Lymphoma is a malignant tumor of the immune system originating from lymph nodes and extralymphatic tissues. Its occurrence is believed to be associated with various immune cells due to the proliferation and differentiation of lymphocytes during the immune response. It has been found in many studies that B-cell activating factor (BAFF), as a member of the tumor necrosis factor (TNF) superfamily, could specifically activate B lymphocytes and promote their proliferation.

**Material and Methods.** The protein expression of BAFF and its receptors in serum and BAFF mRNA expression in peripheral blood mononuclear cells (PBMCs) of 47 NHL patients and 20 healthy subjects were detected by ELISA and RFQ-PCR and compared with LDH and β2M levels.

**Results.** BAFF mRNA expression in the PBMCs of NHL patients was significantly higher than in healthy controls. The expression levels of serum BAFF and the three receptors (TACI, BCMA and BAFF-R) in NHL patients were significantly higher than in healthy controls, and were not significantly correlated with β2M and LDH levels.

**Conclusions.** The serum protein concentration of BAFF and the expression level of BAFF mRNA in PBMCs of NHL patients underwent abnormal changes, indicating that BAFF and its receptors may play some role in the pathogenesis of NHL. (Adv Clin Exp Med 2016, 25, 5, 837–844).

**Key words:** BAFF, RFQ-PCR, NHL.

* These authors contributed equally to this work.

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BAFF can trigger the occurrence of some malignant tumors, including NHL. BAFF plays its biological roles by binding with its receptors. Three BAFF receptors have been identified: B-cell maturation antigen (BCMA), transmembrane activator and CAML-interactor (TACI) and BAFF receptor (BAFF-R, or BR3) [10].

The purpose of the present study was to explore how BAFF and its receptors correlate with the occurrence and prognosis of NHL by detecting BAFF levels in NHL patients and analyzing correlations between serum concentrations of BAFF and its receptors with clinical parameters in NHL patients.

Material and Methods

Specimen Collection

Twenty healthy subjects were selected from among blood donors who underwent physical examinations at the Affiliated Hospital of Nantong University (Nantong, China). They included 13 females and 7 males, and ranged in age from 50 to 65 years, with a mean age of 59 ± 5 years. Forty-seven NHL patients were selected from among hospitalized NHL patients who received treatment at the same hospital between August 2010 and August 2013. They included 27 males and 20 females, and ranged in age from 48 to 73 years, with a mean age of 62 ± 7 years. From each patient and healthy subject, 3 mL of peripheral venous blood was withdrawn and centrifuged at 2000 rpm for about 10 min to separate peripheral blood mononuclear cells (PBMCs). Cell deposits were collected by centrifuge and stored at –70°C until use. At the same time, an additional 2 mL of peripheral venous blood was drawn, centrifuged at 2000 rpm for about 20 min to collect 0.5 mL serum in a 0.5 mL EP tube, and stored at –70°C until use.

Pathological NHL specimens were selected from the paraffin wax-embedded tissue sections. The 47 cases of pathological NHL specimens included 23 cases of diffuse large B cell lymphoma (DLBCL), 6 cases of mucosa-associated lymphoid tissue (MALT), four cases of mantle cell lymphoma (MCL), eight cases of small lymphocytic lymphoma (SLL) and six cases of follicular lymphoma (FL). Clinical staging was done according to the Union for International Cancer Control (UICC) NHL TNM classification. All the histological specimens were pathohistologically confirmed as NHL.

Main Reagents and Instruments

The reagents and instruments used in this study were SuperScript® III Kit (Life Technologies, USA); BAFF, BAFF-R, TACI and BCMA ELISA Kits (Aquatic Diagnostics Ltd., USA); PTC-200 PCR Amplifier (MJ Research, USA); LightCycler Quantitative PCR Amplifier (Roche, Germany); U-0080D Nucleic Acid UV detector (Hitachi, Japan); Dolphin-Doc Gel Imaging System (Wetalket, USA); Alisei Automatic ELISA Instrument (Seac, Italy); Hitachi 7600-020 Automated Biochemical Analyzer (Hitachi, Japan); and Image Auto Immunological Analyzer (Beckman-Coulter, USA).

SYBR Green I Real Time PCR

Primers of BAFF (registration number AY129225) and the internal reference GAPDH (registration number NC_000012.10) were designed by Shanghai Sangon Biological Engineering Co., Ltd. Real-time quantification was performed in triplicate with a FastStart Universal SYBR Green Master (Rox) kit (Roche, Germany). The BAFF primer sequences were 5'-CACGCCTTACTTCTTGCC-3' (forward primer), and 5'-CTTGGAGGATCGGACAG-3' (reverse primer), which yield products of 102 bp. The GAPDH primer sequences were 5'-CGGAGTCAACGGATTTGCATTGTAAT-3' (forward primer), and 5'-AGGCCTTCTCCAATGGTGGAAGAC-3' (reverse primer), which yield products of 193 bp. Each reaction was performed in a final volume of 20 μL, containing 10 μL SYBR Green I mix (Rox), 3 μL cDNA, 0.5 μL forward primer, 0.5 μL reverse primer and RNase-free H2O. The mix was incubated at 94°C for 3 min, followed by 33 cycles of 94°C for 30 s, 55°C for 40 s, and 72°C for 31 s.

Detection of BAFF Receptor Protein Expression by ELISA

BAFF, BAFF-R, TACI and BCMA receptor protein levels were detected by ELISA according to the kit manufacturer’s instructions (Aquatic Diagnostics Ltd., USA).

Detection of Serum LDH and β2M Concentrations

Serum LDH concentrations were measured continuously using the Hitachi-7600-020 automatic biochemical analyzer, and β2M concentrations were measured using the AxSYM automatic immune analyzer.

Immunoblot Analysis

Total protein was extracted using RIPA lysate containing 1% PMSF. SDS-polyacrylamide gel electrophoresis was performed on 100 μg of total protein (80 V, 40 min; 100 V, 60 min), and
the protein was transferred from the SDS-polyacrylamide gel electrophoresis to a polyvinylidene fluoride (PVDF) membrane (300 mA, 120 min). After blocking with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween 20 (TBST), the PVDF membrane was incubated with the primary antibody in 5% bovine serum albumin in TBST overnight at 4°C, washed three times with TBST, and incubated with the secondary antibody in 5% milk in TBST. After three washes with TBST, the membrane was developed with enhanced chemiluminescence (ECL, Amersham Pharmacia, UK).

Statistical Analysis
Using SPSS 18.0 statistical software, the mean (x) and standard deviation (SD) were calculated in both the healthy and patient groups. The results were compared by t-test. P < 0.05 was considered to be statistically significant.

Results

BAFF Expression in Peripheral Blood and Tumor Tissue of NHL Patients

Peripheral PBMCs in the 47 NHL patients and 20 healthy subjects were detected by real-time fluorescence quantitative polymerase chain reaction (RFQ-PCR). The results showed that the amount of BAFF mRNA expression in PBMCs was 0.48 ± 0.023 in the NHL patients and 0.25 ± 0.023 in the healthy subjects, and the difference was statistically significant (t = 17.75, p = 0.0001, Fig. 1 a). BAFF protein expression in the serum and PBMCs of the NHL patients were detected by ELISA and the Western blot technique. The results are shown in Figures 1b and 1c, and showed that BAFF protein expression was higher in the NHL patients than in the healthy subjects. Among the 47 NHL patients, BAFF was elevated in DLBCL (20/23), SLL (6/8), MALT (4/6), MCL (3/4) and FL (6/6). BAFF expression levels were also detected by immunohistochemistry in tumor tissue specimens of the NHL patients (Fig. 1 d). The results showed that BAFF mRNA and protein expression were elevated in the NHL patients.

Protein Expression of Soluble BAFF and Its Receptors in the Serum of NHL Patients

To ensure the consistency of the specimen sources, the protein expression of soluble BAFF and its receptors – TACI, BCMA and BAFF-R – were assessed in the serum of the NHL patients and the healthy subjects, to check whether these levels had any correlation with the clinical parameters. ELISA was used to detect the protein expression of the
three BAFF receptors in the serum of the NHL patients and the healthy subjects (Table 1). The results showed that the levels of serum BAFF and its receptors in the NHL patients (BAFF 19.18 ± 1.29, TACI 212.74 ± 7.98, BCMA 102.98 ± 6.18, BAFF-R 2.95 ± 0.19) were significantly higher than those in the healthy controls (BAFF 10.94 ± 1.56, TACI 139.00 ± 12.51, BCMA 59.66 ± 7.06, BAFF-R 1.34 ± 0.26; t-values were 7.05, 8.61, 7.99 and 8.66, respectively; p < 0.05). These results suggest that the expression of BAFF and its receptors may be helpful in the auxiliary diagnosis of NHL.

### Correlations Between Serum Protein Expression of BAFF and Its Receptors and BAFF mRNA Expression

Correlations between serum BAFF/receptor protein expression and BAFF mRNA expression in the NHL patients were analyzed. The results showed that there were no significant correlations between serum BAFF, BAFF-R and TACI protein expression and the concentration of LDH and β<sub>2</sub>M were investigated. As can be seen from the data in Table 2, no significant association was found between serum BAFF protein concentration and the patients’ age, gender or the concentration of β<sub>2</sub>M (p > 0.05). However, the association between different TNM stages (I–II vs. III–IV) and the serum BAFF protein concentration was statistically significant. The LDH group also had similar results (p < 0.05).

### Correlations Between Serum BAFF Protein Expression Levels and Clinical Parameters

The association between serum BAFF protein concentration and various clinicopathological parameters such as age, sex, histological grading and the concentration of LDH and β<sub>2</sub>M were investigated. As can be seen from the data in Table 2, no significant association was found between serum BAFF protein concentration and the patients’ age, gender or the concentration of β<sub>2</sub>M (p > 0.05). However, the association between different TNM stages (I–II vs. III–IV) and the serum BAFF protein concentration was statistically significant. The LDH group also had similar results (p < 0.05).

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>n</th>
<th>Median (25&lt;sup&gt;th&lt;/sup&gt;–75&lt;sup&gt;th&lt;/sup&gt; percentiles)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>19.87 (14.27–25.18)</td>
<td>0.4710</td>
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<tr>
<td>Female</td>
<td>20</td>
<td>17.35 (11.13–25.15)</td>
<td></td>
</tr>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 60</td>
<td>21</td>
<td>21.51 (16.40–25.02)</td>
<td>0.1642</td>
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<tr>
<td>&gt; 60</td>
<td>26</td>
<td>17.74 (11.02–25.17)</td>
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<tr>
<td>TNM stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–II</td>
<td>20</td>
<td>15.89 (8.09–21.09)</td>
<td>0.0079</td>
</tr>
<tr>
<td>III–IV</td>
<td>27</td>
<td>21.08 (14.38–27.98)</td>
<td></td>
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<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal LDH group</td>
<td>28</td>
<td>16.03 (7.78–17.35)</td>
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</tr>
<tr>
<td>Elevated LDH group</td>
<td>19</td>
<td>20.93 (13.98–26.41)</td>
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<tr>
<td>β&lt;sub&gt;2&lt;/sub&gt;M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal β&lt;sub&gt;2&lt;/sub&gt;M group</td>
<td>22</td>
<td>18.25 (10.51–22.83)</td>
<td>0.2163</td>
</tr>
<tr>
<td>Elevated β&lt;sub&gt;2&lt;/sub&gt;M group</td>
<td>25</td>
<td>18.95 (13.25–27.72)</td>
<td></td>
</tr>
</tbody>
</table>
Expression of BAFF and Receptors in NHL

Correlations Between Serum Protein Expression of BAFF and Its Receptors and Serum β₂M Concentration

Knowing that β₂M is of some value in predicting disease prognosis, the β₂M concentration in the NHL patients was assessed and its correlation with BAFF/receptor expression was analyzed. The results showed that there was no significant correlation between them (BAFF and β₂M, r = 0.014, p > 0.05; BAFF-R and β₂M, r = 0.127, p > 0.05; TACI and β₂M, r = 0.003, p > 0.05; BCMA and β₂M, r = 0.0005 p > 0.05) (Fig. 3). These results suggest that serum BAFF/receptor protein expression may not be related to the prognosis of NHL.

Correlations Between Serum Protein Expression of BAFF and Its Receptors and LDH Concentration

Knowing that LDH is often used as an indicator of disease activity, the LDH concentration in the serum of the NHL patients was assessed. Based on the concentration of LDH, the NHL patients were divided into two groups: The elevated LDH group (310.96 ± 108.00 U/L) and the normal LDH group (42.65 ± 36.00 U/L). BAFF mRNA and serum BAFF/receptors protein expression were compared between the two groups (Table 3).

Table 3. Expression levels of serum BAFF/receptors protein and BAFF mRNA in elevated the LDH group and normal LDH group

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>LDH (U/L)</th>
<th>BAFF mRNA (ratio)</th>
<th>BAFF (ng/mL)</th>
<th>BCMA (pg/mL)</th>
<th>TACI (pg/mL)</th>
<th>BAFF-R (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal LDH group</td>
<td>28</td>
<td>142.65 ± 36</td>
<td>0.35 ± 0.02</td>
<td>13.48 ± 0.69</td>
<td>91 ± 6.24</td>
<td>203 ± 31</td>
<td>2.98 ± 0.13</td>
</tr>
<tr>
<td>Elevated LDH group</td>
<td>19</td>
<td>310.96 ± 108*</td>
<td>0.68 ± 0.16*</td>
<td>22.45 ± 0.50*</td>
<td>124.36 ± 25*</td>
<td>220 ± 37</td>
<td>3.99 ± 1.07</td>
</tr>
</tbody>
</table>

*compared with normal LDH group.
It was found that there were significant differences in BAFF mRNA and the expression of BAFF and BCMA serum protein between the two groups (t-values were 6.658, 21.06, 7.254, p < 0.05). The results showed that BAFF and BCMA expression might be related to NHL disease activity. However, when linear correlation analyses of LDH concentration with the expressions of BAFF mRNA, BAFF and BCMA serum protein were used, there was no significant correlation between them (R values were 0.201, 0.093, 0.031, p > 0.05) (Fig. 4).
Correlation of Serum BAFF Protein Concentration with the Clinicopathological Stage of NHL

Staging of the NHL patients was performed according to UICC NHL TNM classification, and differences in BAFF expression at different stages were compared. It was found that serum BAFF concentration in NHL patients at stages II and III–IV was significantly higher than in the healthy subjects (p < 0.05) (Fig. 5 a). In addition, serum BAFF protein levels in NHL patients at stage III–IV were significantly higher than those in stage I NHL patients (p < 0.05) (Fig. 5 b), indicating that high serum BAFF protein levels in NHL patients may be indicative of an adverse prognosis.

Discussion

B-cell activating factor regulates the survival, proliferation and differentiation of B cells mainly through binding with the three cell-surface receptors (TACI, BCMA and BAFF-R). In addition, BAFF also promotes the generation of immunoglobulin and regulates the immune response [11]. Studies in recent years have shown that BAFF/receptor protein and/or mRNA expression underwent abnormal changes in patients with systemic lupus erythematosus (SLE), Sjögren’s syndrome (SS), multiple myeloma (MM) and NHL [12, 13]. Therefore, it is of clinical significance to accurately quantify the level of BAFF expression.

The mortality of lymphoma in China is 1.5/100,000, ranking 11th–13th among all malignant tumor deaths. NHL accounts for more than 90% of lymphomas. Most NHL originates from extralymphatic tissues and disseminates by skipping across adjacent lymph nodes to distal ones, which is the reason there are many cases in which NHL has already spread throughout the body at the time clinical diagnosis is confirmed. It is therefore necessary to explore the role of BAFF in NHL. It was found in some studies [14] that BAFF mRNA was expressed in the peripheral blood and tumor tissue of NHL patients. Ju et al. [15] found that the expression level of BAFF mRNA in the peripheral blood of NHL patients was no higher than that of normal controls; however, their blood specimens were selected from NHL patients who had received medical treatment. The true relationship between BAFF and clinical parameters, and whether BAFF could be used as an accessory clinical marker for the diagnosis of NHL, remains unclear. In the present study, the blood specimens were taken from NHL patients who had not used any medication before admission. RNA was extracted from PBMCs and assessed for BAFF mRNA level by reverse transcription. The results showed that BAFF mRNA was expressed in the peripheral blood of NHL patients, and that the expression level was significantly different from that in the healthy controls, indicating that BAFF may play some role in the development and progression of NHL.

The present study also showed that soluble BAFF/receptor levels were elevated in the serum of NHL patients, but the elevation of receptor levels was not linearly correlated with the elevation of the BAFF level, probably because the expression levels varied among the receptors. Serum BAFF/receptor protein concentration was elevated in the NHL patients, indicating that BAFF/receptors play a role in the pathogenesis of NHL. LDH and β2M, two clinical indexes related to NHL, were also assessed. As the LDH level may reflect tumor-bearing status, it could be used as an index for monitoring the therapeutic effect and prognosis. β2M is a variable for predicting the survival of NHL patients. The results of the present study showed that there was no significant correlation between BAFF, TACI, BAFF-R protein levels and serum LDH and β2M levels in the NHL patients. However, there was a correlation be-
between serum BCMA protein expression and BAFF mRNA, showing that BCMA might play a major role in NHL. In addition, the BAFF serum protein concentration of stage II and III–IV NHL patients was significantly higher than in the healthy subjects, indicating that increased serum BAFF protein concentration might be indicative of an adverse prognosis, and could be used as a marker of clinical NHL staging. In short, BAFF/receptors may have significant impact on the auxiliary diagnosis and prognosis of NHL.

In summary, the results of this study showed that serum BAFF/receptor protein concentration and BAFF/mRNA expression level in the PBMCs of NHL patients underwent abnormal changes, indicating that BAFF/receptors may play some role in the pathogenesis of NHL. In the study, the BAFF/receptor protein level and BAFF mRNA level in the PBMCs and serum of NHL patients were assessed. More experimental and clinical studies are needed to clarify their actual roles in the progression, prognosis and medical treatment of NHL.

References


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