Neutrophil function in the patient with common variable immunodeficiency – case report

ALEKSANDRA LEWANDOWICZ-USZYŃSKA[1], BOŻENA POLAŃSKA[1], JERZY ŚWIERKOT[2], MARYLA KAZIMIEROWSKA-NIEMCZUK[1], JACEK SZECHIŃSKI[2], ADAM JANKOWSKI[1]

[1]3th Department and Clinic of Pediatrics, Immunology and Rheumatology of Developmental Age, Medical University of Wrocław, Poland; [2]Department of Rheumatology, Medical University of Wrocław, Poland

Abstract

Chosen parameters assessing neutrophil function (myeloperoxidase – MPO concentration, elastase concentration, phagocytic test and oxygen metabolism of neutrophils) in 38 year old patient diagnosed with CVID are presented. The examinations were carried out before the first infusion of IVIG and on the second day after infusion. Before IVIG substitution increased MPO serum concentration was found, enhanced total phagocytic skills and strongly suppressed oxygen metabolism of neutrophils measured by the chemiluminescency test. IVIG substitution improved both spontaneous and stimulated neutrophil chemiluminescence (CL).

Key words: *metabolic activity of neutrophils, common variable immunodeficiency.*

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Introduction

Common variable immunodeficiency (CVID) belongs to the group of primary immune deficiencies occurring both in children and in adults. Characteristic feature is antibody deficiency (deficiency in at least two classes of immunoglobulin and lack of isohaemagglutinin) and frequent occurrence of recurrent infections as well as enhanced tendency to autoimmune diseases and certain types of neoplasm [1-7]. The main cause of infection is antibodies deficit which directly impedes pathogen elimination. Immunoglobulin deficit can as well indirectly influence neutrophil function rendering the phagocytosis and oxygen burst process less effective. The aim of the study was to estimate the metabolic activity of neutrophils in the 38 year old patient with CVID treated among others with IVIG.

Material and Methods

The patient had long medical history (recurrent tonsillitis, pneumonia and bronchitis, pernicious anaemia, septic arthritis, rheumatoid arthritis) and at the age of 38 was diagnosed with CVID on the basis of clinical and laboratory data (which was described in detail by Swierkot and al. in 2006) [8]. Regular, monthly substitution of IVIG preparations was introduced, what brought about significant clinical response.

Before the first IVIG infusion we carried out the following tests in patient's peripheral blood: standard laboratory tests and MPO concentration, neutrophil elastase concentration, IL-8 and phagocytic capacity of neutrophils as well as chemiluminescence (CL). Some of the tests were repeated (phagocytosis, CL) 2 days after IVIG infusion. Blood used to the tests was taken in the morning together with other routine or control tests from cubital vein to the plastic test-tube with heparin (Sarsted Monovette®) – the amount of 0.5 ml and 1.4 ml on the clot. The study was approved by the local Bioethics Committee.

MPO and IL-8 concentration were assessed by ELISA method with use of the commercial kits by BIOCOM. The concentration of elastase was measured in plasma in the complex form with α1-proteinase inhibitor (E-α_1PI) by commercially available ELISA test manufactured by Merck.

Phagocytic test was carried out in 200 µl of heparinised peripheral blood incubated with 200 µl of Staphylococcus aureus 209P suspension (concentration of 10^8 cell/ml) during 30 minutes in the temperature of 37°C. Subsequently, blood smears were prepared and stained with May-Grunwald

Correspondence: Aleksandra Lewandowicz-Uszyńska, 3th Department and Clinic of Pediatrics, Immunology and Rheumatology of Developmental Age, Medical University of Wroclaw, Al. Kasprowicza 64/66, 51-137 Wrocław, Poland. Phone number: +48 71 323 64 50, +48 601 696 344, Email: alusz1@poczta.onet.pl

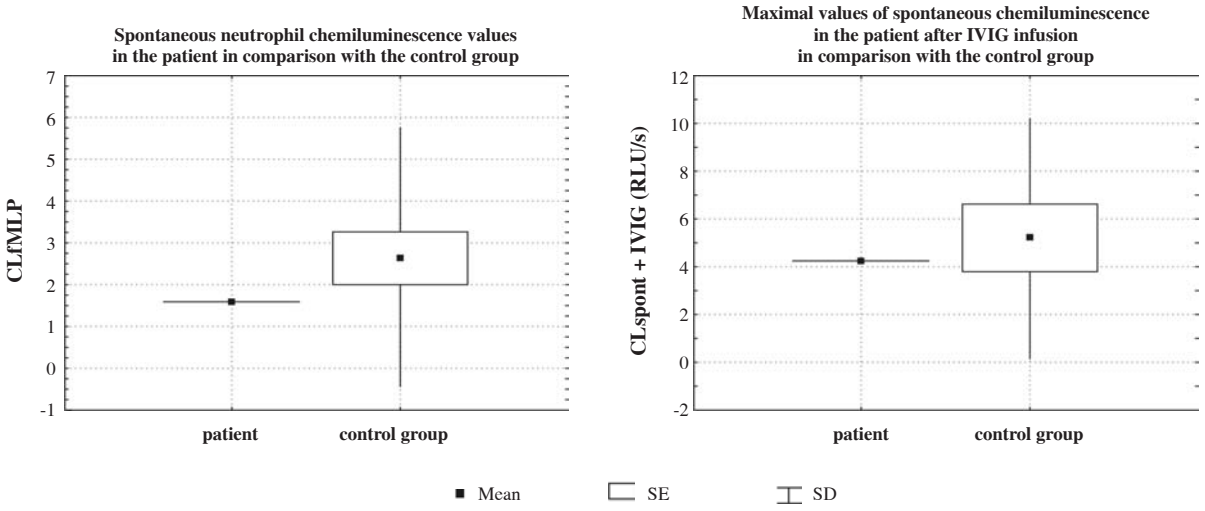

Fig. 1. Spontaneous neutrophil chemiluminescence values in the patient before (A) and after (B) IVIG infusion in comparison with the control group

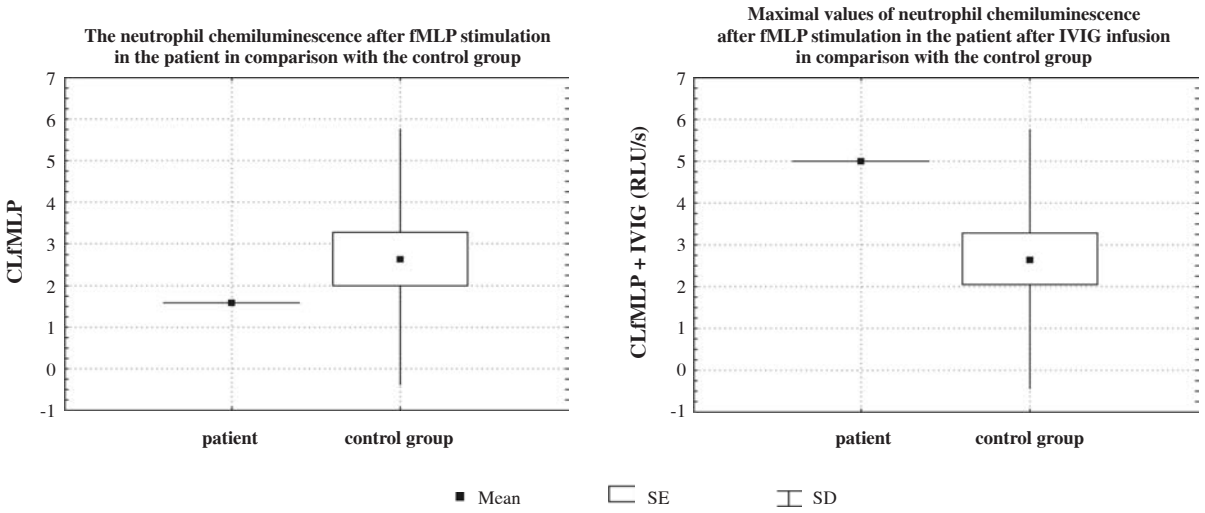

Fig. 2. Maximal values of neutrophil chemiluminescence after fMLP stimulation in the patient before (A) and after (B) IVIG infusion in comparison with the control group

method. Results were estimated in the light microscope with 100 × magnification and immersion. The number of bacteria was counted in 100 neutrophils and subsequently the index of granules was calculated, the so-called phagocytic index (PI):

PI = the number of bacteria in 100 phagocytes/100.

On the basis of the leukocyte count, the neutrophil percentage in the blood smear PI and total phagocytic capacity (TPC) was calculated:

TPC = leukocyte count × % of neutrophils × PI/100.

Oxygen-dependent metabolic activity of neutrophils was assessed by chemiluminescence method [9, 10]. Luminol-dependent chemiluminescence was estimated – both spontaneous and stimulated [11], with use of MicroLumat LB 96P device (EG&G BERTHOLD, Germany) on microplates (LB96P-WMP) in the 8 × 12 wells system [12]. The examination was carried out by the kinetic method during 45 minutes in the temperature of 37°C, and point measurement of CL during 0.2 sec every 60 sec. Results were obtained in the RLU (relative light units). As a CL stimulator fMLP solution was used (formylo-methionylo-leucylo-phenyloalanine) in

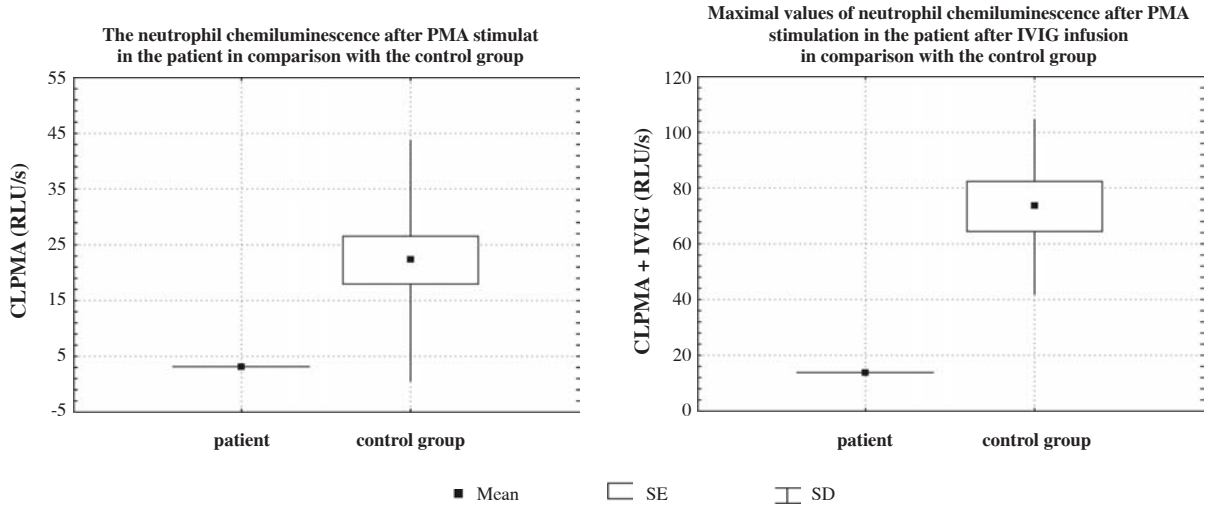

Fig. 3. Maximal values of neutrophil chemiluminescence after PMA stimulation in the patient before (A) and after (B) IVIG infusion in comparison with the control group

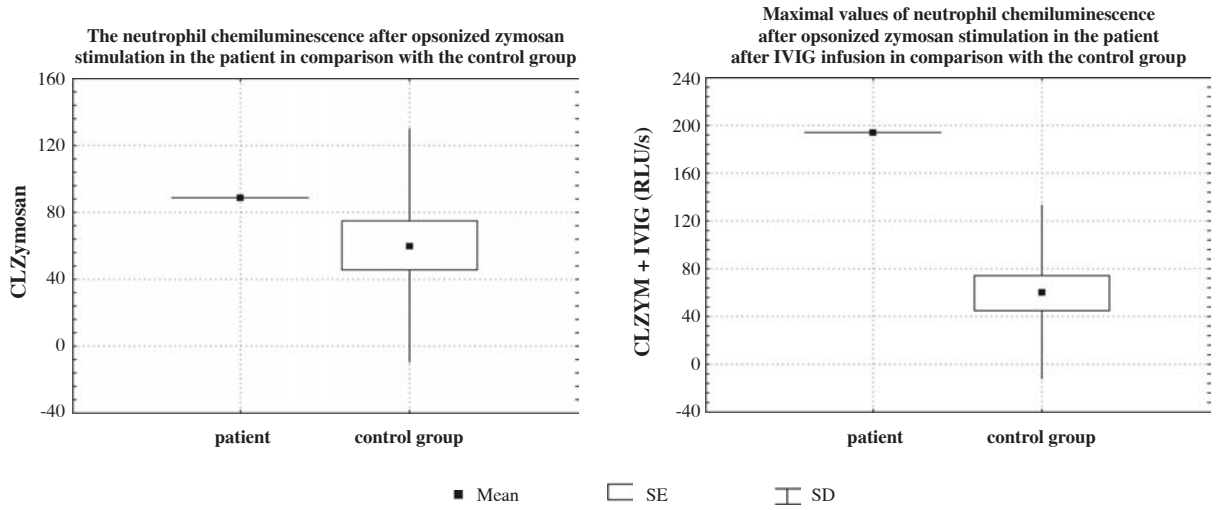

Figure 4. Maximal values of neutrophil chemiluminescence after opsonized zymosan stimulation in the patient before (A) and after (B) IVIG infusion in comparison with the control group

the concentration of 2×10^6 (5 mg of fMLP was diluted in 4.56 ml DMSO (Dimethyl sulfoxide, Sigma) and subsequently in PBS (Liquid Bal Salt Sol, Alab) zymosan (ZAS) (5 mg of zymosan was suspended in 1 ml of PBS and 10 ml of 0,9% NaCl), PMA (phorbol-12-myristate 13 acetate) concentrated at 1 u/ml.

Substrates at the respective volumes were added to the wells on the plates in the following order:

Without stimulation: fMLP: zymosan: PMA:
1. PBS (40 µl) 1. PBS (20 µl) 1. PBS (20 µl) 1. PBS (20 µl)
2. Luminol (20 µl) 2. fMLP (20 µl) 2. Zymosan (20 µl) 2. PMA (20 µl)
3. Blood (40 µl) 3. Luminol (20 µl) 3. Luminol (20 µl) 3. Luminol (20 µl)
4. Blood (40 µl) 4. Blood (40 µl) 4. Blood (40 µl)

In order to reach higher accuracy of the measurement each of the systems was repeated three times and measured simultaneously. From the three values obtained arithmetic average was calculated. The results were presented as the maximal value of CL after the induction with a defined stimulator, registered during 45 minutes of the measurement (CL max).

Results

Results are summarised in two tables and on four figures. Normal IL-8 serum concentration and very high concentration of MPO and neutrophil elastase was shown (increased E-α_1PI). Total phagocytic capacity was significantly increased (the result typical for antigen stimulation, in this case typical for chronic infection) while phagocytic index and the percentage of neutrophils capable of phagocytosis was decreased (Table 1). Spontaneous CL value of neutrophils as well as induced neutrophils CL after fMLP and zymosan stimulation was significantly decreased in comparison with the results obtained in the control group and increased significantly after the infusion of IVIG. Neutrophil chemiluminescence after PMA stimulation was decreased both before and after IVIG treatment (although got partially corrected).

Discussion

CVID is one of the primary immunodeficiency syndromes (PID) which relatively often is not diagnosed before adulthood [13]. It is due not only to doctors unawareness of the fact, that some of PID may manifest in patients over 18 y. of age, but as well to the variability of manifestations with often very eventful clinical course (in our patient: inflammatory states of tonsils, Eustachian tube, left frontal sinus and maxillary sinuses, bronchial and pulmonary infections, gastrointestinal infections, flaccid paresis of upper and lower extremities, pernicious anaemia, reactive arthritis, rheumatoid arthritis and fevers) which hindered the right diagnosis. All those diseases were treated intensively, but unfortunately – as separate entities. Out of all the specialists consulting the patient the clinical immunologist was the last one to have seen her. Variability of the laboratory tests does not make that task easier. The measurement of the main immunoglobulin classes concentration (and sometimes of the isohaemagglutinin, IgG subclasses) is of the uttermost importance to formulate the correct diagnosis. The lack or very low concentrations of IgG class antibodies in this disease cause that the organism is unable to protect itself from infections [14]. Immunoglobulin deficiency caused by different abnormalities in the number and function of T and B lymphocytes makes that deficit even deeper [15]. The assessment of neutrophil function in this syndrome does not get enough attention. However neutrophils are the important element of immunity taking part in combating infectious agents [16]. With their oxygen-dependent and oxygen-independent bactericidal potential they in great part provide anti-infectious protection.

In this study in we have shown in our patient considerable enhancement of neutrophil phagocytic processes (TPC) which is typical for infection. The measurement of the granulocyte enzymes concentration – MPO and elastase (E-α_1PI) – showed as well the significant increase, which is typical for the inflammatory and infective processes present in this patient [17]. The presence of increased concentrations of neutrophil elastase in her blood may be in favour of chronic inflammatory processes running in the patient (rheumatoid arthritis). The measurement of plasma E-α_1PI concentration may serve as a new and sensitive indicator of the acute and chronic disease course and neutrophil activation in different also rheumatoid disease states [18, 19].

Neutrophil function expressed by the free radical production measured by chemiluminescence test showed deep suppression of the oxygen metabolism of these cells, both spontaneous and after activation with stimulators (fMLP, PMA, opsonized zymosan). The reason for this can be the exhaustion of the "aerobic" potential of neutrophils. In consequence of recurrent infections it may come to the suppression of the oxygen metabolism of neutrophils [20, 21], what may additionally favour consequent infectious diseases and deepen already existing immunity defect. As it has been shown in the studies of many authors low values of neutrophils chemiluminescence strongly promote infections [22-26]. On the other hand low values of CL (before IVIG infusion) can be influenced as well by the concomitant rheumatoid arthritis. The suppressed chemiluminescence has been shown in patients with this disease as well [27-30]. Low oxygen metabolism of neutrophils correlated strongly with low concentrations of IL-8, which is one of he strongest inducer influencing NADPH oxidase [31, 32].

After IVIG substitution we observed improved oxygen metabolism of neutrophils in the following range: spontaneous neutrophil CL, after fMLP and opsonized zymosan stimulation, and they were maintained above the values of control group. They reminded of neutrophil CL activity in healthy subjects with symptoms of infection, what was in favour of regaining the capability of free radical generation, and subsequently to intracellular killing of pathogens. The immunological abnormalities concerning neutrophil chemiluminescence observed in our patient can be primary or secondary.

The IVIG substitution improved neutrophil CL value after PMA stimulation, but still it remained below the values in healthy subjects. The IVIG infusion did not influence extrareceptor pathway of neutrophil activation. Introducing of IVIG supplementation, which is the cornerstone of CVID treatment [33] significantly decreased the number of the subsequent infections (during a 12-months follow-up) in the patient described, by supplying "ready to use" antibodies against multiple pathogens. It enhanced as well the neutrophil function in the field of free radicals generation, which are the important part in immunity against infective agents. The decreasing of the number of infections will contribute to sparing FR reserves by neutrophils. The IVIG substitution had as well beneficial effect on RA course, which is in accordance with other authors observations [34, 35].

CVID, owing to its variable clinical course and observed immunological abnormalities still belongs to not completely explored PID. The correct treatment depends on the cooperation of many representatives of different medical and laboratory specialities. And observed abnormalities still demand the follow up and explanation.

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