INFECTIOUS MONONUCLEOSIS – DIAGNOSTIC POTENTIALS

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SUMMARY:

Infectious mononucleosis is a disease in children and adolescents. It is common mainly in countries with temperate and cold climate. Patients usually present with fever, sore throat, lymphadenopathy, often hepatosplenomegaly. Haematologic abnormalities include a peripheral blood lymphocytosis, more than 10 % of the leucocytes in blood consisit of atypical lymphocytes. Epstein-Barr virus (EBV) is an etiologic agent. In organism it leads to characteristic immunopathogenetic process which is visualized by various diagnostic tests. Aims: To analyze the possibilities of flowcytometric immunophenotypization of lymphocyte populations and subpopulations in peripheral blood in order to differentiate the infectious mononucleosis from other benign lymphocytoses and malignant lymphoprolific diseases. Materials and metods: Lymphocyte subsets from whole blood were examined in 25 acute infectious mononucleosis patients with FACSort flow cytometer. We used monoclonal antibodies against CD3 (T-cells), CD4 (T-helper cells), CD8 (T-cytotoxic cells), CD19 (B cells), CD56 and CD16 (natural killer cells), CD3/HLA-DR (activated T cells), CD2 (T-cells), CD5 (T-cells), CD7 (T-cells). Results: The levels of CD8 T-cytotoxic cells were significantly increased in all 25 patients. The T-cells in all cases expressed activation antigen HLA-DR and displayed down-regulation of CD7 in the CD8+ population. Òhree cases showed down-regulation of CD5 in the CD8+ population. We found also that CD19 B cells and CD4/CD8 ratio were significantly decreased in all patients with acute infectious mononucleosis. Conclusion: The data in the present study show that acute infectious mononucleosis is characterized by an activated CD8+ T-cell population with antigenic aberrancy (down-regulation) of CD7 and occasionally of CD5, in addition to a decrease in B-cells and CD4/CD8 ratio. There is specific flow cytometric constellation which makes possible for the patients to be differentiated by infectious mononucleosis and other lymphocytosis.

Key words: infectious mononucleosis, Epstein-Barr virus, flow cytometric analysis

INTRODUCTION:

Infectious mononucleosis is a acute infectious disease, caused by Epstein-Barr virus (EVB) that belongs to the Herpesviridae. It is mostly common among the teenagers and young people. The main mechanism of infection is the droplet one, through an infected saliva. Therefore, the disease is also known as "kissing disease". EBV can also be transferred through blood transfusion, blood products and contact-consumer way, through objects contaminated with infected saliva. The secondary attack rates of infectious mononucleosis are low (about 10%). Serologic researches proved that 90% of the people in Europe had contact with the virus and its healthy carriers. In some geographic areas the infection with the virus is connected with a number of malignant diseases such as nasopharyngeal carcinoma, Burkitt lymphoma, Hodzhkin's lymphoma and other lymphoproliferative disorders.

The virus penetrates through the epithelium of the oropharynx. It replicates in the oropharyngeal epithelium cells and B-lymphocytes. EBV adheses to B-lymphocytes, through CD21-their antigen provokes their transformation and proliferation. In the course of infection, in the peripheral blood there appear a large number of atypical lymphocytes resulting from the polyclonal activation of cytotoxic-suppressor CD8 cells. They limit the excessive transformation and proliferation of B-cells. In the event of inefficacious T-cell immune response, one can develop persistent infection and uncontrolled B-cell proliferation that is in the basis of the EBV oncogenic potential. The virus possesses a number of antigens against which in the course of immunogenesis antibodies are formed: early antigen (EA), virus capsid antigen (VCA), nuclear antigen (NA).

Clinically, the infectious mononucleosis is characterised with high temperature, flue, lympho-nodylopathy, frequently hepatosplenomegaly. The process is observed and registered when there are data about active EBV infection for more than 6 months.

The disease diagnosis is complex:

Clinical and epidemiological - predominantly young patients, most often male, during cold months of the year,

characterized with febrility, oropharynx inflammation, larger lymph nodes (neck, axillaric) and hepatosplenomegaly ;

Laboratory – from the differential blood count is typical lymphomonocytosis of over 50-60% and atypical lymphocytes of over 10% which are CD8 and CD4 T-lymphocytes. Morphologic heterogenity of the lymphoid population – normal lymphocytes and monocytes and atypical lymphoplasmotic cells with wide cytoplasm, peripheral basophilia and eccentrically positioned nuclear are observed. A blood chemistry test may reveal abnormalities in liver function.

Serologic – a routine test to find out infectious mononucleosis is the Paul-Bunell test, proving heterophilic antibodies (as compared to sheep erythrocytes). It is considered positive if titer is over 1:10. It is positive after the first week of the disease and remains positive for a few weeks. The test gives false negative result with children or during the first week of the disease. False positive samples can be observed with lymphoma, system lupus, HIV and other virus infections. There exist express tests (Monospot test) to prove heterophilic antibodies that become positive with 80%-90% of the patients. Diagnostic difficulties increase with patients of atypical symptoms and negative Paul-Bunell test. Then, serologic tests have to be made proving the existence of specific antibodies against EBV, more precisely: anti-EBV, VCA-IgM/IgG, anti-ÅA-IgM/IgG and anti-EBNA-IgG [1,2].

Histologic – patients with lymphadenopathy have to undergo hystologic test, specifying the reason for the enlarged lymph node. The research is useful in order to exclude lymphoproliferative diseases with another etiology. Infectious mononucleosis is characterized with paracortical hyperplasia and proliferation of cell elements.

Flow cytometric research of peripheral blood – this test is relevant for patients, suspected for infectious mononucleosis, who were not subjected to serologic diagnostics, with atypical clinical disease forms and differentiating the infectious mononucleosis from other lymphocytoses.

The objective of this study was to analyze the possibilities of flowcytometric immunophenotypization of lymphocyte populations and subpopulations in peripheral blood in order to differentiate the infectious mononucleosis from other benign lymphocytoses and malignant lymphoprolific diseases.

MATERIALS AND METHODS:

The research of 25 patients: 17 (68%) men and 8 (32%) women between the age of 3 and 58 (average age 21) was made in the MDL (Medical Diagnostic Laboratory) of Immunology at University Multiprofile Hospital of Active Treatment "Dr. G. Stranski" – Pleven during 2003-2007, having received the patients' consent. The 25 patients to be tested with suspicion for infectious mononucleosis according to clinical data, have been directed from a Clinic of

Hematology and Clinic of Infectious Diseases. All patients had positive reaction to Paul-Bunell.

Flow cytometry (FCM) - 5 ml of peripheral blood (K2 EDTA vacutainer) were drawn from each patient. Two-color immunophenotype analyses of lymphocyte populations and subpopulations were performed on a FACSort flow cytometer (Becton Dickinson, CA, USA) using CellQuest Software for acquisition, as previously described [3]. Peripheral blood was stained with fluorescein isothiocyanate (FITC) and phycoerythrin (PE)-labeled monoclonal antibodies (MoAbs), according to the Becton Dickinson staining protocol. MoAbs to CD3 (T cells), CD4 (T-helper cells), CD8 (T-suppressor-cytoxic cells), CD19 (B cells), CD16+56+ (natural killer cells), CD3/HLA-DR (activated T cells) and pan-T-cell antigens (CD2, CD5, CD7) were used. Isotype-matched MoAbs were used as a non-specific control. Fluorescence data were obtained at logarithmic settings. Cells within the lymphocyte cell gate as determined by light scatter, were evaluated for fluorescence after reaction with the antibodies and were presented as percentages (**Fig. 1**).

RESULTS:

Lymphomonocytosis is observed in the differential blood count of all patients. The data from the flow cytometric reaserch show the following changes in the differential blood count: lymphocytosis is registered with 13 patients (52%), 4 have increase of monocytes (32%). Immunophenotypic analysis of all infectious mononucleosis cases did not show any pan–T-cell antigen deletion. The levels of CD8 T-cytotoxic cells were significantly increased in all 25 patients. The T-cells in all cases expressed activation antigen HLA-DR and displayed down-regulation of CD7 in the CD8+ population (**Fig. 2**).

Assignation of intensity was performed according to published criteria [4]. Òhree cases showed down-regulation of CD5 in the CD8+ population. We found also that CD19 B cells and CD4/CD8 ratio were significantly decreased in all patients with acute infectious mononucleosis. CD16+56+ natural killer (NK) cells were increased in six of the cases (**Table 1**).

DISCUSSION:

Infectious mononucleosis usually is characterized by relative and absolute lymphocytosis. At least a subset of the lymphocytes are atypical, Downey-type cells, with abundant pale cytoplasm and a basophilic cytoplasmic rim, as well as insinuation between neighboring erythrocytes [5].

The lymphocytes in infectious mononucleosis are activated (as connoted by HLA-DR expression) and are composed of a mixture of CD8+ cytotoxic-suppressor T-cells, NK cells, and CD4+ helper T-cells. The dominant population by far is the CD8+ T cells, which have a role in the suppression of viral replication and have cytotoxic activity

against virally infected B cells [6-9]. Increased numbers of CD8+ cytotoxic-suppressor T-cells also have been seen in other viremias, including HIV and cytomegalovirus infection [10], as well as in hepatitis C.

In patients with unexplained T-cell lymphocytosis, the possibility of a T-cell lymphoproliferative disorder must be considered. This diverse group of T-cell neoplasms often is difficult to characterize and diagnose [11,12]. Immunophenotyping by FCM has become a widely used tool for the analysis of both B- and T-cell lymphoid populations. Unlike the situation with B-cell lymphoproliferative disorders, clonality of T-lymphocytes cannot be determined definitively by FCM. However, aberrant antigen expression can be detected by FCM and represents a useful diagnostic clue that a T-cell lymphoproliferative disorder may be present. Down-regulation of CD7 is one of the most commonly seen antigenic aberrations in T-cell lymphoproliferative disorders [11-13] and down-regulation of CD5 also is relatively common. T-cell antigenic aberrancy must be interpreted with caution, especially given the spectrum of nonneoplastic T-cell antigen expression. Down-regulation or absence of CD7 expression on T-cells can be seen in a variety of reactive conditions, including inflammatory dermatoses and rheumatoid arthritis [14,15].

CD7 is a 40-kd glycoprotein member of the immuno-globulin superfamily and is expressed on the cell surface early in T-cell ontogeny and persists during differentiation. It is expressed not only on precursor and peripheral T cells but also on NK cells and during early stages of B and myeloid cell development. Its exact function is largely unknown; it facilitates early activation of T and NK cells and is thought to function as a signal-transducing receptor on NK cells [20]. CD7-mediated signals may augment the function of adhesion molecules on NK cells, possibly by delivering signals to the cell interior and inducing tyrosine and lipid kinase activities.

The data in the present study show that acute infectious mononucleosis is characterized by an activated CD8+ T-cell population with antigenic aberrancy (down-regulation) of CD7 and occasionally of CD5, in addition to a decrease in B-cells and CD4/CD8 ratio. A clue to the correct diagnosis is that peripheral T-cell neoplasms often are negative for the activation antigen HLA-DR. HLA-DR is expressed heterogeneously in T-cell lymphoproliferative disorders [21] and is expressed more commonly in cutaneous T-cell lymphoma than in other types. If an HLA-DR+ T-cell population displays antigenic down-regulation of CD7 and possibly also of CD5, it is important to correlate the flow cytometric findings with the presence or absence of atypical lymphocytosis on the peripheral blood smear and with appropriate viral serologic findings in consid- eration of infectious mononucleosis.

CONCLUSION:

The data in the present study show that acute infectious mononucleosis is characterized by an activated CD8+ T-cell population with antigenic aberrancy (down-regulation) of CD7 and occasionally of CD5, in addition to a decrease in B-cells and CD4/CD8 ratio. Careful clinicopathologic correlation always is warranted in interpreting all laboratory data, including flow cytometric analysis.

Table 1. Flow cytometric parameters among sick with infectious mononucleosis

Flowcytometric parametres	n=25	%
CD 3 (T-cells) - increased	16	64
CD 3+DR - increased	25	100
CD 4 (Th) – decreased	23	92
CD 8 (Ts) - increased	25	100
Th/Ts – decreased	25	100
CD 19 (B-cells) – decreased	25	100
CD 16/56 (NK-cells) - increased	6	24
CD 7 (T-cells) – doun-regulation	25	100
CD 5 (T-cells) - doun-regulation	3	12

Fig. 1. Flow cytometric analysis of periferial blood in a patient with infectious mononucleosis. Initial gate set around Lymphocytes (Small size, no granules) by FSC/SSC (A). Then further gates applied to identifield changes in B-Ly by CD3/CD19 dot plot (B); Ts-Ly by CD3/CD8 (C); Th-Ly by CD3/CD4 (D); NK by CD3/16+56 (E); activated-Ly by CD3/HLA-DR (F).
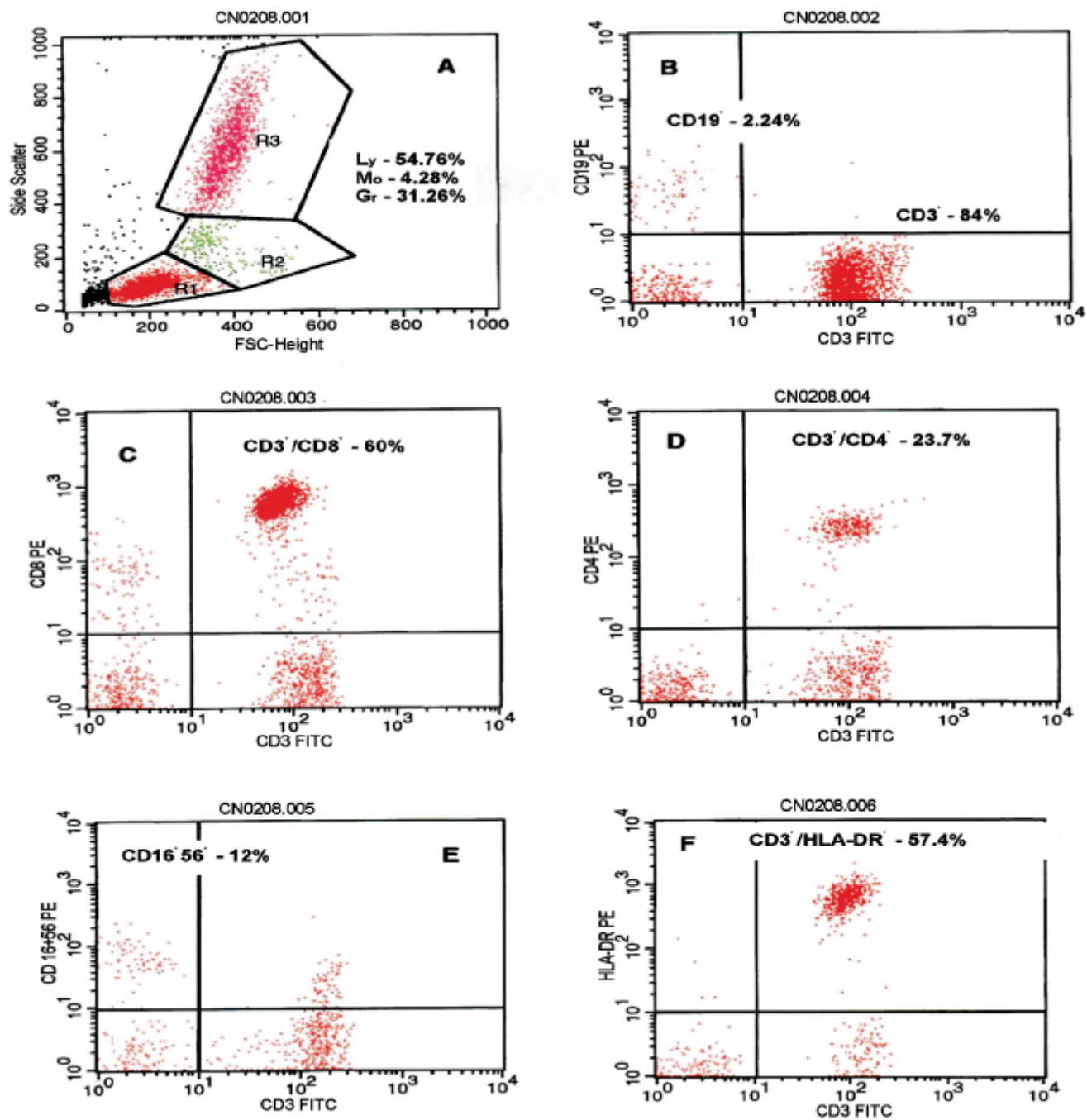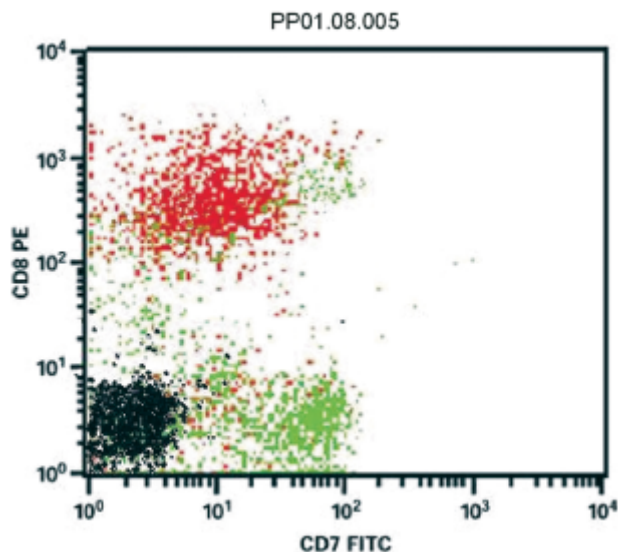
Fig 2. Down-regulation of CD7 by the CD8 T-cell population (red) in a case of infectious mononucleosis.

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