Hematologic Differences in Heterophile-Positive and Heterophile-Negative Infectious Mononucleosis

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INTRODUCTION

Infectious mononucleosis (IM) due to all causes is characterized by atypical lymphocytosis. We sought to compare hematologic parameters of infectious mononucleosis due to Epstein–Barr virus (EBV) infection (heterophile antibody (HA) positive) with mononucleosis due to other causes. Mono-Latex Slide Agglutination Test results and complete blood counts (CBC) of 147 patients with mononucleosis were retrospectively analyzed. Leukocyte count, absolute lymphocyte count, and presence of atypical lymphocytes in EBV-positive and EBV-negative groups were statistically compared. We analyzed 68 EBV-positive and 79 EBV-negative cases. EBV-positive patients were significantly younger than EBV-negative patients were. Mean total WBC count and mean absolute lymphocyte count were significantly higher in EBV-positive patients. Absolute lymphocytosis, absolute leukocytosis, and atypical lymphocytosis were also significantly more frequent in EBV-positive patients. Leukopenia was more frequently seen in EBV-negative patients. Am. J. Hematol. 76:315–318, 2004. © 2004 Wiley-Liss, Inc.

Key words: infectious mononucleosis; heterophile antibody; atypical lymphocytosis

The characteristic hematologic findings in IM include lymphocytosis defined as a differential count > 50% or an absolute count > 4,500/mm³, and atypical lymphocytosis (defined as atypical lymphocytes more than 10% of total lymphocytes) [3]. Mild thrombocytopenia and neutropenia are also seen but to a lesser extent [3].

Only a few studies have specifically addressed the relationship between quantitative hematologic features and serologic status in IM patients [10–13], with even fewer reports demonstrating and comparing quantitative hematologic abnormalities in both HA-negative and HA-positive patients [12–14]. This study was undertaken to compare the frequency of quantitative hematologic abnormalities in a large

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series of HA-positive and HA-negative mononucleosis patients.

MATERIALS AND METHODS

One hundred forty-seven consecutive patients with clinical features suspicious for infectious mononucleosis who had heterophile antibody tests were selected for the study. HA test results, as well as simultaneously performed complete blood counts (CBC), were retrieved from the laboratory computer database of the University of Texas Medical Branch (UTMB).

Heterophile antibody tests were performed in the UTMB Microbiology division, Serology unit, using the Mono-Latex Slide test (Wampole Laboratories/Carter-Wallace, Cranbury, NJ). This is a qualitative latex agglutination test that utilizes bovine erythrocyte mononucleosis antigen. CBC were performed in the UTMB Hematology laboratory on a Coulter Star-S instrument (Coulter Corp., Hialeah, FL). Differential counts were manually performed by the medical technology staff of the UTMB hematology laboratory.

All cases were classified as either HA-positive or HA-negative based on the Mono-Latex test results. Hematologic profiles of each patient were tabulated and analyzed. Using chi-square analysis and Student’s t-tests, the frequency of quantitative hematologic abnormalities—leukocytosis, leukopenia, lymphocytosis, lymphopenia and atypical lymphocytosis—in both HA-negative and HA-positive groups were statistically compared.

RESULTS

A total of 147 patients with ages ranging from 2 to 97 years old were included in the study. The mean age was 20 ± 12 years. There were slightly more females than males. HA-positive patients were significantly younger than HA-negative patients (Table I).

The HA-positive group exhibited a significantly higher (P < 0.05) mean white blood cell count compared to the HA-negative group (Table II, Fig. 1). The mean leucocyte count of the HA-positive group was above the normal reference range.

Even more significantly (P < 0.001), the HA-positive group exhibited higher mean absolute lymphocyte counts compared to the HA-negative group, although both groups had a mean lymphocyte count that was within the normal reference range (Table II, Fig. 2).

Lymphocytosis was more commonly seen in the HA-positive group (Table III, Fig. 3). Lymphocytosis, defined as a lymphocyte count > 4.4 × 10^3/mm^3, was present in 32% of the HA-positive patients and in only 3% of the HA negative patients. This difference was noted to be significant by chi-square analysis. Although a greater number of HA-negative patients exhibited lymphopenia (lymphocyte count < 1.3 × 10^3/mm^3), the difference in frequency of

### Table I. Case Demographics

<table>
<thead>
<tr>
<th>Heterophile antibody</th>
<th>No. of cases</th>
<th>Mean age (years)</th>
<th>Age range (years)</th>
<th>Male:female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>68</td>
<td>15^a</td>
<td>2–97</td>
<td>0.84</td>
</tr>
<tr>
<td>Negative</td>
<td>79</td>
<td>22^a</td>
<td>3–61</td>
<td>0.61</td>
</tr>
</tbody>
</table>

^aP < 0.05.

### Table II. Mean Absolute Leukocyte and Lymphocyte Counts

<table>
<thead>
<tr>
<th>Heterophile antibody</th>
<th>WBC</th>
<th>Absolute lymphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11.4 ± 5.4^a</td>
<td>4.0 ± 3.2^b</td>
</tr>
<tr>
<td>Negative</td>
<td>8.8 ± 7.6^a</td>
<td>2.0 ± 0.9^b</td>
</tr>
</tbody>
</table>

^aP < 0.05.  
^bP < 0.001.

### Table III. Frequency of Lymphocytosis and Lymphopenia

<table>
<thead>
<tr>
<th>Lymphocyte count</th>
<th>HA-positive (n = 68)</th>
<th>HA negative (n = 79)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated</td>
<td>32%</td>
<td>3%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal</td>
<td>60%</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>8%</td>
<td>19%</td>
<td></td>
</tr>
</tbody>
</table>
lymphopenia between the two groups was not statistically significant. Of note, most of the patients, whether HA-positive or HA-negative, appeared to present with normal lymphocyte counts.

Leukopenia was uncommon in HA-positive patients while more frequent in HA-negative patients. Leukocytosis, on the other hand, was evident in 21% of HA-positive patients and in 6% of HA-negative patients, marking a statistically significant difference between the two groups (Table IV).

Atypical lymphocytosis was significantly more frequent in the HA-positive group compared to the HA-negative group. Only 21% of HA-positive cases presented with atypical lymphocytosis, which is defined as atypical lymphocytosis greater than 10% of the lymphocytes (Table V). Another six HA-positive patients had atypical lymphocytes but not enough to fulfill diagnostic criteria of IM. In contrast, only two of the 79 HA-negative cases had increased atypical lymphocytes.

**DISCUSSION**

Lymphocytosis, defined as a differential count >50% or an absolute count >4,500/mm³, has been a relatively constant feature of IM patients [10,13,14].

In our series, only 32% of the HA-positive patients presented with absolute lymphocytosis. In contrast, Brigden et al. demonstrated lymphocytosis in 50% of their HA-positive cases [13]. It is possible that this difference may be due to inherent differences in sensitivity of the heterophile antibody assays. However, as previously reported [13], absolute lymphocytosis was far more commonly seen in the HA-positive population compared to the HA-negative group (32% vs. 3%).

The diagnosis of IM relies on the presence of atypical lymphocytes comprising at least 10% of the total lymphocytes. In most cases, a differential count of 20–40% of atypical lymphocytes is highly suggestive of infectious mononucleosis, while a count of above 40% of atypical lymphocytes is considered pathognomonic of EBV-associated IM [12]. The presence of atypical lymphocytes, however, is not specific for EBV-associated IM. Atypical lymphocytosis may also be seen in other viral infections such as viral hepatitis, herpes, and influenza [15]. In one study of 70 HA-negative patients with (atypical) lymphocytosis, 40% were seropositive for EBV, 39% for CMV, 25% for HHV-6, 3% for Toxoplasma, and none (0%) for HIV [16].

Atypical lymphocytosis is a relatively constant finding in HA-positive IM patients [10–12]. In our study, atypical lymphocytosis was statistically more frequent in HA-positive cases compared to HA-negative cases, although atypical lymphocytosis was present in only 21% of our HA-positive cases. Varying frequencies of atypical lymphocytosis in HA-positive IM patients have previously been reported in the literature. Aronson et al. [14] reported a 26% association between atypical lymphocytosis and HA-positivity, a result similar to the present results. In contrast, a much higher association between atypical lymphocytosis and HA-positivity, a result similar to the present results. In contrast, a much higher association between atypical lymphocytosis and HA-positivity (61%; mean age 17 years) was reported by another group [12]. This difference may be due to the fact that, in the previous study, sera were tested with both a sheep red cell tile test and a Monospot (Ortho, Raritan, NJ) test. In cases of EBV antibody positive IM, a high correlation (93%) between serology and atypical lymphocytosis has been demonstrated, indicating the high sensitivity of atypical lymphocytosis for EBV-positive infectious mononucleosis [10].

Our data indicates that absolute leukocytosis in IM is significantly more often associated with HA-positivity than with HA-negativity. Of our HA-positive population, 21% exhibited absolute leukocytosis as compared with 38% reported by Brigden et al. [13]. Half of our cases with absolute leukocytosis also presented with absolute lymphocytosis. Leukocytosis peaks in the second to third week of IM, but it may

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**TABLE IV. Frequency of Leukocytosis and Leukopenia**

<table>
<thead>
<tr>
<th>WBC</th>
<th>HA-positive (n = 68)</th>
<th>HA-negative (n = 79)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated</td>
<td>21%</td>
<td>6%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Normal</td>
<td>77.8%</td>
<td>85%</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.2%</td>
<td>9%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**TABLE V. Frequency of atypical Lymphocytosis**

<table>
<thead>
<tr>
<th>Atypical lymphocytosisa</th>
<th>HA-positive</th>
<th>HA-negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>21%</td>
<td>2%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Absent</td>
<td>47%</td>
<td>77%</td>
<td></td>
</tr>
</tbody>
</table>

*a >10% of total lymphocyte count.
be depressed in the earlier weeks [7]. The significance of leukocytosis therefore may be difficult to assess unless the cases compared are studied at similar times post-infection.

CONCLUSION

In this large series of patients with mononucleosis, we have conclusively demonstrated that certain quantitative hematologic abnormalities, specifically leukocytosis, absolute lymphocytosis, and atypical lymphocytosis, are significantly more frequent in HA-positive than HA-negative IM. Since heterophile antibody positivity is specific for EBV etiology, these results suggest that absolute leukocytosis, absolute lymphocytosis, and atypical lymphocytosis are more characteristic of EBV-induced IM than non-EBV associated mononucleosis syndromes. Future studies utilizing IM cases confirmed by EBV serology are planned to more thoroughly investigate this matter.

REFERENCES