

CLINICAL SCIENCE

Two-week methotrexate discontinuation in patients with rheumatoid arthritis vaccinated with inactivated SARS-CoV-2 vaccine: a randomised clinical trial

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ABSTRACT

Objective To evaluate the effect on immunogenicity and safety of 2-week methotrexate (MTX) discontinuation after each dose of the Sinovac-CoronaVac vaccine versus MTX maintenance in patients with rheumatoid arthritis (RA).

Methods This was a single-centre, prospective, randomised, investigator-blinded, intervention study (NCT04754698, CoronavRheum) including adult patients with RA (stable Clinical Disease Activity Index (CDAI) ≤ 10 , prednisone ≤ 7.5 mg/day) randomised (1:1) to withdraw MTX (MTX-hold) for 2 weeks after each vaccine dose or maintain MTX (MTX-maintain), evaluated at day 0 (D0), D28 and D69. Coprimary outcomes were anti-SARS-CoV-2 S1/S2 IgG seroconversion (SC) and neutralising antibody (NAb) positivity at D69. Secondary outcomes were geometric mean titres (GMT) and flare rates. For immunogenicity analyses, we excluded patients with baseline positive IgG/NAb, and for safety reasons those who flared at D28 (CDAI > 10) and did not withdraw MTX twice.

Results Randomisation included 138 patients with 9 exclusions (5 COVID-19, 4 protocol violations). Safety evaluation included 60 patients in the MTX-hold and 69 patients in the MTX-maintain group. Further exclusions included 27 patients (13 (21.7%) vs 14 (20.3%), $p=0.848$) with positive baseline IgG/NAb and 10 patients (21.3%) in MTX-hold with CDAI > 10 at D28. At D69, the MTX-hold group ($n=37$) had a higher rate of SC than the MTX-maintain group ($n=55$) (29 (78.4%) vs 30 (54.5%), $p=0.019$), with parallel augmentation in GMT (34.2 (25.2–46.4) vs 16.8 (11.9–23.6), $p=0.006$). No differences were observed for NAb positivity (23 (62.2%) vs 27 (49.1%), $p=0.217$). At D28 flare, the rates were comparable in both groups (CDAI, $p=0.122$; Disease Activity Score in 28 joints with C reactive protein, $p=0.576$), whereas CDAI > 10 was more frequent in MTX-hold at D69 ($p=0.024$).

Conclusion We provided novel data that 2-week MTX withdrawal after each dose of the Sinovac-CoronaVac vaccine improves anti-SARS-CoV-2 IgG response. The increased flare rates after the second MTX withdrawal may be attributed to the short-term interval between vaccine doses. This strategy requires close surveillance and shared decision making due to the possibility of flares.

Key messages**What is already known about this subject?**

⇒ Temporary methotrexate (MTX) withdrawal for 2 weeks after influenza vaccine improved immunogenicity in patients with rheumatoid arthritis (RA) without worsening disease activity.

What does this study add?

⇒ This is the first randomised study showing that 2-week MTX withdrawal after each 28-day interval Sinovac-CoronaVac vaccine dose improves anti-SARS-CoV-2 immunogenicity.
⇒ However, the strategy was associated with higher flare rates (Clinical Disease Activity Index (CDAI) > 10) after the second dose of vaccine.

How might this impact on clinical practice or future developments?

⇒ These novel results reinforce the recommendation of temporary MTX withdrawal after Sinovac-CoronaVac vaccine in patients with RA with CDAI ≤ 10 , with close disease activity surveillance.
⇒ The increased flare rates after the second MTX withdrawal may be due to the short-term interval between vaccine doses.

INTRODUCTION

The SARS-CoV-2 virus has caused worldwide health, social and economic crisis with death toll reaching millions.¹ Brazil has been one of the most impacted countries, with mortality surpassing 600 000 subjects in October 2021.² The WHO has recommended the emergency use of the Sinovac-CoronaVac vaccine (Sinovac Life Sciences, Beijing, China),³ an inactivated vaccine against SARS-CoV-2 CN02 strain and also one of the first approved vaccines in Brazil, accounting for over 70 million doses as of October 2021.⁴ The effectiveness of this vaccine was demonstrated in a large study with 10.2 million people in whom the protective effect for hospitalisation, intensive care unit admission and COVID-19-related death was over 85%.⁵

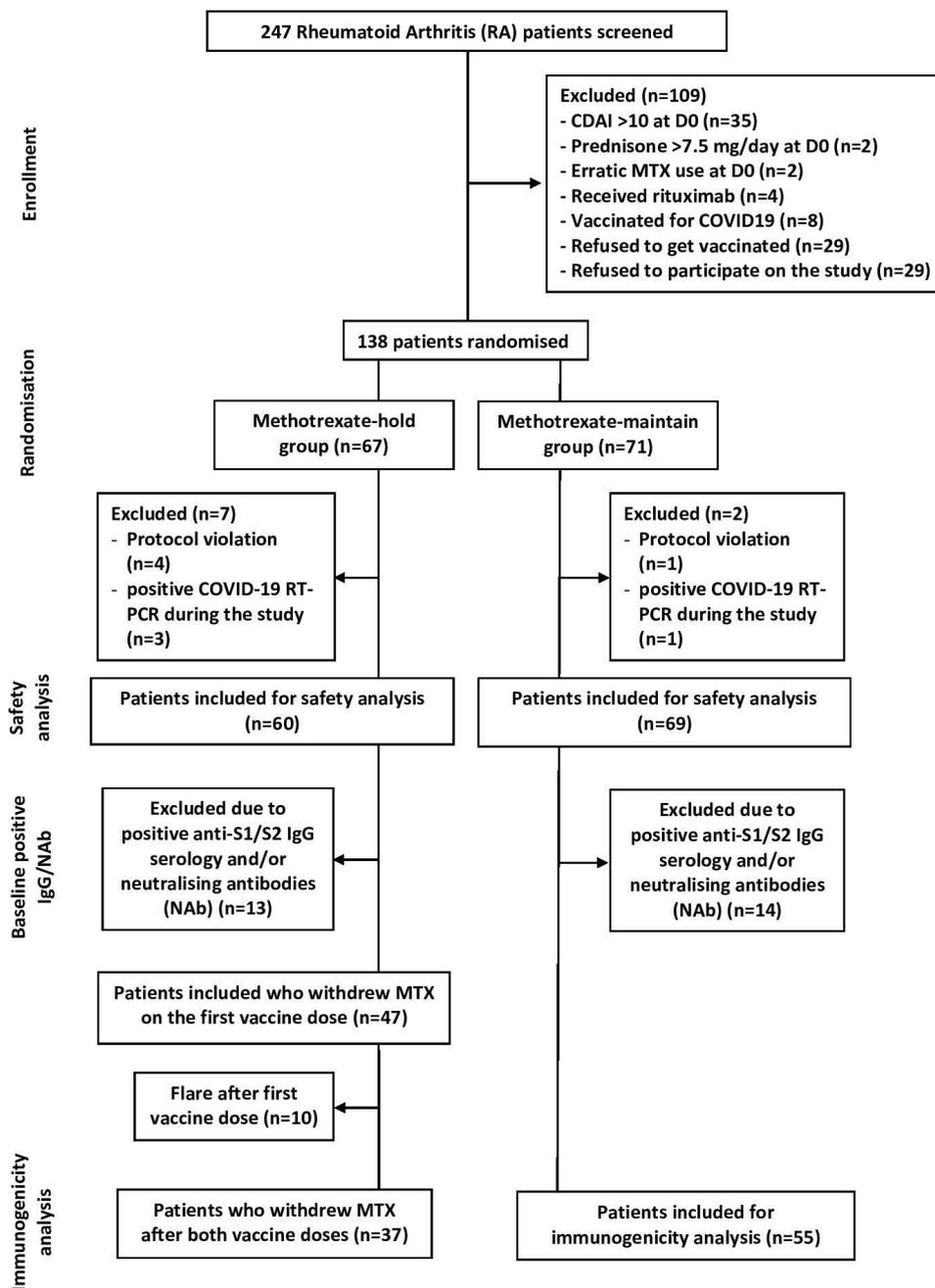


Figure 1 Modified CONSORT flow diagram. CDAI, Clinical Disease Activity Index; CONSORT, Consolidated Standards of Reporting Trials; D0, day 0; RT-PCR, reverse transcriptase PCR.

Patients with rheumatoid arthritis (RA) are at higher risk of hospitalisation and death by COVID-19 due to comorbidities⁶⁻⁸ or immunosuppressive treatments.⁶⁻⁹ Moreover, patients with RA have reduced immunogenicity to COVID-19 vaccines¹⁰⁻²¹ when using rituximab,¹¹⁻¹⁷ abatacept,^{11 12 17} methotrexate (MTX)^{11-13 18-21} and glucocorticoids.^{10-12 18}

Temporary immunosuppressant withdrawal is suggested as a possible strategy to enhance vaccine immunogenicity in patients with autoimmune rheumatic diseases (ARD).^{19 20 22} In this context, Park *et al*^{19 20} demonstrated that the discontinuation of MTX improved immunogenicity of the annual influenza vaccines in patients with RA, concluding that the interruption of two MTX doses after vaccination was safe and equally effective as holding four MTX doses.^{23 24} Due to these results, recommendations have emerged favouring the withdrawal of MTX for 1–2 weeks after COVID-19 vaccines.^{25 26}

However, to this date, no comparative study has assessed the impact of this intervention on immunogenicity and disease activity after any SARS-CoV-2 vaccination schedule.

Therefore, this trial aimed to evaluate the immunogenicity and safety of a 2-week MTX discontinuation after each dose of the Sinovac-CoronaVac vaccine in patients with RA with remission/low disease activity compared with age and sex balanced RA group who maintained the drug.

METHODS

Study design

This was a single-centre, randomised, investigator-blind, intervention study performed at the rheumatology outpatient clinic of a tertiary centre. All patients with RA fulfilled the American

Table 1 Baseline characteristics of patients with rheumatoid arthritis according to MTX interruption (MTX-hold) or MTX maintenance (MTX-maintain)

	Patients for safety analyses			Patients for immunogenicity analyses		
	MTX-hold (n=60)	MTX-maintain (n=69)	P value	MTX-hold (n=37)	MTX-maintain (n=55)	P value
Current age, years	61 (52–70.5)	58 (49–68)	0.395	59 (45–68)	59 (51.5–68)	0.828
Female sex	51 (85.0)	63 (91.3)	0.265	34 (91.9)	50 (90.9)	>0.999
Caucasian race	28 (46.7)	29 (42)	0.597	19 (51.4)	25 (45.5)	0.579
Disease parameters						
Disease duration	17 (10–30.5)	17 (10–24)	0.801	19 (10–28)	17 (10–25)	0.616
RF positivity	48 (80)	53/68* (77.9)	0.776	31 (83.8)	44 (80)	0.647
Anti-CCP positivity	27/37* (73)	35/47* (74.5)	0.877	17/22* (77.3)	26/36* (72.2)	0.670
CRP, mg/dL	4.4 (1.1–9.3)	3.7 (1.6–7.0)	0.655	5.6 (1.0–9.6)	4.0 (2.5–7.5)	0.847
CDAI	7.0 (4.0–9.0)	6.0 (3.0–8.0)	0.485	6.0 (2.0–8.0)	6.0 (3.0–8.0)	0.548
SDAI	7.1 (4.1–10.1)	6.3 (3.9–9.3)	0.380	6.8 (3.1–9.4)	6 (3.8–8.3)	0.618
DAS28-CRP	2.72 (2.10–3.15)	2.33 (1.96–2.82)	0.178	2.43 (1.80–3.15)	2.34 (2.02–2.87)	0.927
CDAI ≤2.8	11 (18.3)	10 (14.5)	0.556	10 (27)	8 (14.5)	0.139
SDAI ≤3.3	12 (20)	14 (20.3)	0.967	12 (32.4)	11 (20)	0.177
DAS28-CRP ≤2.6	30 (50)	46 (66.6)	0.055	21 (56.8)	36 (65.5)	0.400
Boolean criteria	9 (15)	14 (20.3)	0.434	9 (24.3)	12 (21.8)	0.779
Current therapy						
Prednisone	33 (55)	24 (34.8)	0.021	19 (51.4)	18 (32.9)	0.074
Prednisone dose, mg/dL	5 (4.38–5)	5 (5–5)	0.495	5 (2.5–5)	5 (5–5)	0.336
MTX dose	20 (17.5–25)	20 (15–25)	0.397	20 (15–25)	20 (15–25)	0.314
MTX 10–15 mg/week	16 (26.7)	23 (33.3)	0.411	11 (29.7)	20 (36.4)	0.509
MTX 17.5–25 mg/week	44 (73.3)	46 (66.7)		26 (70.3)	35 (63.6)	
MTX monotherapy	13 (21.7)	18 (26.1)	0.558	9 (24.3)	16 (29.1)	0.614
MTX in combination	47 (78.3)	51 (73.9)		28 (75.7)	39 (70.9)	
Leflunomide	8 (13.3)	17 (24.6)	0.105	6 (16.2)	13 (23.6)	0.389
Other sDMARD	10 (16.7)	19 (27.5)	0.140	6 (16.2)	16 (29.1)	0.156
Abatacept	7 (11.7)	9 (13.0)	0.813	6 (16.2)	7 (12.7)	0.638
Other bDMARD	18 (30.0)	14 (20.3)	0.203	11 (29.7)	9 (16.4)	0.128

Results are expressed in median (IQR) and n (%).
 Continuous data are compared using Mann-Whitney U test and categorical variables with χ^2 or Fisher's exact test, as appropriate, as two-sided analyses.
 For safety analyses, all patients who adhered to the protocol were included. For immunogenicity analyses, patients with baseline positive S1/S2 IgG were excluded from both groups and patients with CDAI >10 at day 28 who withdrew MTX only once were excluded only from the MTX-hold group.
 *Percentages calculated according to available data.
 Anti-CCP, anticyclic citrullinated peptides; bDMARD, biologic disease-modifying antirheumatic drugs; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28-CRP, disease activity score with 28 joints and C reactive protein; MTX, methotrexate; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; sDMARD, synthetic disease-modifying antirheumatic drugs.

College of Rheumatology/European League Against Rheumatism criteria for classification of RA,²⁷ agreed to participate in the study and signed informed consents. The protocol is part of a larger study of immunosuppressed patients with ARD (ClinicalTrials.gov: NCT04754698).¹² Patients and the public were not involved in the design, conduct, reporting or dissemination plans of the present research.

The coprimary outcomes were seroconversion (SC) rates for anti-SARS-CoV-2 S1/S2 IgG and neutralising antibody (NAb) positivity at day 69 (D69). Secondary immunogenicity outcomes were SC rates for anti-S1/S2 IgG and NAb positivity at D28, geometric mean titre (GMT) and factor increase of GMT (FI-GMT) for anti-SARS-CoV-2 S1/S2 IgG, and NAb activity at D28 and D69.

Secondary safety outcomes were longitudinal variations in disease activity scores: Clinical Disease Activity Index (CDAI),²⁸ Simplified Disease Activity Index (SDAI),²⁸ Disease Activity Score in 28 joints with C reactive protein (DAS28-CRP)²⁹ and frequency of adverse events related to vaccine. Exploratory outcomes were the frequency of patients with flare at D28 and D69, defined by CDAI >10^{30 31} or by an increase in DAS28-CRP >1.2 (or >0.6 if the baseline DAS28 was >3.2).^{32 33} Moreover, patient perception of disease activity worsening was also evaluated.

Participants

We recruited adult (≥ 18 years old) patients with RA diagnosis²⁷ with low disease activity or remission (CDAI ≤ 10)²⁸ at first vaccination day and with stable MTX dose for at least 4 weeks, both in monotherapy or in association with synthetic or biologic disease-modifying antirheumatic drugs (DMARD). The maximum allowed prevaccination oral prednisone dose was 7.5 mg/day. Patients were invited to participate after review of their electronic records in the last 3 months (recruitment up to 3 weeks before enrolment).

Exclusion criteria were acute febrile illness/symptoms of COVID-19 at vaccination, history of anaphylaxis to vaccine components, demyelinating disease, decompensated heart failure (class III/IV), blood transfusion ≤ 6 months, inactivated virus vaccine ≤ 14 days, live virus vaccine <4 weeks, denial to participate, hospitalisation, previous vaccination with any SARS-CoV-2 vaccine, reverse transcriptase PCR (RT-PCR)-confirmed COVID-19 during the study and rituximab use in the previous 12 months. Patients with prevaccination positive COVID-19 serology (anti-S1/S2 IgG and/or NAb) were excluded from immunogenicity analysis but kept for safety evaluation.

Visit schedule

Patients were evaluated in three visits: D0 (first dose of the vaccine), D28 (second dose) and D69 (6 weeks after the second

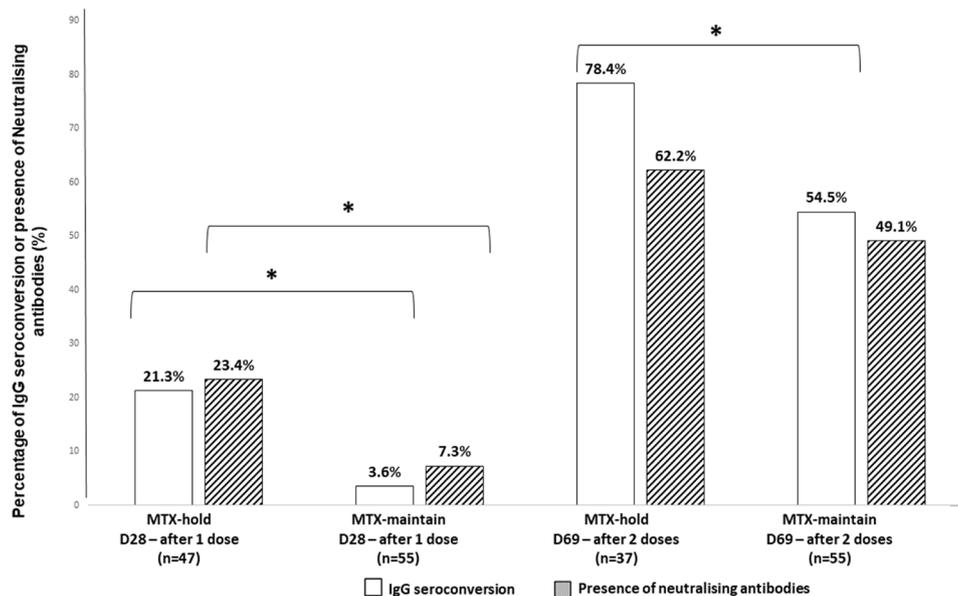


Figure 2 Frequencies of anti-SARS-CoV-2 IgG seroconversion S1/S2 and presence of neutralising antibodies at D28 and D69 in the MTX-hold and MTX-maintain groups compared using a two-sided χ^2 or Fisher's exact test, as appropriate. Data are shown as percentages. MTX-hold: baseline seronegative patients randomised to discontinue MTX after the first dose (n=47) and second dose (n=37; due to the exclusion of patients who had CDAI >10 at D28 and withdrew MTX only once). MTX-maintain: baseline seronegative patients randomised to maintain methotrexate throughout the study (n=55). *P<0.05 in comparison between groups. The number of patients in the groups is described under their designations. CDAI, Clinical Disease Activity Index; D28, day 28; D69, day 69; MTX, methotrexate.

dose). The first dose was given on 9–10 February 2021 (D0), while the second dose was on 9–10 March 2021 (D28).

The vaccination protocol included two doses of ready-to-use syringes with Sinovac-CoronaVac vaccine (batch #20200412; Sinovac Life Sciences), consisting of 3 μ g in 0.5 mL of β -propiolactone inactivated SARS-CoV-2 with aluminium hydroxide adjuvant. The vaccine was administered in the deltoid muscle.

Randomisation and masking

Investigators responsible for disease activity measures, statisticians and laboratory personnel were blinded to the allocation groups. Only two researchers (CSRA and MSRS) were not blinded and were responsible for safety surveillance and patient follow-up by telephone for adherence purposes. These two investigators were not involved in disease activity measures, laboratory analysis or patient vaccination.

At D0, before the first dose of vaccine, patients were evaluated by blinded experienced rheumatologists who assessed disease activity by CDAI and rechecked the inclusion and exclusion criteria. Patients with CDAI \leq 10 proceeded to the enrolment station, where the unblinded researchers revised the protocol, explained the procedures, collected the informed consent and conducted the randomisation, which was performed on the web-based software 'The REDcap Project version 10.5.0'. Allocation was generated instantaneously in a 1:1 ratio to one of the following groups: withdraw MTX for 2 weeks after each dose of the CoronaVac (MTX-hold group) or to maintain MTX continuously (MTX-maintain group).

At D28 and D69, patients were initially assessed by the unblinded researchers, checked for protocol violation and instructed not to inform their allocation groups to anyone else. Then, they proceeded for the blinded disease activity evaluation. Subsequently, they returned to the unblinded researchers and were instructed accordingly.

Intervention

At D0, the two unblinded researchers instructed patients in the MTX-hold group not to take two doses of MTX after vaccination, according to the last MTX dose. They provided a date diagram (online supplemental figure 1) informing the dates in which they would skip MTX and the date to resume its usage. At D28, patients in the MTX-hold group with CDAI \leq 10 were instructed to withdraw MTX again and a new date diagram was produced. Patients with CDAI >10 in the MTX-hold group were instructed not to withdraw MTX again after the second dose of vaccine. Patients in the MTX-maintain group were instructed to continue MTX on the same day and dose throughout the study. The two unblinded researchers checked adherence to protocol by telephone contact with all patients in the weeks following both vaccine doses.

Adding or changing DMARD therapy was not allowed until D69, although patients were permitted to use analgesics, non-steroidal anti-inflammatory drugs or prednisone up to 10 mg/day in case of disease activity worsening.

Laboratory analyses

Blood samples (30 mL) were collected immediately before each vaccine dose (D0 and D28) and 6 weeks after the second dose (D69). Serum samples were stored at -70°C . IgG antibodies against the SARS-CoV-2 S1/S2 proteins were measured using a chemiluminescent immunoassay (Indirect ELISA, LIAISON, DiaSorin, Italy). SC was defined as positive serology, measured in arbitrary units per milliliter (AU/mL) (\geq 15.0 AU/mL).^{34 35} GMT and 95% CI were calculated attributing the value of 1.9 AU/mL to undetectable levels (<3.8 AU/mL). FI-GMT was calculated as the ratio of the IgG titre after vaccination to the IgG titre before vaccination. Detection of NAb was performed using the SARS-CoV-2 surrogate virus neutralisation test (sVNT) Kit (GenScript, Piscataway, New Jersey, USA).³⁶ NAb activity was defined as the percentage of inhibition of the interaction between the

receptor-binding domain of the viral spike glycoprotein with the ACE-2 cell surface receptor. Positivity was defined as $\geq 30\%$ inhibition of this linkage.³⁶ The median (IQR) of the percentage of neutralising activity was only calculated for positive samples. C reactive protein (CRP) (by the nephelometric method) was also measured.

Safety outcomes

Disease activity was checked by experienced rheumatologists, blinded to allocation groups, who assessed the following parameters: number of tender joints and number of swollen joints (both in 28 joint count), patient global assessment of disease activity (by Visual Analogue Scale), and evaluator global assessment of disease activity (by Visual Analogue Scale). With these data, CDAI was calculated. With CRP from sera collected on the same day, SDAI and DAS28-CRP were also calculated.

Patients were instructed to fill a structured diary of local and systemic symptoms after each vaccination to explore potential vaccine side effects. Adverse effect severity was classified according to the WHO definition.³⁷ Patients who had suggestive symptoms of COVID-19 infection had nasopharyngeal RT-PCR tests done.

Statistical analysis

The sample size calculation was based on the 2009 non-adjuvanted influenza A/H1N1 primary vaccination in a large cohort of patients with RA under MTX, which induced an SC rate of 46%.³⁸ Expecting an increment of 20% in the MTX-hold group,^{19,20} which should achieve a 66% SC rate, with a 5% alpha error and 80% power (1:1 ratio), the minimum sample would be 96 patients per group.

Categorical variables were presented as number (percentage) and compared using χ^2 or Fisher's exact test, as appropriate. Continuous general data were presented as median (IQR) and compared using t-test or Mann-Whitney test, as appropriate. Data regarding IgG titres and disease activity scores at different time points were analysed using generalised estimating equations with normal marginal distribution and gamma distribution, respectively, and identity binding function assuming first-order autoregressive correlation matrix between moments in the comparison of the two groups, followed by Bonferroni's multiple comparisons. IgG titres were analysed as Napierian

logarithm-transformed data. Multiple regression analyses were performed including SC or presence of NAb at D69 as the dependent variable and as independent variables those with $p < 0.2$ in the univariate analyses. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed using Statistical Package for the Social Sciences V.20.0.

RESULTS

A total of 247 patients with RA fulfilled the profile on electronic chart review and were preselected. After exclusion criteria, 138 patients remained and were randomised, 67 in the MTX-hold group and 71 in the MTX-maintain group (figure 1). During the study, there were nine further exclusions: five RT-PCR-confirmed COVID-19 and four protocol violations. Therefore, the final group for all safety analyses and disease activity evaluation consisted of 60 patients in the MTX-hold and 69 in the MTX-maintain group. For the immunogenicity analyses, patients with positive anti-SARS-CoV-2 serology/NAb (13 (21.7%) vs 14 (20.3%), respectively, $p = 0.848$) at D0 were excluded and the groups consisted of 47 (MTX-hold) vs 55 (MTX-maintain) patients for D0 and D28 analyses. Out of the 47 patients in the MTX-hold group, 10 (21.3%) had a flare on D28 and did not stop MTX the second time; thus, 37 patients finished the complete MTX withdrawal protocol and were regarded for D69 immunogenicity analyses.

The MTX-hold and MTX-maintain groups had similar age and female sex frequencies ($p > 0.05$). Other demographic characteristics, comorbidities, disease duration, baseline disease activity, rheumatoid factor and anticyclic citrullinated peptide positivity, and current therapy did not differ between the two groups ($p > 0.05$), except for the association with prednisone which was more frequent in the MTX-hold group in the safety analyses ($p = 0.021$) (table 1).

Immunogenicity outcomes

Baseline anti-SARS-CoV-2 S1-S2 IgG GMT (2.1 (1.9–2.3) vs 2.0 (1.9–2.1), $p > 0.999$) were similar among the groups. At D69, patients who withdrew MTX after both vaccine shots (MTX-hold, $n = 37$) had higher SC ($p = 0.019$) with a parallel augmentation in GMT ($p = 0.006$) and a higher FI-GMT ($p = 0.007$) in comparison with the MTX-maintain group ($n = 55$) (table 2, figures 2 and 3). For NAb positivity, the difference was not

Table 2 Data on anti-S1/S2 IgG seroconversion rates, anti-SARS-CoV-2 S1/S2 IgG GMT, FI-GMT in titres, frequency of NAb and median percentage of neutralising activity in MTX-hold and MTX-maintain groups at baseline (D0) and after first (D28) and second (D69) dose of vaccine

	After first dose (D28)			After second dose (D69)		
	MTX-hold (n=47)	MTX-maintain (n=55)	P value	MTX-hold (n=37)	MTX-maintain (n=55)	P value
Anti-S1/S2 IgG						
Seroconversion, n (%)	10 (21.3)	2 (3.6)	0.011	29 (78.4)	30 (54.5)	0.019
GMT	5.7 (4.3–7.5)	2.8 (2.3–3.5)	0.002	34.2 (25.2–46.4)	16.8 (11.9–23.6)	0.003
FI-GMT	2.9 (2.2–3.7)	1.4 (1.1–1.7)	<0.001	17.1 (12.6–23.1)	8.1 (5.8–11.4)	0.007
Neutralising antibodies						
NAb positivity, n (%)	11 (23.4)	4 (7.3)	0.027	23 (62.2)	27 (49.1)	0.217
Neutralising activity	41.7 (37.0–46.0)	57.7 (51.8–65.9)	0.133	53 (42–68.8)	51.7 (37.8–62.2)	0.335
MTX-hold: baseline seronegative patients randomised to interrupt MTX after the first and second dose ($n = 47$ at D28 and $n = 37$ at D69 due to 10 patients who flared at D28 and did not withdraw MTX-twice). MTX-maintain: baseline seronegative patients randomised to maintain methotrexate stable throughout the study and who adhered to the protocol ($n = 69$). Seroconversion (SC) is defined as postvaccination titre ≥ 15 AU/mL by Indirect ELISA (LIAISON SARS-CoV-2 S1/S2 IgG). Positivity for NAb was defined as neutralising activity $\geq 30\%$ (cPass sVNT Kit). Frequencies of subjects with SC or positive NAb are presented as number (%) and were compared using a two-sided χ^2 test at prespecified time points (D28 and D69). IgG antibody titres and FI-GMT are expressed as GMT with 95% CI. Data on IgG titres were analysed in logarithm-transformed data using generalised estimating equations with normal marginal distribution and gamma distribution, respectively, and identity binding function assuming first-order autoregressive correlation matrix between moments in the comparison of the two groups, followed by Bonferroni's multiple comparisons. Percentages of neutralising activity among subjects with positive NAb are expressed as median (IQR). FI-GMT and neutralising activity were compared using a two-sided Mann-Whitney U test for comparison between the two groups, at prespecified time points (D28 and D69). All analyses were two-sided. D28, day 28; D69, day 69; FI-GMT, factor increase of geometric mean titre; GMT, geometric mean titre; MTX, methotrexate; NAb, neutralising antibody; RA, rheumatoid arthritis.						

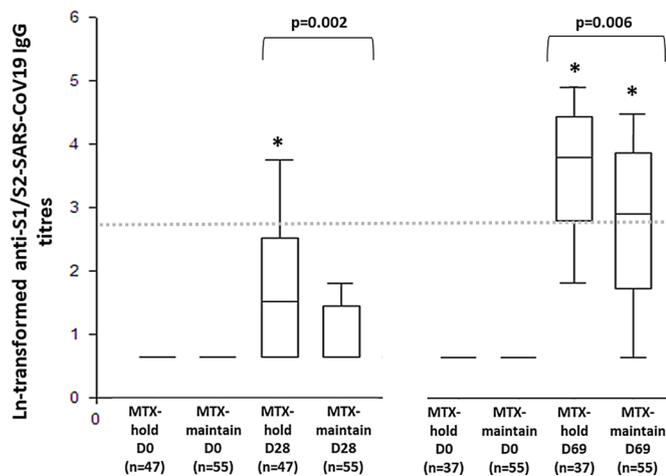


Figure 3 Box plots of anti-S1/S2 IgG titres at baseline after the first and second dose of Sinovac-CoronaVac vaccine in patients with rheumatoid arthritis according to MTX interruption (MTX-hold) or MTX maintenance (MTX-maintain). MTX-hold: baseline seronegative patients randomised to interrupt MTX after the first dose (n=47) and second dose (n=37; due to exclusion of patients who had CDAI >10 at D28 and withdrew MTX only once). MTX-maintain: baseline seronegative patients randomised to maintain methotrexate throughout the study (n=55). Analyses were performed with Napierian logarithm (ln)-transformed data using generalised estimating equations with normal marginal distribution and gamma distribution, respectively, and identity binding function assuming first-order autoregressive correlation matrix between moments in the comparison of the two groups (MTX-hold and MTX-maintain), followed by Bonferroni's multiple comparisons. The mean behaviour of the ln-transformed IgG titres was different in MTX-hold and MTX-maintain (p interaction=0.003 from D0 to D28 and p<0.001 from D0 to D69). Groups were comparable at baseline (p>0.999), but the MTX-hold group had higher mean titres at D28 (p=0.002) and D69 (p=0.006). Mean titres increased at each time point for the MTX-hold group (*p<0.001 from D0 to D28 and from D0 to D69). For the MTX-maintain group, the titres did not increase from D0 to D28 (p=0.423), but increased from D0 to D69 (*p<0.001). All analyses were two-sided. The dotted line denotes the cut-off level for positivity (ln 15 AU/mL=2.71 by Indirect ELISA, LIAISON SARS-CoV-2 S1/S2 IgG). CDAI, Clinical Disease Activity Index; D0, day 0; D28, day 28; D69, day 69; MTX, methotrexate.

significant (p=0.217), as also occurred for NAb activity (p=0.335) (table 2, figure 2).

The additional analysis at D28, after only one dose of vaccine, prior to exclusion due to flares (MTX-hold, n=47; MTX-maintain, n=55), showed that the MTX-hold group had higher SC rates (p=0.011), NAb positivity (p=0.027), GMT (p=0.002) and FI-GMT (p<0.001) (table 2, figures 2 and 3).

Assessment of factors associated with immunogenicity

In a further analysis combining both groups, the comparison of patients who had SC and those who did not seroconvert showed that older age, age ≥ 60 years and combination with leflunomide were negatively associated with SC, while completing the MTX withdrawal protocol (withdrawing MTX twice) was positively related to it. For NAb, only older age and age ≥ 60 years were negatively associated with presence of NAb (table 3). In multivariate analyses, older age (OR 0.71 (0.56–0.89) for each 5-year interval, p=0.003) and age ≥ 60 years (OR 0.16 (0.05–0.50), p=0.001) persisted negatively associated with SC, while MTX

withdrawal twice (OR 4.6 (1.43–15.04), p=0.010) was positively associated with SC.

Disease activity

For these evaluations, the groups consisted of 60 (MTX-hold) and 69 (MTX-maintain) patients, including those with positive baseline IgG/NAb. Longitudinally, CDAI (p=0.144), SDAI (p=0.117), DAS28-CRP (p=0.718) and CRP (p=0.410) had the same behaviour in the MTX-hold and MTX-maintain groups, with worsening at D28 (p<0.001 for CDAI, SDAI and DAS28-CRP; p=0.027 for CRP), but not from D28 to D69 (p>0.999 for CDAI, SDAI and CRP; p=0.602 for DAS28-CRP) (figure 4).

At D28, no differences appeared regardless of flare definition (p>0.05) (table 4). At D69, the groups had similar rates of flares based on DAS28 variations (p=0.188). However, the MTX-hold group had more flares according to the CDAI >10 criteria (p=0.011) and more patients reported disease worsening (p=0.044). Evaluating flares at any time during the study, the same pattern was observed: no differences between the groups based on DAS28 variation (p=0.094) and a higher number of flares based on CDAI (p=0.024) and patient perception of disease worsening (p=0.022). The magnitude of variation of CDAI among patients who flared was similar between the groups (9 (4–13.5) vs 7 (4.8–10.8), p=0.456).

Vaccine side effects

Approximately half of the patients reported mild side effects, without differences between the groups. After the second dose of vaccine, myalgia (10 (16.7%) vs 3 (4.3%), p=0.037) and vertigo (7 (11.7%) vs 1 (1.5%), p=0.024) were more frequent in the MTX-hold group (online supplemental table 1).

DISCUSSION

To the best of our knowledge, this is the first randomised study to compare the impact of MTX withdrawal on the immunogenicity and disease activity of any COVID-19 vaccine in patients with RA. We demonstrated that temporary suspension is effective in increasing IgG SC and GMT levels. The observed comparable disease activity variation in the MTX-hold and MTX-maintain groups suggests that this strategy is effective in improving anti-SARS-CoV-2 IgG response, however with an increase in flare rates after the second dose of vaccine.

The study has some strengths, such as the inclusion of patients in remission/low disease activity and low prednisone doses, providing a safer condition for MTX withdrawal.²⁴ In addition, the randomised clinical design with allocation concealment, the blind evaluation of disease activity status and use of validated RA scores^{28 29} allowed a precise analysis of flares. Moreover, the balanced distribution of demographic profile, disease features and treatment was relevant since these are known factors to influence vaccine immunogenicity and flares.^{10–22} The final small sample size of the study is related to the high rate of refusals to participate and the rigorous exclusion criteria. Such small sample size underpowered the trial and is an important limitation, precluding a definite conclusion about our findings. However, the larger than expected benefit of MTX withdrawal on IgG serology allowed the identification of a significant difference between groups for SC and GMT.

We provide herein novel evidence of an increment of approximately 25% in anti-SARS-CoV-2 antibodies induced by the Sinovac-CoronaVac vaccine with temporary MTX withdrawal. Such improvement is very similar to the 20% increase first

Table 3 Baseline characteristics of patients with rheumatoid arthritis who finished the study protocol with regard to IgG antibodies and NAB after two doses of the Sinovac-CoronaVac vaccine (n=92)

	Positive IgG after two doses (n=59)	Negative IgG after two doses (n=33)	P value	Positive NAB after two doses (n=50)	Negative NAB after two doses (n=42)	P value
Demographics						
Current age, years	55 (42.5–64.5)	66 (59–69)	0.001	52.5 (40.5–67)	62.5 (55.25–69)	0.006
Age >60 years	20 (33.9)	24 (72.7)	<0.001	18 (36.0)	26 (61.9)	0.013
Female sex	53 (89.8)	31 (93.9)	0.707	47 (94.0)	37 (88.1)	0.462
Caucasian race	29 (49.2)	15 (45.5)	0.733	23 (46.0)	21 (50)	0.702
Baseline disease activity						
CDAI	5.0 (3.0–8.0)	6.0 (4.0–8.0)	0.469	6.0 (3.0–8.0)	6.0 (3.0–8.0)	0.829
SDAI	6.1 (3.1–9.1)	7.1 (4.1–9.3)	0.339	6.1 (3.2–9.4)	7.0 (3.8–9.2)	0.763
DAS28-CRP	2.43 (1.82–3.05)	2.27 (2.11–2.90)	0.843	2.45 (1.83–3.10)	2.33 (1.98–2.97)	0.742
CRP	3.3 (1.0–9.2)	8.9 (7.3–9.8)	0.154	1.6 (0.9–9.2)	7.6 (3.8–9.8)	0.161
TJC	1 (0–2)	0 (0–1)	0.086	1 (0–2)	0 (0–1)	0.259
SJC	0 (0–1)	1 (0–1)	0.297	1 (0–1)	1 (0–1)	0.727
PGA	3 (1–4)	3 (2–4)	0.491	3 (1–4)	3 (1–4)	0.570
EGA	1 (1–2)	1 (1–2)	0.223	1 (1–2)	1 (1–2)	0.766
Current therapy						
Prednisone	25 (42.4)	12 (36.4)	0.573	18 (38.0)	19 (45.2)	0.368
Prednisone dose	5 (2.5–5)	5 (5–5)	0.406	5 (2.5–5)	5 (5–5)	0.278
MTX-hold protocol	29 (49.2)	8 (24.2)	0.019	23 (46.0)	14 (33.3)	0.217
MTX monotherapy	20 (33.9)	5 (15.2)	0.053	15 (30.0)	10 (23.8)	0.506
Leflunomide	8 (13.6)	11 (33.3)	0.024	7 (14.0)	12 (28.6)	0.086
Other sDMARD	16 (27.1)	6 (18.2)	0.335	15 (30.0)	7 (16.7)	0.135
Abatacept	6 (10.2)	7 (21.2)	0.145	4 (8)	9 (21.4)	0.066
Other bDMARD	12 (20.3)	8 (24.2)	0.663	13 (26.0)	7 (16.7)	0.280

Results are expressed in median (IQR) and n (%).

Continuous data were compared using Mann-Whitney U test and categorical variables with χ^2 or Fisher's exact test, as appropriate, as two-sided analyses.

Flare was defined as CDAI >10.

bDMARD, biologic disease-modifying antirheumatic drugs (tumour necrosis factor inhibitors and tocilizumab); CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28-CRP, Disease Activity Score with 28 joints and C reactive protein; EGA, evaluator global disease assessment; MTX, methotrexate; NAB, neutralising antibody; PGA, patient global disease assessment; SDAI, Simplified Disease Activity Index; sDMARD, synthetic disease-modifying antirheumatic drugs (sulfasalazine, hydroxychloroquine, tofacitinib); SJC, swollen joint count; TJC, total joint count.

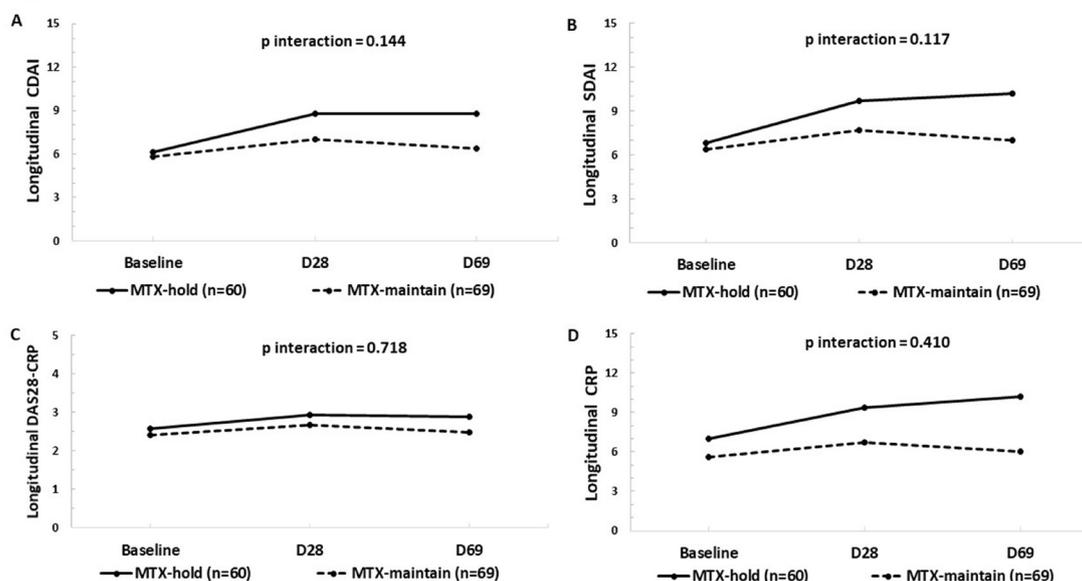


Figure 4 Analyses of continuous disease activity parameters at baseline and after the first and second dose of the Sinovac-CoronaVac vaccine in patients with rheumatoid arthritis according to MTX interruption (MTX-hold) or MTX maintenance (MTX-maintain). MTX-hold: baseline seronegative patients randomised to interrupt MTX after the first and second dose, excluding those who had CDAI >10 at D28 and withdrew MTX only once. MTX-maintain: baseline seronegative patients randomised to maintain MTX throughout the study and who adhered to the protocol. Data regarding disease activity parameters are shown as means and were analysed using generalised estimating equations with normal marginal distribution and gamma distribution, respectively, and identity binding function assuming first-order autoregressive correlation matrix between moments (D0, D28 and D69) in the comparison of the two groups (MTX-maintain and MTX-hold), followed by Bonferroni's multiple comparisons. The mean behaviour of CDAI (A), SDAI (B), DAS28-CRP (C) and CRP (D) was similar in MTX-hold and MTX-maintain throughout the study ($p=0.144$, $p=0.117$, $p=0.718$ and $p=0.410$, respectively), increasing after the first dose ($p<0.001$, $p<0.001$, $p<0.001$ and $p=0.021$, respectively) and remaining stable after the second dose ($p>0.999$, $p>0.999$, $p=0.602$ and $p>0.999$, respectively). CDAI, Clinical Disease Activity Index; CRP, C reactive protein; D28, day 28; D69, day 69; DAS28-CRP, Disease Activity Score with 28 joints; MTX, methotrexate; SDAI, Simplified Disease Activity Index.

Table 4 Disease activity analyses after the first and second dose of the Sinovac-CoronaVac vaccine in patients with rheumatoid arthritis

		After first dose (D28)	P value	After second dose (D69)	P value	At any moment	P value
Δ DAS28-CRP \geq 1.2 or Δ DAS28-CRP \geq 0.6 + DAS28-CRP $>$ 3.2	MTX-hold (n=60)	7 (11.7)	0.576	12 (20)	0.188	22 (36.7)	0.094
	MTX-maintain (n=69)	6 (8.7)		8 (11.6)		16 (23.2)	
CDAI $>$ 10	MTX-hold (n=60)	13 (21.7)	0.122	19 (31.7)	0.011	23 (38.3)	0.024
	MTX-maintain (n=69)	8 (11.6)		9 (13)		14 (20.3)	
Patient impression of disease flare	MTX-hold (n=60)	6 (10)	0.577	8 (13.3)	0.044	14 (23.3)	0.022
	MTX-maintain (n=69)	5 (7.2)		2 (2.3)		6 (8.7)	

For safety analyses, all patients who adhered to the protocol were included.

Results are expressed in n (%) and compared with χ^2 or Fisher's exact test, as appropriate, as two-sided analyses.

CDAI, Clinical Disease Activity Index; D28, day 28; D69, day 69; DAS28-CRP, Disease Activity Score with 28 joints and C reactive protein; MTX, methotrexate; Δ DAS28-CRP, variation of DAS28-CRP score.

described regarding MTX discontinuation for 2 weeks after influenza vaccine,²⁰ and could therefore partially reduce the deleterious effects in SC induced by MTX reported for the Sinovac-CoronaVac vaccine¹² and BNT162b2 mRNA COVID-19.^{18, 21} This immunogenicity enhancement was observed even with a high frequency of combined DMARD therapy and corticosteroids, factors that could further impair immune response to COVID-19 vaccine.^{12, 18} Importantly, MTX dose was comparable between the groups and all patients had doses above 10 mg/week, in line with the observation that only patients with doses greater than 7.5 mg/week benefited from MTX withdrawal after influenza vaccine.²⁰

Concerning combination therapy, the distribution of drugs was alike between the groups, equalising possible additional harmful effects of different DMARDs. We also deliberately excluded patients under rituximab due to well-known effect on humoral immunogenicity and the heterogeneity of phases of treatment cycles.¹¹⁻¹⁷ In this context, multiple regression analyses revealed that neither combined DMARD nor prednisone impacted the benefit of MTX temporary discontinuation.

Safety related to vaccine and MTX withdrawal intervention was carefully assessed and included several composite measures. Longitudinally, CDAI, SDAI, DAS28-CRP and CRP had similar behaviours between the groups, increasing after the first dose and remaining stable after the second dose. In fact, the increase in disease activity measures, even in the MTX-maintain group, is in accordance with the 20% flare rate of SDAI after BNT162b2 mRNA vaccination.¹¹

Considering the rate of flares, the MTX-hold and MTX-maintain groups were also comparable with regard to the DAS28-CRP criteria. The similar criteria with the Disease Activity Score in 28 joints with erythrocyte sedimentation rate (DAS28-ESR) was used in previous influenza vaccine MTX withdrawal studies^{19, 20} and performed better than other DAS28 flare definitions according to the Outcome Measures in Rheumatology (OMERACT) initiative.³² In our trial, however, CDAI $>$ 10 showed to be more sensitive than DAS28-CRP, detecting significantly more flares in the MTX-hold in comparison with the MTX-maintain group at D69 and at any time. The similar longitudinal behaviour of composite measures/CRP between groups and the rate of flares at D69 in the MTX-hold group may have been downplayed by the safety strategy of not withdrawing MTX twice in patients who flared at D28. However, the short interval between vaccine doses and the close repetition of MTX holding possibly favoured the occurrence of flares.

The Sinovac-CoronaVac vaccine was well tolerated, with no severe side effects. However, the MTX-hold group reported a higher frequency of myalgia and vertigo. The former

manifestation may be associated with the vaccine or related to underlying disease activity.

In conclusion, this study provides novel data that 2-week MTX withdrawal after each vaccine dose improves anti-SARS-CoV-2 IgG response to the Sinovac-CoronaVac vaccine. The increased flare rates after second MTX withdrawal may be due to the short-term interval between vaccine doses. This strategy requires close surveillance and shared decision making due to the possibility of disease activity worsening.

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Contributors CSRA, ACM-R, CGSS, EFNY, NEA and EB conceived and designed the study. EB is responsible for the overall content as the guarantor. CSRA, ACM-R, CGSS, KRB, DSD, AYS, CAS, EFNY, TP, LdVKK, GZ, RMRP, CAS and NEA reviewed the charts and selected and invited potential patients for the study. CSRA and MSRS were the two non-blinded researchers and were responsible for randomisation, enrolment, explanation of the procedures, informed consent, safety surveillance and patient follow-up by telephone for adherence purposes. ACM-R, CGSS, KRB, DSD and AYS were the blinded investigators responsible for disease activity measures. LdVKK, TP and EB organised and supervised blood collection and vaccination protocol. SGP supervised serum processing, SARS-CoV-2-specific antibody ELISA/neutralisation assays and SARS-CoV-2 RT-PCR. ACM-R, CGSS, KRB, DSD, AYS, EFNY, SGP, CAS, TP, LdVKK, GZ, RMRP, NEA and EB participated in data collection and analysis and clinical data management. CSRA, ACM-R, CGSS, EFNY, SGP, CAS, LdVKK, NEA and EB were responsible for writing and revision of the manuscript. All authors helped edit the manuscript.

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