may also produce neutropenia in CLL or low-grade lymphoma patients, pretreated with chemotherapeutic agents [17].

Late-onset neutropenia following RTX therapy is not an unusual finding in both lymphoma and autoimmune disorders patients [18, 19]. In our patient, progressive late-onset neutropenia appeared together with lymphopenia and panhypogammaglobulinemia, what previously was not noticed. Neutropenia is observed with RTX therapy especially in cases of combined treatment with chemotherapy [20-22]. Delayed neutropenia has been described in rituximab-treated CLL patients. However among reported cases, the late onset and progressive leukopenia was not reported.

Hypogammaglobulinemia in chronic lymphocytic leukemia

The majority of patients with CLL develop hypogammaglobulinemia in the course of the disease [23, 24]. Zhu *et al.* [25] revealed that among 83 CLL patients, the IgG, IgA and IgM levels were reduced in 12, 26 and 34 cases, respectively. The incidence of Ig reduction was higher in Binet C stage than that in Binet A and B. Ig levels were not checked in our patient before his admission to Immunological Outpatient Clinic, however before initiation of the treatment he was in Binet stage A and had no clinical manifestations of hypogammaglobulinemia. Therefore it is to be supposed that his humoral immunity state was not affected. According to guidelines prepared by experts in the field of CLL, Prof. Rai and Prof. Keating, in patients who demonstrate a serum IgG < 500 mg/dl and who have had recurrent infections requiring intravenous antibiotics or hospitalization, administration of intravenous immunoglobulin is recommended. The usual dose amounts to 200 to 400 mg/kg, given at three- to four-week intervals. Serum IgG should be monitored in CLL patients due to its importance for evaluating immunologic state and susceptibility for infections. Even in successfully treated patients, there is rarely recovery of Ig levels [26]. Interestingly, Alexandrescu *et al.* suggested that use of high doses of rituximab can improve immune dysfunction and restore, in part, the production of immunoglobulins [27]. This issue has to be clarified in further studies.

Conclusions

The present findings suggests that FCR therapy in some individuals may lead to the induction of progressive alteration of the cellular and humoral immunity leading to the development of pathologies similar to those found in common variable immunodeficiency. Therefore the levels of immunoglobulins should be regularly and frequently monitored before, during and after RTX treatment and in case of reduced IgG concentration, replacement therapy have to be considered especially when symptomatic infections appear. The lasting effects of rituximab on both the humoral and cellular arm of the immune system need further investigation. A long-term prospective study on patients who were treated with FCR regimen due to CLL have to be conducted for explaining the issue of post-FCR lymphopenia and hypogammaglobulinemia. Research should clarify how often progressive and late-onset aforementioned adverse events appear and which predictors are responsible for the observed effect.

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