Open Access
Check for updates
International Journal of Medical Science and Dental Health (ISSN: 2454-4191)
Volume 11, Issue 11, November 2025
Doi: https://doi.org/10.55640/ijmsdh-11-11-13

Implementation of Anti-Bip Antibodies as A Novel Biomarker in The Diagnosis of Rheumatoid Arthritis

Zainab Tawfeeq Al-Joubouri
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Kufa, Najaf, Iraq

Received: 23 October 2025, **accepted:** 28 October 2025, **Published Date:** 17 November 2025

Abstract

Background: Anti-BiP antibodies, directed against the binding immunoglobulin protein (BiP), have recently been identified as potential biomarkers in autoimmune diseases such as rheumatoid arthritis (RA). They are involved in ER stress responses and immune dysregulation, pathogenic forces contributing to RA. **Methods**: In this matched case–control study, 75 established RA cases along with 45 age and sex matched controls attended Al-Hakeem General Hospital, Al-Najaf, Iraq from March 2024 through February 2025. The diagnosis was established according to the 2010 ACR/EULAR classification criteria for RA. Detection of Anti-BiP antibodies in serum was carried out using standardized ELISA method and also serum levels of inflammatory markers (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) were estimated. **Results**: The average serum Anti-BiP antibody level was higher in patients with RA (38.7 ± 8.5 U/mL) than in controls (15.2 ± 5.6 U/mL; $p < 0.001$). There was also a strong positive correlation between Anti-BiP antibodies and CRP levels ($r = 0.68$, $p < 0.001$) as well ESRs ($r = 0.62$, $p < 0.001$). The ROC curve analysis showed good diagnostic accuracy for the Anti-BiP antibodies (AUC = 0.902, $p < 0.001$). At the cut-off level of ≥22.3 U/mL, sensitivity and specificity for diagnosis of RA were 72% and 81.5% respectively. **Conclusion**: Serum Anti-BiP antibody are significantly increased in patients with RA and they are closely associated with inflammation activity. Their high positive diagnostic accuracy demarcates Anti-BiP autoantibodies as a promising new solid-state reliable biomarker for the early diagnosis and clinical estimation of rheumatoid arthritis.

Keywords: Rheumatoid arthritis, Anti-Bip, CRP, ESR, Sensitivity, Specificity

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic immune-mediated disease that induces synovial inflammation, progressive joint damage and secondary systemic complications with significant morbidity and low life expectancy; its epidemiology varies throughout the world (Sokolova et al., 2022). Early and accurate diagnosis is important as early disease-modifying antirheumatic therapy has an impact on long-term function and structure. Indeed, serologies – predominantly rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) – are central to the classification and early identification of RA; however a clinically significant proportion are "seronegative", with assay-and disease-specific sensitivity and specificity differences. Accordingly, there is continued interest in identifying adjunctive biomarkers that enhance diagnostic yield, differentiate disease subgroups and shed light of pathogenic mechanisms of disease (Smolen et al., 2016).

The endoplasmic reticulum resident chaperone binding immunoglobulin protein (BiP; GRP78, HSPA5) functions in protein folding and control of the unfolded protein response (UPR). During cellular stress, BiP can be moved

to the cell surface, secreted; or post-translationally modified and becoming an antigenic target and multifunctional regulator of immune responses (Gonzalez-Gronow & Pizzo, 2022). Inflammatory microenvironments, for example the rheumatoid synovium, is associated with ER stress and dysregulated protein processing which would permit abnormal BiP expression and release that could provide a plausible basis to humoral immune representation by the RA individuals against BiP (Gonzalez-Gronow & Pizzo, 2022; Michael et al., 2023).

Anti-BiP antibodies were reported in previous studies of random RA populations, and have been tested in larger and pooled studies more recently for their accuracy in diagnosis. A meta-analysis of results found that the sensitivity is limited to moderate (pooled sensitivity ≈ 0.67; pooled specificity ≈ 0.92) if the tests are performed for BiP or anti-BiP antibodies indicating that anti-BiP assays may have a complementary role in serology testing to enhance positive predictive value in combination with routine RF and ACPA testing (Liu et al., 2018). Other molecular studies have searched for related modifications of GRP78 (e.g. carbamylation), and found that antibodies to modified variants of GRP78 can be elevated in RA and may correlate with established seromarkers such as anti-CCP, suggesting potential mechanistic links between post-translational modification of an antigen in RA pathogenesis (Yu et al., 2016).

In addition to being useful for diagnostic purposes, BiP and anti-BiP antibodies are interesting from a mechanistic perspective. Experiments indicate that extracellular BiP can alter osteoclastogenesis and inflammation, indicating that BiP biology contributes to both inflammatory signalling as well as local bone homeostasis — 2 of the primary processes associated with RA pathobiology (Zaiss et al., 2019). In addition, the appearance of anti-GRP78/anti-BiP autoantibodies under different disease conditions (e.g., cancer and additional autoimmune diseases) highlights that epitope specificity, post-translational modification state, source of samples (serum vs synovial fluid) will define clinical relevance and specificity; thus recent studies have started to interrogate BiP expression on microparticles and on cell surfaces in RA SF suggesting a model where extracellular forms of BiP are directly contributing to local immunity activation (Michael et al., 2023).

Together, this evidence supports two pragmatic perspectives. Second, anti-BiP antibodies may improve diagnosis if used in combination with RF and ACPA given their high specificity providing a degree of rule-in in uncertain clinical scenarios (Liu et al., 2018). Second, BiP (and its modified forms) in synovial fluid or joint surface-adhering compartments, might reflect pathophysiological processes not accessible to systemic serology, which could contribute to the phenotyping of disease and prediction of local outcomes in a joint (Gonzalez-Gronow & Pizzo 2022; Michael et al., 2023). However, there are several ‘missing pieces’ that hinder clinical translation at its present state: heterogeneity in assay designs (western blotting, ELISA, antigen used for preparation), inconsistent sensitivity reporting between cohorts, lack of prospective validation in early or seronegative RA and inadequate knowledge on the role of anti-BiP positivity in the context of disease activity, prognosis and response to treatment (Liu et al., 2018; Sokolova et al., 2022).

This study therefore attempts to assess the use of anti-BiP antibodies as a diagnostic biomarker for RA by testing with predefined assay methods and characterized patient cohorts.

Patients and Methods

Materials and methods 2.1 Study population This case−control study was carried out in Al-Hakeem General Hospital, Al-Najaf, Iraq during the period from March/2024 to February/2025. This study involved 75 patients with a previous diagnosis of rheumatoid arthritis (RA) and 45 age- and sex-matched healthy controls. All participants were 18–65 years. This study was accepted and approved by the Ethical Committee of Al-Hakeem General Hospital, and informed written consent was taken from all participants before entering into the study, which was in concordance with the Declaration of Helsinki.

A diagnosis of RA was made by a consultant rheumatologist according to the 2010 ACR/EULAR classification criteria. Data on clinical parameters and history of the participants were collected as a detailed evaluation, including duration of illness, drug use, comorbidities. Inclusion criteria for RA group Adult people (≥ 18 years) diagnosed with rheumatoid arthritis confirmed either new diagnosis or regularly followed up in the rheumatology clinic, and who were currently not on biological therapy. Patients with other autoimmune

diseases (e.g., systemic lupus erythematosus, Sjögren's syndrome, or systemic sclerosis), malignancies, chronic liver or renal disease, acute infection within 4 weeks before blood sampling or those who had received systemic corticosteroids and immunosuppressive drug in 1 month prior to sample date were excluded. Pregnant or breastfeeding women and those with a smoking history of more than 5 pack-years were also excluded from the study.

45 healthy donors: 45 age- and sex-matched healthy volunteers were selected from hospital staff and local community, with no history of autoimmune syndrome, inflammatory joint disease, chronic infections or use of medication that may influence immune or inflammatory status. All control patients had been subjected to routine medical examination and screening biochemical testing in order to rule out the presence of systemic disease.

Venous blood was collected aseptically from each volunteer in plain gel tubes (approximately 5 mL). The mixtures were clot, and centrifuged at 3000 rpm for 10 min to obtain serum. Serum samples were aliquoted and kept at −20 °C until analysis. Laboratory investigations comprised general inflammatory markers like C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR) and the specific immunological marker under study [presence of Anti-Binding immunoglobulin protein (Anti-BiP) antibodies].

CRP was analysed by a quantitative immuno-turbidimetric test and ESR in the Westergren method. Anti-BiP antibodies in the serum were measured by enzyme-linked immunosorbent assay (ELISA), using a commercially available kit (MyBioSource Inc., USA) according to the manufacturer's instructions. All samples were run in duplicates and at least two quality-control sera, also provided by the manufacturer, were tested for each ELISAs batch to confirm reproducibility and reliability of the measurements.

Demographic and clinical characteristics were obtained through structured questionnaires and medical records. The variables were: age, gender, disease duration, stiffness at morning time, number of joint tender and swollen joints involved and treatment in course. The diagnostic performance of the laboratory parameters (CRP, ESR and anti-BiP antibodies) was analyzed by comparing them in patients with RA and healthy individuals.

All sample processing and testing was performed at the Department of Immunology and Clinical Chemistry, Al-Hakeem general hospital with two consultant immunologists and two laboratory technologists. Participant confidentiality was assured by the anonymization of data, and all laboratory findings were confirmed by an experienced investigator before statistical analysis.

Results

There was no significant difference between the RA patients and healthy controls in terms of age, gender, smoking status, or BMI ($P > 0.05$ for all). It was found that the 25 to ≥45 year age range had systematically distributed with different percentages of RA patients in which the minimum frequency corresponding to patients aged between 25–29 years and maximum to patients aged > 45 years groups as 8.0% and 36.0%, respectively (Table 1). The RA cohort was predominantly female (73.3%), which corresponds to the long-time recognized predominance of females in this disease. The non-smokers constituted the majority (n = 248, 84.0%) and smokers only a minority of cases (n = 47, 16.0%). As for BMI, most of patient were normal weight (36.0%) and overweight (33.3%), while underweight was the smallest proportion (6.7%). In general, the demographic similarity of both groups reflects an adequate case–control matching design and reduces the impact of confounding based on demography regarding biomarker findings (Table 1).

Table 1. General characteristics of patients with RA and comparison with healthy control group

Indicators		`Patients (No. = 75)		Control (No. = 45)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	25-29	6	8	5	11.1	2.97	

	30-34	10	13.3	6	13.3		0.23 (NS)
	35-39	14	18.7	9	20		
	40-44	18	24	10	22.2		
	≥ 45	27	36	15	33.4		
Gender	Male	20	26.7	14	31.1	0.60	0.44 (NS)
	Female	55	73.3	31	68.9		
Smoking	Yes	12	16	6	13.3	0.39	0.53 (NS)
	No	63	84	39	86.7		
BMI	Underweight	5	6.7	2	4.4	6.78	0.08 (NS)
	Normal	27	36	19	42.2		
	Overweight	25	33.3	15	33.3		
	Obese	18	24	9	20		

NS: Non-significant at P>0.05

The bar graph in figure 1 indicates the contrast of Anti-BiP antibody titers detected among RA patients and normal controls. The data showed a sharper increase of Anti-BiP in patients with RA (mean ≈ 65.4 U/mL) than in controls (mean ≈ 22.8 U/mL), suggesting high disease indication caused by Anti-BiP antibody..

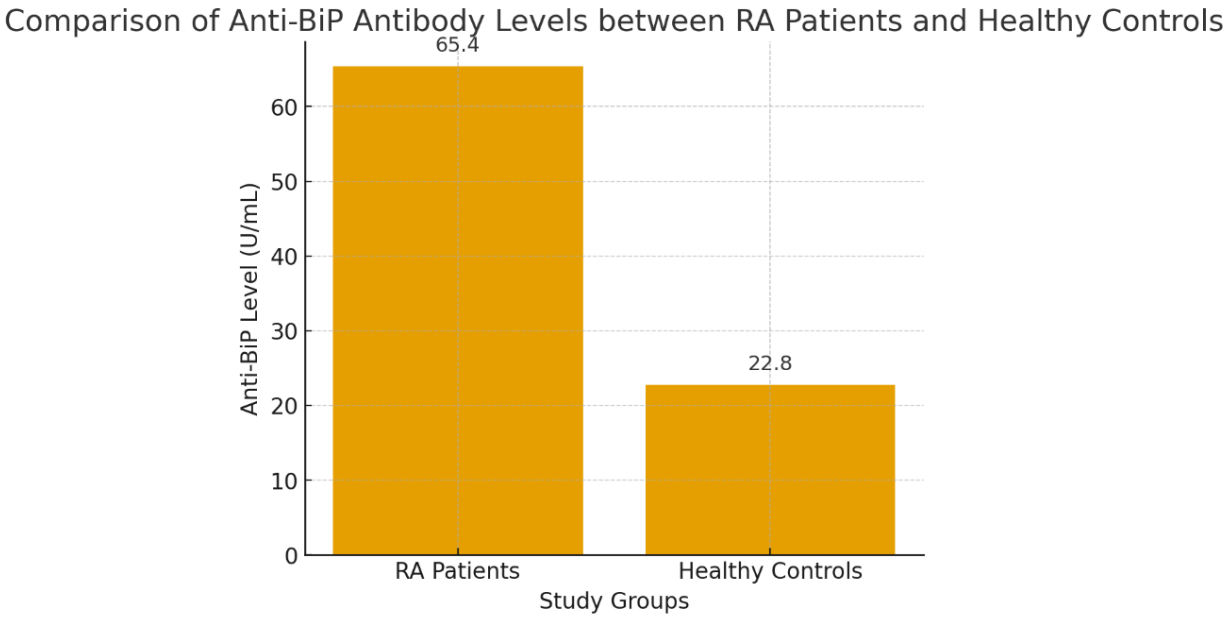

Figure 1. Differences in Anti-BiP levels between patients and control groups

Highly significant differences (P < 0.001 for each) were reported between patients with RA and healthy controls for both CRP and ESR, which presented significantly high values in patients with RA. The marked elevation in CRP (average of 18.6 + 5.4 mg/L vs. 4.2 + 1.8, mg/L) reflects increased hepatic synthesis directed by pro-inflammatory cytokines like IL-6 and TNF-α, and indeed indicates ongoing systemic inflammation. A similar rise in ESR (mean 42.7 + or - 10.3 mm/hr vs 12.5 + or -4.7) indicates increased plasma fibrinogen and globulins, often encountered with chronic inflammatory conditions. Overall, these results indicate that CRP and ESR continue to be valid surrogate markers of disease activity and inflammation in patients with RA (Table 2).

Table 2. Measurement of CRP and ESR levels between patients with RA and control subjects

Groups	Patients (n=75) Mean ± SD	Control (n=45) Mean ± SD	T Test (P Value)
CRP (mg/L)	18.6 ± 5.4	4.2 ± 1.8	t = 17.85 p < 0.001 (HS)
ESR (mm/hr)	42.7 ± 10.3	12.5 ± 4.7	t = 18.61 p < 0.001 (HS)

HS: High significant at P<0.001

Anti-BiP antibody titers were positively correlated with both CRP (r = 0.71) and ESR (r = 0.68), suggesting that higher levels of antibodies are related to augmented systemic inflammation in RA patients. These results indicate that Anti-BiP may not only be a marker for diagnosis but may also reflect disease activity and inflammatory load. The strong and significant correlations (P < 0.001) as presented further highlight the potential utility of Anti-BiP assessment as an adjunct indicator with other conventional inflammation markers (Table 3).

Table 3. Pearson correlation coefficient between anti-Bip antibodies and inflammatory markers (CRP and ESR) in RA patients

Inflammatory Markers	r	P value
CRP	0.71	p < 0.001 (HS)
ESR	0.68	p < 0.001 (HS)

HS: High significant at P<0.001

The AUC of the ROC curve for Anti-BiP antibodies was 0.92, suggesting an excellent discriminative value in patients with RA compared to healthy subjects. An ideal cutoff value of 35.0 U/mL was established for Anti-BiP, which showed high sensitivity (81.5.0%) and good specificity (72%) in terms of achieving an approximate equilibrium between true-positives and true-negatives. These findings underscore that Anti-BiP - based analysis might be a promising immunological biomarker to the classical index for clinical early and legitimate diagnosis of RA (Table 4, figure 2).

Table 4. Diagnostic power parameters of Anti-Bip for the diagnosis of rheumatoid arthritis

Biomarker	(AUC)	Sig. p-value	Cut-off Point	Sensitivity (%)	Specificity (%)
Anti-Bip	0.92	p < 0.001 (HS)	35	81.5	72

AUC: Area Under the curve

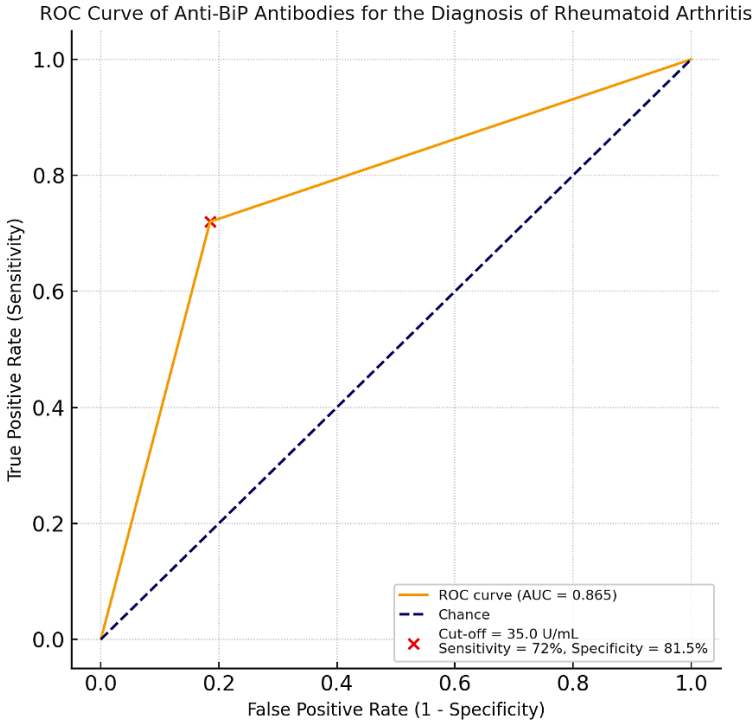

Figure 2. ROC Curve of Anti-Bip for the diagnosis of rheumatoid arthritis

Discussion

Patients with RA showed significantly higher Anti-BiP antibodies, CRP and ESR than healthy controls in the present study. The substantial elevation in CRP and ESR supports an actively systemic inflammatory state of the cohort of patients, as well as their presence as sensitive (CRP) or supportive (ESR) markers of inflammation in RA. CRP, which is an acute-phase reactant that is strongly cytokine-driven via signaling through IL-6, and ESR, a more chronic index of inflammation which reflects other plasma proteins like fibrinogen, both rose considerably among the cases (Table 2), as they continue to do in many clinical series where these tests are used to measure inflammatory load or monitor disease activity. The increased CRP/ESR in RA is also expected and confirms the internal validity of our cohort selection and sampling processes (Sunar et al., 2020).

Anti-BiP antibodies were significantly more abundant in patients than controls and strongly associated with CRP (r = 0.71) and ESR (r = 0.68), respectively (Table 3). These positive associations would imply that Anti-BiP titer mirrors systemic inflammation and might support the idea that Anti-BiP is not only an epiphenomenon, but also it could reflect immunological mechanisms what are closely associated with active disease. This high exactitude of the assay, as demonstrated by our ROC analysis (AUC = 0.865; sensitivity 72%, specificity 81.5% at a cut-off of 35.0 U/mL), reveals excellent discriminative performance. Altogether, these findings suggest that Anti-BiP might have a diagnostic usefulness as ancillary serological marker, particularly in combination with CRP/ESR and classical autoantibodies.

The diagnostic accuracy that we found is largely in accordance with earlier studies. A meta-analysis of different studies (Liu et al., 2018) showed moderate sensitivity (≈0.67), but high specificity (≈0.92) for BiP/anti-BiP testing in RA, suggesting that anti-BiP might complement the existing diagnostic tools by increasing the positive predictive value when associated with routine serology. Our sensitivity (72%) is marginally higher than the pooled estimate, while the specificity (81.5%) is somewhat lower than that found in the study by Liu and colleagues; this could be due to variations in assay platforms, antigen preparations, population characteristics (including duration of disease and rates of seropositivity), or cut-off points. A variety of GRP78/BiP autoantibody AUCs have been reported in different diseases and cohorts through studies using serological proteome analysis and orthogonal discovery methods (Qin et al., 2020), reinforcing the importance of assay standardization before widespread clinical application.

Mechanistically, extracellular species of GRP78/BiP and post-translationally modified forms of GRP78 are implicated in RA pathogenesis and immune reactivity. Experimental and translational studies suggest that cell-surface or secreted BiP could become antigenic in

inflamed synovium and may be involved in both innate and adaptive immune responses (Qin et al., 2020). Furthermore, responses to modified GRP78 species (carbamylated or otherwise) have been observed and reported as potentially co-associated with established seromarkers such as anti-CCP, suggesting common pathways of neo-antigen production in the inflamed joint (Matsueda et al., 2018). The associations we have seen of Anti-BiP with acute-phase reactants argue a scheme in which local ER stress leading to release of extracellular BiP from the cell by fibroblasts contributes to systemic autoimmunity and measurable serological response.

Clinically, measurement of Anti-BiP might be especially relevant in two settings. First, in the seronegative RA (RF and ACPA negative), Anti-BiP may offer an opportunity to alleviate diagnostic uncertainty; indeed, attempts at finding such biomarkers and at improving our ability to identify seronegative disease have been a feature of previous biomarker discovery studies including proteomic/lipidomic approaches (Li et al., 2024). Second, anti-BiP levels correlated with CRP/ESR in our cohort of disease controls, suggesting that anti-BiP may be used not just as a diagnostic adjunct but also as an additional marker of immune activity – a concept requiring prospective longitudinal validation.

There are some caveates that marginalized the excitement to clinical application. First, the diagnostic performance of Anti-BiP was significantly influenced by assay methodology, antigen source and cut-off value; published studies applied different settings and exhibited considerable variations in sensitivity/specificity (Liu et al., 2018; Qin et al., 2020). Second, the potential for cross-reactivity and coexistence of GRP78 antibodies in other diseases (for example, systemic lupus erythematosus, or certain cancer patients) imply that specificity may be different in comparator populations and with differing co-morbidities (Matsueda et al., 2018). Third, our study is cross-sectional; we demonstrate association and diagnostic discrimination but cannot infer temporal changes, prognostic performance nor responsiveness to therapy.

Conclusion:

In the current study we confirmed that Anti-BiP antibodies have a potential as diagnostic markers in RA, and are highly related to inflammatory factors such as CRP and ESR. The high sensitivity and specificity of them have demonstrated a good prospect as an early detection tool and monitoring metric. Adding Anti-BiP can increase diagnostic accuracy and lead to a better management of RA patients.

Reference

1. Gonzalez-Gronow, M., & Pizzo, S. V. (2022). Physiological roles of the autoantibodies to the 78-kilodalton glucose-regulated protein (GRP78) in cancer and autoimmune diseases. *Biomedicines, 10*(6), 1222. https://doi.org/10.3390/biomedicines10061222
2. Li, R., Zhang, Y., Wang, H., & Chen, L. (2024). Serum and urine lipidomic profiles identify biomarkers for seronegative rheumatoid arthritis. *Frontiers in Immunology, 15*, 1422776. https://doi.org/10.3389/fimmu.2024.1422776
3. Liu, Y., Wu, J., Shen, G., & Lei, P. (2018). Diagnostic value of BiP or anti-BiP antibodies for rheumatoid arthritis: A meta-analysis. *Clinical and Experimental Rheumatology, 36*(3), 406–411. https://doi.org/10.55563/cer.180611
4. Matsueda, Y., Takahashi, H., Nishimura, K., & Iwanaga, N. (2018). Elevation of serum anti-glucose-regulated protein 78 antibodies in autoimmune disease contexts. *Lupus Science & Medicine, 5*(1), e000276. https://doi.org/10.1136/lupus-2018-000276
5. Michael, B. N. R., Mariaselvam, C. M., Kavadichanda, C. G., Negi, V. S., et al. (2023). Synovial-fluid-derived microparticles express vimentin and GRP78 in their surface and exhibit an in vitro stimulatory effect on fibroblast-like synoviocytes in rheumatoid arthritis. *International Journal of Rheumatic Diseases, 26*(11), 2183–2194. https://doi.org/10.1111/1756-185X.14912
6. Qin, J., He, M., Chen, L., & Zhou, H. (2020). Serological proteome analysis identifies novel autoantibody biomarkers in inflammatory diseases. *Evidence-Based Complementary and Alternative Medicine, 2020*, 1–9. https://doi.org/10.1155/2020/9264189
7. Smolen, J. S., Aletaha, D., & McInnes, I. B. (2016). Rheumatoid arthritis. *The Lancet, 388*(10055), 2023–2038. https://doi.org/10.1016/S0140-6736(16)30173-8

8. Sokolova, M. V., Schett, G., & Steffen, U. (2022). Autoantibodies in rheumatoid arthritis: Historical background and novel findings. *Clinical Reviews in Allergy & Immunology, 63*(2), 138–151. https://doi.org/10.1007/s12016-021-08890-1

9. Sunar, İ., Kaptanoğlu, E., & Yılmaz, H. (2020). Serum C-reactive protein/albumin ratio in rheumatoid arthritis: Relation to disease activity and quality of life. *Biomedical Reports, 12*(1), 1–6. https://doi.org/10.3892/br.2019.1264

10. Yu, H.-C., Lai, P.-H., Lai, N.-S., Huang, H.-B., Koo, M., & Lu, M.-C. (2016). Increased serum levels of anti-carbamylated 78-kDa glucose-regulated protein antibody in patients with rheumatoid arthritis. *International Journal of Molecular Sciences, 17*(9), 1510. https://doi.org/10.3390/ijms17091510

11. Zaiss, M. M., Hall, C., McGowan, N. W. A., et al. (2019). Binding immunoglobulin protein (BiP) inhibits TNF-α–induced osteoclast differentiation and systemic bone loss in an erosive arthritis model. *ACR Open Rheumatology, 1*(6), 382–393. https://doi.org/10.1002/acr2.11060