Serum Antinuclear Antibodies as Indicator of Relapse in Patients with Non-Hodgkin's Lymphoma

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Abstract
Background: The association of antinuclear antibodies (ANA) has been reported in Non-Hodgkin’s lymphoma (NHL). This study was conducted to assess the serum levels of ANA in NHL patients and its relationships with prognosis.

Subjects and methods: This study included 64 patients with NHL and 30 healthy controls. Enzyme-linked immunosorbent assay (ELISA) was performed to determine serum levels of ANA.

Results: There were significant differences in the serum ANA between NHL patients and controls (P<0.001). ANA positivity was detected in 19 NHL patients (29.6%). Positive ANA levels were observed in 17 patients in relapse of 19 (89.4%). No significant association was found between serum ANA levels and various clinicopathological and immunohistochemical features. There was a significant association between ANA levels and leukocyte common antigen (LCA) (P=0.010). ROC curve was applied to assess the diagnosis of relapse in NHL with cutoff >0.8 (AUC: 0.876).

Conclusion: Our data argue that positive ANA levels can be useful for diagnosis of relapse in NHL patients.

Keywords: Antinuclear antibody; Non-Hodgkin's lymphoma

Introduction
Non–Hodgkin Lymphoma (NHL) are a group of lymphoproliferative disorders with different biology and prognosis [1,2]. NHL comprises a group of cancers, which have both indolent and aggressive types [3]. In spite of NHL treatment advances, many patients are immune to treatments or relapse.

Antinuclear antibodies (ANA) are antibodies that bind to several nuclear antigens. ANA have been reported in cancers, suggesting that they may have a role in the pathogenesis of malignancy [4,5]. Many studies have reported that NHL patients could display ANA antibodies [6,7]. This study assessed serum levels of ANA in NHL patients and examined its association with hematological, clinicopathological, and immunophenotyping parameters of NHL and assessed whether high ANA titres are related to relapse in NHL.

Subjects and Methods
The present work was carried out on 64 NHL patients aging from 13 to 75 years old and thirty healthy controls were included in the analysis for ANA. NHL patients were selected from the Minia Oncology Center in the period from October 2015 to March 2016. Minia Oncology Center committee gave approval and informed consent, which was obtained from all patients in this study.

Laboratory Investigations
Complete blood picture was assessed by Cell Dyn 1700 (USA). Serum levels of creatinine, blood urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Lactate dehydrogenase (LDH) and uric acid were assessed using ACE automated chemistry Analyzer (Schiaparelli Biosystems, INC, USA).

Measurement of antinuclear antibodies
An ELISA assay was used for the detection of ANA in the serum of all patients and controls (Sunred Biological Technology Co., Ltd, Shanghai). ELISA reader was used at 450 nm for reading. ANA index ≥ 1.2 was considered positive. Detection of ANA by ELISA is used as a method for different diseases [8].

Statistical methods
Data analyses were performed using SPSS program (SPSS–20, Chicago, IL, USA). Data were expressed as mean ± SD. Mann-Whitney test, Chi-square test, ROC curve and the Fisher Exact test were done for analysis of the data. Spearman’s and Pearson’s correlation coefficient were calculated to determine the relations between the variables. P values ≤ 0.05 was considered significant.

Results
Serum levels of ANA in NHL
Serum ANA was detected in 19 NHL patients, and the mean was 2.07 ± 2.56 (range 0.2-8.5) which was significantly higher than that of controls (P <0.001) (Table 1). A total of 19 patients out of 64 (29.6%) showed a positivity for ANA antibodies (Table 2).

Table 1: ANA levels in NHL patients. Mann-Whitney tests for nonparametric quantitative data between the two groups.

<table>
<thead>
<tr>
<th>ANA</th>
<th>NHL (n=64)</th>
<th>Control (n=30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>(0.2-8.5)</td>
<td>(0.1-0.8)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2.07 ± 2.56</td>
<td>0.41 ± 0.21</td>
<td></td>
</tr>
</tbody>
</table>

Highly statistically significant differences (P ≤ 0.001) are indicated with asterisks (**). NHL: Non-Hodgkin’s Lymphoma; N: Number; ANA: Antinuclear Antibodies.

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Table 2: Prevalence of antinuclear antibodies in 64 NHL patients.

<table>
<thead>
<tr>
<th>Treatment outcome</th>
<th>Negative ANA (n=45)</th>
<th>Positive ANA (n=19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>23 (51.1%)</td>
<td>0 (0%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Complete remission</td>
<td>8 (17.8%)</td>
<td>1 (5.3%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Partial remission</td>
<td>9 (20%)</td>
<td>1 (5.3%)</td>
<td>0.257</td>
</tr>
<tr>
<td>Relapse</td>
<td>5 (11.1%)</td>
<td>17 (39.5%)</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Negative ANA was <1.2; high, ≥ 1.2. Highly statistically significant differences (P<0.001) are indicated with asterisks (**). NHL: Non-Hodgkin’s Lymphoma; N: Number; ANA: Antinuclear Antibodies.

Table 3: Correlation of ANA with relapse in NHL patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>ANA</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>0.135</td>
<td>0.288</td>
<td></td>
</tr>
<tr>
<td>WBCs</td>
<td>-0.038</td>
<td>0.769</td>
<td></td>
</tr>
<tr>
<td>PLT</td>
<td>-0.101</td>
<td>0.428</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>-0.096</td>
<td>0.452</td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>-0.016</td>
<td>0.898</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>-0.159</td>
<td>0.211</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>0.085</td>
<td>0.503</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>-0.095</td>
<td>0.454</td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>-0.045</td>
<td>0.727</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>0.071</td>
<td>0.578</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Correlation of ANA levels with features in NHL patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Optimal Cut-off</th>
<th>AUC</th>
<th>P value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td>0.876</td>
<td>0.886</td>
<td>&lt;0.001</td>
<td>77.27</td>
<td>95.24</td>
<td>89.3</td>
<td>98.9</td>
<td>90.1</td>
</tr>
</tbody>
</table>

Figure 1: Diagnostic performances of ANA for discriminating NHL patients with relapse (A) ROC curve obtained by plot at different cutoffs for ANA in NHL patients with relapse. (B) The most discriminative cutoff concentration of serum ANA was >0.8 with an AUC value of 0.876. The sensitivity and specificity were 77.27 and 95.24%, respectively. Highly statistically significant differences (P ≤ 0.001) are indicated with asterisks (**).
patients with NHL were positive for ANA, the cause for that is many patients had diffuse large B-cell lymphoma (DLBCL) at presentation. Alituntas et al. [12] evaluated the prevalence of ANA antibodies in patients with NHL. 13.4% of these patients were positive for ANA antibodies. They did not evaluate serial ANA levels in terms of relapse.

ANA positivity was absent in newly diagnosed patients with NHL. This may be explained by antigen binding of overall available antibodies [13]. Complete remission and partial remission occurred less in ANA antibody-positive patients in comparison to ANA antibody-negative patients that may be due to suppression of antibody production by treatment.

In this study, it was demonstrated that positive ANA was associated with relapse in NHL patients. ANA positive patients had the advanced disease in comparison with negative patients, thus ANA may support its use as a poor prognostic marker for monitoring relapse in NHL. One report suggested that median survival was longer in patients who did not demonstrate autoimmune markers than in those who did [14]. Although the reason for ANA-positivity in relapse remains unknown. It is possible that the antibodies are produced by the immune system, in response to a release of tumor-associated antigens. Immunoregulatory disturbances of the immune system, chronic stimulation of lymphocytes and genetic susceptibility increase the likelihood of initiating mutations [15]

In this study, clinical and laboratory data have been analyzed for 19 patients with ANA. There was a lack of significant correlation between ANA positivity and these features, demonstrating the lack of strict correlation between ANA and autoimmune symptoms. Another study reported a prevalence of ANA without clinical manifestation [16], there was a significant association between ANA and LCA as the most cases with negative LCA are negative ANA and most cases +ve LCA, are +ve ANA. High expression of LCA was previously observed in metastasis [17]. This finding confirms our results with the presence of ANA in relapse. According to the data, the serum level of ANA could be a valuable biomarker for diagnosing relapse in NHL. The AUC of ANA for distinguishing NHL with a relapse from all controls was 0.876 and was higher than that of complete remission and partial remission (0.670, 0.659 respectively) with a cutoff value for ANA >0.8. High levels of anti-nuclear antibodies have been found in many human disorders of the immune system, such as systemic lupus erythematosus (SLE) [18]. As hypomethylation at the T and B-lymphocyte-associated gene, loci is a general feature in SLE patients [19,20], loss of DNA methylation at certain loci might strongly associate with these human disorders. Therefore, it is important to study the links between the alteration of epigenetic markers, such as DNA methylation and initiation/occurrence of NHL. In addition, as DNA methyltransferases (DNMTs) and some histone methyltransferases (HMTs) function as the main enzymes that required for the establishment and maintenance of DNA methylation [21-23], it is also essential to investigate the correlation between the mutation/instability of these enzymes and NHL in patients. Follow-up studies of ANA in NHL will help to identify its role in the mechanisms of relapse in NHL.

There is a limitation of the present study. The number of the patients evaluated was relatively small. Some of the insignificant differences in might become significant with a larger number of patients.

Conclusion
Antinuclear antibodies are associated with lymphomas. The finding of a positive ANA in NHL patients strongly suggests the presence of relapse. According to our data, ANA could be used as a marker to discriminate NHL with relapse.

References