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Review

The effects of methotrexate on the immune responses to the COVID-19 vaccines in the patients with immune-mediated inflammatory disease: A systematic review of clinical evidence

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ABSTRACT

COVID-19 vaccines exhibit high levels of immunogenicity in the overall population. Data on the effects of immunomodulators on the consequences of COVID-19 in patients with Immune-mediated inflammatory diseases (IMIDs) remains scarce. This systematic review aimed to evaluate the immune responses to the COVID-19 vaccines in IMID patients receiving methotrexate (MTX) compared to healthy individuals. A comprehensive literature search was carried out using electronic databases such as PubMed, Web of Science, Scopus, Google Scholar, and Embase up to August 2022 to identify eligible RCTs evaluating the effect of MTX on immune responses in patients with COVID-19. The PRISMA checklist protocol was applied for the quality assessment of the selected trials. Our findings demonstrated that MTX lowered the responses of T cells and antibodies in IMID patients compared to healthy controls. We also discovered that young age (<60 years) was the main parameter influencing the antibody response after vaccination, while MTX had little effect. Following vaccination, MTX-hold and age were considered the main factors influencing the antibody response. In patients older than 60 years of age, the time point of 10 days of MTX discontinuation was critical to boosting the humoral response to anti-SARS-CoV-2 IgG. Because many IMID patients did not have adequate humoral and cellular responses, our findings highlighted the importance of second or booster doses of vaccine and temporary MTX discontinuation. As a result, it implies that individuals with IMIDs should be subjected to more research, particularly humoral and cellular immunity efficiency trials after COVID-19 vaccination, until credible information is achieved.

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1. Introduction

Immune-mediated inflammatory diseases (IMIDs), including rheumatoid arthritis (RA), psoriasis (PSO), and inflammatory bowel disease (IBD) affect a total of 3 to 7% of the population in Europe and North America [1–3]. Patients with IMID are inherently more vulnerable to infection and, therefore, may be at a higher risk of developing COVID-19. Interestingly, the degree of responsiveness to virus vaccines (such as influenza and hepatitis B) and their long-term protection in IMID patients who received conventional disease-modifying anti-rheumatic drugs (DMARDs) like methotrexate (MTX) may not have been as strong as those in the overall population after vaccination [4,5]. MTX used alone or in combination with biologic DMARDs, such as tumor necrosis factor (TNF) inhibitors, is highly efficient at inhibiting common pathogenic immunological mechanisms in IMID; however, it significantly raises the potential for severe infections, especially respiratory tract infections [6,7]. Immunosuppressive medications have been shown to impair immune responses following COVID-19 immunization, and immunocompromised individuals account for 40–44% of hospitalized outbreak infections [8,9]. The impact of immunosuppressive drugs such as MTX on COVID-19-related consequences is mainly unclear. During the COVID-19 pandemic, the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) did not establish any guidelines on MTX use.

The first report of a decreased vaccination response in the presence of MTX was published in 2016 for the influenza vaccine [10]. Follow-up findings demonstrated that when MTX was stopped two weeks before and after the immunization, or simply two weeks after vaccination, an improvement was observed in humoral immunity [11]. Therefore, studies have shown that the timing of MTX discontinuation after vaccination, rather than before vaccination, is critical [12]. In research examining the immunogenicity of influenza and pneumococcal vaccines, MTX disrupted humoral responses based on decreased antibody titers in patients who took MTX compared to healthy individuals [13]. As a result, all existing recommendations for COVID-19 immunizations while on MTX are predicted based on influenza vaccine experience and do not consider the mRNA-based approach employed for COVID-19 vaccinations [14].

Vaccine-induced host protection is facilitated by a complicated interaction between the humoral and cellular immune systems, which is essential for efficacious and long-lasting protection against SARS-CoV-2 [15]. For the immunocompetent adult population, data on the safety, immunogenicity, and efficacy of messenger RNA (mRNA) COVID-19 vaccines is quickly evolving, with >90% of participants getting the desired humoral response [16]. However, the ability of patients with IMID to respond effectively to these vaccinations, as well as the disparities in both humoral and cellular immune responses to SARS-CoV-2 immunization, are unknown, presenting a vast knowledge gap that hampers optimum patient care. Considering the trend toward elongated vaccination schemes to optimize global coverage, assessing immunological responses to a single or second dose of vaccine is very valuable. Therefore, it is critical to identify the immunogenicity of COVID-19 vaccines mediated by both functional antibodies and antigen-specific T cells in IMID patients receiving MTX. Because therapeutic immunosuppression with MTX is typically provided as monotherapy without accompanying systemic corticosteroids, we hypothesized that MTX-treated IMID patients would respond less to mRNA COVID-19 vaccines than non-IMID participants [17]. In the present systematic review, we aimed to illustrate how MTX monotherapy in IMID patients affects both cellular and humoral immune responses following the first and second doses of COVID-19 vaccinations.

Reviewing studies were conducted based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [18]. Studies were found using a thorough systemic literature search strategy of PubMed, Google Scholar, Web of Science, EMBASE, and Cochrane Library databases for all clinical trial articles related to SARS-CoV-2 vaccines published from the inception of each database and with no language or regional restriction up to August 2022. The search terms were used according to the combined text and medical subject heading (MeSH) terms in the title and abstract: ("COVID-19" OR "2019 novel coronavirus" OR "2019 nCoV") AND ("Covid-19 Vaccine" OR "SARS-CoV-2 Vaccine" OR "BNT162b2 mRNA vaccination" OR "Pfizer-BioNTech" OR "Sinovac CoronaVac vaccine" OR "AZD1222" OR "Moderna" OR "Janssen/Johnson & Johnson") AND ("Immune-mediated inflammatory disease" OR "IMID" OR "rheumatoid arthritis" OR "RA" OR "psoriasis" OR "PSO" OR "inflammatory bowel disease" OR "IBD") AND ("clinical trial" OR "phase trial" OR "randomized" OR "hospital staff" OR "medical staff") AND ("methotrexate" OR "MTX") AND ("targeted immunosuppression" OR "immunotherapy" OR "csDMARD") AND ("Humoral response" OR "Vaccine response" OR "Immunogenicity" "anti-spike IgG titer "OR "anti-RBD-IgG" OR "Neutralizing activity" OR "Anti-spike S1 IgG" OR "geometric mean titers" OR "GMT" OR "mean IgG titer"). Fig. 1 presents a PRISMA flow diagram detailing the systematic search.

2. Result and discussion

2.1. Review of the literature and study characteristics

A search of 420 articles was conducted. Among them, six articles were identified that were relevant to the research topic and met inclusion criteria. The six eligible studies represented retrospective chart reviews and were published up to August 2022. One study reported two cohort studies, so we reviewed seven reports from a total of six studies. The data came from different regions, including New York, Germany, London, and Brazil. Overall, there were 604 participants. Of these, 316 patients with IMID received MTX treatment, and 288 were healthy controls. The percentage of female patients with IMID ranged from 35% to 91%, with an overall mean percentage of 51.75% ($n = 223$). The age range of patients varied from 48 to 63.2 years. All patients and control subjects had no prior COVID-19 exposure, and all groups received two SARS-CoV-2 vaccine doses. Of these seven reports, three used only the BNT162b2 Pfizer-BioNTech vaccine; two were BNT162b2 Pfizer-BioNTech, mRNA-1273 Moderna, and AZD1222 AstraZeneca vaccinations; one was both the BNT162b2 Pfizer-BioNTech and mRNA-1273 Moderna vaccines; and one was the Sinovac CoronaVac vaccine. Five studies evaluated the immunogenicity of SARS-CoV-2 after the second dose of vaccination. Two were the first doses of vaccination. One limitation of the included studies was that individuals no longer received combined therapy with different immunosuppressants or glucocorticoids. The included studies also had insufficient immunogenicity data after a second dose of the vaccine. We did not evaluate the safety of MTX discontinuation in our systematic review; however, cutting-edge records no longer display a notably accelerated prevalence of flares or disorder activity in association with 2-week MTX discontinuation. In another included study, decreased anti-spike IgG tiers were detected in IMID sufferers compared to controls. It is currently unclear whether or not this titer could lead to decreased levels of protection. In addition, because of the inclusion of sufferers with preceding COVID-19 contamination, the effects can also have preferred the ones no longer taking MTX. However, apart from all patients with previous contamination, the outcomes remained comparable. The characteristics of the included reviews are summarized in Table 1.

2.2. The effect of MTX on humoral response

MTX is the first-line or even gold-standard therapy for several autoinflammatory diseases. Several mechanisms may contribute to MTX's anti-inflammatory activities, ultimately influencing normal immunological responses [19]. Accordingly, MTX reduced humoral immunity after vaccination as it diminished vaccine serological responses to seasonal influenza and pneumococcal diseases [13]. MTX

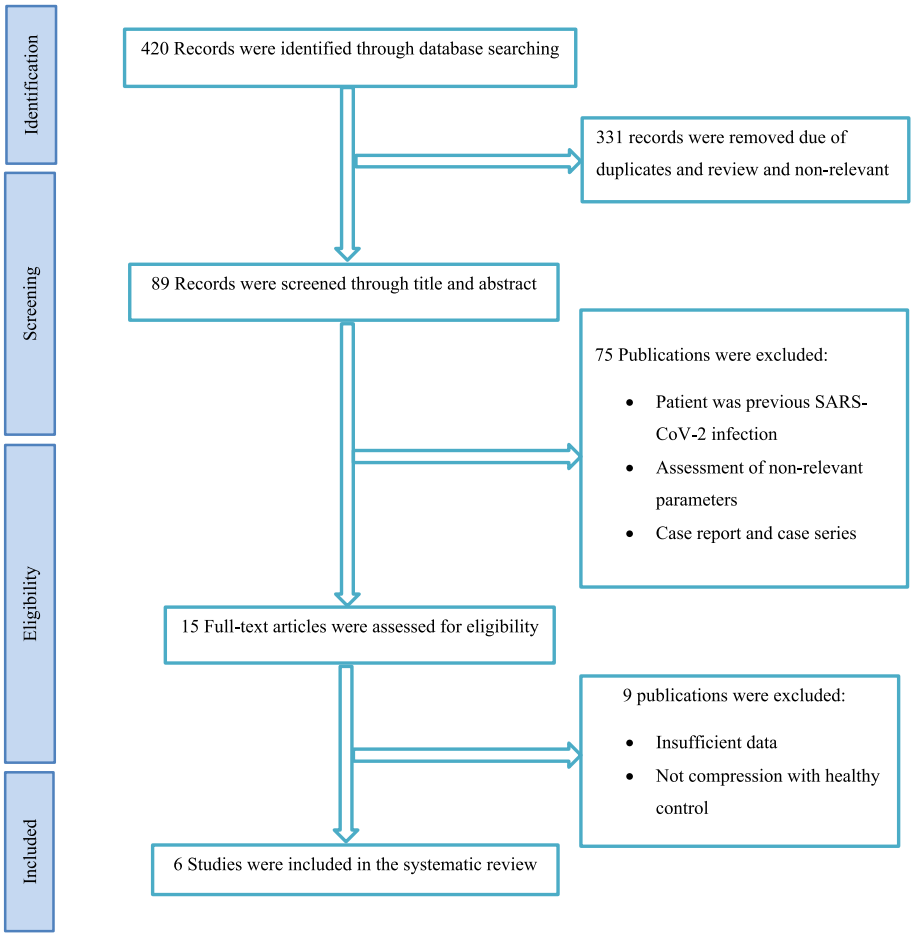

Fig. 1. The diagram of study identification and selection.

Table 1
Characteristics of included studies in this systematic review.

Author/year (reference)	Region	Disease	Population		Age-mean, female-n (%)	Vaccine type	Days after second vaccination	Description of response
			Healthy control	Patients with MTX				
Haberman A/ 2021 [5]	New York	PsO, PsA, RA	26	25	63.2 yrs., 18 (72%)	Pfizer–BioNTech		Using direct ELISA, measuring IgG antibody responses to the S protein and cellular immune response
Haberman B/ 2021 [5]	Germany	IMID	182	20	54.5 yrs., 7 (35%)	Pfizer–BioNTech		using the commercial ELISA from Euroimmun, measuring, IgG antibodies against the S1 domain and Cellular immune responses
Mahil/2021 [2]	London	PsO	17	17	48 yrs., 6 (35%)	Pfizer–BioNTech	28 days after first dose	using ELISAs, Measuring neutralizing antibody responses to wild-type SARS-CoV-2, and spike-specific T-cell responses
Mahil/2022 [4]	London	PsO	15	14	49.5 yrs., 6 (43%)	Pfizer–BioNTech	14 days after second dose	using ELISAs, measuring IgG specific for the SARS-CoV-2 spike and spike-specific T-cell production of interferon-γ and IL-2.
de Silva A.N. 2022 [7]	Germany	RA, PsA	21	64	61.1 yrs., 45 (70%)	Pfizer–BioNTech, mRNA-1273, AZD1222	2 week after second dose	Using surrogate virus neutralization test, measuring anti-RBD-IgG levels
Araujo 2022 [6]	Brazil	RA	–	92	59 yrs., 84 (91%)	SinovacCoronaVac vaccine	69 day after second vaccination	using a chemiluminescent immunoassay, measuring IgG antibodies against the SARS-CoV-2 S1/S2 proteins
Haberman/ 2022 [3]	New York	PsA, RA, IBD	27	84	54.8 yrs., 57 (68%)	Pfizer–BioNTech, mRNA-1273	4 week, 3, and 6 month after first dose	using the QuantiVac ELISA test system, testing IgG antibodies against the S1 domain of SARS-CoV-2 and percent of antibodies with neutralizing capacity

Abbreviation: yrs., year; RA, Rheumatoid arthritis; PsO, psoriasis; PsA, psoriatic arthritis; IMID, immune-mediated inflammatory disease;, IBD, inflammatory bowel disease.

treatment reduces the rate of polysaccharide-protein conjugate vaccine-specific antibody responses. The lower level was most likely not caused by decreased activation of B cells in lymphoid tissue because there was no decrease in the number of particular plasmablasts [20]. MTX could inhibit plasmablast activation and switch to memory B cell (12). Thus, while the pathophysiology of the disease seems to be involved in lowering the degree of humoral response, MTX usage dramatically accelerates the suppression of antibody responses. This is the first systematic review designed to compare the effect of MTX on the immunogenicity and immune responses of COVID-19 vaccines in patients with IMID. Correspondingly, to demonstrate the effect of MTX therapy on vaccine immunogenicity, the included studies evaluated the IgG antibody response against the SARS-CoV-2 spike protein. The current study discovered that MTX reduced humoral responses in patients with IMID following COVID-19 vaccination. According to previous research, the amount of specific antibodies in MTX-treated IMID patients reaches a sufficient level about three months after the first dose of the vaccine [21]. Bugatti et al. observed SARS-CoV-2 S1/S2 IgG positivity in 39.4% of chronic inflammatory arthritis patients using MTX [22]. They stated that MTX increases the likelihood of nonresponse to the first dose of BNT162b2 Pfizer-BioNTech COVID-19 vaccination by an eight-fold proportion [22]. In addition, Mahil et al. discovered a lower seroconversion rate in MTX-treated psoriasis patients [23]. In a similar manner, in the cohort study, Mahil et al. demonstrated that the seroconversion rate was statistically reduced in patients with psoriasis receiving MTX compared to healthy controls (47% vs. 100%) at 28 days after the first vaccination [23]. Similarly, Haberman et al. reported that MTX treatment attenuated adequate humoral response in patients with the IMID compared to healthy controls at 4 weeks after the first dose of vaccination (78.6% vs. 96.3%, $P = 0.002$) but did not significantly differ at 3 and 6 months (85.7% vs. 100%, $P = 0.100$; 45.9% vs. 65.4%, $P = 0.074$). They also showed that patients who had not received MTX had a similar rate of adequate humoral response to healthy controls at 4 weeks as well as 3 and 6 months after the second dose of vaccination (93.2% vs. 96.3%, 90.7% vs. 100%, and 62.4% vs. 65.4%) [21]. After two COVID-19 vaccine doses, the spike-specific IgG titers were statistically reduced in patients receiving MTX compared to healthy controls at 14 days after vaccination [25]. In the New York City and Erlangen cohorts, the rate of adequate humoral response was also lowered in patients on MTX therapy than in healthy controls (72% vs. 96.1% ($P = 0.023$), 50% vs. 98.4% ($P < 0.001$), respectively) [26]. We also validated that transient MTX is powerful in increasing IgG responses. The result illustrated that patients who held MTX therapy could achieve a greater adequate humoral response to COVID vaccination than those who did not hold MTX at 4 weeks, as well as 3 and 6 months after the first dose of vaccination [21]. Taken together, these results suggest that the MTX-hold group, which was instructed not to retake MTX after the second dose of the vaccine, could significantly increase the level of anti-spike SARS-CoV-2 antibody. At 69 days after the second dose of vaccination, the anti-SARS-CoV-2 S1/S2 IgG seroconversion rate was significantly higher in MTX-hold compared to MTX-maintain (78.4% vs. 54.5%, $P = 0.019$) [27]. The similar disease activity found in both MTX-hold and MTX-maintain cohorts implies that this method is promising in enhancing IgG levels but with increased rates of flare following the second dose of vaccine. Additionally, de Silva A. N. et al. outlined that MTX decreases the age-dependent humoral response to SARS-CoV-2 vaccination. Patients aged ≥60 years had a significant improvement in antibody response after holding MTX treatment for at least 10 days after vaccination [28]. It is demonstrated that the therapeutic efficacy of MTX was sustained for several weeks after discontinuation and showed a long biological half-life of MTX (Pharmacokinetics of oral methotrexate in patients with rheumatoid arthritis (https://pubmed.ncbi.nlm.nih.gov/18975321/). This suggests that short-term discontinuation of MTX does not enhance the response of RA patients to vaccines. Park et al. indicated that temporary MTX-holding improved the vaccine response of patients who had been on a stable dose of MTX (−Effect of methotrexate discontinuation on the efficacy of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomized clinical trial (https://pubmed.ncbi.nlm.nih.gov/28468794/). The analyses illustrated that during SARS-CoV-2 vaccination, age and MTX-hold are regarded as major factors influencing the antibody response. However, studies have suggested that 10 to 14 days of MTX withdrawal after vaccination can improve the humoral response to anti-SARS-CoV-2 IgG in patients older than 60 years [27,28].

In contrast, despite only receiving one dose of the SARS-CoV-2 mRNA vaccine, 10 of 13 individuals with rheumatic and musculoskeletal disorders on baseline MTX showed detectable anti-RBD levels. However, it had a small sample size and used a semi-quantitative ELISA method [29]. The studies, as mentioned earlier, only described the reaction after the first dose of the vaccination. A two-fold dose administration can compensate for a loss of humoral immunity. We observed that anti-spike-specific IgG median titers were quantitatively lower in patients taking MTX after the first dosage than in those receiving targeted biologics and healthy controls. On the other hand, a positive seroconversion rate was observed in all psoriasis patients receiving MTX 14 days after a second dose of immunization [25]. Indeed, the second dose successfully boosted spike-specific IgG titers. The difference in spike-specific IgG levels between the first and second doses was more remarkable in patients receiving MTX than in healthy controls and patients on targeted biologics [25]. In research by Haberman et al., anti-spike IgG was positive in 72% of IMID patients on MTX in New York and 50% of the Erlangen cohorts. Although the results of the two investigations differ, the use of two different ELISA kits makes comparing humoral responses between cohorts challenging.

In our review, it has been illustrated that, in addition to the importance of two doses of vaccination, IMID patients on MTX were typically older, which likely explains some of the differences in immunogenicity. Current research has shown differences in the clinical outcomes of antibody responses to COVID-19 mRNA vaccines in the older population compared to those younger than 60 years. As a result, considering immunosenescence in vaccine immunogenicity evaluation is critical, as it may reduce immunological responses to vaccine dosages. Accordingly, age ≥ 60 years was inversely associated with seroconversion and neutralizing antibodies [27]. Further studies revealed that older age for each 5-year interval, particularly age ≥ 60 years, was negatively linked to seroconversion. Besides, individuals under the age of 55 had significantly lower rates of anti-spike IgG after COVID-19 vaccination [31]. However, neither study investigated neutralizing activity or cellular immunity. Additionally, there was only one-time point during the investigation, making it difficult to hypothesize response dynamics [31]. Similarly, in a case report study about a 50-year-old female with RA receiving MTX, the anti-SARS-CoV-2 antibody was negative after the first dose of the BNT162b2 Pfizer-BioNTech vaccine but was positive after booster dose on days 8 and 14 [32]. They declared that the antibody levels continued to increase, although slower than those who did not receive immunomodulatory treatment. The other vaccines have also demonstrated that a booster dose has better efficacy than a first dose. A single HAV vaccine dose was insufficient to produce seroprotection in half of the JIA patients on MTX who had never been exposed to HAV; however, they were immunogenic to two doses of inactivated HAV vaccination. [33]

2.3. The effect of MTX on neutralization activity

To identify the impact of the antibody response on MTX in patients following vaccination, we reviewed studies identifying neutralization activity in response to vaccination. One study outlined that neutralizing activity against wild-type SARS-CoV-2 was significantly lowered in patients with psoriasis who received methotrexate 28 days after the first dose of vaccination ($P = 0{\cdot}0032$) [23]. The other study by Haberman et al. demonstrated that the neutralizing antibodies were lower in patients on MTX than in healthy controls after 4 weeks of the first dose of

vaccination (72.2% vs. 90.9%, $P < 0.001$) [21]. Furthermore, de Silva A. N. et al. reported that neutralization levels differed between patients who held MTX therapy and continued MTX after the second vaccination dose (83.1% vs. 61.2%, $p = 0.001$). This effect was more notable in patients older than 60 years (80.8% vs. 51.9%, $p = 0.001$) [28]. On the other hand, neutralizing antibody titers across the second dose of vaccination were detected at similar levels in patients receiving MTX and control groups ($P = 0{\cdot}63$) [25].

Whether seroconversion alone appropriately represents vaccination immunogenicity and protects is still unknown. Virus neutralization assays, the gold-standard measure of humoral immunogenicity, are thus an important measurement [34,35]. The association between serological immunoassays and virus neutralization tests is being studied. Mahil et al. discovered that MTX-treated psoriasis patients had a lower seroconversion and neutralizing capacity against the wild-type SARS-CoV-2 strain than either targeted biological therapy or healthy controls. However, neutralizing activity against the alpha variant was poor in all groups [36]. Interestingly, another trial found that MTX or targeted biologics such as TNF inhibitors (TNFi) did not neutralize antibody responses two weeks after a second dose [25].

TNFi, like MTX, could suppress the immunological response to vaccines against viral infections. Remarkably, most early time-point studies found little effect of TNFi on humoral response. The effect of TNFi appears to be related to the short interval time of the study. TNFi promotes a 6-month decrease in immunogenicity compared to earlier time points [21]. Furthermore, the activity of TNFi is amplified when combined with MTX. Anti-SARS-CoV-2 antibody reactivity has been found to be lower in IBD patients receiving TNFi rather than vedolizumab. It was also reduced with thiopurine, or MTX [37]. According to Mahil et al., following the first dosage, spike-specific IgG median titers and neutralizing activity were quantitatively lowered in psoriasis patients receiving MTX compared to patients receiving TNFi, interleukin [IL]-17 inhibitors, or IL-23 inhibitors, and healthy controls [25]. However, the fold change in spike-specific IgG titer between doses 1 and 2 was most significant in MTX-treated patients compared to healthy controls and patients on targeted biologics [25]. The other study showed a higher seropositivity rate following the initial COVID-19 vaccination in autoimmune disease patients receiving MTX or anti-CD20 therapy. Prednisone or TNFi levels were lowered in subjects who did not receive immunosuppressive therapy and in the control group. However, the majority of patients who received two doses of the COVID-19 vaccine were seropositive, and seroconversion rates for all treatment regimens were comparable to controls except those using anti-CD20 therapies [38]. The action of anti-CD20 drugs implies that anti-CD20 drugs have an enormous inhibitory effect on humoral immunity through particular suppression of B cells.

It was primarily noticed that patients who had recently received B-cell depletion therapy (BCDT) or glucocorticoids had low or absent anti-spike IgG and neutralizing antibody titers after mRNA-based SARS-CoV-2 vaccination compared to MTX-treated patients [39]. In another study, however, even with concurrent MTX, most individuals receiving JAK inhibitors became seropositive [40]. MTX emerged as a risk factor for hospital admission but no longer for mortality, at the same time as different cDMARDs did not show variations. Additionally, patients on glucocorticoids had worse survival [41]. However, the mortality rate among hospitalized patients decreased as compared with the standard population. Our initial outcomes advise that COVID-19 no longer has a prime impact on mortality in sufferers with IMID. However, glucocorticoids appear to increase the risk of mortality, while MTX might additionally increase the risk of hospital admission. These findings advocate variations in drug mechanisms, which may also affect COVID-19 direction, and emphasize the significance of investigating the effect of immunosuppressive treatment in addition. There is a possibility that the complexity of the individuals when evaluating their effects on immunogenicity was influenced by other variables such as disease status or concurrent immunosuppressive treatment [42]. As a result, it appears to be difficult to analyze and compare the precise effects of the medications.

2.4. T cell response

The cellular immune response is one of the main determinants of the clinical outcome for evaluating vaccine efficacy. Only two studies found a long-term impact of MTX therapy on the cellular immunogenicity of the COVID-19 vaccine BNT162b2 Pfizer-BioNTech in patients with IMID. Indeed, the studies identified that the cellular responses were comparable between the healthy controls and the patients receiving MTX after the second vaccine dose. The importance of cellular immunity in preventing SARS-CoV-2 infection with low humoral immunity remains debatable. Our findings also showed that a second dose of vaccine, delivered in conjunction with a long-term scheduling regimen, was effective in inducing antibody responses in subjects taking MTX. However, as compared with controls, a lower percentage of sufferers receiving immunosuppressive drugs had detectable T-cell responses or increased T-cell immunity after the second dose. Findings from our time-to-intervention evaluation confirmed that a proportion of the control and MTX groups had undetectable T-cell responses to the primary vaccine dose. After the second dose, all subjects in the control group showed T-cell responses, whereas a proportion of immunosuppressed patients had undetectable responses. Haberman et al., using high-dimensional spectral flow cytometry, mentioned that activation of CD4+ T cells and circulating T follicular helper cells (cTfh) cells by Spike-specific B cells was the same in patient and healthy control groups before vaccination and after the second dose of vaccination. In comparison, the poor induction of activated CD8+ T cells expressing granzyme B may represent reduced cytotoxic functionality of these cells in IMID patients receiving MTX [26]. As well, Mahil et al. outlined that treatment with MTX could not induce or boost the total T-cell responses following the first and second vaccination doses. In contrast, the induction of T-cell responses was increased in healthy controls across vaccinations [23,25].

According to some evidence, specific cellular immunity may be a better criterion than humoral immunity for protection against respiratory viruses in immunocompromised individuals [43,44]. Cellular immunity plays an essential role in COVID-19 protection and recovery, and robust activation of specific T cells provides long-lasting neutralizing antibody responses [45,46]. Patients with COVID-19 had activated SARS-CoV-2-specific CD4- and CD8-T cells, Tfh cells, and memory B cells [47]. Therefore, post-vaccination cellular immune responses likely influence vaccine immunogenicity against SARS-CoV-2. Two investigations focused on cellular immunity following COVID-19 immunization, both with BNT162b2 Pfizer-BioNTech. After the first dose of vaccination, Mahil et al. observed comparable cellular immunity rates in MTX-treated patients and controls [36]. In another trial, researchers found that after the initial dosage, some control subjects and psoriasis patients exhibited untraceable spike-specific T-cell responses (interferon-γ, Interleukin (IL)-2, or IL-21), but all controls had measurable T-cell responses within two weeks. After the second dose, control subjects showed a more potent induction of T-cell responses than the MTX-treated group. There was no difference in interferon-γ, IL-2, or IL-21 production between the first and second vaccination doses in MTX-treated patients. According to the findings, even with a second vaccine dosage, cellular immunity in MTX recipients may diminish over time [25]. Haberman et al. discovered a similar activation of CD4+ T cells and cTfh cells in the groups, as well as an unusual decrease in activated CD8+ T-cell responses in the MTX-receiving group. Weak induction of activated CD8+ T lymphocytes producing granzyme B likely indicates lower cytotoxic activity [31]. According to the critical role of CD8+ T cells and antibodies against viral infection, patients with IMID on MTX are at a higher risk of insufficient vaccine response. The study also mentioned that T-cell responses increased two weeks following the second dose of vaccination. Our study demonstrated lowered anti-COVID-19 T-cell responses in MTX-treated patients, revealing the

impact of age and preserving the first records on MTX-hold following COVID-19 vaccination. We identified young age, MTX-hold, and a longer vaccine interval as the principal agents for the improved antibody response following vaccination. The poor impact of age on vaccination reactions has already been recognized. However, consideration of age was no longer distinguishable in previous studies examining the immune response to MTX treatment. Therefore, our data suggest that the continuous use of MTX and old age aggravate the negative elements. Therefore, the temporary discontinuation of MTX intake could promote immunological responses to COVID-19 vaccinations. As a result, recommendations have stated that MTX should be withheld for 1–2 weeks after COVID-19 immunizations [48,49]. It is critical to monitor disease activity after discontinuing MTX therapy. Because this policy effectively increases the immunological responses to the influenza vaccination, a two-week break in MTX following the booster dose of COVID-19 is now being investigated in multiple studies [50]. According to the study, short MTX cessation for two weeks following influenza vaccination had better seroprotection rates when compared to MTX maintenance [50]. Indeed, the MTX-hold group had higher antibody levels against influenza antigens. Importantly, it is linked to any change in disease activity. However, only the humoral immune response was evaluated, and the clinical impact of more significant antibody responses on the incidence of infections is unclear [50]. A randomized clinical trial assessed the effect of discontinuing MTX for two weeks after each vaccination dose versus continuing MTX on humoral response in RA patients on days 0, 28, and 69. Seroconversion titers were higher in the MTX-hold group than in the MTX-maintain group. However, there were no changes in neutralizing activity [27]. The disease activity scores were the same in the MTX-hold and MTX-maintain groups, worsening on day 28 (after the first dose) but not on day 69 (after the second dose). They hypothesized that the greater flare rates following the second MTX cessation could be attributed to the short time between vaccination doses [27]. Then, MTX cessation after immunization is offered as a potentially beneficial strategy for expanding vaccine immunogenicity.

3. Limitation

This study has some limitations. Since records concerning MTX consumption at some point in the future were evaluated retrospectively, bias cannot be eliminated. Further data on immunogenicity after a booster dose of vaccine in participants receiving MTX without infection is expected. While worldwide mass vaccination programs for COVID-19 are underway, there is concern about the effectiveness of the vaccine in immunosuppressed sufferers, combined with new variants of SARS-CoV-2 that pose a risk of immune escape. Similar developments have been recognized in unique cohorts of sufferers with IMID, and these findings have raised questions about whether modification of MTX or brief discontinuation ought to enhance immune responses to COVID-19 vaccines. Taken collectively, our outcomes advocate that the top-rated safety of sufferers with IMID in opposition to COVID-19 would require similarly rigorous research to decide whether extra doses of vaccine, dose modification of MTX, or even brief discontinuation of this drug can raise immune reactions, as has been confirmed for specific viral vaccines in this affected patient population. Measures of the immune responses that correlate with the post-vaccination risk of COVID-19 are currently unknown, and a growing body of research in immunosuppressed individuals has focused solely on serological responses. Although our data indicating preserved mobile immunogenicity across MTX, independent of neutralizing interest, is reassuring, ongoing pharmacovigilance studies will determine whether this finding interprets into the scientific effectiveness of vaccines. The magnitude of T-cell responses may also be reduced by additional vaccine doses, so analysis of cell dynamics after booster doses is critical.

4. Conclusion

Pending clinical effectiveness data, up to now, our study informed the importance of second or booster vaccine doses and temporary MTX withdrawal since a proportion of AID patients did not have enough humoral and cellular responses. However, studies on the immunogenicity of SARS-CoV-2 vaccine in AID patients are very limited, and people with AID should be subject to further investigations, especially humoral and cellular immunity effectiveness experiments after COVID-19 vaccination, as long as a reliable result can be reached. More research with a larger sample size will be required to elucidate the correlation between age, MTX, and COVID-19 vaccine immunogenicity and to determine whether additional immunosuppressive medicines influence MTX-induced attenuation of the humoral and cellular responses following COVID-19 vaccination.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript is approved by all authors for publication.

Authors' contributions

M.A. and T.M performed the research. A.J. and A.K. designed the research study. A.K. and F.F. contributed essential reagents or tools. F.F., S.Kh. and S.T. analyzed the data, and M.A., T.M and A.J wrote the paper. SH.D., I.P. and J.C·C contributed to structuring and arranging the content of the revised article. M.A., M.D. and R.A. validated, reviewed and edited the paper.

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Declaration of Competing Interest

The authors declared that they have no competing interests.

Data availability

The data and materials used in this study are available from the corresponding author on reasonable request.

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