J Rheum Dis 2023;30(1):58-64
https://doi.org/10.4078/jrd.22.0027
pISSN: 2093-940X, eISSN: 2233-4718

Case Report

Development of Spondyloarthritis After COVID-19 in HLA-B27-Positive Monozygotic Twins: Case Reports With Single Cell Transcriptome Profiling

Minae Oh, M.D.[1]*, Jung Gon Kim, M.D., M.S.[2]*, In-Pyo Baek, M.S.[3], Ji Hyeon Ju, M.D., Ph.D.[1,3]

[1]Division of Rheumatology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, [2]Division of Rheumatology, Department of Internal Medicine, Inje University Ilsan Paik Hospital, Goyang, [3]YiPSCELL, Inc., Seoul, Korea

A subset of spondyloarthritis (SpA) called 'reactive arthritis' is triggered by causal pathogens, usually bacteria related to venereal disease or gastrointestinal infection. During the outbreak of coronavirus disease 2019 (COVID-19), there have been case reports about SpA after COVID-19, but the causality is still elusive. We described cases of 23-year-old monozygotic twins both diagnosed with SpA after COVID-19. The probable linkage between SpA and COVID-19 was elaborated with our cases as well as literature reviews. Of note, shared genetic traits by monozygotic twins, particularly HLA-B27 positivity, might have contributed to their susceptibility to COVID-19-induced SpA. Moreover, single-cell transcriptome analysis revealed that the transcriptomic profile of peripheral compartment of SpA after COVID-19 was distinctive from that of typical radiographic axial SpA as shown by differential expression of ribosomal protein S26 (RPS26) and small nucleolar RNA host gene 5 (SNHG5) in nearly all subsets of peripheral blood mononuclear cells.

Keywords: Arthritis, reactive; COVID-19

INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) in December 2019 has caused the global crisis in public health [1]. Although COVID-19 is a form of respiratory infection, associations between COVID-19 and rheumatic features have also been reported [2]. Several studies have investigated the association of spondyloarthritis (SpA) with COVID-19 [3], but the information about SpA triggered by COVID-19 is still limited.

Here we present cases of 23-year-old human leukocyte antigen B27 (HLA-B27)-positive monozygotic twin brothers manifesting typical features of SpA after COVID-19. Furthermore, we investigated single-cell transcriptome profiles using their peripheral blood mononuclear cells (PBMCs).

This study was reviewed and approved by the Institutional Review Board of Seoul St. Mary's Hospital of the Catholic University of Korea (approval number: KC21RISI0360). Written informed consent was obtained from each patient.

CASE REPORT

Twin A and Twin B were monozygotic twins living in the same place. On January 14, 2021, they had a meal with a COVID-19-confirmed case at the same restaurant.

Received July 26, 2022; **Revised** August 30, 2022; **Accepted** September 6, 2022, **Published online** September 23, 2022

Corresponding author: Ji Hyeon Ju, https://orcid.org/0000-0002-1381-5466
Division of Rheumatology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea. **E-mail:** juji@catholic.ac.kr
*These authors contributed equally to this work.

Case 1 (Twin A)

A 23-year-old male patient who had no known medical illnesses or family history took a nasopharyngeal and throat swab test for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection on January 18, 2021 (day 1). The swab by real-time reverse transcription-polymerase chain reaction (RT-PCR) confirmed that the patient was positive for SARS-CoV-2. He was admitted to a community hospital accommodating COVID-19 patients. During 10 days of hospitalization from January 21 to January 30 (day 4 to 13), his clinical manifestations (fever, sore throat) was mild. On February 8 (day 22), he started to complain of swelling and pain in left knee and both ankles.

On March 19 (day 61), he was referred to Seoul St. Mary's Hospital, a tertiary care university hospital and referral centre. On examination, his left knee and left ankle were tender and swollen. Laboratory investigations showed C-reactive protein (CRP) level of 48.8 mg/L, and erythrocyte sedimentation rate of 92 mm/hour. HLA-B27 was positive, but tests for other rheumatic diseases, bacterial, and viral pathogens were all negative. Simple radiographs of knees, ankles, and spine did not show any abnormal findings. Ultrasound evaluation and magnetic resonance imaging (MRI) documented the presence of left knee synovitis and coexisting left achilles tendinopathy (Figure 1A). Despite the absence of chronic inflammatory back pain, MRI of the sacroiliac joints demonstrated obvious bone marrow edema at both sides of sacroiliac joints (Figure 1B).

To be brief, he had inflammatory arthritis of the left knee, left achilles tendinopathy, positive HLA-B27, sacroiliitis on MRI, and elevated CRP. He was diagnosed with peripheral SpA according to the Assessment of SpondyloArthritis international Society (ASAS) criteria [3]. Because of the typical pattern of SpA plus the evidence of COVID-19 in the preceding two weeks, he was considered to have SpA triggered by COVID-19. He was treated with naproxen, sulfasalazine, and prednisolone and administered intra-articular triamcinolone in the left knee joint. He had good responses to naproxen. He was discharged in a good general condition on March 30 (day 72).

Case 2 (Twin B)

He (Twin B) was 23-year old monozygotic twin brother of Case 1 (Twin A). He was exposed to COVID-19 case with his brother (Twin A) on January 14, 2021. His RT-PCR for SARS-CoV-2 was equivocal on January 18 (day 1). He was quarantined for 14 days. During quarantine, he had malaise without fever. On February 5 (day 19), he suddenly started to experience fever and pain in the left buttock. On February 10 (day 24), he complained of swelling and pain in the right sternoclavicular joint, let knee, and the left ankle.

On March 16 (day 58), he was referred to Seoul St. Mary's Hospital, a tertiary care university hospital and referral centre for unremitting symptoms. He complained of fever, back pain, and swelling and pain in his left knee, both ankles, and the right sternoclavicular joint. At admission, he had fever up to 38.5°C (tympanic temperature). On examination, his left knee and both ankles were tender and swollen. Laboratory investigations showed raised CRP level of 85.3 mg/L, and HLA-B27 was positive. Ultrasound evaluation and MRI documented the presence of synovitis in the left knee and both ankles (Figure 1C). MRI of sacroiliac joints demonstrated bone marrow edema at both sacroiliac joints (Figure 1D). Furthermore, bone scan demonstrated arthritis in both sternoclavicular joints, both sacroiliac joints, ankles, left 5th metacarpophalangeal joint, and left knee. Tests for other rheumatic diseases, bacterial, and viral pathogens were

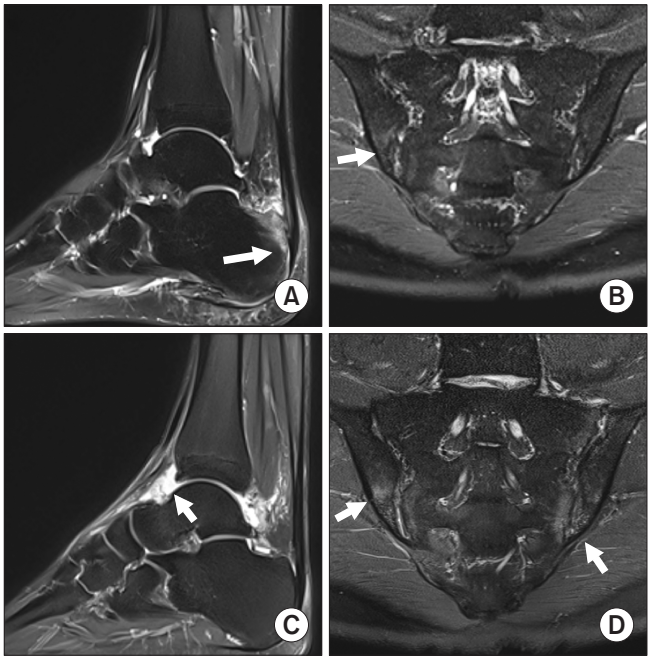

Figure 1. MRI of the ankle and sacroiliac joints in Twin A and Twin B. (A) Sagittal plain T2-weighted images of Twin A displaying tendinopathy of the left Achilles tendon (white arrow). (B) STIR images of Twin A displaying the sacroiliac bone marrow edema (white arrow). (C) Sagittal plain T2-weighted images of Twin B displaying synovial proliferation and joint effusion (white arrow). (D) STIR images of Twin B displaying bilateral sacroiliac bone marrow edema (white arrows). MRI: magnetic resonance imaging, STIR: short tau inversion recovery.

negative. He was diagnosed with peripheral SpA according to ASAS criteria. He was treated with naproxen, sulfasalazine, and prednisolone and administered intra-articular triamcinolone in the left knee joint and the right sternoclavicular joint. He also had a good response to naproxen. He was discharged in good general conditions on March 30 (day 72).

In this case, a SARS-CoV-2 RT-PCR test was equivocal. However, the diagnosis of COVID-19 was highly suspicious, as he had the contact history with the confirmed patient and alternative causes were ruled out. A temporal relation between SpA and COVID-19 was considered. The timing of disease onset was consistent with his monozygotic twin's COVID-19 infection.

Single-cell RNA sequencing (scRNA-seq)

Furthermore, we investigated single-cell transcriptome profiles of PBMCs from the twins in comparison with a 45-year old male patient with typical radiographic axial spondyloarthritis (axSpA) whose HLA-B27 was positive as well. Peripheral blood was obtained from the twins on March 29, 2021 (the 10th and 13th day of treatment with naproxen, sulfasalazine, and prednisolone, respectively). Peripheral blood was drawn from the patient with typical axSpA on March 30, 2021. He had been receiving naproxen and sulfasalazine for 8 years, but adalimumab was newly added due to the acute deterioration of axSpA a month before the blood sampling for scRNA-seq. Mononuclear cells were isolated from the peripheral blood using Ficoll-Paque gradient centrifugation. $CD45^+$ cells were isolated using magnetic beads. The single-cell samples were loaded on Chromium Controller (10X Genomics) for library preparation, and the barcoded libraries were constructed using the Chromium Single Cell 3' Reagent Kit. The libraries were ran multiple times on Illumina Novaseq 6000 for sequencing and following the manufacturer's instructions. PBMCs analyzed for genetic profiles were clustered using Uniform Manifold Approximation and Projetion using the R package Seurat version 4.0.6 [4]. Fourteen clusters were identified (Figure 2A). Difference in frequency of cell population is displayed in bar graphs (Figure 2B). The most obvious difference between SpA after COVID-19 and typical axSpA was found in T lymphocytes. The population of naïve $CD4^+$ T cells and naïve $CD8^+$ T cells was more expanded in SpA after COVID-19. Memory $CD4^+$ T cells and cytotoxic $CD8^+$ T cells were more frequent in typical axSpA. Differentially expressed genes of each cell subset were investigated. The mRNA expression of ribosomal protein S26 (RPS26) was systematically down-regu-

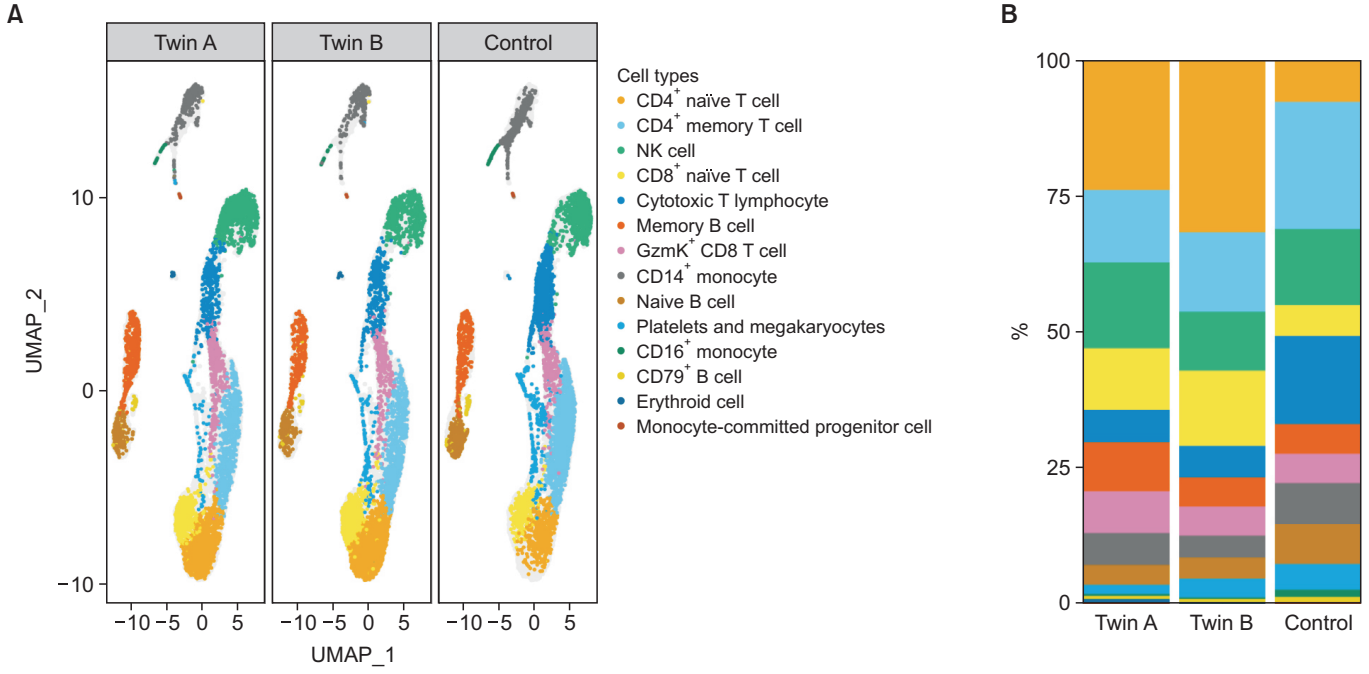

Figure 2. Cell clusters identified by single-cell RNA sequencing analysis. (A) Identification of 14 clusters in pooled peripheral blood mononuclear cells from twins who developed spondyloarthritis after COVID-19 and one patient with typical radiographic axial spondyloarthritis. (B) Difference in frequency of cell population is displayed in bar plots. COVID-19: coronavirus disease 2019, UMAP: Uniform Manifold Approximation and Projetion.

lated in most subsets of SpA after COVID-19 compared to that in typical axSpA (Figure 3A). The expression of small nucleolar RNA host gene 5 (SNHG5) was consistently upregulated in SpA after COVID-19 compared to that in typical axSpA (Figure 3B).

DISCUSSION

This study described two cases of HLA-B27-positive monozygotic twin brothers who almost simultaneously developed SpA after COVID-19. To the best of our knowledge, this is the first report on SpA in monozygotic twins after COVID-19. Our results suggest a causal relation between SpA and COVID-19 and genetic susceptibility to COVID-19-triggering SpA, particularly stressing out HLA-B27.

To study more about SpA after COVID-19, we performed a comprehensive review of the literature. A total 8 cases from 7 papers were classified as SpA according to ASAS criteria (Table 1) [3,5-10]. The median age of these cases was 44 years old (range: 21~65 years). Males were predominant (5 cases out of 8). Axial involvement was detected in 4 cases [3,6-8]. Enthesitis was reported in one case. There were no cases with dactylitis [9]. Psoriasis was present in 2 cases [6,10]. Oral non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroid, and sulfasalazine were administrated to treat SpA. In only one case, tumor necrosis factor-alpha inhibitor was administered. Our two cases were also males with axial involvement and elevated CRP. HLA-B27 was positive in 5 cases out of 8 [3,5,7,9]. Individuals with the HLA-B27 allele or with a family history of SpA have a higher

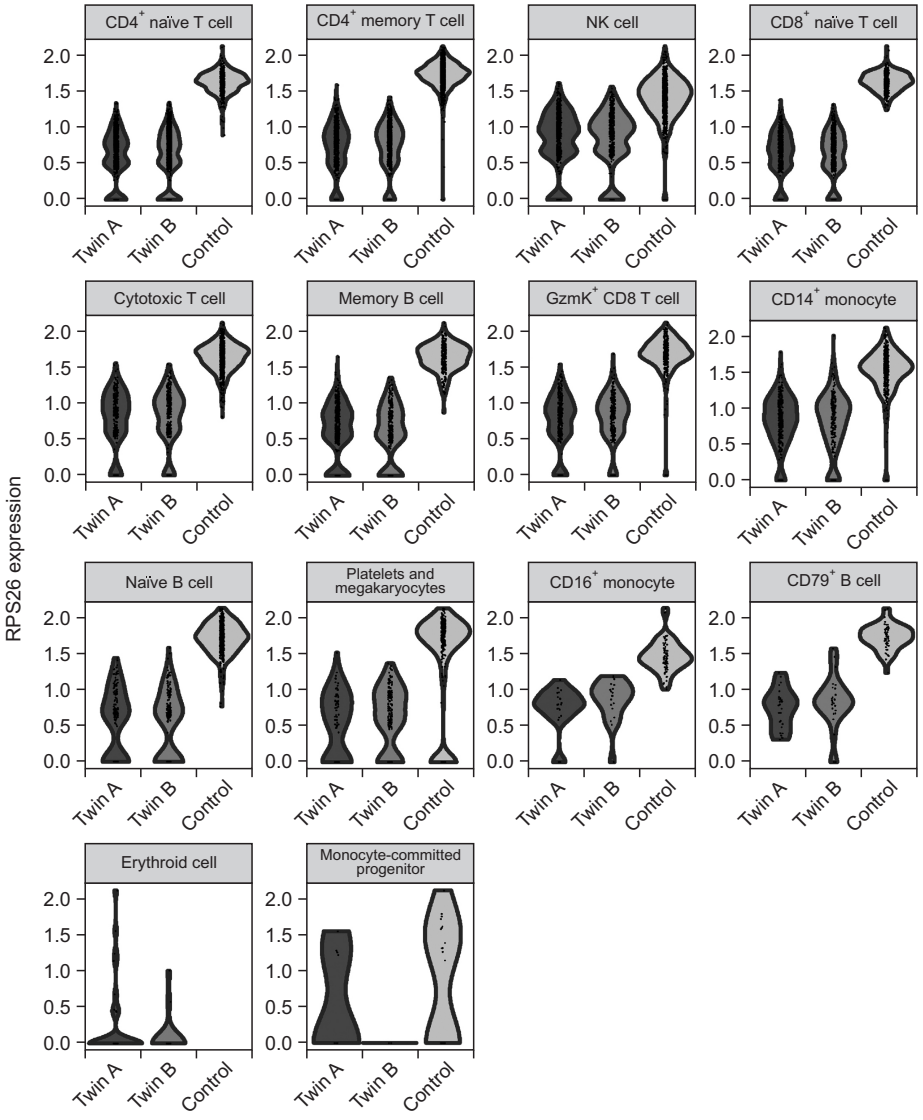

Figure 3. The differentially expressed genes from single-cell RNA sequencing analysis. (A) The mRNA expression of ribosomal protein S26 (RPS26) is systematically down-regulated in most subsets of spondyloarthritis (SpA) after coronavirus disease 2019 (COVID-19) compared to that in typical axial spondyloarthritis. (B) The expression of small nucleolar RNA host gene 5 (SNHG5) is consistently upregulated in most subsets of SpA after COVID-19 compared to that in typical axial spondyloarthritis.

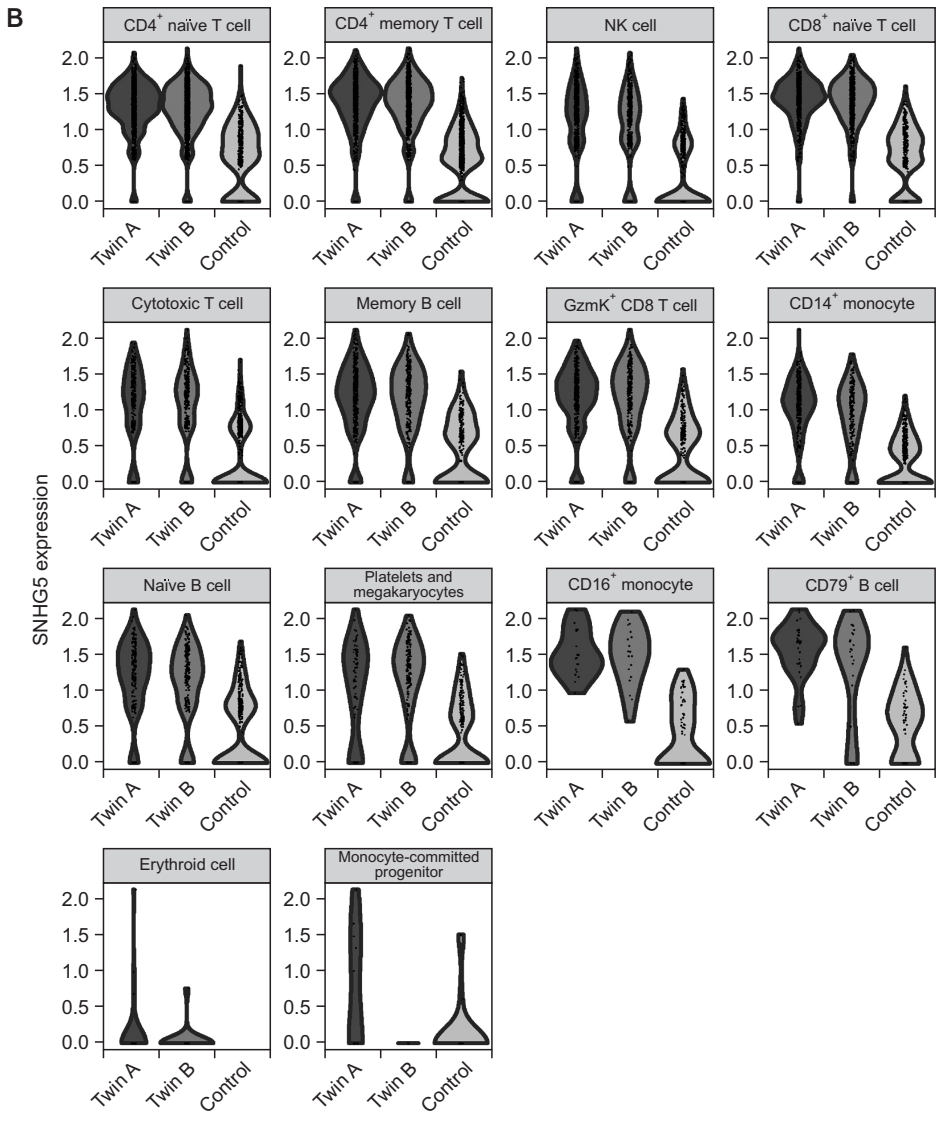

Figure 3. Continued.

risk of developing reactive arthritis [11]. Therefore, shared genetic traits by monozygotic twins, particularly HLA-B27 positivity, might have contributed to their susceptibility to COVID-19-induced SpA.

The transcriptomic profiling has not drawn any conclusion in our analysis, and the result might be attributable to inter-individual differences and medication status. The downregulated gene, RPS26, is a ribosomal protein gene that regulates mRNA-specific translation by recognizing Kozak sequence elements [12]. RPS26 is commonly mutated in Diamond–Blackfan anemia [13]. As suggested in a previous expression quantitative trait loci analysis of T cells, RPS26 gene expression is associated with autoimmune diseases [14]. The upregulated gene, SNHG5, is known as one of cytoplasmic long non-coding RNA genes. It is closely associated with various cancers by exerting tumorigenic or suppressive actions [15]. However, the role of SNHG5 in autoimmune conditions is not well-defined yet.

SUMMARY

In this study, we presented cases of HLA-B27-positive monozygotic twins developing SpA after COVID-19. To the best of our knowledge, this is the first report on SpA in monozygotic twins after COVID-19. Our results suggest a possible causal relation between SpA and COVID-19, emphasizing genetic susceptibility to COVID-19-triggering SpA, especially HLA-B27. Moreover, we first compared the transcriptomic landscape of PBMC between SpA after COVID-19 and typical radiographic

Table 1. Demographic and clinical characteristics of each case of spondyloarthritis after COVID-19

Author	Sex/Age	Onset (after COVID-19)	Clinical features	Treatment
Case 1 (Twin A)	M/23	22 days	Peripheral arthritis Achilles tendinitis Inflammatory back pain HLA-B27(+) Sacroiliitis on MRI	NSAIDs, prednisolone, sulfasalazine, IA corticosteroid
Case 2 (Twin B)	M/23	19 days	Peripheral arthritis Inflammatory back pain HLA-B27(+) Sacroiliitis on MRI	NSAIDs, prednisolone, sulfasalazine, IA corticosteroid
Schenker et al. [5]	F/65	10 days	Peripheral arthritis Cutaneous vasculitis HLA-B27(+)	Prednisolone
Novelli et al. [6]	F/27	5 months	Peripheral arthritis Inflammatory back pain Psoriasis HLA-B27(−) Sacroiliitis on MRI	-
Coath et al. [7]	F/53	-	Peripheral arthritis Inflammatory back pain HLA-B27(+) Sacroiliitis on MRI	NSAIDs, IM methylprednisolone
Saikali and Gharib [8]	F/21	3 months	Inflammatory back pain HLA-B27(−) Sacroiliitis on MRI	NSAIDs, TNF inhibitor
El Hasbani et al. [3] (Case 1)	M/25	19 days	Peripheral arthritis Inflammatory back pain HLA-B27(+) Sacroiliitis on MRI	NSAIDs, prednisolone, sulfasalazine
El Hasbani et al. [3] (Case 2)	M/57	1 month	Peripheral arthritis HLA-B27(+)	NSAIDs, prednisolone
Ciaffi et al. [9]	M/55	2 months	Peripheral arthritis Achilles tendinitis HLA-B27(+)	-
De Stefano et al. [10]	M/30s	24 days	Peripheral arthritis Psoriasis HLA-B27(−)	NSAIDs, topical steroids

COVID-19: coronavirus disease 2019, HLA-B27: human leukocyte antigen B27, M: male, F: female, NSAID: non-steroidal anti-inflammatory drugs, IA: intra-articular, MRI: magnetic resonance imaging, IM: intramuscular, TNF inhibitor: tumor necrosis factor inhibitor.

axSpA. Further studies are needed to elucidate the mechanistic role of these differentially expressed genes in SpA after COVID-19.

FUNDING

None.

ACKNOWLEDGMENTS

We would like to express our gratitude to all the members of Division of Rheumatology at Seoul St. Mary's Hospital.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Study concept and design: J.H.J., M.O., J.G.K., I.P.B. Acquisition of data: M.O., J.G.K. Writing—original draft: J.G.K., M.O. Writing—review & editing: M.O., J.H.J., I.P.B., J.G.K. Analysis and interpretation of the scRNA-seq data: I.P.B., J.G.K. All authors approved the final manuscript.

ORCID

Minae Oh, https://orcid.org/0000-0001-9046-7322
Jung Gon Kim, https://orcid.org/0000-0002-3661-4974
In-Pyo Baek, https://orcid.org/0000-0002-5779-4345
Ji Hyeon Ju, https://orcid.org/0000-0002-1381-5466

REFERENCES

1. Chams N, Chams S, Badran R, Shams A, Araji A, Raad M, et al. COVID-19: a multidisciplinary review. Front Public Health 2020;8:383.
2. Ciaffi J, Meliconi R, Ruscitti P, Berardicurti O, Giacomelli R, Ursini F. Rheumatic manifestations of COVID-19: a systematic review and meta-analysis. BMC Rheumatol 2020;4:65.
3. El Hasbani G, Jawad A, Uthman I. Axial and peripheral spondyloarthritis triggered by sars-cov-2 infection: a report of two cases. Reumatismo 2021;73:59-63.
4. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. Nat Biotechnol 2018;36:411-20.
5. Schenker HM, Hagen M, Simon D, Schett G, Manger B. Reactive arthritis and cutaneous vasculitis after SARS-CoV-2 infection. Rheumatology (Oxford) 2021;60:479-80.
6. Novelli L, Motta F, Ceribelli A, Guidelli GM, Luciano N, Isailovic N, et al. A case of psoriatic arthritis triggered by SARS-CoV-2 infection. Rheumatology (Oxford) 2021;60:e21-3.
7. Coath FL, Mackay J, Gaffney JK. Axial presentation of reactive arthritis secondary to COVID-19 infection. Rheumatology (Oxford) 2021;60:e232-3.
8. Saikali W, Gharib S. The first non-radiographic axial spondyloarthrits with COVID-19. Immun Inflamm Dis 2021;9:628-31.
9. Ciaffi J, Mancarella L, Borlandelli E, Facchini G, Meliconi R, Ursini F. May polyenthesitis follow COVID-19? Joint Bone Spine 2021;88:105158.
10. De Stefano L, Rossi S, Montecucco C, Bugatti S. Transient monoarthritis and psoriatic skin lesions following COVID-19. Ann Rheum Dis 2020 Aug 4 [Epub]. DOI:10.1136/annrheumdis-2020-218520.
11. Bentaleb I, Abdelghani KB, Rostom S, Amine B, Laatar A, Bahiri R. Reactive arthritis: update. Curr Clin Microbiol Rep 2020;7:124-32.
12. Ferretti MB, Ghalei H, Ward EA, Potts EL, Karbstein K. Rps26 directs mRNA-specific translation by recognition of Kozak sequence elements. Nat Struct Mol Biol 2017;24:700-7.
13. Doherty L, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Clinton C, et al. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. Am J Hum Genet 2010;86:222-8. Erratum in: Am J Hum Genet 2010;86:655.
14. Kasela S, Kisand K, Tserel L, Kaleviste E, Remm A, Fischer K, et al. Pathogenic implications for autoimmune mechanisms derived by comparative eQTL analysis of CD4+ versus CD8+ T cells. PLoS Genet 2017;13:e1006643.
15. Han W, Shi J, Cao J, Dong B, Guan W. Latest advances of long non-coding RNA SNHG5 in human cancers. Onco Targets Ther 2020;13:6393-403.