Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Infection and Public Health 15 (2022) 685–688

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: www.elsevier.com/locate/jiph

COVID-19 breakthrough infections in rheumatic diseases patients after vaccination

Abeer N. Alshukairi[a,b,*], Awad Al-Omari[b,c], Abdurahman Albeity[a], Thamir A. Alandijany[d,e], Ahmed M. Hassan[d,e], Sherif A. El-Kafrawy[d,e], Ashraf Dada[b,f], Mohammad K. Al Hroub[g], Aiman El-Saed[h], Lina S. Bissar[a,i], Radwan M. Daghmush[a], Saeed M.G. Al-Ghamdi[a], Stanley Perlman[j], Esam I. Azhar[d,e,**], Hussein Halabi[a]

[a] Department of Medicine, King Faisal Specialist Hospital and Research Center, Jeddah, Kingdom of Saudi Arabia
[b] College of Medicine, AlFaisal University, Riyadh, Kingdom of Saudi Arabia
[c] Department of Critical Care, Dr Sulaiman Al Habib Medical Group, Riyadh, Kingdom of Saudi Arabia
[d] Special Infectious Agents Unit – BSL3, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
[e] Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia
[f] Department of Pathology and Laboratory Medicine, King Faisal Specialist Hospital and Research Center, Jeddah, Kingdom of Saudi Arabia
[g] Department of Infection Control and Hospital Epidemiology, King Faisal Specialist Hospital and Research Center, Jeddah, Kingdom of Saudi Arabia
[h] Department of Infection Prevention and Control, King Abdulaziz Medical City, Riyadh, Kingdom of Saudi Arabia
[i] Volunteer Faculty, Irvine School of Medicine, University of California, California, USA
[j] Department of Microbiology and Immunology, Department of Pediatrics, University of Iowa, Iowa City, USA

ARTICLE INFO

Article history:
Received 3 February 2022
Received in revised form 27 April 2022
Accepted 9 May 2022

Keywords:
Breakthrough infection
COVID-19
Outcome
Rheumatic diseases
Vaccine

ABSTRACT

Background: Rheumatic diseases patients receiving Rituximab had severe COVID-19 disease. Although they had impaired humoral immune responses following COVID-19 vaccine, they had preserved cellular immune responses. Waning of COVID-19 antibody responses was observed within six months post vaccination among immunocompromised patients. Recent reports showed fatal outcome of breakthrough SARS-CoV-2 infections among vaccinated high-risk rheumatic diseases patients receiving Rituximab. SAR-CoV-2 serological tests were not performed.
Objective: Evaluation of COVID-19 vaccine humoral responses and breakthrough infections among low risk fully vaccinated rheumatic patients during the Delta Variant Era.
Methods: A case series of 19 fully vaccinated patients with rheumatic diseases were followed to determine post vaccine SARS-CoV-2 neutralizing antibody titers and to monitor the development of breakthrough infections up to eight months post vaccine at our tertiary care center in Jeddah, Saudi Arabia from 1st April until 30th November 2021.
Results: The mean age of patients was 49 years old. 10% of patients were receiving Rituximab. 73% of patients had positive SARS-CoV-2 serological testing post second vaccine. Two mild breakthrough COVID-19 infections were diagnosed six months post second dose of vaccine. Patients were less than 65 years, did not receive Rituximab, did not have interstitial lung diseases and had positive post vaccine serological testing.
Conclusions: We demonstrated high SARS-CoV-2 neutralizing antibodies seroprevalence and self-limiting breakthrough infections in low risk rheumatic diseases patients during the Delta Era. Future studies are needed to study the outcome of rheumatic diseases patients in the Era of Omicron in view of viral immune escape responses.

Introduction

The effect of COVID-19 on patients with rheumatic diseases is complex; while age and comorbidities are associated with severe COVID-19 disease, the effect of biological therapies on COVID outcome remains unclear [1]. The use of Rituximab is associated with severe disease and prolonged hospitalization in patients with

* Correspondence to: Adult Infectious Diseases Section, Department of Medicine, King Faisal Specialist Hospital and Research Center,PO BOX 40047, Jeddah 21499, Kingdom of Saudi Arabia.
** Correspondence to: Special Infectious Agents Unit – BSL3, King Fahd Medical Research Center, Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.
E-mail addresses: abeer.alshukairi@gmail.com (A.N. Alshukairi), eazhar@kau.edu.sa (E.I. Azhar).

https://doi.org/10.1016/j.jiph.2022.05.005
1876-0341/

rheumatic diseases infected with COVID-19 [2]. COVID-19 vaccine is recommended for patients with autoimmune inflammatory diseases being safe and immunogenic; even in patients receiving B cell depleting agents, they had impaired humoral but preserved cellular immune responses to COVID-19 vaccine [3]. As more cases of SARS-CoV-2 variants of concerns are being detected worldwide, more cases of post COVID-19 vaccine breakthrough infections are being diagnosed in patients with variable disease severity depending on the extent of immunosuppression [4]. Cook et al. described the clinical characteristics and outcomes of 16 breakthrough COVID-19 infections in BioNTech BNT162b2 vaccinated patients with high risk rheumatic diseases (31% of patients received Rituximab). 6 (38%) patients required hospitalization, 1 (6%) patient required ventilation and 2 (13%) patients died. SARS-CoV-2 serological status was not described [5].

Our objective was to determine post vaccine SARS-CoV-2 neutralizing antibody titers in a case series of patients with low risk rheumatic diseases and follow them for breakthrough COVID-19 infection, 8 months post second dose of vaccine, during the Delta variant Era.

Methods

Study type

Our study was a prospective descriptive analysis of a case series of 19 patients in a single center, a tertiary care hospital, King Faisal specialist hospital and research center in Jeddah, Saudi Arabia, from 1st April 2021 until 30th November 2021.

Inclusion criteria

Patients with systemic rheumatic diseases who completed two doses of BioNTech BNT162b2, those who agreed to participate in the study and patients who provided blood for SARS-CoV-2 antibody testing at least 14 days following the second dose of BioNTech BNT162b2 were included. Patients who were fully immunized were followed for the development of breakthrough infections, eight months post second dose of vaccine.

Exclusion criteria

We excluded patients who received non BioNTech BNT162b2COVID-19 vaccines, those who received one dose BioNTech BNT162b2COVID-19 of and patients who presented for blood collection less than 14 days post second dose of vaccine.

It was difficult to have the same time frame for post vaccine serological tests as patients were vaccinated at different time points in different hospitals and most of them were not available in town for blood collection.

Laboratory tests

5 ml of whole blood were collected from the patients, transported directly to our CAP accredited lab, where the blood centrifuged for 5 min by 3500 rpm using Eppendorf centrifuge, Hamburg/Germany. Sera were manually pipetted each in 1.5 ml Eppendorf tube and stored immediately on − 30 C for serology testing.

SARS-CoV-2 neutralizing antibodies were detected using in house ELISA and Micro-neutralization (MN) assay as previously described [6].

In-house ELISA

Antigen coating of flat bottom microtiter plates (Immulon® 2 HB, USA) was performed overnight at 4 °C with 100 ng per well of SARS-CoV-2 (2019-nCoV) spike S1 +S2 ECD-His recombinant protein (Sino Biological, China). Subsequently, the plates were subjected to three washes with PBS containing 0.1% Tween 20 (PBST) prior to blocking in PBST containing 5% skimmed milk for 1 h at room temperature. This step was followed by three washes with PBST. Sera were diluted at 1:100 dilutions in PBST containing 5% skimmed milk, added at 100 µl volume, and incubated for an hour at 37 °C. Following three washes with PBST, 100 µl of secondary antibody (goat KPL peroxidase-labeled antibodies to human IgG; Seracare, USA) at a dilution of 1:64,000 were added and allowed to incubate for an hour at 37 °. The plates were subjected to three washes with PBST. Then, 100 µl of substrate (3,3′,5,5′-Tetramethylbenzidine (TMB); Seracare, USA) were added for 5 min for color development prior to addition of 100 µl of 1 N hydrochloric acid (HCL) to stop the reaction. Using Elx 800 bioelisa Reader (Biokit, Spain), the optical density was measured at 450 nm (OD_{450}). OD_{450} values of > 0.27 were considered positive. Negative and positive controls utilized in this assay were sera of a healthy blood donor and a recovered COVID-19 patient known to have neutralizing IgG antibodies, respectively. The in-house ELISA provides 100% sensitivity, 98.4% specificity, 98.8% agreement, and high overall accuracy.

Micro-neutralization (MN) assay

MN assay was used as a confirmatory test to determine the presence of SARS-CoV-2-specific neutralizing antibodies. The local SARS-CoV-2 clinical isolate (SARS-CoV-2/human/SAU/85791 C/2020) (Genbank accession number MT630432.1) was utilized in this assay. The virus stock was propagated and titrated by Median Tissue Culture Infectious Dose ($TCID_{50}$) on African green monkey kidney cells Vero E6 (ATCC® CRL-1586™). Sera were subjected to heat inactivation at 56 °C for 30 min. Then, samples were serially diluted in DMEM containing 2% fetal calf serum (DMEM-FCS) and added with equal volume of DMEM-FCS containing 100 $TCID_{50}$ of SARS-CoV-2 on confluent cells. The cells were incubated at 37 °C in 5% CO2 until extensive cytopathic effect was observed (typically for 3–4 days). Uninfected cells and SARS-CoV-2 infected cells in the absence of human serum were utilized as controls. MN titers of ≥ 1:20 were considered positive.

Data sources

Electronic charts were reviewed for patients' demographics and clinical characteristics. Ministry of health data base was reviewed for details of vaccination, type of vaccine, timing of vaccine and breakthrough COVID-19 infections. Patients with breakthrough COVID-19 infections were called by phone to determine disease severity and outcome.

Statistical analysis and variables

Categorical data were presented as frequencies and percentages while continuous data were presented as mean and standard deviation (SD). Mann Whitney test was used to examine differences in continuous variables between patients with positive and negative serological testing. SPSS (Version 25.0. Armonk, NY: IBM Corp) was used for all statistical analyses.

Results

A total of 19 patients was included in our study. The mean age was 49.1 ± 11.1 years and 89.5% were females. 37% of patients had

Table 1
Summary of the patients' characteristics.

	N (%) Mean ± SD
Age	
Mean ± SD	49.1 ± 11.1
≤ 50 years	9 (47.4%)
> 50 yaers	10 (52.6%)
Gender	
Males	2 (10.5%)
Females	17 (89.5%)
SARS-CoV-2 Antibody tests (ELISA and Neutralizing antibodies)	
Negative	5 (26.3%)
Positive	14 (73.7%)
Days from the second dose of BioNTech BNT162b2 vaccine to SARS-CoV-2 antibody tests	37.0 ± 23.8
Titer of SARS-CoV-2 neutralizing antibodies	462.9 ± 483.0
Breakthrough SARS-CoV-2 infection after BioNTech BNT162b2 vaccine	
No	17 (89.5%)
Yes	2 (10.5%)
COVID-19 before BioNTech BNT162b2 vaccine	
No	18 (94.7%)
Yes	1 (5.3%)
Diagnosis	
Rheumatoid arthritis	7 (36.8%)
Systemic lupus erythematosus	6 (31.6%)
Psoriatic arthritis	2 (10.5%)
Behcet disease	1 (5.3%)
Giant cell arteritis	1 (5.3%)
Relapsing polychondritis	1 (5.3%)
Spondyloarthritis	1 (5.3%)
Medications	
Prednisolone	7 (36.8%)
Methotrexate	6 (31.6%)
Tocilizumab	3 (15.8%)
Hydroxychloroquine	3 (15.8%)
Adalimumab	2 (10.5%)
Rituximab	2 (10.5%)
Other medications	4 (21.1%)

rheumatoid arthritis and 32% had systemic lupus erythematosus. Two (10.5%) patients received rituximab and 6 (31.6%) patients received methotrexate. All patients received two doses of BioNTech BNT162b2 vaccine. The mean time between the second vaccine and serological testing was 37.0 ± 23.8 days. A positive COVID-19 serological test was identified in 14 (73.7%) of patients. The titer of neutralizing antibodies ranged between 40 and 1280, and the mean titer was 462.9 ± 483.0. Five out 19 (26.3%) patients had negative serological testing. The mean age of patients who had positive serological testing was 46.7 ± 10.3 years, while the mean age of patients who had negative serological testing was 55.6 ± 11.9 years (p = 0.164). The mean time between the second vaccine and serological testing was 36.9 ± 22.0 days in patients who had positive serological tests while the mean time between the second vaccine and serological testing was 37.2 ± 31.5 days in patients who had negative serological testing (p = 0.889). Two (10.5%) patients developed mild SARS-CoV-2 infection, 6 months post second dose of vaccine. These patients were less than 65 years old, did not receive rituximab and had detectable antibody titers post vaccine. None of the patients with negative serological testing developed breakthrough infections (Tables 1 and 2).

Discussion

In our case series of low risk rheumatic diseases, we demonstrated 73% seroprevalence of SARS-CoV-2 and mild breakthrough infections in the Era of Delta variant.

A prospective cohort study in Netherlands showed that 92% of patients with auto immune diseases had positive SARS-CoV-2 serological testing after two doses of vaccine [7]. Our results showed that 10% of patients developed breakthrough COVID-19 infections six months following the second dose of vaccine. Levin et al. described waning of antibody response post BioNTech BNT162b2 vaccination over six months especially in immunocompromised patients [8]. A third booster dose of COVID-19 vaccine is currently recommended for immunocompromised patients, 28 days post second dose vaccine [9].

In our study, self-limiting breakthrough infections were observed in patients who were less than 65 years old, did not have interstitial lung diseases, did not receive B cell depleting agents and had positive SARS-CoV-2 serological testing post second vaccine dose. On the other hand, Cook et al. described fatal breakthrough infections in patients who received ritoximab and had chronic lung diseases [5].

We did not observe any breakthrough infections among patients with negative SARS-CoV-2 serological testing. We postulated that the adherence of patients to the use of universal face masks

Table 2
Demographics, clinical and serological characteristics of patients.

Number	Age	Gender	Days between vaccine and serology	Neutralizing SARS-CoV-2 Antibody results	Titer	Post-vaccine COVID 19 infection	Pre-vaccine COVID 19 infection	Diagnosis	Medication
1	42	Female	21	Positive	1280	No	No	RA	MXT, HQ
2	31	Female	21	Positive	1280	No	No	SA	ADL
3	50	Female	16	Positive	1280	No	Yes	SLE	PRD
4	53	Female	20	Positive	640	No	No	RA	PRD, TCZ
5	54	Female	20	Positive	640	No	No	SLE	HQ
6	52	Male	55	Positive	320	Yes	No	PA	SKB
7	53	Male	47	Positive	320	Yes	No	SLE	HQ
8	36	Female	25	Positive	160	No	No	SLE	MXT
9	55	Female	47	Positive	160	No	No	GCA	PRD, MXT
10	47	Female	25	Positive	160	No	No	RA	ETC
11	55	Female	52	Positive	80	No	No	SLE	None
12	41	Female	56	Positive	80	No	No	BD	PRD, AZN
13	25	Female	19	Positive	40	No	No	SLE	PRD, MXT, RXB
14	60	Female	92	Positive	40	No	No	RA	TCZ
15	60	Female	34	Negative	NA	No	No	RA	RXB
16	49	Female	14	Negative	NA	No	No	RP	MMF
17	60	Female	24	Negative	NA	No	No	PA	MXT
18	39	Female	22	Negative	NA	No	No	RA	PRD, TCZ
19	70	Female	92	Negative	NA	No	No	RA	PRD, MXT, ADL

Abbreviation: Days between vaccine and serology: days from second dose of COVOD-19 vaccine to SARS-CoV-2 serology, NA: not applicable, RA: rheumatoid arthritis, SLE: systemic lupus erythematosus, PA: psoriatic arthritis, BD: Behcet disease, GCA: giant cell arteritis; RS: relapsing polychondritis, SA: spondyloarthritis, PRD: prednisolone, MXT: methotrexate, TCZ: tocilizumab, HQ: hydroxychloroquine, ADL: adalimumab, MMF: mycophenolate mofetil, AZN: azathioprine, ETC: Etanercept, RXM: Rituximab, SKB: Secukinuma

contributed to the prevention of COVID-19 transmission in Saudi Arabia [10].

Our study was limited by small sample size, lack of a control group, lack of serial serological testing for each patient and unavailability of results of whole genome sequence for COVID-19 isolates of the breakthrough infections. The sample size was small to determine risk factors for breakthrough COVID-19 infections such as age, gender, type of rheumatological diseases, comorbidities, medications, and SARS-CoV-2 neutralizing antibodies.

Delta variant was the predominant COVID-19 strain in Saudi Arabia at the time of our study [11]. Although breakthrough infections were mild in our study of low risk rheumatic patients during the Delta variant Era, future studies are needed to evaluate the outcome of breakthrough infections in rheumatic disease patients during the Omicron variant in view of viral immune escape responses [12].

Ethical approval

This research was approved by the institutional research board, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia: IRB-2020-54. Patients were consented and agreed to participate in the study.

Funding

The research was funded by Jameel Fund for Infectious Disease Research and Innovation in Saudi Arabia, grant number (8-3) 2120191.

Conflict of interest

None.

Data Availability

Details of data can be provided when requested.

Acknowledgment

We would like to thank our patients who contributed to this research. The authors acknowledge the generous charitable donation from the Late Sheikh Ibraheem Ahmed Azhar in the form of reagents and supplies as a contribution to the scientific research community. The authors would like to thank the funding support of Jameel Fund for Infectious Disease Research and Innovation in Saudi Arabia, grant number (8-3)2120191. We appreciate the technical support of King Abdulaziz University and King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia. Special thanks for Dr Edward Cupler and Dr Ashraf Dada for their tremendous efforts to support our research projects during COVID-19 pandemic at King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia. We would like to thank Lama Hefni, research coordinator, department of Medicine, King Faisal Specialist Hospital and Research Centre, Jeddah, Saudi Arabia for her outstanding care to supervise the progress of our research projects.

Contribution

All authors contributed equally to this research; study concept, data acquisition, data analysis, performing the experiment, writing and reviewing the manuscript.

Patient consent for publication

Not applicable.

References

[1] Hyrich KL, Machado PM. Rheumatic disease and COVID-19: epidemiology and outcomes. Nat Rev Rheuma 2021;17(2):71–2.
[2] Avouac J, Drumez E, Hachulla E, et al. COVID-19 outcome in patients with inflammatory rheumatic and musculoskeletal diseases treated with rituximab: a cohort study. Lancet Rheuma 2021;3:e419–26.
[3] Prendecki M, Clarke C, Edwards H, et al. Humoral and T-cell responses to SARS-CoV-2 vaccination in patients receiving immunosuppression. Ann Rheum Dis 2021;80(10):1322–9.
[4] Lipsitch M, Krammer F, Regev-Yochay G, et al. SARS-CoV-2 breakthrough infections in vaccinated individuals: measurement, causes and impact. Nat Rev Immunol 2021:1–9. https://doi.org/10.1038/s41577-021-00662-4
[5] Cook C, Patel NJ, D'Silva KM, et al. Clinical characteristics and outcomes of COVID-19 breakthrough infections among vaccinated patients with systemic autoimmune rheumatic diseases. Ann Rheum Dis 2021. annrheumdis-2021-221326.doi.10.1136.
[6] Alshukairi AN, El-Kafrawy SA, Dada A, et al. Re-infection with a different SARS-CoV-2 clade and prolonged viral shedding in a hematopoietic stem cell transplantation patient. Int J Infect Dis 2021;110:267–71.
[7] Boekel L., Steenhuis M., Hooijberg F. et al. Antibody development after COVID-19 vaccination in patients with autoimmune diseases in the Netherlands: a substudy of data from two prospective cohort studies. Lancet Rheumatol 2021August; doi.org/10.1016/S2665–9913(21)00222–8.
[8] Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 COVID-19 vaccine over 6 months. N Eng J Med 2021;385(24):e84https://doi.org/10.1056/NEJMoa2114583. Epub 2021 Oct 6.
[9] Prevention CfDCa. COVID-19 Vaccines for Moderately to Severely Immunocompromised People. Accessed 17 August, 2021. ⟨https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/immuno.html⟩.
[10] Meo SA, Alqahtani SA, Aljedaie GM, et al. Face masks use and its role in retaining the spread of COVID-19 pandemic in Saudi Arabia: knowledge, attitude and practices based cross sectional study. Front Public Health 2022(9). https://doi.org/10.3389/fpubh.2021.818520
[11] Alhamlan FA-QA, Obeid D, et al. SARS-CoV-2 delta variant predominant at a tertiary-care hospital in Saudi Arabia. Res Sq 2021. https://doi.org/10.21203/rs.3.rs-779549/v1
[12] Hu J, Peng P, Cao X, et al. Increased immune escape of the new SARS-CoV-2 variant of concern Omicron. Cell Mol Immmunol Online 2022;11. https://doi.org/10.1038/s41423-021-00836-z