B-cell activating factor as a serological biomarker for polymyositis and dermatomyositis

Aim: To investigate serum levels of B-cell activating factor (BAFF) in the patients with polymyositis (PM) and dermatomyositis (DM), and to systematically examine the association between serum BAFF levels and disease activity in PM/DM patients.

Patients & methods: A cross-sectional analysis included 92 PM/DM patients and 25 healthy control subjects. A longitudinal study followed 24 patients. Serum BAFF concentrations were detected by the ELISA method.

Results: Serum BAFF levels in PM/DM patients were significantly higher than those in healthy controls. A cross-sectional assessment revealed a modest correlation between serum BAFF levels and global disease activity and a mild correlation between serum BAFF levels and muscle disease activity. The longitudinal study showed that serum BAFF levels modestly correlated with global disease activity and muscle disease activity.

Conclusion: Resulting data showed high serum BAFF levels in PM/DM patients and suggested BAFF as a serological biomarker for PM/DM disease activity.

Keywords: B-cell activating factor • cytokine • dermatomyositis • disease activity • polymyositis

Background
Idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of muscle diseases characterized by symmetrical proximal muscle weakness, inflammatory infiltrates in skeletal muscle tissue and elevation of serum muscle enzymes and autoantibodies [1,2]. In addition to affecting muscles, IIMs also present cutaneous, joint, gastrointestinal, pulmonary, heart and renal manifestations [3]. Polymyositis (PM) and dermatomyositis (DM) are two common subtypes of IIM. Several serum biomarkers have been identified as possible indicators of disease activity, such as creatine kinase (CK), the anti-Jo-1 antibody [4] and KL-6 [5]. CK levels are very useful for tracking disease activity but do not correlate with systemic manifestations such as lung disease. Considering that only about 20% of IIM patients were found positive for the anti-Jo-1 antibody [6,7], serum anti-Jo-1 antibody levels cannot be a reliable indicator for IIM patients who test negative for the anti-Jo-1 antibody. Recently, KL-6 was found to be a promising biomarker for PM/DM patients with interstitial lung disease, but it is unsuitable for patients without interstitial lung disease (ILD). Therefore, no suitable biomarker has been found for monitoring general IIM disease activity. A better understanding of biomarkers that are present in the duration of the disease would significantly help to determine disease activity and inspire optimal treatment for each individual patient.

B-cell activating factor (BAFF), belonging to the tumor necrosis factor family, plays a crucial role in B-cell maturation and survival [8]. Serum BAFF levels have been found to be elevated in patients with various autoimmune diseases, such as rheumatoid arthritis, Sjögren’s syndrome and systemic lupus erythematosus. These findings implied that BAFF may play a role in autoimmune diseases and suggest that BAFF could be a potential therapy target [9]. Considering the critical role that BAFF plays in the adaptive immune system, BAFF was
suspected to be involved in the immune-mediated damage caused by IIM. Krystufkova et al. found increased serum levels of BAFF in Caucasian IIM patients [10]. Despite these studies, BAFF concentrations in Chinese patients with PM/DM and the nature of their association with IIM disease activity were still unknown. Additionally, longitudinal data from Chinese PM/DM patients are scarce.

The aim of this study was to investigate whether Chinese patients with PM/DM have elevated serum levels of BAFF compared with healthy controls and to systematically examine the correlation between serum BAFF levels and the disease activity in these patients. Therefore, we designed a cross-sectional study and a longitudinal study involving Chinese PM/DM patients to examine the association between serum BAFF levels determined by ELISA and disease activity measured by the Myositis Disease Activity Assessment Visual Analog Scales (MYOACT).

**Patients & methods**

**Study population**

During inpatient and outpatient encounters from 2003 through 2011, 92 Chinese patients with PM/DM at China–Japan Friendship Hospital were recruited for this study. Their diagnoses of PM/DM were based on the Bohan and Peter criteria [11]. Additionally, 25 healthy age- and sex-matched volunteers were selected to be the control group during the same time period. This study was performed with approval of the Human Ethics Board of China–Japan Friendship Hospital (China) and written informed consent was obtained from all participating individuals.

This study was conducted in two parts. First, a cross-sectional study was designed to examine the correlation between serum levels of BAFF and MYOACT disease activity scores. Serum samples were collected from all 92 PM/DM patients, and disease activity was evaluated by an experienced physician at the time when the serum sample was collected. The data of the clinical parameters were collected from patient charts. The correlation between serum BAFF levels and the clinical parameters was also analyzed. Second, a longitudinal study was conducted to investigate the variation in serum BAFF levels over time in relation to disease activity. When patients came to the hospital for follow-up visits, serum samples were collected and disease activities were assessed.

**Assessment of disease activity**

Disease activity was measured by the 2005 Myositis Disease Activity Assessment Tool established by the International Myositis Assessment and Clinical Studies (IMACS) group [12], which consists of the MYO-ACT and the Myositis Intention-to-Treat Index. In the previous studies, our data showed that the MYOACT could be used to reliably evaluate disease activity in Chinese patients with PM/DM [13]. Therefore, we used MYOACT to evaluate the disease activity of PM/DM patients following the guidelines for physician scoring of disease activity.

**Measurement of serum BAFF levels**

Serum levels of BAFF were determined in stored samples of blood from Chinese PM/DM patients by using a commercially available ELISA kit (R&D Systems, MN, USA). Tests were done according to the manufacturer’s instructions. Every sample was analyzed in triplicate.

**Statistical analysis**

Statistical analysis was performed using GraphPad Prism V.4.03 (GraphPad Software, CA, USA) and SPSS V.16.0 (SPSS, IL, USA). Tests were compared by using the Mann–Whitney U test. Spearman’s correlation analysis was used to test for correlations. The \( \chi^2 \) test was carried out to compare binary data. The Wilcoxon signed rank test was used on paired data when appropriate. A p-value ≤ 0.05 was considered statistically significant.

**Results**

**Clinical characteristics & treatment of PM/DM patients**

Of the 92 patients with PM/DM enrolled in this study, 41 patients were diagnosed with PM, and the other 51 patients were diagnosed with DM, according to the criteria established by Bohan and Peter. Among the 92 patients, nine had rheumatoid arthritis complications, three had systemic lupus erythematosus complications and two had Sjögren’s syndrome complications. The mean age of these patients at onset of PM/DM was 45.2 years of age. Females outnumbered males in the cohort. The mean duration of symptoms at initial evaluation was 5.6 years (range: 0.25–27.5 years). The clinical features that the patients presented are summarized in Table 1.

The treatments received by the patients in our cohort varied according to the severity of their disease and the experience of their attending physician. All patients received corticosteroid at doses between 0.5 and 1 mg/kg as part of their initial therapy after their diagnosis of PM/DM. Meanwhile, approximately 70% of our patients (64 out of 92) also received at least one or more immunosuppressants, among which were methotrexate, cyclophosphamide, azathioprine, intravenous immunoglobulin, hydroxychloroquine and mycophenolate mofetil.
Serum levels of BAFF in PM/DM patients were determined using a commercial ELISA kit. As shown in Figure 1, we found that the median value of serum BAFF concentration in PM patients was 1627.5 pg/ml (range: 239.2–19950 pg/ml), and the median value of serum BAFF concentration in DM patients was 1582.5 pg/ml (range: 506.5–18360 pg/ml), while the median value of that in healthy controls was 842 pg/ml (range: 512–1354 pg/ml). Therefore serum levels of BAFF were significantly higher in PM patients (p < 0.001) and DM patients (p < 0.001) than those in the healthy control subjects, while there was no significant difference between the serum levels of BAFF in PM patients and DM patients (p > 0.05). Horizontal bars indicate median levels.

BAFF: B-cell activating factor; DM: Dermatomyositis; PM: Polymyositis.

**Serum levels of BAFF in PM/DM patients**

Serum levels of BAFF in PM/DM patients were determined by using a commercial ELISA kit. As shown in Figure 1, we found that the median value of serum BAFF concentration in PM patients was 1627.5 pg/ml (range: 239.2–19950 pg/ml), and the median value of serum BAFF concentration in DM patients was 1582.5 pg/ml (range: 506.5–18360 pg/ml), while the median value of that in healthy controls was 842 pg/ml (range: 512–1354 pg/ml). Therefore serum levels of BAFF were significantly higher in PM patients (p < 0.001) and DM patients (p < 0.001) than those in the control subjects. Furthermore, there was no significant difference between the serum levels of BAFF in PM patients and those in DM patients (p > 0.05).

**Cross-sectional study**

For the 92 patients, the correlation between their serum
BAFF levels and their disease activity scores was analyzed by using Spearman’s correlation analysis. We found significant correlations between serum BAFF levels and global disease activity scores both in PM patients ($r = 0.481$; $p < 0.01$) and in DM patients ($r = 0.536$; $p < 0.01$), as shown in Figure 2A & D. Interestingly, a mild correlation between serum BAFF levels and muscle disease activity scores was found in PM patients ($r = 0.257$; $p < 0.05$), as well as in DM patients ($r = 0.228$; $p < 0.05$) (Figure 2B & E). As shown in Figure 2C & F, serum BAFF levels were found to be significantly correlated with serum CK levels in PM ($r = 0.675$; $p < 0.01$) and DM patients ($r = 0.723$; $p < 0.01$). In addition, we also found a mild correlation between serum BAFF levels and cutaneous disease activity scores in DM patients ($r = 0.354$; $p < 0.05$) (Figure 2G). However, there was no significant correlation of BAFF levels with pulmonary activity scores ($r = 0.071$; $p > 0.05$) or with cardiac activity scores ($r = 0.154$; $p > 0.05$).

Although no significant correlation between BAFF levels and pulmonary activity scores was found, Krystufkova et al. [10] and our previous study did observe that patients with ILD had significantly higher serum BAFF levels than patients without ILD. Therefore, we analyzed the association between serum BAFF levels and the presence of ILD. First, we defined a cut-off value of BAFF levels as the mean plus two standard deviations (SDs) of the control subjects, which was 1172 pg/ml. Then, we grouped the 92 PM/DM patients into two subgroups. One subgroup consisted of 66 patients whose serum BAFF levels were above 1172 pg/ml. Of these patients, 45 patients had ILD, and 21 patients did not have ILD. The other group consisted of 26 patients whose serum BAFF levels were below 1172 pg/ml. Of these patients, four patients had ILD and 22 patients did not have ILD. The $\chi^2$ test was used to analyze the frequency of ILD incidence in the two subgroups, and the subgroup with serum BAFF levels above the cut-off value showed a significantly higher frequency of ILD incidence ($\chi^2$ value = 20.886; $p < 0.001$).

In a previous study [14], we found that IIM patients had statistically higher levels of CD19⁺CD5⁻ cell count than healthy controls, and the percentages of CD19⁺CD5⁻ cells in IIM patients were significantly higher than those in healthy controls [14]. In order to reveal possible relationships between serum BAFF levels and CD19⁺CD5⁻ cell counts, we analyzed the correlation between serum levels of BAFF and levels of peripheral blood lymphocyte subsets. Among the 92 patients in our cross-sectional study, 49 patients have detailed records of levels of their peripheral blood lymphocyte subsets. Thus, the correlation between BAFF levels

### Table 1. Demographic and clinical features of polymyositis/dermatomyositis patients.

<table>
<thead>
<tr>
<th>Demographic &amp; clinical features</th>
<th>Parameters</th>
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<tbody>
<tr>
<td><strong>General features</strong></td>
<td></td>
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<tr>
<td>Age of onset, mean ± SD (range), years</td>
<td>45.2 ± 15.4 (19.5–72.6)</td>
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<tr>
<td>Number of females/males</td>
<td>72/20</td>
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<tr>
<td>Number of PM patients/number of DM patients</td>
<td>41/51</td>
</tr>
<tr>
<td>Duration of symptoms at initial evaluation, mean ± SD (range), years</td>
<td>5.6 ± 5.4 (0.25–27.5)</td>
</tr>
<tr>
<td>Levels of creatine kinase at time BAFF was measured (mean ± SD)</td>
<td>1503.4 ± 1529.9</td>
</tr>
<tr>
<td><strong>Clinical features, number of patients affected (% of cohort)</strong></td>
<td></td>
</tr>
<tr>
<td>Interstitial lung disease</td>
<td>47 (51.1)</td>
</tr>
<tr>
<td>Oropharyngeal dysphagia</td>
<td>37 (40.2)</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>18 (19.6)</td>
</tr>
<tr>
<td>Mechanic hand</td>
<td>13 (14.1)</td>
</tr>
<tr>
<td>Arthritis</td>
<td>42 (45.7)</td>
</tr>
<tr>
<td><strong>MYOACT disease activity scores (mean ± SD)</strong></td>
<td></td>
</tr>
<tr>
<td>Global disease</td>
<td>6.9 ± 1.9</td>
</tr>
<tr>
<td>Muscle disease</td>
<td>6.1 ± 2.7</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>3.0 ± 2.8</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>1.3 ± 1.9</td>
</tr>
<tr>
<td>Cutaneous disease of DM patients</td>
<td>3.4 ± 3.4</td>
</tr>
</tbody>
</table>

BAFF: B-cell activating factor; DM: Dermatomyositis; MYOACT: Myositis Disease Activity Assessment Visual Analog Scales; PM: Polymyositis; SD: Standard deviation.
and peripheral blood lymphocyte subsets counts was analyzed by using Spearman’s correlation analysis. As shown in Table 2, serum BAFF levels of PM/DM patients was negatively correlated with the following: CD3+ cell count \((r = -0.334; p < 0.05)\), CD3+CD4+ cell count \((r = -0.302; p < 0.05)\), CD3+CD8+ cell count \((r = -0.320; p < 0.05)\) and CD19+CD5− cell count \((r = -0.322; p < 0.05)\). However, no significant correlation was found between serum BAFF levels and NK cell count \((r = 0.047; p > 0.05)\).

**Longitudinal study**

In order to investigate the variation in serum BAFF levels over time and in relation to disease activity, we designed a longitudinal study. Patients in the cross-sectional study were included if they had at least two serum samples collected and disease activity evaluated at the same time. In total, 24 patients were included and each of them had between two and seven serum samples collected. The time intervals between the first and last follow-up visits ranged from 0.5 to 6 years.

*Figure 2. Correlation of serum levels of BAFF with myositis disease activity.* Serum BAFF levels were found significantly correlated with global disease activity scores both in (A) PM patients and in (D) DM patients, and correlated with serum creatine kinase in (C) PM patients and (F) DM patients. A mild correlation between serum BAFF levels and muscle disease activity scores was found in (B) PM patients, as well as in (E) DM patients. In addition, a mild correlation between serum BAFF levels and cutaneous disease activity scores was found in (G) DM patients.

BAFF: B-cell activating factor; DM: Dermatomyositis; PM: Polymyositis.
Among the 24 patients, 20 were followed up for longer than 2 years and more than three longitudinal serum samples were obtained from each patient.

In total, we collected 73 samples from the 24 patients involved in the longitudinal study, and these serial patient samples were analyzed by using Spearman’s correlation analysis. A modest correlation of serum levels of BAFF was found with global disease activity scores ($r = 0.68; p < 0.001$) as well as with muscle disease activity scores ($r = 0.533; p < 0.001$).

Among the 24 patients enrolled in the longitudinal study, 22 patients were newly diagnosed, and their serum samples and clinical data before and after treatment were obtained. By applying the Wilcoxon signed rank test, a significant decrease was observed in the patients’ serum BAFF levels ($p < 0.001$), as well as in global disease activity scores ($p < 0.001$), as shown in Figure 3A & B. Furthermore, the change in BAFF levels and the change in global disease activity scores were compared by calculating effect size. As a result, for BAFF levels before treatment and after treatment, we found that the Cohen’s d value was 0.86, and the effect-size r-value was 0.40. Furthermore, for global disease activity before treatment and after treatment, the Cohen’s d value was 2.82, and the effect-size r-value was 0.82. Therefore, both serum BAFF levels and global disease activity were decreased significantly after treatment. In addition, we analyzed the correlation between the change in BAFF levels and the change in global disease activity before and after treatment, and a modest correlation was found ($r = 0.434; p < 0.05$), as shown in Figure 3C.

**Discussion**

In this study, we have confirmed that serum levels of BAFF are significantly increased in Chinese patients with PM/DM compared with healthy control subjects. Both cross-sectional and longitudinal studies demonstrated that serum levels of BAFF correlated with disease activity in PM/DM patients.

Serum BAFF levels have been identified as a vital regulator for both innate and adaptive immune responses [9,16–17]. Increased serum BAFF levels have been found in a number of different autoimmune diseases. Increased serum BAFF levels can be found in rheumatoid arthritis patients [18] and are associated with anticollagen type II antibodies in collagen-induced arthritis, an animal model of rheumatoid arthritis [19,20]. Similarly, BAFF levels were found to be elevated in patients with Sjögren’s syndrome and correlated with autoantibody levels [21]. Moreover, in some studies on systemic lupus erythematosus, serum BAFF levels correlated with the disease activity [15,22–23]. All these studies suggest that BAFF may be involved in the autoimmune diseases.

In a study by Krystufkova et al., serum BAFF levels were found to be significantly higher in Caucasian patients with IIM than in normal controls. The study also observed that serum BAFF levels correlated with CK level [10]. However, it was still unclear whether BAFF levels could provide information about disease activity. Consistent with Krystufkova’s study, our findings suggested that Chinese patients with PM/DM also had elevated serum BAFF levels compared with control subjects, and a positive correlation between serum BAFF level and CK level was also found in Chinese PM/DM patients. Most importantly, for the first time, our study demonstrated a correlation between serum BAFF levels and PM/DM disease activity.

In the longitudinal study, we found a moderate correlation between serum BAFF levels and PM/DM disease activity, suggesting that BAFF could be a biomarker for myositis and that serial measurements of serum BAFF levels may be useful in follow-ups of myositis patients. The strength of our study is that 20 of our patients have been followed up for more than 2 years and more than three longitudinal serum samples were obtained from each patient.

Recently, it is reported by Lopez De Padilla CM et al. that BAFF mRNA levels of peripheral blood lymphocyte subsets (Table 2)

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Patients (n)</th>
<th>r-value</th>
<th>p-value</th>
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<tbody>
<tr>
<td>CD3+ cell count†</td>
<td>49</td>
<td>-0.334</td>
<td>0.019</td>
</tr>
<tr>
<td>CD3+CD4+ cell count†</td>
<td>49</td>
<td>-0.302</td>
<td>0.035</td>
</tr>
<tr>
<td>CD3+CD8+ cell count†</td>
<td>49</td>
<td>-0.320</td>
<td>0.025</td>
</tr>
<tr>
<td>CD19+CD5− cell count†</td>
<td>49</td>
<td>-0.322</td>
<td>0.024</td>
</tr>
<tr>
<td>NK cell count†</td>
<td>49</td>
<td>0.047</td>
<td>0.750</td>
</tr>
</tbody>
</table>

† Analyzed by Spearman’s correlation analysis.‡ Analyzed by a multiple labeling method [15].NK: Natural killer.
mononuclear cells were positively correlated with disease activity measures in IIM [24]. Our findings are consistent with their results. Therefore, our study provides further evidence for BAFF as a serological biomarker of PM/DM disease activity.

To our knowledge, our study was the first to reveal a negative correlation between serum levels of BAFF and peripheral blood lymphocyte subsets in PM/DM patients. In our previous study, our data showed that counts of CD3+ cells, CD3+CD4+ cells, CD3+CD8+ cells and CD19+CD5− cells correlated with global disease activity scores as determined by MYOACT [14]. Specifically, CD19+CD5− cell counts were found to be significantly higher in IIM patients than those in healthy controls. In addition, CD19+CD5− cell counts in inactive DM patients were statistically higher than those in active DM patients. According to the present study, BAFF levels correlated negatively with CD19+CD5− cell counts, which is well in accordance with our previous findings. However, these current findings seem to be inconsistent with the function of BAFF for promoting B cell differentiation, proliferation and survival. Currently, we do not have a good explanation for this finding. However, as it is unknown whether all the B cells in peripheral blood could express receptors for BAFF, and whether there is a positive correlation between serum BAFF levels and autoreactive B-cell counts, remain unclear and require further investigation.

Perhaps more important than the implication of BAFF as a biomarker for clinical disease activity, our findings may have some implications for the potential role of BAFF in the immunopathogenesis of PM and DM. Recently, Ahmi Baek et al. found that BAFF expression was markedly increased in muscle fibers in the perifascicular area but not in blood vessels. They also found that the BAFF receptor was expressed in inflammatory cells in the skeletal muscle tissue of DM patients, indicating that BAFF may play an important role in DM pathogenesis [25]. Our current understanding of the pathogenesis of IIM suggests an interplay between adaptive immune, innate immune and nonimmune mechanisms in the damage and dysfunction that occur in myopathic muscle tissue [26]. Evidence has indicated the presence of B cells and plasma cells in muscle tissue in PM, DM and IBM patients, according to muscle tissue biopsies [27,28]. It was also reported that marked upregulation of BAFF transcripts presented in the muscle biopsies of IIM patients [27]. These findings suggest that the maturation of B cells to plasma cells occurs locally in muscle tissue and that myositis-affected muscle tissue provides a permissive environment for the myeloid dendritic cells to activate Th cells [27]. Our study has
demonstrated a correlation between BAFF levels and muscle disease activity, thus providing indirect evidence supporting the potential role of B cells in the pathogenesis of PM/DM.

Conclusion & future perspective
In conclusion, our data demonstrates that Chinese patients with PM/DM have high serum levels of BAFF and that, furthermore, serum BAFF levels correlates with global disease activity and muscle disease activity therefore BAFF could be a potential biomarker for disease activity. In the future, serum BAFF evaluation could be a useful tool for disease activity assessment of PM/DM patients. Moreover, even though the precise mechanism of BAFF in the pathogenesis of PM/DM remains to be elucidated, the establishment of the correlation between BAFF and disease activity indicates a probability that BAFF may play a role in the pathogenesis of PM/DM.

Financial & competing interests disclosure
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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

References
Papers of special note have been highlighted as:
• of interest
•• of considerable interest

- Demonstrates increased serum B-cell activating factor (BAFF) levels in Caucasian patients with myositis.


- Provides disease activity evaluation tools for polymyositis/dermatomyositis (PM/DM).


- Demonstrates that Myositis Disease Activity Assessment Visual Analog Scales could be used to reliably evaluate disease activity in Chinese PM/DM patients.


- Demonstrates the clinical significance of peripheral blood lymphocyte subsets in PM/DM patients.


- Supports our hypothesis of the correlation between BAFF levels and muscle disease activity.


- Demonstrates local maturation of B cells into plasma cells in myositis muscle.


- Total mRNA was extracted from peripheral blood mononuclear cells and BAFF mRNA levels were measured by quantitative real-time PCR. The results showed that BAFF mRNA levels were positively correlated with disease activity measures in idiopathic inflammatory myopathies.