The expression of BAFF in the muscles correlate with refractory autoimmune necrotizing myopathy with anti-signal recognition particle antibodies

Yawen Zhao
Peking University First Hospital
Wei Zhang
Peking University First Hospital
Yilin Liu
Peking University First Hospital
Zhaoxia Wang
Peking University First Hospital
Yun Yuan (yuanyun2002@126.com)
Peking University First Hospital https://orcid.org/0000-0003-3282-9123

Research

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Abstract

Background

Autoimmune necrotizing myopathy with anti-signal recognition particle antibodies (ANM-SRP) is considered as a refractory myositis; some patients respond poorly to conventional immunosuppression required the treatment of B lymphocyte clearance. We aimed to identify the factors related to refractory ANM-SRP.

Results

48 patients with ANM-SRP were included to identify the factors related to refractory ANM-SRP. We monitored the clinical symptoms and image changes over 12 months. Refractory ANM-SRP appeared in 32.5% patients, who showed no or minimal improvement after 12 months with steroid therapy. The clinical risk factors for refractory patients were male (19.57, 1.49-256.53), severe muscle weakness (7.51, 41.03-54.88) and concurrent interstitial lung disease (39.70, 3.04-518.38). The fatty infiltration rate of thigh muscles over 3 months was more severe (P=0.022) in refractory patients. Muscles of all patients underwent routine histology, enzyme histochemistry and immunohistochemistry staining. The first antibodies used for immunohistochemistry include anti-B cell activating factor (BAFF) and BAFF receptor (BAFF-R) antibodies. Immunohistochemical staining and western bolt showed that the expression of BAFF and BAFF-R in muscles of the patients were higher than that of the normal controls. The positive BAFF-R cells (P=0.036) and CD19 positive lymphocytes (P=0.002) in refractory patients were higher than in patients with good clinical response to therapy.

Conclusion

The expression of BAFF and BAFF-R in muscles of ANM-SRP were upregulated. Male, severe muscle weakness, concurrent interstitial lung disease, quick development of muscle fatty infiltration and more BAFF-R and B-lymphocytes infiltration in muscle indicate a poor response to immunosuppressive therapy.

Background

Autoimmune necrotizing myopathy with anti-signal recognition particle antibodies (ANM-SRP) is one subtype of idiopathic inflammatory myopathy [1]. It is often treated as refractory myositis due to its poor response to immunosuppressive agents including glucocorticoid and second-line immunosuppressive therapy [2, 3], especially in patients with other myositis-specific autoantibodies [4, 5] or other autoimmune diseases [6], often requires combination medication [2, 7]. Japanese studies also used glucocorticoids as basic drugs, 77% of which required additional immunosuppressants [8]. Some studies had applied rituximab to refractory patients, suggesting that it should be used in the case of poor effect to glucocorticoid or combined with other autoimmune diseases [3, 9, 10].

Several investigations demonstrated that early-onset disease, severe myasthenia, dysphagia, muscular atrophy, combined with concurrent interstitial lung disease (ILD) are risk factors for poor prognosis of ANM-SRP [2,8]. In addition, patients with chronic disease course often show more severe myasthenia symptoms and muscular atrophy, as well as worse treatment effect and prognosis [11]. In our previous muscle MRI study of 12 patients, we found that four patients presented with severe thigh muscle fatty infiltration of posterior group, and muscle fatty infiltration was one of the factors contributing to refractory [12]. The proportion of patients with refractory ANM-SRP and which clinical and pathological indicators could be used for evaluating the response to conventional immunosuppression are lacking due to few systematic follow-up studies. Here, we performed a followed-up study for the therapeutic effect of conventional immunosuppression in a large cohort of Chinese patients with ANM-SRP and analyzed the risk factors related to refractory patients.

Methods

Patient registry

This is a single-center, retrospective, observational cohort study including 48 patients who were diagnosis as ANM-SRP by clinical, serological, and pathological criterion. Demographic data, clinical features, and initial drug treatment information were collected (Supplementary materials). The procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation and approved by the Institutional Review Board. Informed consent for all examinations was obtained from the patients or their guardians.

Serum myositis antibody test

An immunoblot kit was used to determine the serum antibodies to SRP by detecting the 54kD subunit. The membrane strips were pretreated, incubated with patient serum, followed by enzyme binding. The results were scanned using EUROlineScan software, and were recorded as negative, weak positive (+), positive (++) and strong positive (+++). All the patients recruited were strong positive (+++).

Muscle MRI

Thirty-six patients underwent bilateral thigh MRI (tMRI) (3.0 T GE 1.5 Sigma Twin Speed; GE Healthcare, Waukesha, WI, USA). Axial T1-weighted MRI was performed to evaluate the degree of fatty infiltration according to the modified Mercuri scale (0–5 scale). Axial Short T1 inversion recovery sequences were used to assess the degree of edema (0–5 scale) [11]. Edema and fatty infiltration scores were calculated in the gluteus maximus at the pelvic level and thigh muscles (vastus intermedius, vastus medialis, vastus lateralis, rectus femoris, biceps femoris, semitendinosus, semimembranosus, adductor magnus, sartorius, long adductor, and gracilis) at the mid-thighs. We calculated and summed the total fatty infiltration and edema scores of the gluteus maximus and thigh muscles between adolescent patients and adult patients. Additionally, we compared the thigh MRI of 36 patients with 36 controls (autoimmune necrotizing myopathy patients with negative anti-SRP antibody) to observe the damage in the tMRI.

Muscle pathology

Muscle biopsies were taken from the biceps brachii or quadriceps femoris of all the patients. Serial frozen sections were stained with hematoxylin and eosin (HE), modified Gomori trichrome, periodic acid-Schiff, Oil Red O, adenosinetriphosphate (ATP) enzyme (pH 4.5 and 10.8), NADH-tetrazolium reductase, Succinate dehydrogenase (SDH), and cytochrome C oxidase (COX) stains. The sections were immunohistochemically stained with primary antibodies against human CD3, CD4, CD8, CD19, CD20, CD68, B cell activating factor (BAFF) and its receptor, major histocompatibility complex class -I (MHC-I), membrane attack complex (MAC), dystrophin, sarcoglycans, and dysferlin. The positive cellular expression of BAFF, BAFF-R and CD19 positive cells (numbers of BAFF, BAFF-R, CD19 positive cells / numbers of muscle fibers) were calculated per high-power microscopic field. Correlation of the positive cellular expression of BAFF, BAFF-R, CD19 positive cells was analyzed.

BAFF and BAFF-R measurement

Muscle tissue of 14 patients with ANM-SRP and four healthy control were sampled. RIPA lysate (including protease inhibitor and phosphatase inhibitor) was added to the tissue samples for homogenization, and the protein was extracted after lysis on ice for 30min. An equal amount of protein (80 μg) was suspended in loading buffer, denatured at 100°C for 5 minutes, and loaded on an SDS-PAGE gel. After being electrophoresed in 10% polyacrylamide gel, transferred electrophoretically to nitrocellulose membrane, the membrane was blocked with nonfat milk buffer for 1 hour and then incubated with the primary antibodies stay overnight at 4°C. The primary antibodies were IgG specific for BAFF (Abcam, ab16081, 1:500), BAFF-R (Abcam, ab5965, 1:500), Actin (Abcam, ab8226, 1:3000). After three 5-minute washes (20-mmol/L Tris, pH 7.6, 8g sodium chloride, 0.05% Tween-20), blots were incubated for 50 minutes with horseradish peroxidase–conjugated goat anti–rabbit IgG or rabbit anti-rat IgG(1:5000). After washing, bound IgG was detected autoradio-graphically by enhanced chemiluminescence (Quantity One v.4.6.2).

Follow up

The primary outcome of this study was the achievement of complete or partial remission, the patients were divided into non-refractory and refractory groups according to the clinical follow-up indicators at 3, 6, and 12 months after treatment.

Patients who still had obvious myasthenia and high levels of serum CK on the basis of glucocorticoid therapy combined with other immunosuppressive agents or intravenous immunoglobulins (IVIg) in the study period were considered refractory. The therapeutic effect evaluation method adopted in this study is the modified Rankin Scale score (mRS). If patients' muscle strength returned to normal or was close to normal, the mRS were 0-2 scores, they were placed in the non-refractory group. If there was still obvious limb weakness after 12 months of treatment, the mRS were 3-5 points, or recurrence, the patients were placed into the refractory group [8, 13]. We monitored the clinical symptoms, the muscle fatty infiltration and edema changes by tMRI for 3, 6, 12, 18 and 24 months after treatment. Clinical follow-up indicators were mRS [8], imaging indicators were the average change rates of thigh muscle fatty infiltration and

edema (the scale gap of tMRI / interval time between before and after treatment) for 3, 6, 12, 18 and 24 months after treatment.

Statistical analysis

All analyses were performed using SPSS 17.0 software. Single factor and binary logistic regression analysis were used to compare the clinical, tMRI changes and pathological features between the two groups. The Mann-Whitney *U* or Kruskal-Wallis test was used for the continuous variables and Chi-square test for categorical variables. Multivariate logistic regression analysis was performed on related predictors for refractory disease. Explanatory variables were selected using a liberal criteria ($p<0.10$) for inclusion in the multivariate regression model. For all statistical analyses, significance was accepted as $p<0.05$.

Results

Clinical refractory related factors: Male, severe muscle weakness, concurrent ILD

In our cohort, the ratio of male to female was 14:34, including 6 teenagers and 42 adults. The mean onset age was 40.9±17.2 years old and the time to admission was six (4, 18) months. Patients with acute, subacute, and chronic disease were numbered 5, 24 and 19, respectively. According to the Medical Research Council (MRC) classification, there were 18 patients (37.5%) with severe weakness (MRC 1-2/5), 20 patients (41.7%) with moderate weakness (MRC 3/5), and 10 patients (20.8%) with mild weakness (MRC 4-5/5). The severe weakness appeared in the lower limbs of 24 patients (50.0%), in the upper limbs of 6 patients (12.5%), and in all limbs of 18 patients (37.5%). Additional clinical baseline data can be seen in supplementary materials.

The follow-up study was completed in 40 out of 48 enrolled patients. The mean follow-up duration was 3.85 ± 2.49 years (12 months to 9 years). After 12 months of treatment, 27 patients (67.5%) had normal or nearly normal muscle strength, mRS 0-2 (the non-refractory group), and 13 patients (32.5%) still had muscle weakness, mRS 3-5 (the refractory group). The mean onset age in the non-refractory group and refractory group were 37.26 ± 18.06 and 50.77±15.77 years old, respectively (P=0.029). The proportion of weight lost after the onset of disease was 40.7% in the non-refractory group and 76.9% in the refractory group (P=0.032). Furthermore, the percentage of interstitial lung disease was 22.2% in the non-refractory group and 69.2% in the refractory group (P=0.004) and the proportion of patients initially treated with methotrexate was 59.3% for the non-refractory group and 23.1% for refractory group (P=0.032). Binary logistic regression analysis verified the risk factors for refractory patients were being male (OR=19.57, 95%CI=1.49-256.53), severe muscle weakness (OR=7.51, 95%CI=41.03-54.88) and the presence of interstitial lung disease (OR=39.70, 95%CI=3.04-518.38) **(Table 1)**.

Imaging refractory related factors: Quick development of muscle fatty infiltration

Thirty-six patients underwent tMRI, which showed fatty infiltration in 29 patients (80.6%) and edema in 32 patients (88.9%). The fatty infiltration and edema mainly affected the gluteus maximus, adductor magnus, semimembranosus, semitendinosus and the long head of the biceps both in both the refractory and non-refractory groups (Table 2). The average fatty infiltration score in the adductor magnus was higher in adults compared with adolescents (P=0.028), while the average edema score in the adductor magnus (P=0.028) and the long head of the biceps (P=0.041) were higher in adolescent group. There was no statistically significant difference between the thigh MRI of patients with ANM-SRP and 36 ANM patients with negative anti-SRP antibody.

Thigh MRI follow-up showed an increasing rate of fatty infiltration at 3, 6, 12, 18 and 24 months was 3.44, 1.45, 0.68, 0.41 and 0.11, respectively (**Figure 1**). The reducing rate of edema at 3, 6, 12, 18 and 24 months was 2.37, 2.72, 1.07, 0.55, 0.57, respectively. The mean fatty infiltration rate of the thigh muscles at 3 months was higher in the refractory group compared with the non-refractory group (P=0.022). However, there was no statistically significant difference between the two groups in the increasing rate of fatty infiltration and the reducing rate of edema at the remaining periods.

Pathology refractory related factors：High expression of BAFF and BAFF-R in muscle

Muscle biopsies revealed a large variation in fiber size in all patients except two patients with terminal changes. Muscle fiber hypertrophy appeared in 10 patients (22.7%). Muscle fiber necrosis with myophagocytosis appeared in 40 patients (90.9%) and muscle fiber regeneration appeared in 43 patients (97.7%). $CD68^+$ macrophages appeared in necrotic fibers in the perimysium in 41 patients (93.2%). Ragged blue fibers in SDH staining were found in 3 patients (7.5%), and COX negative muscle fibers were found in 6 patients in cox staining (15%). Mild to moderate connective tissue proliferation appeared in 13 patients (29.5%). Perivascular lymphocyte infiltration was noted in the perimysium was observed in 9 patients (20.5%), while CD3, CD4 and CD8 lymphocytes appeared in 22 (50%), 26 (59.1%) and 23 (52.3%) patients, respectively. $CD20^+$ lymphocytes appeared in 4 patients (9.1%) and $CD19^+$ lymphocytes appeared in 21 patients (72.4%) of 29 tested patients. MAC deposition in muscle fibers was seen in 32 of 37 patients (86.5%). MHC-I positive myofibrils appeared in 37 of 40 patients (92.5%) (**Figure 2**).

Pathological indicators like muscle fiber necrosis, regeneration, atrophy, hypertrophy, connective tissue proliferation, infiltration of CD3, CD4, CD8 and CD20 positive lymphocytes and infiltration of CD68 positive macrophages, MAC deposition and MHC-I positive expression were not significantly different between the refractory and non-refractory groups.

BAFF staining revealed 10 of 29 patients (34.5%) with positive deposition in necrotic tissue, regenerated muscle fibers and individual lymphocytes (**Figure 3**). The positive expression of BAFF-R was found in 24 of 29 patients (82.8%), mainly expressed in necrotic muscle fibers, muscle perimysium, muscle underwear and by lymphocytes infiltrating around blood vessels (**Figure 4**). The expression level of CD19 positive lymphocytes overlapped with BAFF-R (**Figure 5**). Spearman correlation tests showed a correlation between BAFF-R and CD19 ($R = 0.818$, $P=0$).

The positive cellular expression of BAFF-R in muscles were 0.27 ± 0.14 for the non-refractory group and 0.42 ± 0.23 for the refractory group (P=0.036). The positive cellular expression of CD19 in skeletal muscle was 0.18 ± 0.08 for the non-refractory group and 0.36 ± 0.21 for the refractory group (P=0.002). There was no statistically significant difference in the expression of BAFF. The western bolt of BAFF and BAFF-R in skeletal muscle of patients and healthy control also showed that the expression of BAFF-R in skeletal muscle of the patients was significantly higher than that of the healthy control. BAFF were expressed in skeletal muscle of both patients and healthy control (**Figure 6**).

Discussion

This study found that male patients with ANM-SRP have a worse prognosis, our previous study followed up three early onset patients with good prognosis, all of whom were female [14], while most studies had not found correlation between gender and refractory [8,13]. We also found that severe muscle weakness before the initiation of therapy indicated poor response which has also been reported by Aggarwal in a cohort of 25 patients with ANM-SRP [15]. More than 90% of the patients in Brazil showed mild and moderate muscle weakness, and the refractory rate was 42.9% [16]. Whether there is a racial difference between the degree of limb weakness and the therapeutic effect needs further analysis. In the refractory group, the proportion of patients with ILDs was high (34.8%), and its incidence is close to that of Brazilian patients (36%) [16], which is higher than 25% reported in Japan, Europe and America [8, 17, 18]. The combination of ILDs is an important factor affecting the quality of life and prognosis of patients [2, 19, 20], while the use of mycophenolate or tacrolimus may improve the therapeutic effect in patients with the combination of ILDs [21, 22].

Our study added skeletal muscle MRI to monitor disease activity and severity and was used for long-term follow-up of patients [23]. The fatty infiltration exacerbation of skeletal muscle MRI in the early treatment period often suggests refractory disease. Similar conclusions can be seen in other MRI studies of skeletal muscle in idiopathic inflammatory myopathy [24]. Previous study showed muscle fatty infiltration may be closely related to the prognosis of ANM-SRP and is often a marker of glucocorticoid resistance or refractory treatment [12]. Therefore, it is recommended that patients with ANM-SRP should have skeletal muscle MRIs within 3 to 6 months after the initiation treatment to monitor the efficacy of immunotherapy and the progression of the disease. For patients with early fatty infiltration changes, immunotherapy should be strengthened and more frequently follow-up should be conducted. Whether preventing fatty infiltration of skeletal muscle will reduce the poor response to treatment remains for further studies.

In this study, we observed that the expression of BAFF and BAFF-R were upregulated in the muscle of patients with ANM-SRP. Previous studies have detected the upregulation of BAFF in the serum of dermatomyositis, polymyositis and anti-synthase antibody syndrome patients [25, 26], and its level correlated to the activity of IIM [27]. Patients with ANM-SRP had high expression of BAFF-R and CD19, and the refractory patients had significantly higher expression of BAFF-R and CD19 than non-refractory patients, suggesting that BAFF and its receptors may cause muscle fiber injury and lymphocyte

proliferation. The expression of CD19 and BAFF-R in skeletal muscle might be effective pathological indicators to predict the treatment effects in patients. A number of small sample studies suggest that removal of B cells is effective for treating refractory ANM-SRP [9, 10], while this study also provided pathological evidence for the use of B lymphocyte clearance drugs, and suggested that BAFF inhibitors [28] may be effective in the treatment of patients with ANM-SRP.

Conclusion

The expression of BAFF and BAFF-R in skeletal muscle of ANM-SRP were upregulated, and the expression level of BAFF-R overlapped with CD19 positive lymphocytes. Male, severe muscle weakness, concurrent interstitial lung disease, quick development of muscle fatty infiltration also indicate a poor response to immunosuppressive therapy.

Abbreviations

Abbreviations	Full names
ANM-SRP	autoimmune necrotizing myopathy with anti-signal recognition particle antibodies
BAFF	B cell activating factor
BAFF-R	B cell activating factor receptor
ILD	concurrent interstitial lung disease
thigh MRI	tMRI
HE	hematoxylin and eosin
ATP	adenosinetriphosphate
SDH	succinate dehydrogenase
COX	cytochrome C oxidase
MHC-I	major histocompatibility complex class -I
MAC	membrane attack complex
IVIg	intravenous immunoglobulins
mRS	the modified Rankin Scale score
MRC	Medical Research Council

Declarations

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Author's contributions

All authors were involved in drafting the article and in revising it critically for intellectual content. Yun Yuan conceived and designed the study. Yawen Zhao analyzed the data, wrote the first version of the manuscript. Yawen Zhao and Yilin Liu evaluated the muscle MRI images. Wei Zhang, Zhaoxia Wang and Yun Yuan revised the manuscript.

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Availability of data and materials

The patients data used in this study are included in this publish article and its supplementary files.

Ethics approval and consent to participate

The study was performed in accordance with the ethical standards of the responsible committees on human experimentation and approved by the Institutional Review Board (no. 2011[419]). Informed consent for all examinations was obtained from the patients or their guardians. The protocol in the study strictly followed the rules of the Declaration of Helsinki.

Consent for publication

Applicable.

Competing interests

The authors declare no conflicts of interest.

Authors' information

Department of Neurology, Peking University First Hospital, No.8 Xishiku Street, West District, Beijing 100034, China.

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Tables

Table 1. Binary logistic analysis of patients with refractory versus responsive disease

Indicator	OR	95%CI	P
Gender（male）	19.57	1.49-256.53	0.024
Severe muscle weakness	7.51	41.03-54.88	0.047
With ILD	39.70	3.04-518.38	0.005

: interstitial lung disease

le 2. The different of muscle imaging between patients with refractory and responsive disease

		GM	VL	VM	RF	Sa	Gr	AL	AM	BF	ST	SM	
FI	Sum	1.92	1.08	0.81	0.81	0.72	1.14	0.94	0.83	1.92	1.86	1.75	1.89
	Re	2	1.44	1	1.22	1.1	1.56	1.33	1.44	2.44	2.55	2.44	2.44
	N-Re	1.6	0.8	0.5	0.45	0.45	0.75	0.6	0.4	1.5	1.45	1.3	1.55
Edema	Sum	2.67	2.14	1.33	1.58	2.03	1.28	1.28	0.94	2.5	2.67	1.67	2.19
	Re	2.56	2.11	1.44	1.44	2.22	1.11	1.33	1	3	2.89	1.67	2.44
	N-Re	2.6	2.05	1.1	1.45	1.75	1.15	1.05	0.8	2.2	2.2	1.6	2

Re：Refractory group；N-Re：Non-refractory group; AL: adductor longus; AM: adductor magnus; BF: biceps femoris long head; GM: gluteus maximus; Gr: gracilis; RF: rectus femoris; Sa: sartorius; SM: semimembranosus; ST: semitendinosus; VI: vastus intermedius; VL: vastus lateralis; VM: vastus medialis

Figures

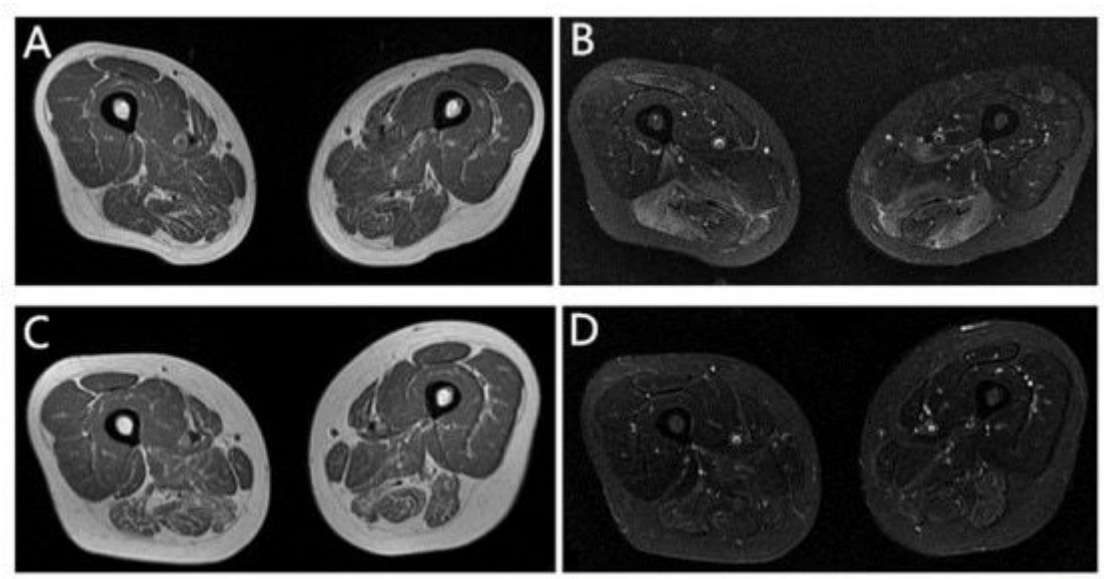

Figure 1

Thigh MRI follow-up showed an increasing rate of fatty infiltration at 3, 6, 12, 18 and 24 months was 3.44, 1.45, 0.68, 0.41 and 0.11, respectively.

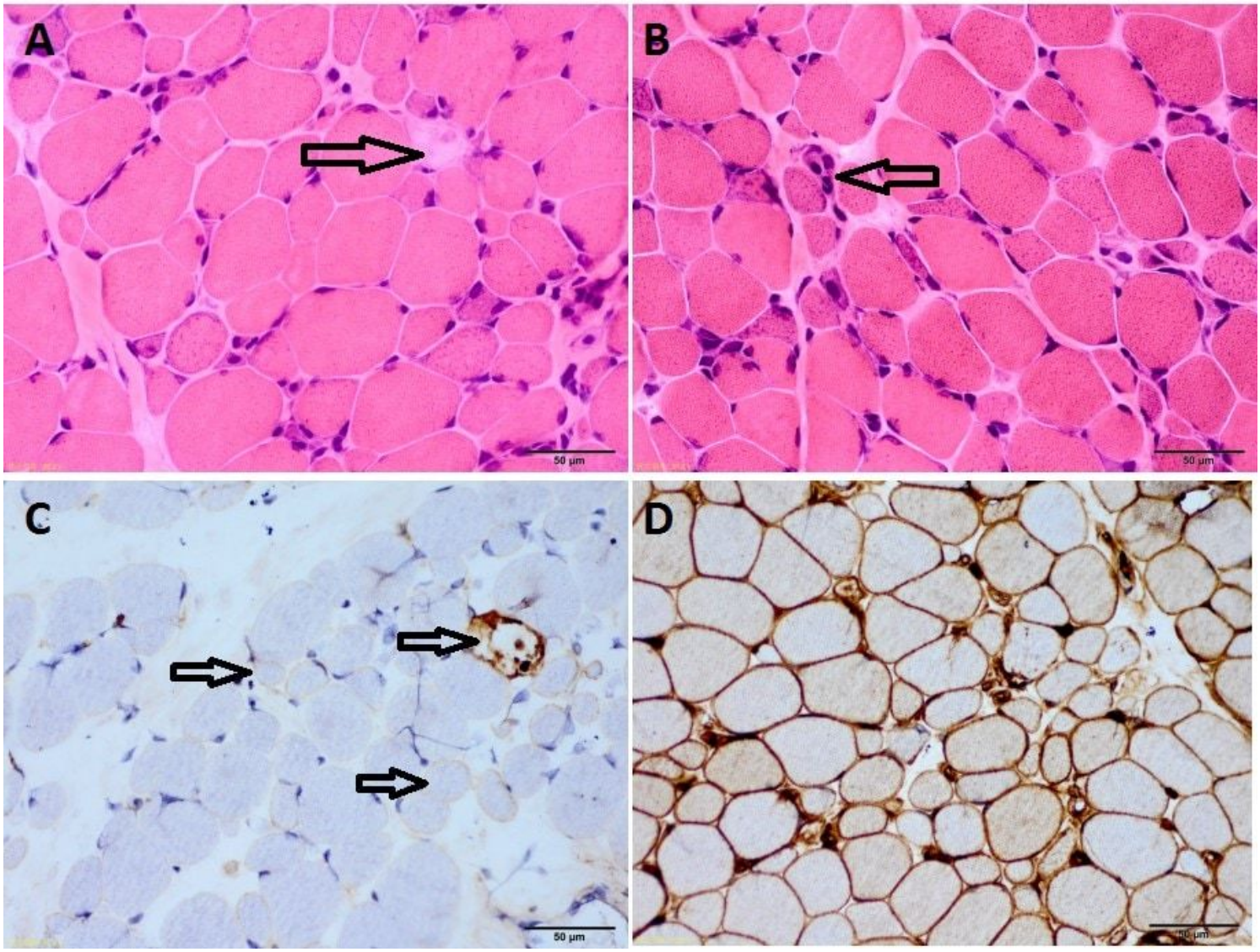

Figure 2

MAC deposition in muscle fibers was seen in 32 of 37 patients (86.5%). MHC-I positive myofibrils appeared in 37 of 40 patients (92.5%).

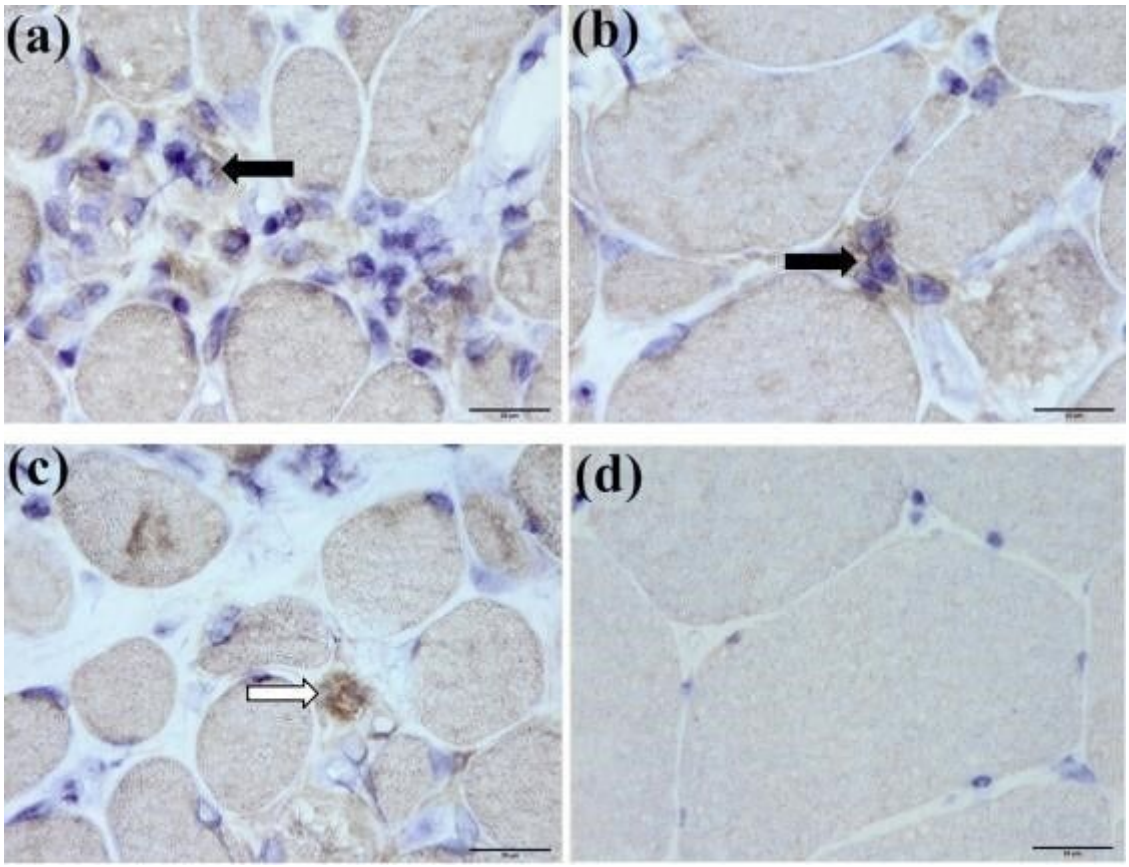

Figure 3

BAFF staining revealed 10 of 29 patients (34.5%) with positive deposition in necrotic tissue, regenerated muscle fibers and individual lymphocytes.

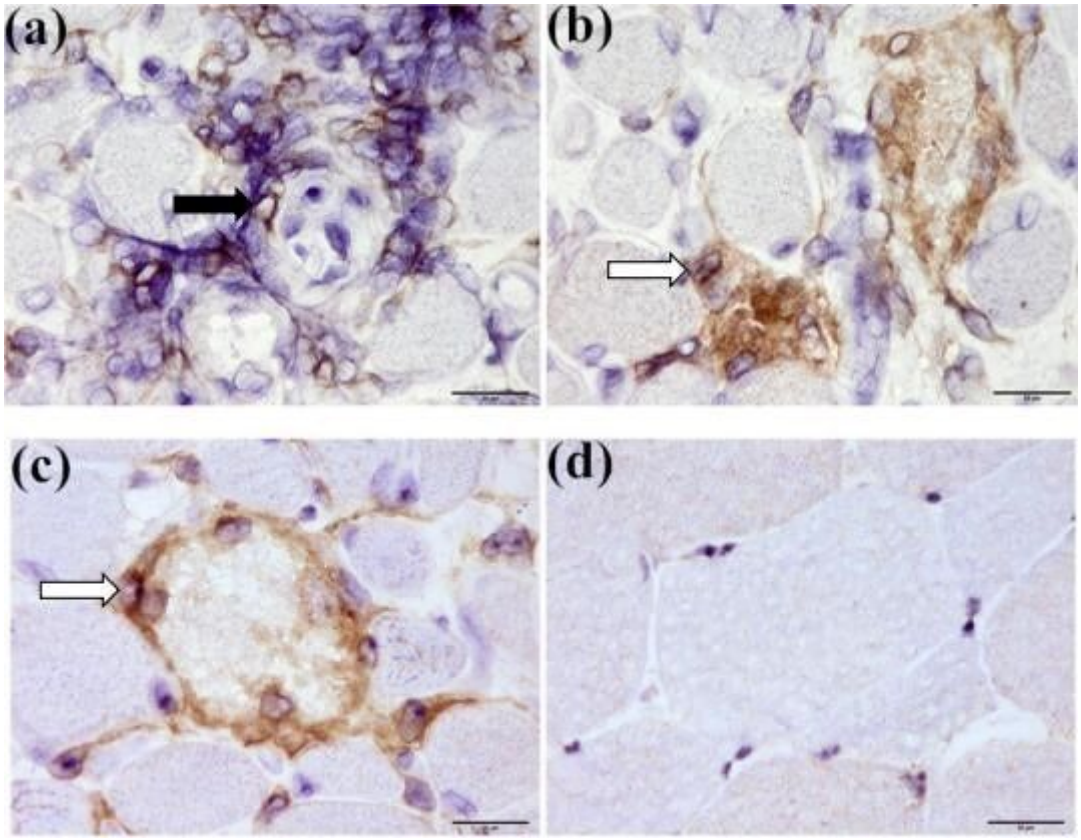

Figure 4

The positive expression of BAFF-R was found in 24 of 29 patients (82.8%), mainly expressed in necrotic muscle fibers, muscle perimysium, muscle underwear and by lymphocytes infiltrating around blood vessels.

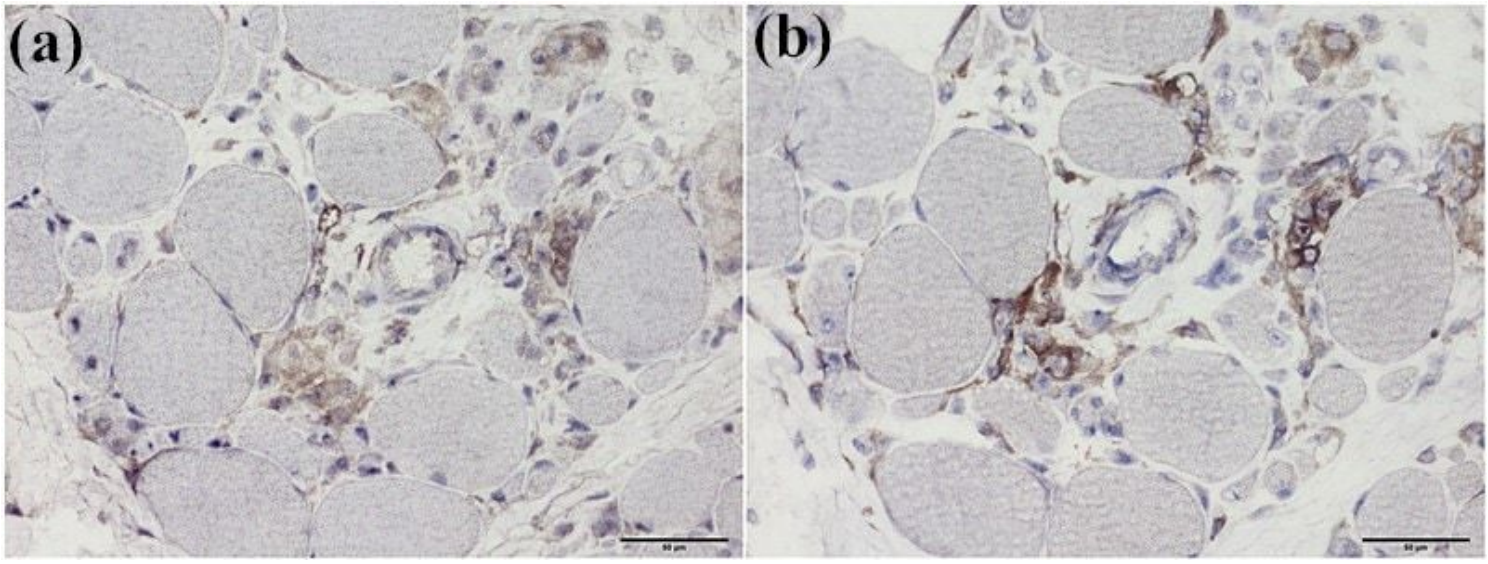

Figure 5

The expression level of CD19 positive lymphocytes overlapped with BAFF-R.

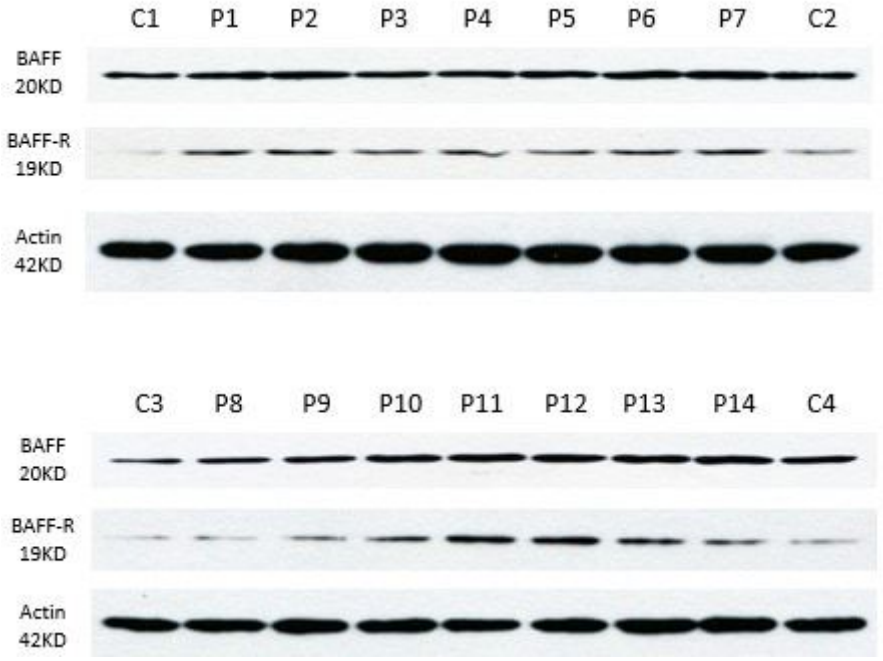

Figure 6

The western bolt of BAFF and BAFF-R in skeletal muscle of patients and healthy control also showed that the expression of BAFF-R in skeletal muscle of the patients was significantly higher than that of the healthy control. BAFF were expressed in skeletal muscle of both patients and healthy control.