Change of serum B-cell activating factor level in patients with positive antiphospholipid antibodies and previous adverse pregnancy outcomes and its significance

Xin-Yi Li, Hong-Ji Duan, Xiang-Yuan Liu, Xiao-Li Deng

Department of Rheumatology and Immunology, Peking University Third Hospital, Haidian District, Beijing 100191, China.

Abstract

Background: B-cell activating factor (BAFF) is vital for B cell survival. Serum BAFF levels are elevated in thrombotic antiphospholipid syndrome, but little is known about levels in patients with positive antiphospholipid antibodies (aPLs) and previous adverse pregnancy outcomes (APOs). We aimed to analyze serum BAFF concentrations of these patients in early pregnancy along with different pregnancy outcomes.

Methods: Thirty-six pregnant patients positive for aPLs and previous APOs (patient group), 25 healthy pregnant females (HP group) and 35 healthy non-pregnant females (HNP group) from the Peking University Third Hospital, between October 2018 and March 2019, were enrolled in this study. Serum of HNP and serum of patients as well as HP in the first gestational trimester were collected. Enzyme-linked immunosorbent assay kits were used to measure serum BAFF and interferon-alpha (IFN-α) concentrations. Cytometric bead array analysis was used to measure serum concentrations of cytokines. The patient group was further divided into APOs and non-APOs (NAPOs) group, fetal loss and live birth group according to pregnancy outcomes. The Mann-Whitney *U*-test was used to assess significance between and within groups. Spearman rank-order was used to evaluate correlation coefficients between BAFF and related cytokines.

Results: The serum BAFF level in HP group was significantly lower than HNP group (245.24 [218.80, 265.90] *vs.* 326.94 [267.31, 414.80] pg/mL, $Z = -3.966$, $P < 0.001$). The BAFF level was obviously elevated in patient group compared to that in HP group (307.77 [219.86, 415.65] *vs.* 245.24 [218.80, 265.90] pg/mL, $Z = -2.464$, $P = 0.013$). BAFF levels in APOs group tended to be higher than that in NAPOs group (416.52 [307.07, 511.12] *vs.* 259.37 [203.59, 375.81] pg/mL, $Z = -2.718$, $P = 0.006$). Compared to HP group, concentrations of IFN-α, interleukin (IL-6) and tumor necrosis factor were higher in patient group (33.37 [18.85, 48.12] *vs.* 13.10 [6.85, 25.47] pg/mL, $Z = -2.023$, $P = 0.043$; 39.16 [4.41, 195.87] *vs.* 3.37 [2.92, 3.90] pg/mL, $Z = -3.650$, $P < 0.001$; 8.23 [2.27, 64.46] *vs.* 1.53 [1.25, 2.31] pg/mL, $Z = -3.604$, $P < 0.001$, respectively). Serum BAFF levels had a positive correlation with the concentrations of both IL-6 and IL-10 (IL-6: $r = 0.525$, $P = 0.002$; IL-10: $r = 0.438$, $P = 0.012$).

Conclusions: Serum BAFF levels are increased in patients with positive aPLs and previous APOs as compared to healthy pregnant females and tend to be higher in individuals with current APOs. The BAFF levels have a positive correlation with serum IL-6 and IL-10.

Keywords: Antiphospholipid syndrome; B-cell activating factor; Cytokine; Inflammation; Obstetrics

Introduction

Obstetric antiphospholipid syndrome is an autoimmune disease whose pathogenic mechanisms remain to be elucidated. Several studies have shown that heterogeneous antiphospholipid antibodies (aPLs) can disrupt various cellular functions through the activation of endothelial cells and placental tissue, leading to obstetric complications.[1] Therefore, aPL-mediated pathological changes are one of the characteristics of obstetric antiphospholipid syndrome (OAPS).

B-cell activating factor (BAFF), also known as B-lymphocyte stimulator (BLyS), is an important growth factor for B cells. Various cells can produce BAFF, including antigen-presenting cells, neutrophils, epithelial cells, and activated T lymphocytes.[2] It plays an important role in homeostasis, survival, and plasma cell differentiation of B cells, and in the transformation of transitional type I/II B cells.[3]

An increase in serum BAFF levels has been detected in patients with systemic lupus erythematosus (SLE) and correlates with disease activity.[4,5] However, little is known about the role of BAFF in antiphospholipid

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000948

Correspondence to: Dr. Xiao-Li Deng, Department of Rheumatology and Immunology, Peking University Third Hospital, 49 North Garden Rd, Haidian District, Beijing 100191, China
E-Mail: 13683657545@163.com

Chinese Medical Journal 2020;133(19)

Received: 20-04-2020 **Edited by:** Peng Lyu

syndrome (APS). Few studies have looked at serum BAFF concentrations in catastrophic APS and thrombotic APS patients, revealing elevated levels of serum BAFF that correlate with higher adjusted global APS scores.[6,7] To our knowledge, studies have not looked at BAFF concentrations in patients with positive aPLs and previous adverse pregnancy outcomes (APOs). Therefore, the aim of this study is to assess and comparatively analyze the concentrations of BAFF in aPLs positive patients with previous APOs, along with different pregnancy outcomes.

Methods

Ethical approval

This study followed the ethical standards for research using human subjects established in the *Declaration of Helsinki*, and was approved by the Ethics Committee of Peking University Third Hospital (No. M2018238). All patients provided written informed consent for their inclusion in the study.

Patients and healthy controls

Patients positive for aPLs and previous APOs, healthy pregnant, and non-pregnant females from the Peking University Third Hospital, between October 2018 and March 2019, were enrolled in the study.

Clinical criteria of the patients with positive aPLs and APOs included: (1) early fetal loss (<10th week of gestation)[8] or/and late fetal loss or/and APOs due to pre-eclampsia, eclampsia or placental abruption or intrauterine growth restriction; (2) in the first gestational trimester. Laboratory inclusion criteria were as follows: tested positive of IgG/IgM anti-cardiolipin antibodies (aCL) (>95th percentile) or/and IgG/IgM anti-β2 glycoprotein I antibodies (aβ2GPI) (>95th percentile) and/or lupus anticoagulants (LA) for at least two times (not necessarily 12 weeks apart). Previous APOs of patients caused by well-known reasons were excluded (including abnormal reproductive anatomy, reproductive endocrine dysfunction, chromosomal abnormalities, and male sperm abnormalities). Patients complicated with other connective tissue disease (like SLE, primary Sjogren syndrome), hypertension, chronic kidney disease or thyroid disease, aged less than 20 years or older than 40 years, and having received assisted reproduction were also excluded.

Healthy non-pregnant females aged 20 to 40 years with no previous history of chronic diseases and no history of acute infectious diseases within the past month were included as a control group.

Inclusion criteria of the healthy pregnant female were as follows: aged 20 to 40 years and in the first gestational trimester. Exclusion criteria included: previous APOs history, abnormal reproductive anatomy, reproductive endocrine dysfunction, chromosomal abnormalities, and acute infectious diseases history within the past month.

The enrolled patients included the criteria OAPS and non-criteria OAPS (NC-OAPS). The criteria OAPS was diagnosed according to the updated Sydney classification criteria.[9] The NC-OAPS in this study was diagnosed according to the following standards: (1) ≥2 times positive for IgG/IgM aCL (95th–99th percentile) or/and IgG/IgM aβ2GPI (95th–99th percentile) with any manifestation of clinical inclusion criteria; (2) ≥2 times positive for IgG/IgM aCL (≥99th percentile) or/and IgG/IgM aβ2GPI (≥99th percentile) or/and LA with one or two times early fetal loss.

Collection of general information and clinical data

The following demographic and clinical data were recorded: age, history of APOs, serological manifestations (aPLs, anti-nuclear antibody [ANA] and autoantibody profiles), and the follow-up pregnancy outcomes (obtained by reviewing obstetricians' documents). All laboratory indicators were from the Peking University Third Hospital. ANA was detected by indirect immunofluorescence method, aCL and aβ2GPI were detected by chemiluminescence method, and LA was detected by clotting assay method.

Laboratory assessments

Serum separated from peripheral venous blood of eligible subjects was obtained from the remaining specimen samples at the clinical laboratory of the Peking University Third Hospital, and stored at −80 °C until analyzed.

Serum interferon-alpha (IFN-α) and BAFF levels were determined using the Human IFN-α enzyme-linked immunosorbent assay (ELISA) Kit (4ABiotech, Beijing, China, CHE0084-096), Human BAFF/BLyS/TNFSF13B DuoSet ELISA Kit (R&D Systems, Minneapolis, MN, USA, DY124-05), respectively. Analysis was performed according to the manufacturer's instructions. The minimum level of detection for IFN-α was less than 4 pg/mL. The assay range of BAFF was 39.1 to 2500 pg/mL. Cytokines, including interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17A, and tumor necrosis factor-alpha (TNF-α), were measured by the Human Th1/Th2 Cytokine Cytometric Bead Array (CBA) Kit II (Bioscience, Franklin Lakes, NJ, USA, 551809), from the Hematology Research Laboratory of Peking University Third Hospital. The fluorescence produced by CBA beads was measured on a FACS Calibur flow cytometer equipped with Cell Quest software (BD Bioscience) and analyzed using BD™ CBA Software (BD Bioscience, Cat. No. 550065). The detection limits of IL-2, IL-4, IL-6, IL-10, IL-17A, and TNF-α were 2.6, 2.6, 2.4, 2.8, 18.9, and 2.8 pg/mL, respectively.

Definitions in this study

APOs: definitions of APOs in this study included the following:

(1) Fetal loss: including early fetal loss and late fetal loss (<10 gestational week and ≥10 gestational week, respectively); (2) preterm delivery: birth before the 37th gestational week due to pre-eclampsia or eclampsia or placental insufficiency (decreased amniotic fluid, fetal growth restriction) or placental abruption.[10] Pre-eclampsia or eclampsia refers to new-onset persistent hypertension (blood pressure >140/90 mmHg) after the 20th

gestational week in association with new-onset proteinuria (300 mg or more of protein in a 24-h urine collection) or hypertension without proteinuria but associated with organ dysfunction. Fetal growth restriction refers to estimated fetal weight below the 10th percentile for a given gestational age, associated with Doppler abnormalities;[11] (3) others, including birth after the 37th gestational week but complicated by pre-eclampsia, eclampsia, or other placental insufficiency.

aPLs positive: patients positive for IgG/IgM aCL, IgG/IgM aβ2GPI, and/or LA were defined as aPLs positive. Patients positive for one, two, or all three of these parameters were defined as single-aPLs positive, double-aPLs positive, and triple-aPLs positive, respectively.

Grouping in this study

In this study, patients were divided into two groups according to the follow-up pregnancy outcomes: (1) APOs and non-APOs (NAPOs) group and (2) live birth and fetal loss group.

Statistical analysis

The SPSS 23.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. The Shapiro-Wilk test was used to determine normality of data distribution for sample sizes ($n < 50$). Data with normal distribution were expressed as mean and standard deviation, whilst data with non-normal distribution were expressed as the median (Q1, Q3). The Mann-Whitney *U*-test was used for comparisons between and within groups including: BAFF in positive aPLs and previous APOs patient and healthy pregnant women (HP) groups, BAFF in patient and healthy non-pregnant women (HNP) groups, BAFF in HP and HNP groups, BAFF between APOs and NAPOs groups, as well as fetal loss group and live birth group; cytokines in patient and HP groups, cytokines in APOs and NAPOs groups. Correlation coefficients between BAFF levels compared to IL-4, IL-6, IL-10, IL-17A, IFN-α, and TNF-α were obtained by Spearman rank-order method. A two-tailed P value < 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics

A total of thirty-six, twenty-five, and thirty-five subjects in the patient group (aged 27–39 years, mean 32.0 years), HP group (aged 21–38 years, mean 33.0 years) and HNP group (aged 23–38 years, mean 31.0 years) respectively, were included in this study. All the subjects from the patient group had recorded outcomes at the end of the study and were further divided into APOs, NAPOs groups and fetal loss, live birth groups, according to the pregnancy outcomes. Characteristics for all subjects are shown in [Table 1].

Serum BAFF concentrations in all subjects and different pregnancy outcome groups

Serum BAFF levels in pregnant subjects (both in the patient group and the HP group) were lower than the HNP control

Table 1: Baseline characteristics of study participants.

Parameters	Patients group ($n = 36$)	HP group ($n = 25$)	HNP group ($n = 35$)
Age (years)	32.6 ± 3.9	32.7 ± 4.5	30.8 ± 4.6
ANA positivity	6 (16.7)	–	–
Positive aPLs serology		–	–
Positive for one Ab	29 (80.6)	–	–
Positive for two Ab	5 (13.9)	–	–
Positive for three Ab	2 (5.5)	–	–
APLs serological profile		–	–
Anti-cardiolipin antibody	9 (25.0)	–	–
aβ2GPI antibody	6 (16.7)	–	–
Lupus anti-coagulant	20 (55.6)	–	–
Adverse pregnancy outcome history*		–	–
Early miscarriage (<10 weeks)	32 (88.9)	–	–
APOs (≥10 weeks)	11 (30.6)	–	–
Pregnancy outcome followed		–	–
Full-term delivery	24 (66.7)	–	–
Adverse outcomes†	12 (33.3)	–	–
Eclampsia	2 (16.7)	–	–
Early or late miscarriage	3 (25.0)	–	–
Placental abruption	1 (8.3)	–	–
Fetal asphyxia	1 (8.3)	–	–
Low birth weight	3 (25.0)	–	–
Pre-mature birth	4 (33.3)	–	–

Data are presented as mean ± standard deviation or *n* (%). *7 patients had both the early miscarriage and APOs (≥10 weeks) histories. †There were overlaps between the listed adverse outcomes. HP group: Healthy pregnant group; HNP group: Healthy non-pregnant group; ANA: Anti-nuclear antibody; APLs: Antiphospholipid antibodies; Ab: Antibody; aβ2GPI: anti-β2 glycoprotein I antibodies; APOs: Adverse pregnancy outcomes; –: No data.

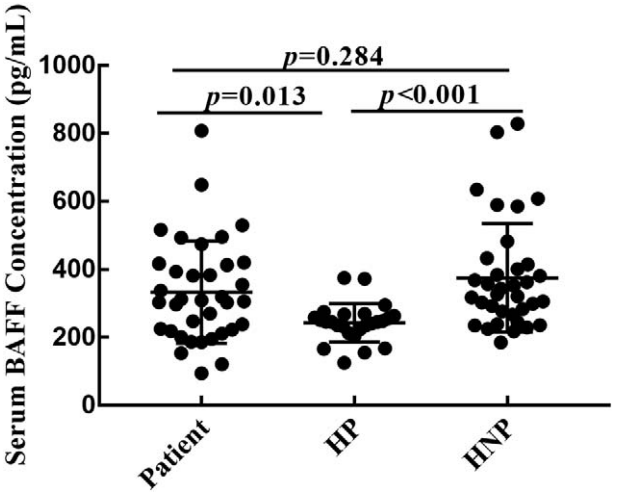

Figure 1: Serum BAFF concentrations in patients group, healthy pregnant (HP) group and healthy non-pregnant (HNP) group. Patient group: $n = 36$; HP group: $n = 25$; HNP group: $n = 35$. Serum BAFF concentrations in HP group was lower than that in HNP group ($P < 0.001$). Serum BAFF concentration in patient group was higher than the HP group ($P = 0.013$). BAFF: B cell-activating factor.

group (307.77 [219.86, 415.65] *vs.* 245.24 [218.80, 265.90] *vs.* 326.94 [267.31, 414.80] pg/mL, respectively). Significant differences in serum BAFF concentrations were observed between patient and HP group (307.77 [219.86, 415.65] *vs.* 245.24 [218.80, 265.90] pg/mL, $Z = -2.464$, $P = 0.013$). Moreover, BAFF levels in HP group were significantly lower than in the HNP group (245.24 [218.80, 265.90] *vs.* 326.94 [267.31, 414.80] pg/mL, $Z = -3.966$, $P < 0.001$). No differences were observed in serum BAFF levels between patient group and HNP group (307.77 [219.86, 415.65] *vs.* 326.94 [267.31, 414.80] pg/mL, $Z = -1.081$, $P = 0.284$) [Figure 1]. BAFF levels in APOs group tended to be higher than in NAPOs group (416.52 [307.07, 511.12] *vs.* 259.37 [203.59, 375.81] pg/mL, $Z = -2.718$, $P = 0.006$). There was no difference in serum BAFF level between fetal loss group and live birth group (412.56_{median} [195.66_{min}, 648.95_{max})] pg/mL *vs.* 306.36 [220.85, 405.34] pg/mL, $Z = -0.715$, $P = 0.512$) [Figure 2].

Cytokine levels in the patient, HP, and different pregnancy outcome groups

Some subjects within the patient and HP groups (12/28 and 14/25, respectively) had undetectable levels of human IFN-α according to the minimum concentration recommended by the kit (<4 pg/mL). Compared to HP group, the concentrations of IFN-α, IL-6, and TNF-α were higher in patient group (33.37 [18.85, 48.12] *vs.* 13.10 [6.85, 25.47] pg/mL, $Z = -2.023$, $P = 0.043$; 39.16 [4.41, 195.87] *vs.* 3.37 [2.92, 3.90] pg/mL, $Z = -3.650$, $P < 0.001$; 8.23 [2.27, 64.46] *vs.* 1.53 [1.25, 2.31] pg/mL, $Z = -3.604$, $P < 0.001$, respectively). The serum IL-17A concentration in patient group was lower than the HP group (4.90 [0.11, 8.28] *vs.* 11.72 [9.61, 13.93] pg/mL, $Z = -3.820$, $P < 0.001$) [Table 2]. There were no statistical differences in the levels of IFN-α and other cytokines, including IL-2, IL-4, IL-6, IL-10, IL-17A, and TNF-α, in patient group based on pregnancy outcomes [Table 3].

Correlations of serum IFN-α, IL-4, IL-6, IL-10, TNF-α, IL-17A with serum BAFF in patients group with pregnancy outcomes

Concentrations of IL-6 and IL-10 positively correlate with serum BAFF levels (IL-6: $r = 0.525$, $P = 0.002$; IL-10: $r = 0.438$, $P = 0.012$). Correlations were not observed between serum BAFF levels and the concentrations of other cytokines [Figure 3].

Discussion

This is a rare study that the concentrations of serum BAFF and other BAFF-related cytokines were measured and analyzed in patients with positive aPLs and prior APOs. We found that concentrations of serum BAFF were increased in patients as compared to HP subjects, and tended to be higher in individuals with APOs. The concentrations of IFN-α, IL-6, and TNF-α in the patient group were higher than those in the HP group. Additionally, BAFF levels positively correlated with the concentrations of both IL-6 and IL-10.

BAFF, a member of the TNF superfamily, is essential for B-cell survival, homeostasis, and plasma cell differentiation.[12] Although many studies have found possible pathogenic mechanisms of BAFF in SLE, there is still a lack of systemic comparison and evaluation of these molecules in APS, especially in OAPS. Many studies have shown that aPLs can disrupt various cellular functions through the activation of endothelial cells and placental tissue, leading to obstetric complications.[1] Additionally, B cells influence the production of aPLs; therefore, BAFF may participate in the pathogenesis of OAPS.

Previous studies have detected the pregnancy-associated B-cell lymphopenia in animals and humans during pregnancy.[13,14,15] Muzzio *et al*[13] found that B cells in the blood depicted a significant decrease in the total number of CD19+ B cells in pregnant mice compared to non-pregnant control mice. Moreover, the serum levels of BAFF significantly dropped during pregnancy and remained low until birth. Our data also showed that the levels of BAFF in both patients with positive aPLs and HP females were lower than that in HNP females, thereby providing further support to previous findings. Although we did not further investigate the mechanism behind this phenomenon, previous work has shown that BAFF can support the survival and proliferation of auto-reactive B cells, which have a higher BAFF dependence.[16] Indeed, autoimmunity is often associated with elevated levels of BAFF. Therefore, the strong reduction in BAFF levels, in synergy with B cell lymphopenia during pregnancy, may represent an acquired protective mechanism and may be related to the physiology of maternal-fetal immune tolerance.

In our study, we found elevated serum BAFF levels in our patient group when compared to HP group. Based on BAFF pathogenic mechanisms in SLE,[17] we propose that

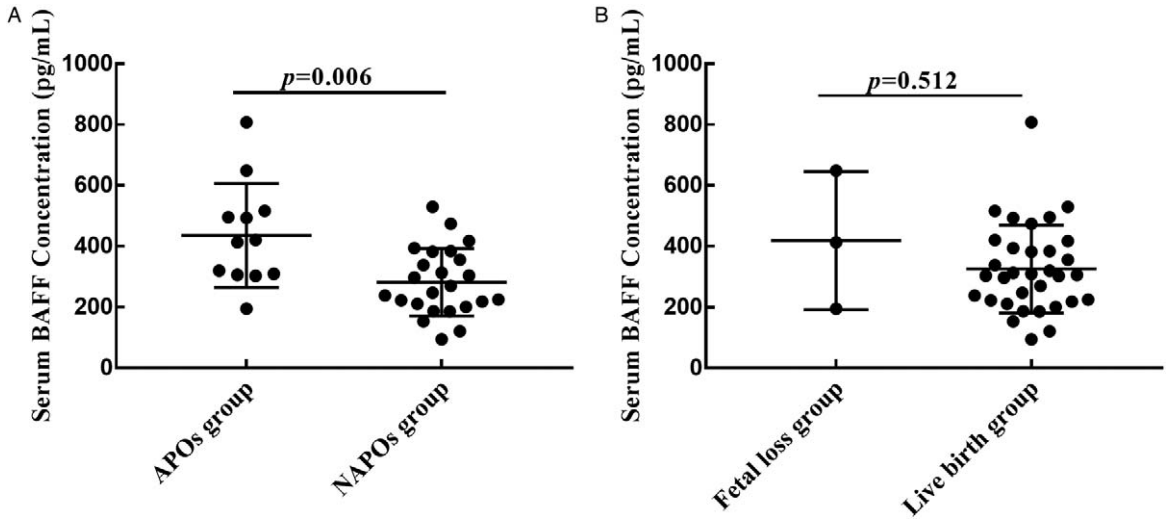

Figure 2: Serum BAFF concentrations in different pregnancy outcome groups. (A) APOs group: $n = 12$; NAPOs group: $n = 24$; (B) Fetal loss group: $n = 3$; Live birth group: $n = 33$. Serum BAFF concentration in APOs group was higher than NAPOs group ($P = 0.006$). No difference was found between fetal loss group and live birth group ($P = 0.512$). APOs: Adverse pregnancy outcomes; BAFF: B cell-activating factor; NAPOs: Non-adverse pregnancy outcomes.

Table 2: Concentrations of different cytokines in patient group and HP group.

Parameters (pg/mL)	Patient group ($n = 32$)	HP group ($n = 20$)	*Z*	*P*
IL-2	1.41 (0.98, 2.33)	1.11 (0.10, 1.22)	−1.658	0.097
IL-4	1.02 (0.64,1.88)	1.57 (1.23, 1.57)	−1.867	0.062
IL-6	39.16 (4.41, 195.87)	3.37 (2.92, 3.90)	−3.650	<0.001
IL-10	1.79 (1.25, 2.73)	1.50 (1.33, 1.75)	−1.516	0.129
IL-17A	4.90 (0.11, 8.28)	11.72 (9.61, 13.93)	−3.820	<0.001
TNF-α	8.23 (2.27, 64.46)	1.53 (1.25, 2.31)	−3.604	<0.001
IFN-α*	33.37 (18.85, 48.12)	13.10 (6.85, 25.47)	−2.023	0.043

Data are presented as median (Q1, Q3). *16 and 11 in the patient and HP groups, respectively. HP group: Healthy pregnant group; IL: Interleukin; TNF-α: Tumor necrosis factor-alpha; IFN-α: Interferon-alpha.

Table 3: Concentrations of different cytokines in APOs and NAPOs groups.

Parameters (pg/mL)	APOs group ($n = 10$)	NAPOs group ($n = 22$)	*Z*	*P*
IL-2	1.25 (0.15, 2.47)	1.41 (1.01, 2.59)	−0.997	0.319
IL-4	0.99 (0.67, 1.20)	1.23 (0.54 2.05)	−0.102	0.919
IL-6	42.22 (4.74, 213.96)	12.90 (3.24, 213.72)	−0.549	0.589
IL-10	1.61 (0.04, 4.03)	1.79 (1.48, 2.57)	−0.793	0.428
IL-17A	1.94 (0, 5.02)	6.81 (0.33, 9.38)	−1.686	0.092
TNF-α	13.59 (1.30, 46.84)	8.23 (3.32, 69.11)	−0.630	0.529
IFN-α*	36.94 (28.66, 52.81)	24.33 (8.82, 40.14)	−1.575	0.115

Data are presented as median (Q1, Q3). *8 and 8 in the APOs and NAPOs groups, respectively. APOs: Adverse pregnancy outcome; NAPOs: Non-adverse pregnancy outcome; IL: interleukin; TNF-α: Tumor necrosis factor-alpha; IFN-α: Interferon-alpha.

the increased BAFF levels in these patients could promote B cell survival and differentiation, leading to aPLs production and further generation of the immune complex. This would give rise to aPL-mediated pathological changes including inflammations, complement activations, and vascular thrombosis in trophoblastic and decidua cells.

Another important finding of this study includes the elevated serum BAFF concentrations in current APOs patients. In addition to the effect on B-cell survival and differentiation, increased BAFF also activates monocytes and other antigen-presenting cells to release IL-6, IL-17, and IL-23,[3,18] which are pro-inflammatory cytokines and pre-dominant in APS. These cytokines can further activate neutrophils thereby sustaining the inflammation status. In OAPS, one of the pathogenic mechanisms includes the production of pro-inflammatory factors. Endothelial cells treated with IgG-aPL can activate cells to increase the expression of tissue factors, IL-6, IL-8, and TNF-α, leading to thrombosis and placental tissue injury.[19] Therefore, higher serum BAFF levels may be indicative of the intensive inflammatory status. Although the specific roles of BAFF in

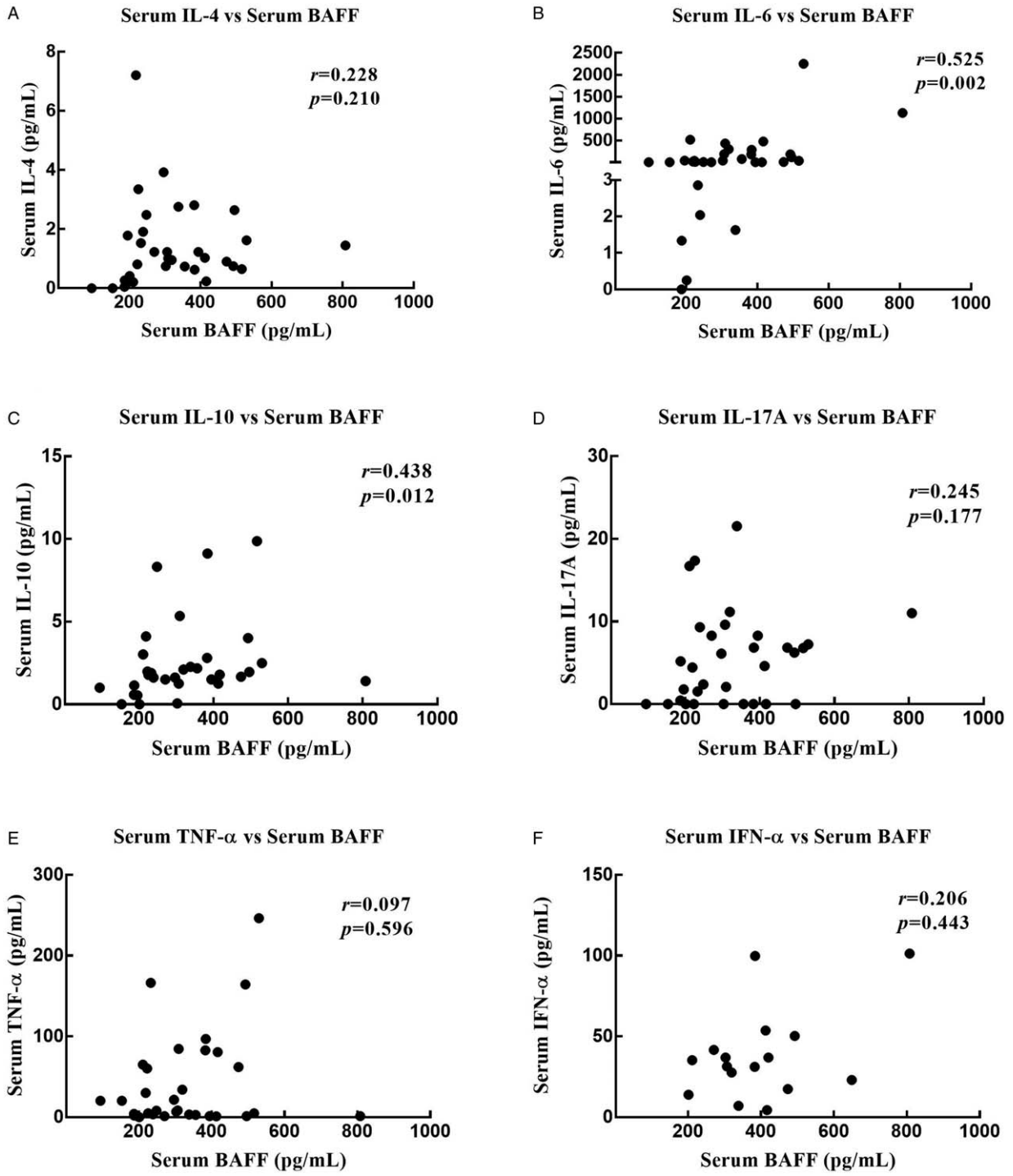

Figure 3: Correlations of serum human cytokines with serum BAFF in patient group. (A–E) (IL-4, IL-6, IL-10, IL-17A, TNF-α, respectively, $n = 32$); (F) (IFN-α: $n = 16$). Concentrations of IL-6 and IL-10 positively correlate with serum BAFF levels (IL-6: $r = 0.525$, $P = 0.002$; IL-10: $r = 0.438$, $P = 0.012$). BAFF: B cell-activating factor; IL: Interleukin; IFN-α: Interferon-alpha; TNF-α: Tumor necrosis factor-alpha.

the pathogenesis of OAPS are still unclear, the above findings suggest that BAFF may be a potential marker to predict pregnancy outcomes of OAPS patients. Treatment responses to Belimumab in SLE and primary APS patients, with higher serum BAFF levels, have been reported in two cases.[20,21] As such, in the future, serum BAFF might be a promising therapeutic target in OAPS.

IFN-α is important in the production and pathological function of BAFF. Lopez *et al*[22] cultured monocytes that were isolated from the peripheral blood of healthy donors; they found that IFN-α was the most efficient trigger of BAFF production. Additionally, in human dendritic cell (DC) cultures, IFN-α up-regulates BAFF expression,[23] whilst treatment with anti-IFN-α monoclonal antibody, in

SLE patients, down-regulates BAFF expression.[24,25] Taken together, these findings all support the fact that IFN-α significantly influences the expression of BAFF. In many systemic autoimmune diseases, a defect in the clearance of apoptotic materials, or an excess of apoptosis, leads to the accumulation of nuclear autoantigens and immune complexes. They are phagocytized by DCs through Fcγ receptor (FcγR) IIa and are delivered into the endosomal compartment triggering the activation of Toll-like receptor (TLR) 7 and TLR9, culminating in the production of IFN-α. High levels of IFN-α promote the activation and induction of BAFF in B cells, monocytes, mature DCs and neutrophils.[17,26] Palli *et al*[27] found that the expression of type I IFN-regulated genes increased in primary APS, indicating that inflammation pathways involving type I IFN may be implicated in the pathophysiology of APS. In our study, we found elevated levels of IFN-α in patients positive for aPLs with APOs compared to the control group, although a positive correlation was not found between the levels of serum BAFF and IFN-α.

Another critical feature in OAPS is the imbalance of cytokines. Th2 cells secrete IL-4, IL-6, and IL-10, which can promote the activation of B-lymphocytes and induce the production of IgG1.[28] The decreased function of Th1 and the hyperfunction of Th2, leads to excessive activation of B cells, generation of autoantibodies and tissue injury.[29] Studies have observed increased serum levels of proinflammatory cytokines, such as IL-6 and TNF-α, in APS patients. Our study revealed similar serum cytokine trends in OAPS patients. Moreover, positive correlations between BAFF and the cytokines, IL-6 and IL-10 were also found, indicating an interacting pathogenic relationship between OAPS, cytokines, and BAFF.

The limitations of this study are as follows: it was only a preliminary study on the level of BAFF in OAPS; the number of aPLs positive patients with APOs was relatively small; the concentrations of serum BAFF and IFN-α, as well as cytokines levels were not dynamically assessed during different gestational periods.

In conclusion, to the best of our knowledge, this is a rare study about BAFF in patients positive for aPLs with previous APOs. Increased serum levels of BAFF in these patients, with APOs, support the idea that BAFF may be a potential biomarker to predict pregnancy outcomes, and may act as a promising therapeutic target for OAPS patients in the future.

Acknowledgements

The authors thank Prof. Wen-Ling Han from Peking University, Human Disease Research Center, for the guidance. The authors also acknowledge the technical support and help from the Hematology Research Laboratory of the Peking University Third Hospital.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81501390).

Conflicts of interest

None.

References

1. van den Hoogen LL, van Roon JAG, Radstake TRDJ, Fritsch-Stork RDE, Derksen RHWM. Delineating the deranged immune system in the antiphospholipid syndrome. Autoimmunity Rev 2016;15:50–60. doi: 10.1016/j.autrev.2015.08.011.
2. Mackay F, Schneider P. Cracking the BAFF code. Nat Rev Immunol 2009;9:491–502. doi: 10.1038/nri2572.
3. Thompson N, Isenberg DA, Jury EC, Ciurtin C. Exploring BAFF: its expression, receptors and contribution to the immunopathogenesis of Sjogren's syndrome. Rheumatology (Oxford) 2016;55:1548–1555. doi: 10.1093/rheumatology/kev420.
4. Vincent FB, Morand EF, Schneider P, Mackay F. The BAFF/APRIL system in SLE pathogenesis. Nat Rev Rheumatol 2014;10:365–373. doi: 10.1038/nrrheum.2014.33.
5. Salazar-Camarena DC, Ortiz-Lazareno PC, Cruz A, Oregon-Romero E, Machado-Contreras JR, Muñoz-Valle JF, *et al*. Association of BAFF, APRIL serum levels, BAFF-R, TACI and BCMA expression on peripheral B-cell subsets with clinical manifestations in systemic lupus erythematosus. Lupus 2016;25:582–592. doi: 10.1177/0961203315608254.
6. van den Hoogen LL, Palla G, Bekker CPJ, Fritsch-Stork RDE, Radstake T, van Roon JAG. Increased B-cell activating factor (BAFF)/B-lymphocyte stimulator (BLyS) in primary antiphospholipid syndrome is associated with higher adjusted global antiphospholipid syndrome scores. RMD Open 2018;4:e000693. doi: 10.1136/rmdopen-2018-000693.
7. Wojdasiewicz P, Wajda A, Haładyj E, Romanowska-Próchnicka K, Felis-Giemza A, Nałęcz-Janik J, *et al*. IL-35, TNF-α, BAFF, and VEGF serum levels in patients with different rheumatic diseases. Reumatologia/Rheumatology 2019;57:145–150. doi: 10.5114/reum.2019.86424.
8. Chauleur C, Galanaud JP, Alonso S, Cochery-Nouvellon E, Balducchi JP, Mares P, *et al*. Observational study of pregnant women with a previous spontaneous abortion before the 10th gestation week with and without antiphospholipid antibodies. J Thromb Haemost 2010;8:699–706. doi: 10.1111/j.1538-7836.2010.03747.x.
9. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, *et al*. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4:295–306. doi: 10.1111/j.1538-7836.2006.01753.x.
10. Palma dos Reis CR, Cardoso G, Carvalho C, Nogueira I, Borges A, Serrano F. Prediction of adverse pregnancy outcomes in women with systemic lupus erythematosus. Clin Rev Allergy Immunol 2019. doi: 10.1007/s12016-019-08762-9. [Ahead of print].
11. Gynecologists ACoOa. Intrauterine growth restriction. Obstet Gynecol 2000;95:1–12x.
12. Vincent FB, Saulep-Easton D, Figgett WA, Fairfax KA, Mackay F. The BAFF/APRIL system: emerging functions beyond B cell biology and autoimmunity. Cytokine Growth Factor Rev 2013;24:203–215. doi: 10.1016/j.cytogfr.2013.04.003.
13. Muzzio DO, Soldati R, Ehrhardt J, Utpatel K, Evert M, Zenclussen AC, *et al*. B cell development undergoes profound modifications and adaptations during pregnancy in mice. Biol Reprod 2014;91:115. doi: 10.1095/biolreprod.114.122366.
14. Kraus TA, Engel SM, Sperling RS, Kellerman L, Lo Y, Wallenstein S, *et al*. Characterizing the pregnancy immune phenotype: results of the viral immunity and pregnancy (VIP) study. J Clin Immunol 2012;32:300–311. doi: 10.1007/s10875-011-9627-2.
15. Lima J, Cambridge G, Vilas-Boas A, Martins C, Borrego LM, Leandro M. Serum markers of B-cell activation in pregnancy during late gestation, delivery, and the postpartum period. Am J Reprod Immunol 2019;81:e13090. doi: 10.1111/aji.13090.
16. Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu HB, *et al*. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. Immunity 2004;20:441–453. doi: 10.1016/s1074-7613(04)00079-2.
17. Lopez P, Rodriguez-Carrio J, Caminal-Montero L, Mozo L, Suarez A. A pathogenic IFNalpha, BLyS and IL-17 axis in systemic lupus

erythematosus patients. Sci Rep 2016;6:20651. doi: 10.1038/srep20651.
18. Chang SK, Arendt BK, Darce JR, Wu X, Jelinek DF. A role for BLyS in the activation of innate immune cells. Blood 2006;108:2687–2694. doi: 10.1182/blood-2005-12-017319.
19. Velásquez M, Rojas M, Abrahams VM, Escudero C, Cadavid ÁP. Mechanisms of endothelial dysfunction in antiphospholipid syndrome: association with clinical manifestations. Front Physiol 2018;9:1840. doi: 10.3389/fphys.2018.01840.
20. Roth DA, Thompson A, Tang Y, Hammer AE, Molta CT, Gordon D. Elevated BLyS levels in patients with systemic lupus erythematosus: associated factors and responses to belimumab. Lupus 2016;25:346–354. doi: 10.1177/0961203315604909.
21. Yazici A, Yazirli B. Erkan D belimumab in primary antiphospholipid syndrome. Lupus 2017;26:1123–1124. doi: 10.1177/0961203316682102.
22. Lopez P, Scheel-Toellner D, Rodriguez-Carrio J, Caminal-Montero L, Gordon C, Suarez A. Interferon-alpha-induced B-lymphocyte stimulator expression and mobilization in healthy and systemic lupus erthymatosus monocytes. Rheumatology (Oxford) 2014;53:2249–2258. doi: 10.1093/rheumatology/keu249.
23. Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, *et al*. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 2002;3:822–829. doi: 10.1038/ni829.
24. Jacob N, Guo S, Mathian A, Koss MN, Gindea S, Putterman C, *et al*. B cell and BAFF dependence of IFN-alpha-exaggerated disease in systemic lupus erythematosus-prone NZM 2328 mice. J Immunol 2011;186:4984–4993. doi: 10.4049/jimmunol.1000466.
25. Yao Y, Richman L, Higgs BW, Morehouse CA, de los Reyes M, Brohawn P, *et al*. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. Arthritis Rheum 2009;60:1785–1796. doi: 10.1002/art.24557.
26. Chasset F, Arnaud L. Targeting interferons and their pathways in systemic lupus erythematosus. Autoimmun Rev 2018;17:44–52. doi: 10.1016/j.autrev.2017.11.009.
27. Palli E, Kravvariti E, Tektonidou MG. Type I interferon signature in primary antiphospholipid syndrome: clinical and laboratory associations. Front Immunol 2019;10:487. doi: 10.3389/fimmu.2019.00487.
28. Forastiero RRMM, de Larrañaga GF. Circulating levels of tissue factor and proinflammatory cytokines in patients with primary antiphospholipid syndrome or leprosy related antiphospholipid antibodies. Lupus 2005;14:129–136. doi: 10.1191/0961203305lu2048oa.
29. Pan L, Lu M-P, Wang J-H, Xu M, Yang S-R. Immunological pathogenesis and treatment of systemic lupus erythematosus. World J Pediatr 2020;16:19–30. doi: 10.1007/s12519-019-00229-3.

How to cite this article: Li XY, Duan HJ, Liu XY, Deng XL. Change of serum B-cell activating factor level in patients with positive antiphospholipid antibodies and previous adverse pregnancy outcomes and its significance. Chin Med J 2020;133:2287–2294. doi: 10.1097/CM9.0000000000000948