Center for Disease Control (CDC) Flow Cytometry Panel for Human Immunodeficiency
Virus Infection Allows Recognition of Infectious Mononucleosis Caused by Epstein-Barr
Virus or Cytomegalovirus

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Aim. To analyze the distribution of lymphocyte subsets in the peripheral blood of patients with infectious mononucleo-
sis caused by Epstein-Barr virus (EBV) and cytomegalovirus (CMV) and to investigate the possible diagnostic usefulness
of flow cytometry panel recommended by the Center for Disease Control and Prevention (CDC) for HIV-1 infection.

Methods. The study included 130 immunocompetent adults with infectious mononucleosis caused by EBV (n=103)
and CMV (n=27) and 50 controls. EBV-infected patients were divided into two groups based on typical (n=92) or
atypical (n=11) clinical presentation of the disease. Lymphocyte subpopulations were determined by flow cytometry
and a panel of monoclonal antibodies recommended by the CDC for the immunophenotyping of patients infected
with human immunodeficiency virus (HIV).

Results. Patients with typical and atypical presentation of EBV-induced infectious mononucleosis showed increased
percentages of total T-cells, cytotoxic-suppressor CD8⁺ T cells and activated HLA-DR⁺ T cells compared to healthy
controls. Percentages of CD4⁺ T cells, as well as CD4/CD8 ratio, were significantly decreased. Absolute counts of
CD4⁺ T cells and percentages of B cells did not differ from healthy controls. Pattern of changes in CMV-infected pa-
tients was completely identical to that in healthy controls, although less pronounced.

Conclusion. Lymphocyte subpopulations represented in the CDC panel for HIV are sufficient for the recognition of pa-
tients with infectious mononucleosis caused by EBV and CMV. Flow cytometry can be useful support for reaching di-
agnosis in patients with atypical clinical presentation of EBV-induced infectious mononucleosis.

Key words: B-lymphocytes; cytomegalovirus; Epstein-Barr virus infections; flow cytometry; infectious mononucleosis; T-lym-
phocytes

The diagnosis of infectious mononucleosis is based on clinical presentation and supportive labora-
tory (hematological and biochemical) findings. Differential diagnosis of infectious mononucleosis and atypical lymphocytosis usually includes acute infec-
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EBV initially replicates in the pharyngeal epithe-
ilium and subsequently (within weeks) establishes a latent infection in B cells. Acute EBV-infection is char-
acterized by a self-limiting proliferation of both helper-inducer CD4⁺ and cytotoxic-suppressor CD8⁺
T cells (18,19). The CD8⁺ T cell immune response is believed to responsible for controlling EBV replica-
tion at the acute stage of infection and maintaining vi-
eral latency. It is reasonable to assume that changes in the numbers and proportions of different lymphocyte subsets might be diagnostically useful (20,21).

The aim of this study was to analyze the distribution of several lymphocyte subsets in patients with EBV-induced infectious mononucleosis and compare it with the lymphocyte subset distribution in the healthy controls. In an attempt to investigate potential diagnostic usefulness of immunophenotyping in infectious mononucleosis, we compared the profiles of patients with typical and those with atypical clinical and/or laboratory presentations of EBV-induced infectious mononucleosis. Furthermore, the results were compared with the immunophenotyping profiles in patients with infectious mononucleosis caused by CMV. Our hypothesis was that even a minimum flow cytometry panel, such as the one recommended by the CDC for HIV-1 infection, could be useful in the recognition of infectious mononucleosis, especially in patients with atypical clinical presentation.

Patients and Methods

Patients

The study included 130 non-hospitalized or hospitalized immunocompetent patients with either clinical symptoms or laboratory findings compatible with infectious mononucleosis. The patients were diagnosed and treated at the Dr. Fran Mihaljević University Hospital for Infectious Diseases, Zagreb, Croatia, between November 1999 and December 2002.

The Ethics Committee of the Hospital approved the study. Informed consent was obtained from all patients and healthy controls.

The study included three groups of patients with infectious mononucleosis classified according to the etiology (acute EBV or CMV infection) and clinical presentation of disease (typical vs atypical presentation of EBV-induced infectious mononucleosis).

A group of patients with EBV-induced infectious mononucleosis consisted of 103 patients, with the male to female ratio 1:1.2. Median age of EBV-infected patients was 26 years (range, 19-36 years).

In the group of patients with serologically verified acute EBV infection, 92 patients had typical clinical and laboratory presentation of infectious mononucleosis.

A smaller group of 11 patients had atypical clinical and laboratory presentation of EBV-induced infectious mononucleosis, which was subsequently serologically verified. The criteria for the inclusion in this group were based on either clinical presentation (fever >40 °C, maculopapular skin rash without a history of recent antibiotic use, neurological disease manifestations, and autoimmune hemolytic anemia) or laboratory findings (>30×10⁹/L of white blood cells/L and small percentages of “atypical” or “reactive” lymphocytes in the peripheral blood (PB) during the first 3 weeks).

The group of CMV-infected patients consisted of 27 patients, with the male to female ratio 1:4:1. Median age of CMV-infected patients was 24 years (range, 20-36 years).

Children younger than 17 years of age, immunodeficient patients, and pregnant women (5 patients) were excluded from the study.

Methods

Routine hematological analysis of the peripheral blood, biochemical tests for liver enzyme activity, and cytological analysis of the peripheral blood were performed in all patients. Cytological analysis of lymph node fine needle aspirate was performed in 37 EBV-infected patients.

Serological Testing

All serum samples were tested for IgM and IgG antibodies to viral capsid antigen (VCA), IgG antibodies to early antigen-diffuse (EA(D)), and EBV nuclear antigen (EBNA), respectively, and for IgM and IgG antibodies to CMV, by using an enzyme-linked immunosorbent assay (ELISA, DiaSorin, Saluggia, Italy).

The method used for determination of specific IgM to EBV, VCA, and CMV was indirect IgM capture assay. Testing and interpretation of the results were performed according to the manufacturer’s recommendations (DiaSorin).

Patients presenting with non-specific viral syndromes were also assessed for HIV risk behavior and, following written consent, tested accordingly.

Flow Cytometry

Flow cytometry was performed on a sample of peripheral blood drawn for hematological analysis within 6 days from the onset of symptoms.

The immunophenotyping of peripheral blood was performed by a whole blood non-wash method on a Coulter Multi-Q-Prep System (ImmunoPrep Reagent System, Whole blood lysing reagents, Beckman Coulter, Inc., Hialeah, FL, USA) and EPICS XL flow cytometer (Beckman Coulter) as recommended by the CDC (22,23).

We performed three-color flow cytometry using a panel of monoclonal antibodies specific for CD3, CD4, CD8, CD14, CD19, CD56, HLA-DR (DAKO, Glostrup, Denmark) conjugated with fluorescein (FITC), phycoerythrin (PE) or phycoerythrin-cyanin 5 (RPE-Cy5) with appropriate isotypic controls (combinations CD3-FITC/CD4-PE/CD45-RPE-Cy5, CD3-FITC/CD4-PE/CD45-RPE-Cy5, CD3-FITC/CD4-PE/CD45-RPE-Cy5, and CD3-PE/CD45-RPE-Cy5, and CD3-FITC/HLA-DR-PE).

The absolute count of CD4+ T cells was performed directly on the cytometer by using Flow-Count Fluospheres (Beckman Coulter).

Statistical Analysis

Data were presented as median and range. Statistical analysis was performed by using SAS package (version 6.12, SAS Institute, Cary, NC, USA) (24). The comparison between the groups was performed by Kruskal-Wallis test. The comparisons between two independent groups were done with Mann-Whitney test; p<0.05 was considered statistically significant. To avoid the increase of type one error rate, we made the adjustments to the p-values taking into account the number of tests performed.

Results

Clinical and Laboratory Findings in EBV-infected (Typical Clinical Presentation) and CMV-infected Patients

The majority of both EBV and CMV patients experienced the usual spectrum of symptoms including fever, headache, malaise, myalgia, arthralgia, weight loss, abdominal pain, diarrhea, sore throat, unproductive cough, splenomegaly, and adenopathy. The most pronounced clinical sign in both patient groups was fever, present in 88 (95.3%) EBV-infected patients and 24 (88.8%) CMV-infected patients, which tended to be higher at night. Fever varied over time in some patients and lasted longer than 10 days in 42 (45.6%) EBV-infected patients. Standard laboratory diagnostic procedures revealed leukocytosis, with lymphocytosis in 59 (64.1%) patients in the EBV group and in 18 (66.6%) patients in the CMV group, atypical lymphocytes were present in the peripheral blood of 69 (67.3%) patients in the EBV group and 23 (85.1%) patients in the CMV group.

More than 90% of patients from both groups had abnormal liver function test findings. In 39 (42.3%) EBV-infected patients, alanine aminotransferase (ALT)
values were above 200 IU/mL (median value 118.3 IU/mL; reference value <50 IU/mL). Lactate dehydrogenase (LDH) values were increased in more than two-thirds of patients from both groups (median values, 540 and 449 IU/mL for EBV and CMV groups, respectively). Lymphadenopathy (2-5 enlarged lymph nodes) was observed in the majority of patients, with cytological examination showing reactive hyperplasia. Patients without complications were treated conservatively with supporting measures.

**Clinical and Laboratory Findings in Patients with Atypical Presentation of EBV-induced Infectious Mononucleosis**

Atypical clinical presentations of infectious mononucleosis included a patient with very high fever (>40°C), a patient with maculopapular skin rash, and three patients with total white blood cells count higher than 30x10^9/L. Lymphadenopathy (2-5 enlarged lymph nodes) was observed in the majority of patients, with cytological examination showing reactive hyperplasia. Patients with autoimmune hemolytic anemia.

**Immunophenotyping Profiles in Patients with Infectious Mononucleosis Caused by EBV or CMV**

We determined percentages of T and B cells, helper CD4+ and cytotoxic-suppressor CD8+ T cells, NK cells, activated HLA-DR+ T cells, CD4+ to CD8+ T cell ratios, and absolute counts of CD4+ T-lymphocytes in patients with typical and atypical clinical presentation of EBV-induced infectious mononucleosis, CMV-induced infectious mononucleosis, and healthy controls (Table 1).

The comparison of selected lymphocyte sub-populations in patients with typical and atypical clinical presentation of EBV-induced infectious mononucleosis with healthy controls revealed an identical pattern of changes. The percentages of total T cells in patients with typical and atypical clinical presentation of EBV-induced infectious mononucleosis were significantly increased in comparison with healthy controls (p<0.001). EBV-induced infectious mononucleosis caused a decrease in the percentages of B cells in patients with typical and atypical disease presentation in comparison with healthy controls, but the difference was not statistically significant. Median percentages of cytotoxic-suppressor CD8+ T cells in EBV-infected persons with typical and atypical disease presentation were also significantly increased, compared with healthy controls (p<0.001). Median percentages of helper CD4+ T cells were significantly decreased in both groups of EBV-infected patients, compared with healthy controls (p<0.001). Decreased percentages of CD4+ T-cells and a parallel increase in the percentages of CD8+ T-cells resulted in the inverted CD4+/CD8+ ratios in EBV-infected patients with both typical and atypical disease presentation, compared with healthy controls (p<0.001). The expression of activation molecule HLA-DR on T cells was significantly higher in both subgroups of EBV-infected patients, compared with healthy controls (p<0.001). The percentages of NK cells in both subgroups of EBV-infected patients did not significantly differ from those in healthy controls. The differences in all selected lymphocyte subpopulations in patients with typical versus atypical clinical presentation of EBV-induced infectious mononucleosis were not statistically significant.

The immunophenotyping profiles of peripheral blood lymphocyte subpopulations in patients with CMV-induced infectious mononucleosis showed a pattern of changes completely identical to that in EBV-infected patients. The percentages of T cells, cytotoxic-suppressor CD8+ T-cells, and activated HLA-DR+ T-cells were significantly higher than those in healthy controls (p<0.001 for all parameters; Table 1). Median percentages of helper CD4+ T cells as well as CD4+/CD8+ T cells ratio in CMV-infected patients were significantly lower than those in healthy controls (p<0.001). The percentages of NK cells and B cells in patients with CMV-induced infectious mononucleosis were not significantly different from those in healthy controls.

Patients with CMV-induced infectious mononucleosis had significantly lower median percentages of T cells, cytotoxic-suppressor CD8+ T cells, and activated HLA-DR+ T cells than both groups of EBV-infected patients (p<0.001, Table 1). The percentages of helper CD4+ T-cells and CD4+/CD8+ T-cell ratio in the CMV group were significantly higher than in both EBV subgroups (p<0.001). The differences in the median percentages of NK cells and B cells between CMV-infected and EBV-infected patients were not significant.

Absolute counts of CD4+ T-lymphocytes in both subgroups of EBV-infected patients and CMV-infected patients were significantly lower than those in healthy controls.

**Table 1. Percentages of peripheral blood subpopulations (median, range) in patients with Epstein-Barr virus (EBV)-induced infectious mononucleosis (typical vs atypical clinical presentation), cytomegalovirus (CMV)-induced infectious mononucleosis, and healthy controls**

<table>
<thead>
<tr>
<th>Cellular subpopulation (%)</th>
<th>Typical (n=92)</th>
<th>Atypical (n=11)</th>
<th>CMV infection (n=27)</th>
<th>Healthy controls (n=50)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells</td>
<td>87.8 (62.1-90.0)</td>
<td>89.7 (79.8-89.1)</td>
<td>81.2 (59.5-87.2)</td>
<td>73.3 (59.0-88.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B cells</td>
<td>1.7 (0.2-4.5)</td>
<td>1.6 (0.8-3.5)</td>
<td>3.3 (0.9-10.5)</td>
<td>9.7 (4.4-26.4)</td>
<td>0.48</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>14.2 (6.8-41.7)</td>
<td>12.5 (6.9-28.1)</td>
<td>21.7 (10.4-35.0)</td>
<td>43.8 (34.9-64.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD4+ T cells (count/µL)</td>
<td>94.3 (412-1482)</td>
<td>934 (508-1423)</td>
<td>950 (612-1468)</td>
<td>928 (533-1457)</td>
<td>0.73</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>65.8 (33.3-83.0)</td>
<td>67.8 (39.4-74.0)</td>
<td>48.8 (33.9-70.6)</td>
<td>23.9 (11.0-37.1)</td>
<td>&lt;0.001</td>
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<td>CD4+/CD8+ T cells</td>
<td>0.2 (0.1-1.3)</td>
<td>0.2 (0.1-1.0)</td>
<td>0.4 (0.1-1.0)</td>
<td>1.9 (1.0-3.6)</td>
<td>&lt;0.001</td>
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<td>NK cells</td>
<td>4.1 (0.5-22.4)</td>
<td>3.0 (0.5-14.8)</td>
<td>3.1 (1.3-19.5)</td>
<td>3.9 (0.4-19.3)</td>
<td>0.41</td>
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<tr>
<td>HLA-DR+ T cells</td>
<td>55.0 (13.7-81.4)</td>
<td>57.4 (46.5-71.4)</td>
<td>26.2 (5.3-52.1)</td>
<td>1.9 (0.5-25.9)</td>
<td>&lt;0.001</td>
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</table>

*Significantly different compared to other patient groups (Mann Whitney test).

†Significantly different compared to the group of healthy controls (Mann Whitney test).

‡Significantly different compared to other patient groups (Kruskal-Wallis test).
patients did not significantly differ from those in healthy controls.

**Discussion**

We showed that infectious mononucleosis caused by infections with EBV or CMV significantly changed the percentages and absolute counts of several peripheral blood lymphocyte subpopulations. The immunophenotyping profile in both EBV- and CMV-infected patients was similar and easily recognizable, suggesting its usefulness in differential diagnosis of infectious mononucleosis. The results of this study showed for the first time that patients with atypical clinical presentation of EBV-infected infectious mononucleosis had the same immunophenotyping profile in the peripheral blood as those with typical clinical presentation of this disease.

Acute EBV infection induces a vigorous CD8+ T cell immune response, which is believed to be responsible for the control of viral replication and establishment of latency (25). Our study showed significantly increased percentages of CD8+ T cells in EBV-infected patients with either typical or atypical clinical presentation and CMV-infected patients, as compared with healthy controls. In addition, increased percentages of T cells and activated HLA-DR+ T cells were observed, as well as decreased percentages of helper CD4+ T cells, and inverted the ratio of CD4+ and CD8+ T-cells. Percentages of NK cells and B-cells did not significantly differ from those in healthy controls. Disproportion between significantly decreased percentages of CD4+ T cells and absolute counts within normal values clearly illustrated the extensive lymphocytosis in patients with infectious mononucleosis.

In our previous study, we described immunophenotyping profiles in the peripheral blood of five patients with EBV-induced infectious mononucleosis (26). The results of much larger group of patients in this study confirmed our preliminary observation that acute EBV-induced infectious mononucleosis (both typical and atypical clinical presentation) changed the numbers and proportions of major lymphocyte subsets included in the CDC immunophenotyping panel for HIV-1. The pattern of these changes appears to be characteristic and easily recognizable.

It has been previously shown that children with EBV-induced infectious mononucleosis (older than 17 months) have reversed CD4/CD8 ratio and increased proportions of activated HLA-DR+ CD4+ and CD8+ T-lymphocytes in the peripheral blood (27). The results of our study have shown that the pattern of lymphocyte changes in infectious mononucleosis caused by EBV are similar in both children and adults.

We also investigated possible diagnostic usefulness of flow cytometric immunophenotyping in differential diagnostics of infectious mononucleosis. Differential diagnostics of infectious mononucleosis usually includes other infectious agents, including human immunodeficiency virus, adenoviruses, parvoviruses, herpesviruses, toxoplasma, and lymphoproliferative diseases (1). However, systematic comparisons of the distribution of different lymphocyte subpopulations in EBV-induced infectious mononucleosis and mononucleosis of different etiologies in larger groups of patients are not available. Our study has shown that despite the similarities in the type of changes in the percentages and numbers of T cells and their subpopulations, B cells and NK cells, there were significant differences in the immunophenotyping profiles induced by EBV vs CMV. The observed difference in the intensity of cellular immune response between EBV- and CMV-induced infectious mononucleosis could contribute to the etiological diagnosis in infectious mononucleosis.

Our conclusion is that flow cytometry panel recommended by the CDC for HIV-1 infection can be useful support in reaching diagnosis in patients with atypical clinical or laboratory presentation of EBV-induced infectious mononucleosis. Lymphocyte subpopulations represented in the CDC panel for HIV are sufficient for the recognition of patients with infectious mononucleosis caused by EBV and CMV.

**References**


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