Immunogenicity of COVID-19 Vaccines in Patients Under Immunosuppressive Therapy: Insights Into Antibody Response and Gender Differences

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Received: 12 May 2025; Accepted: 14 Apr. 2026

Abstract- The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has impacted millions worldwide. Patients with autoimmune rheumatic diseases (ARDs) may exhibit attenuated responses to vaccination secondary to immunosuppressive therapy, potentially resulting in reduced vaccine effectiveness. This study investigates the immunogenicity of COVID-19 vaccines in patients with ARDs and evaluates antibody responses and the factors associated with such responses. We included 91 patients with rheumatologic diseases receiving immunosuppressive therapy and attending rheumatology clinics in Bandar Abbas, Iran. All had received two or three doses of a COVID-19 vaccine 6 months prior to enrollment. Antibody levels were measured using plaque reduction neutralization tests. We also collected information on demographics, clinical status, and current medication use. All statistical analyses were performed using SPSS version 21. Females constituted 89% of the participants. The majority had received the Sinopharm vaccine. Gender differences were observed, with males showing higher neutralizing antibody levels. No significant associations were detected between antibody levels and age, vaccine type, or medication use, except for adalimumab and etanercept, which showed a positive association with neutralizing antibodies. While most ARD patients demonstrated a detectable response, gender appeared to influence antibody levels, with females exhibiting a weaker response. Vaccine type did not influence antibody levels, and medication use did not show consistent correlations with antibody responses. These findings are consistent with the literature suggesting that vaccine efficacy may be reduced in immunocompromised populations, further supporting individualized vaccination strategies. COVID-19 vaccines provide incomplete protection in patients with ARDs, and immunosuppressive therapy and gender may affect vaccine efficacy. Personalized vaccination schedules, including booster doses, are recommended to improve protection in this population. Further studies are needed to refine vaccination strategies for immunocompromised patients, with the aim of reducing the burden of severe illness and improving public health.

Acta Med Iran 2026;64(7):453-459.
https://doi.org/10.18502/acta.v64i7.22422

Keywords: Autoimmune rheumatic diseases; COVID-19; Vaccination; Immunosuppressive therapy; Immunogenicity; Antibody response; Gender differences

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, which emerged in December 2019, has profoundly impacted populations worldwide to date (1). As of April 2024, it had infected over 704 million individuals, resulting in significant mortality (1). COVID-19 presents with diverse clinical manifestations, ranging from mild illness to severe, life-threatening complications, particularly in individuals with underlying comorbidities (2-5).

Despite the availability of several COVID-19 vaccines, the response of patients with autoimmune rheumatic diseases (ARDs) to these vaccines remains poorly understood (6). This may be attributed to the underrepresentation of patients with ARDs in studies investigating vaccine immunogenicity and safety (3,7). This population presents unique clinical challenges; for instance, studies involving organ transplant recipients have demonstrated that immunogenicity following mRNA-based vaccination is attenuated compared to the general population, particularly among patients treated with glucocorticoids and antimetabolites, such as mycophenolate (8). Furthermore, patients with ARDs are typically managed with immunomodulatory agents, including methotrexate (MTX), conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), tumor necrosis factor inhibitors (TNFi), rituximab, interleukin-6 inhibitors (IL-6i), and Janus kinase inhibitors (JAKi) (8). Evidence suggests that the use of these agents may adversely impact the immunogenicity of COVID-19 vaccines (9,10).

Despite numerous inquiries, limited data regarding vaccine immunogenicity in patients with ARDs have been published. Existing literature lacks standardized protocols, complicating the derivation of generalized conclusions, even through meta-analyses. Therefore, it is imperative to conduct studies aimed at investigating the immunogenicity of COVID-19 vaccines in rheumatological patients and exploring the factors that influence this response. This study aims to address existing gaps in understanding vaccine effectiveness among patients with rheumatologic conditions. Ultimately, we seek to inform the refinement of vaccination protocols, potentially reducing the severity of COVID-19 outcomes and strengthening the resilience of healthcare systems.

Materials and Methods

Population and sampling

The study population comprised individuals aged 18 years and older with any rheumatological disease attending rheumatology clinics in Bandar Abbas. The inclusion and exclusion criteria were rigorously established to enhance the specificity and reliability of the findings, particularly concerning the immunogenicity of COVID-19 vaccines in patients with autoimmune rheumatic diseases (ARDs) undergoing immunosuppressive or corticosteroid therapy.

Inclusion and exclusion criteria

Inclusion criteria

Age ≥18 years.

Receipt of two to three doses of a COVID-19 vaccine.

An interval of 2 to 6 months between the last dose and sample collection.

Documented neutralizing, anti-spike, and anti-receptor-binding domain (anti-RBD) antibody levels.

Non-pregnant and non-lactating females.

Absence of malignancy or hepatic failure.

No history of alcohol or illicit drug use.

Exclusion criteria

Incomplete medical data or required parameters.

Patient non-cooperation during data collection.

Withdrawal of consent or post-enrollment withdrawal from the study.

Symptomatic COVID-19 infection within the preceding six months.

Study design and sampling

This cross-sectional study was conducted among patients with various rheumatological diseases receiving immunosuppressive therapy. The sample included 100 patients recruited from rheumatology clinics in Bandar Abbas. Eligible participants who expressed willingness to participate were briefed on the study's purpose, aims, and methodology. Written informed consent was obtained from all participants. Participants were selected via simple random sampling.

Data collection instruments and procedures

Demographic information, clinical status, and medication history were collected using a questionnaire administered by trained interviewers. Following an overnight fast of 12-14 hours, a 5-mL blood sample was collected from each participant. Samples were centrifuged at 3000 rpm for 10 minutes to isolate the plasma, which was subsequently stored at -80° C for

analysis. Antibody levels, including neutralizing, anti-spike, and anti-RBD antibodies, were quantified using plaque reduction neutralization tests (PRNT).

Data analysis

Data were analyzed using SPSS version 21. Descriptive statistics were employed: mean±standard deviation (SD) for quantitative variables, and frequencies and percentages for qualitative variables. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Independent sample t-tests and one-way ANOVA were utilized to compare variables, including age, sex, vaccine type, medication use, and antibody levels (neutralizing, anti-spike, and anti-RBD). Statistical significance was set at $P<0.05$.

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was granted under protocol number [IR.HUMS.REC.1401.226]. All participants were fully informed regarding the research objectives, procedures, potential risks, and benefits. Written informed consent was secured from all participants prior to enrollment.

Results

The study included 91 participants with rheumatological diseases, comprising 81 females (89%) and 10 males (11%).

Regarding vaccine distribution, 72(79.1%) participants had received the Sinopharm vaccine, 15(17.6%) the Co-Barkat vaccine, and 3(3.3%) the AstraZeneca vaccine.

In terms of medication regimens, 8.8% (n=8) of participants utilized only csDMARDs. A total of 35.2% (n=32) utilized csDMARDs in combination with prednisolone, while 31.9% (n=29) utilized both csDMARDs and other immunosuppressive agents. Additionally, 6.6% (n=6) used a combination of csDMARDs and biologic disease-modifying antirheumatic drugs (bDMARDs), and 9.9% utilized prednisolone in conjunction with bDMARDs. Furthermore.

Relationship between age and levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

Based on the results of the test, the comparison of the amount of antibodies between the age groups of the study did not reach a significant level, which is fully displayed in Table 2.

Relationship between sex and levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

Regarding the relationship between the amount of antibodies between males and females, while it reached a significant level in the case of neutralizing antibody (P=0.023), in the case of anti-spike Ab (P=0.99) and anti-RBD Ab (P=0.47) did not reach a significant level, which is fully displayed in Table 3.

The relationship between the type of vaccine and the levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

In examining the relationship between the type of injected vaccine and the levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab, there was no significant difference in the amount of antibodies between people who received different vaccines. The data related to antibodies and injectable vaccine were fully displayed in Table 4.

The relationship between the type of drugs consumed and the levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

Based on the test results, no significant correlation was found between the levels of antibodies and the type of drugs used. The data related to the antibodies and drugs used are fully displayed in Table 5.

The relationship between the dose of drugs used and the levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

In the present study, a significant relationship was found between Adalimumab and Etanercept with the level of neutralizing antibodies, but no significant relationship was found between the levels of antibodies and the average dose of other drugs.

Table 1. The amount of antibodies

Anti-Body	Average	SD	Minimum level	Maximum level
neutralizing antibodies	25.23	16.92	2.15	68.47
anti-spike Ab	118.6	54.06	8.68	216.05
anti-RBD Ab	95.65	69.13	5.85	217.75

Table 2. Relationship between age and levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

Anti-body		Average	SD	*P*
neutralizing antibodies	Under 21	13.57	6.21	0.372
	21-30	31.51	18.00	
	31-40	22.91	16.43	
	41-50	28.44	17.58	
	51-60	22.22	17.58	
	61-70	19.69	13.11	
anti-spike Ab	Under 21	76.49	28.4	0.590
	21-30	135.39	46.37	
	31-40	117.07	54.93	
	41-50	119.28	52.19	
	51-60	100.19	60.72	
	61-70	123.36	59.15	
anti-RBP	Under 21	23.40	14.1	0.731
	21-30	107.12	55.33	
	31-40	88.04	67.75	
	41-50	105.11	79.29	
	51-60	82.03	69.33	
	61-70	99.76	68.06	

Table 3. Relationship between sex and levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

Antibody	Sex		*P*
	Male	Female	
Neutralizing antibodies	37.31±18.67	23.89±16.29	0.023
Anti-spike Ab	117.99 ±60.93	118.07±53.67	0.997
Anti-RBD	111.45±84.73	93.90±67.58	0.473

Table 4. The relationship between the type of vaccine and the levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

Anti-body	Vaccine	Average	SD	F	*P*
Neutralizing Antibodies	Sinopharm	26.35	17.70	0.419=(2 87)	0.419
	COVIran Barekat	19.99	10.14		
	AstraZeneca	24.45	25.35		
Anti-spike Ab	Sinopharm	118.84	54.46	0.924=(2 87)	0.942
	COVIran Barekat	113.70	46.46		
	AstraZeneca	121.07	97.40		
Anti-RBP	Sinopharm	96.58	70.56	0.395=(2 87)	0.395
	COVIran Barekat	82.11	50.47		
	AstraZeneca	141.05	116.46		

Table 5. The relationship between the type of drugs consumed and the levels of neutralizing antibodies, anti-spike Ab and anti-RBD Ab

Anti-body	Type of drug	Average	SD	F	P-value
Neutralizing antibodies	CS DMARD	20.33	13.26	1.09 =(5 84)	0.372
	PREDNISOLONE-CS DMARD	25.17	17.90		
	PRED-CS DMARD- Immunosuppressive	21.56	15.10		
	PREDNISOLONE-CS DMARD-B DMARD	38.50	15.09		
	CS DMARD-B DMARD	31.29	17.98		
	PREDNISOLONE- Immunosuppressive	23.84	20.48		
Anti-spike Ab	CS DMARD	107.64	63.35	0.748 =(5 84)	0.590
	PREDNISOLONE-CS DMARD	116.85	55.90		
	PREDNISOLONE-CS DMARD-Immunosuppressive	113.00	47.82		
	PREDNOSOLONE-CS DMARD-B DMARD	147.12	37.11		
	CS DMARD-B DMARD	128.38	63.27		
	PREDNISOLONE- Immunosuppressive	108.98	77.09		
Anti-RBD	CS DMARD	88.06	74.99	0.559 =(5 84)	0.731
	PREDNISOLONE-CS DMARD	93.44	68.88		
	PREDNISOLONE-CS DMARD-Immunosuppressive	83.98	62.12		
	PREDNISOLONE-CS DMARD-B DMARD	63.08	63.08		
	CS DMARD-B DMARD	85.53	85.53		
	PREDNISOLONE- Immunosuppressive	95.16	95.61		

Discussion

The current study investigated the efficacy of COVID-19 vaccination in patients with rheumatologic disorders receiving immunosuppressive therapies, including corticosteroids. In total, 91 patients were included, the vast majority of whom were women (89%). A similar gender distribution has been reported in other studies, with higher prevalence rates of rheumatologic diseases among females, potentially related to differences in immune system regulation influenced by both sex hormones and genetic factors (11-13).

No association between age and antibody levels was detected in our study, although prior investigations have established a link between advancing age and decreased immunogenicity (7). Several studies have indicated that this age-related decline is due to intrinsic impairment of immune function, underscoring the potential need for tailored vaccination approaches, such as dose adjustments or booster doses, in older individuals (7,11,14,15).

We observed significantly higher neutralizing antibody levels in males compared to females; however, no differences were noted for the other two antibody types, namely anti-spike and anti-RBD antibodies. At first glance, these results appear to contrast with previous studies reporting a greater immune response in females, attributed to enhanced humoral and cellular immunity (11). Contributory factors may include hormonal influences and genetic differences. The apparent inconsistency observed in our study may be attributable to the small sample size of male participants, highlighting the need for larger studies to confirm these findings (15-17).

Vaccine type may have influenced the immune response, as patients who received the AstraZeneca vaccine exhibited higher levels of anti-spike and anti-RBD antibodies compared to those vaccinated with Sinopharm or COVIran Barekat. It is important to note that during the pandemic, the COVIran Barekat vaccine was more widely available regionally, limiting direct comparisons across vaccine groups, and the small sample size further constrained the statistical power of these analyses. Nevertheless, when making an overall comparison among all vaccine types with respect to antibody responses, the differences did not reach statistical significance, likely due to the limited sample sizes. This is consistent with findings by Tang et al., who, although reporting variability in immune responses across different vaccine platforms, emphasized the benefit of complete vaccination regimens (7).

Furthermore, although we did not observe a significant overall association between immunosuppressive medication classes and antibody levels, an interesting association was noted with specific agents, namely adalimumab and etanercept. This finding is consistent with previous research indicating that certain biologic therapies may modulate the immune response to COVID-19 vaccination (18). These drugs target TNF, a key proinflammatory cytokine (19). Numerous studies have compared vaccine responses in individuals receiving anti-TNF agents with those in the general population (20). Some have reported reduced responses, whereas others have demonstrated no significant differences in immunogenicity (21-24).

Various studies have examined the effectiveness and safety of COVID-19 vaccination in patients with ARDs, particularly those undergoing immunosuppressive therapies. Tang et al., conducted a systematic review and meta-analysis evaluating the immunogenicity, efficacy, and safety of COVID-19 vaccines in patients with ARDs (7). Furer *et al*., reported that antibody formation in patients with ARDs was significantly lower compared to the general population. Notable treatments associated with reduced vaccine-induced antibody responses included glucocorticoids, rituximab, and mycophenolate mofetil. They documented that only 39% of patients receiving rituximab developed detectable antibodies (11). These results are in concordance with the findings of Alsaed *et al*., who demonstrated that although the majority of patients developed a satisfactory immune response, those treated with rituximab exhibited lower antibody titers compared to other groups. These observations align with previous reports regarding the suppressive effects of B-cell–depleting therapies (25).

In the study by Shen *et al*., the effectiveness of three FDA-approved COVID-19 vaccines was evaluated in a large cohort of immunocompromised patients. These vaccines were generally effective in reducing the risk of severe COVID-19 and hospitalization, although their protective effect was notably lower compared to that observed in the general vaccinated population; their analysis included patients on corticosteroids and other immune-modulating therapies. Their results showed that, even with vaccination, immunocompromised individuals remained at high risk for breakthrough infection and thus required additional preventive measures, including booster doses, to achieve adequate protection in high-risk groups (26).

These findings suggest that individualized vaccination strategies, such as additional doses or

modification of immunosuppressive regimens, may be necessary to achieve optimal protection in this at-risk population. Future studies with larger patient cohorts, focusing on both humoral and cellular immune responses, will be needed to better elucidate vaccine efficacy in immunocompromised patients.

This study investigated the effectiveness of COVID-19 vaccination in patients with rheumatologic diseases receiving immunosuppressive drugs, including corticosteroids. The primary aim of this study was to determine the levels of neutralizing, anti-spike, and anti-RBD antibodies in these patients after vaccination and to compare their immune responses with those of the general population. The results showed that, although a large proportion of patients developed an adequate antibody response, several factors influenced vaccine outcomes.

Sex appeared to be a major determining factor, as male patients exhibited much higher levels of neutralizing antibodies than female patients. This finding may indicate a potential biological difference in the immune response that warrants further investigation. However, it is important to note that the number of male participants was small, which may have affected the statistical results. No significant associations were observed between antibody levels and patient age, chronic disease status, or vaccination type, including Sinopharm, COVIran Barekat, and AstraZeneca.

Most participants in this study received the Sinopharm vaccine, which was predominantly used in Iran and may have introduced regional bias. However, further investigation is needed to compare the efficacy of different vaccine types in this population. The small sample sizes, particularly among male participants and those who received the AstraZeneca vaccine, may have affected the results. This emphasizes the need for larger-scale studies with greater sample sizes to confirm these findings. Although the responses were not uniform, the vaccines provided some degree of protection, and no severe adverse reactions were reported by the participants. However, it is important to note that patients receiving certain immunosuppressive therapies, particularly corticosteroids and specific DMARDs, exhibited diminished antibody responses, indicating that tailored vaccination strategies should be considered.

These findings underscore the importance of continued monitoring and assessment of vaccine efficacy in immunocompromised patients with autoimmune diseases. Given the complexity of immune modulation in these patients, personalized vaccination schedules, such as additional booster doses, may be necessary to achieve improved protection against COVID-19. Moreover, this study indicates that healthcare professionals should consider not only the benefits but also the potential limitations of COVID-19 vaccines in this vulnerable population.

We therefore conclude that, although COVID-19 vaccines are generally effective in patients with rheumatologic diseases, various clinical factors may still influence immune responses. Further studies involving larger groups of immunocompromised individuals are needed to develop improved vaccination protocols, ensure that high-risk groups receive appropriate immunization strategies against COVID-19, reduce severe illness, and strengthen population-level resilience.

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