

Association between anti-interferon-alpha autoantibodies and COVID-19 in systemic lupus erythematosus: correspondence on 'Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus erythematosus under long-term treatment with hydroxychloroquine' by Mathian *et al*

Sarthak Gupta, MD; Shuichiro Nakabo, MD, PhD; Jun Chu, CRNP; Sarfaraz Hasni, MD; Mariana J. Kaplan, MD

Supplemental Material

Methods:

Subject recruitment and clinical assessment.

Biobanked plasma samples from patients with SLE and healthy controls obtained prior to 2020 and stored at -80C were identified through IRB-approved protocols. Patients with SLE fulfilled the 1997 update of the 1982 American College of Rheumatology classification criteria of SLE.¹ Patients were diagnosed with SARS-CoV-2 infection based on symptoms and a positive RT-PCR (n=6), rapid antigen (n=2) or antibody testing (n=1). One subject (Patient 9) had typical symptoms of COVID-19 with close family members with RT-PCR positive COVID-19 but was not tested during active infection or had antibody testing. COVID-19 disease severity for each patient was assessed in accordance with the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7).² Healthy control samples were from age-matched volunteers that reported no acute or chronic infections.

Enzyme linked immunosorbent assay (ELISA) for anti-IFN α autoantibodies:

ELISA was performed as previously reported.³ In brief, 96-well ELISA plates (Corning, catalog # 9018) were coated overnight at 4°C with 50 μ L of 2 μ g/mL recombinant human IFN α (rhIFN α) (PBL assay science, Inc catalog # 11101-2). Plates were then washed (PBS/0.005% Tween), blocked, washed again by incubation with the same buffer supplemented with 2% bovine serum albumin for 3 hours at room temperature, washed, and incubated with 1:50 dilution of plasma samples from the patients or controls for 3 hours at room temperature. After wash, horseradish peroxidase (HRP)-conjugated Fc-specific anti-human IgG (Millipore Sigma) was added at 1:10,000 dilution. Plates were incubated for 1 hour at room temperature and washed. Substrate was added and OD was measured. Arbitrary units were calculated based on the standard curve generated using plasma from a patient with known high titer anti-IFN α autoantibodies from a prior study.⁴ Values two standard deviations above mean in 119 healthy control samples were considered positive.

Functional evaluation of anti-IFN α autoantibodies:

The blocking activity of anti-IFN α autoantibodies in plasma was determined by assessing phosphorylated signal transducer and activator of transcription 1 (pSTAT1) in healthy control peripheral blood mononuclear cells (PBMCs) following stimulation with rhIFN α in the presence of 10% healthy control or lupus plasma as previously described.⁴ Briefly, PBMCs were isolated from peripheral venous blood from healthy controls using Ficoll-Paque density gradient and incubated with fluorescein isothiocyanate (FITC)-conjugated monoclonal mouse antibodies to CD14 (BD Pharmingen). PBMCs (10⁶/reaction) were then

incubated for 15 minutes with rhIFN α (10 ng/mL) in the presence or absence of 10% healthy control or SLE plasma. Cells were fixed, permeabilized, and stained for intracellular pSTAT1 (Y701, BD Biosciences). Cells were then assessed by flow cytometry using FACSCalibur (BD Biosciences) and analyzed using FlowJo software, version 9.9 (Tree Star).

Statistical Analysis:

Data were plotted and statistical analysis performed using GraphPad Prism version 7.

Supplementary Table 1: Demographics of healthy controls:

	Age (years) median (range)	Gender (M/F)	Race
All healthy controls (n= 119)	49 (20-77)	69/50	C=69, AA=35, A=6, H=6, Uk=3
Heathy controls with anti-IFN α autoantibodies (n=6)	52.5 (20-63)	5/1	C=4, AA=1, H=1

M=male, F=female, C=Caucasian, AA= African-America, A=Asian, H=Hispanic, Uk=Unknown

References:

1. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* 1997;40(9):1725.
2. Medicine NHCNAoTC. Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7). *Chinese Medical Journal.* 2020;133(9):1087-1095.
3. Bastard P, Rosen LB, Zhang Q, et al. Autoantibodies against type I IFNs in patients with life-threatening COVID-19. *Science.* 2020;370(6515).
4. Gupta S, Tatouli IP, Rosen LB, et al. Distinct Functions of Autoantibodies Against Interferon in Systemic Lupus Erythematosus: A Comprehensive Analysis of Anticytokine Autoantibodies in Common Rheumatic Diseases. *Arthritis Rheumatol.* 2016;68(7):1677-1687.