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'

Mathian *et al* described the clinical course and heterogeneity of COVID-19 in 17 systemic lupus erythematosus (SLE) patients.[1] SLE subjects could be at higher risk of developing COVID-19, with more severe symptomatology and need for hospitalisation due to multiple underlying risk factors.[1 2] Type-I interferons (IFN), including IFNα, play fundamental roles in immunity and are crucial in antiviral responses.[3] Defects in IFN signalling pathways, secondary to monogenic inborn errors or to blocking autoantibodies, promote immunodeficiency and recurrent infections.[4 5] Dysregulation in type-I IFN pathway also plays key pathogenic roles in SLE.[6] A recent report showed association between anti-type-I IFN autoantibodies in 10% of subjects with life-threatening COVID-19 in the general population.[7] A comprehensive evaluation of multiple anticytokine autoantibodies showed the presence of anti-type-I IFN autoantibodies in 11% of SLE subjects in the pre-COVID-19 era.[8] We hypothesised that SLE patients having anti-IFNα autoantibodies at baseline (prior to 2020) may be at higher risk of developing COVID-19, and that the presence of these autoantibodies may help in guiding management and preventive strategies.

Ten SLE females who developed COVID-19 between 1 April and 1 October 2020 were identified among lupus subjects followed at the National Institutes of Health, Bethesda, MD, USA under IRB-approved SLE natural history protocol 94-AR-0066 (online supplemental methods, table 1). Seven patients had mild to moderate COVID-19 symptoms that were managed at home with supportive care. Three patients had severe symptoms requiring hospitalisation, supplemental oxygen and/or steroids and convalescent plasma infusion. All patients had full recovery. Eight patients were on daily prednisone (range 5–20 mg/day) when COVID-19 symptoms developed. Seven patients were taking hydroxychloroquine prior to COVID-19 and continued it during the infection. One patient (patient 2) had received rituximab in February 2020 and developed COVID-19 in May 2020. Another patient (patient 9) developed COVID-19 while on belimumab.

Biobanked plasma from healthy controls (HC; n=119) and the 10 SLE subjects were tested for anti-IFNα IgG autoantibodies by ELISA (online supplemental methods). Values 2 SD above mean in HC samples were considered positive. Anti-IFNα autoantibodies was detected in 4 out of the 10 SLE patients (patients 2, 3, 9, 10) who developed COVID-19 (40%; figure 1A). Longitudinal assessments of lupus plasma samples confirmed the presence of anti-IFNα autoantibodies preceding the infection as far back as 2017 (figure 1A). Patients with anti-IFNα autoantibodies had higher rates of hospitalisation requiring oxygen (two out of four) compared with those without (one out of six). Of the two patients (patients 2 and 9) who had received anti-B-cell therapy in the prior years, both had persistent anti-IFNα autoantibodies. These results suggest that the prevalence of anti-IFNα autoantibodies is higher in those patients with confirmed COVID-19 than what has been previously reported in SLE.[8]

We evaluated if the plasma positive for anti-IFNα autoantibodies could block IFNα signalling in vitro (online supplemental methods). Out of the four SLE subjects with anti-IFNα autoantibodies, half of the samples (two subjects; patients 3 and 9) blocked recombinant human IFNα-induced signal transducer and activator of transcription 1 (STAT1) phosphorylation in HC peripheral blood mononuclear cells (PBMCs) at 10% concentration (figure 1B). These patients had the highest anti-IFNα autoantibodies titers. None of the COVID-19 SLE plasma samples negative for anti-IFNα autoantibodies (n=6) inhibited STAT1 phosphorylation by rhIFNα.

In this initial assessment, 40% of SLE patients who developed confirmed COVID-19 were positive for anti-IFNα IgG autoantibodies in samples obtained prior to SARS-CoV-2 infection. In general, positive autoantibodies were present several years before and in some patients persisted despite B-cell targeted therapy. Previous reports in the same cohort showed that SLE subjects had anti-IFNα autoantibodies prevalence of 11%.[8] Therefore, those SLE patients who developed confirmed COVID-19 during this initial wave of the pandemic had enrichment in anti-IFNα autoantibodies. Plasma samples with the highest titers of anti-IFNα autoantibodies inhibited signalling of IFNα in vitro, suggesting that levels of these autoantibodies may affect their blocking ability. A worse outcome in COVID-19 patients positive for anti-IFNα autoantibodies in the general population was recently reported, and suggested that these antibodies may precede infection based on two prestored plasma samples.[7] Our findings support this hypothesis, as SLE patients who

Table 1 Clinical characteristics of SLE subjects with confirmed COVID-19

	Age	Gender	Race	COVID-19 Dx method	COVID-19 symptoms	Admission	Tx for COVID-19	Clinical manifestations of SLE/other autoimmune disease	Serologies	SLE medications	Other comorbidities
1	49	F	C	RT-PCR	Cough	No	HCQ, Zinc	LN, pleuritis, anaemia, lymphopenia, SS	ANA, anti-dsDNA, anti-Smith, anti-RNP, ACA, hypocomplementemia	Azathioprine, prednisone (5 mg/day)	Obese (BMI 40)
2	48	F	H	Rapid antigen	SOB, diarrhoea	Yes	Oxygen, convalescent plasma, azithromycin	LN, neuropsychiatric lupus, APLS	ANA, anti-dsDNA, anti-SSA, ACA, hypocomplementemia	Prednisone (10 mg/day), coumadin, rituximab infusion on 2/2020	Overweight (BMI 26)
3	40	F	H	RT-PCR	Cough, fever, SOB, chest pain	Yes	Oxygen	Arthritis, malar rash, pleuritis, alopecia, APLS	ANA, anti-dsDNA, anti-RNP, anti-Smith, anti-SSA, LA, ACA, anti-B2GP1, hypocomplementemia	Prednisone (6 mg/day), azathioprine, HCQ, coumadin	Obese (BMI 32)
4	49	F	AA	RT-PCR	Fever, chills, cough	No	Supportive care	Arthritis, alopecia, LN, anaemia, leucopenia, thrombocytopenia	ANA, anti-dsDNA, anti-RNP, anti-Smith, anti-SSA, anti-SSB, ACA, hypocomplementemia	Prednisone (7.5 mg/day), rivaroxaban (not APLS), MMF, HCQ	Obese (BMI 42)
5	56	F	C	Rapid antigen	Headache	No	Supportive care	Malar rash, photosensitivity, alopecia, LN, thrombocytopenia	ANA, anti-SSA, hypocomplementemia	Prednisone (5 mg/day), azathioprine, HCQ	None
6	49	F	H	RT-PCR	Fever, chills, headaches, vomiting, diarrhoea, loss of smell, sore throat, cough	No	Supportive care	Arthritis, alopecia, photosensitivity, ITP	ANA, anti-dsDNA, LA	HCQ	Obese (BMI 30)
7	48	F	H	Antibody	Fever, cough, fatigue, myalgias, nasal congestion	No	Supportive care	Malar rash, photosensitivity, alopecia, Raynaud's, arthritis, neuropsychiatric lupus	ANA, anti-dsDNA, anti-smith, anti-RNP, anti-SSA, anti-chromatin, hypocomplementemia	MMF, HCQ	Dyslipidaemia, HTN, DVT, PE, obese (BMI 35), ILD
8	48	F	H	RT-PCR	Cough, SOB, URI symptoms	Yes	Oxygen, steroids, convalescent plasma	Arthritis, Raynaud's, photosensitivity, alopecia, oral ulcers, malar rash, SS	ANA, anti-dsDNA, anti-SSA, anti-B2GP1, hypocomplementemia	Prednisone (5 mg/day), HCQ	Overweight (BMI 28)
9	48	F	H	N/A	Fever, cough, fatigue,	No	Supportive care	Arthritis, lymphopenia, LN	ANA, anti-dsDNA, anti-RNP, anti-Smith, anti-SSA, anti-SSB, LA, ACA, anti-B2GP1, hypocomplementemia	Prednisone (20 mg/day), HCQ, MMF, belimumab	None
10	26	F	H	RT-PCR	Loss of taste and smell	No	Supportive care	Malar rash, alopecia, arthritis, photosensitivity	ANA, anti-dsDNA, anti-RNP, anti-Smith, anti-SSA, LA, ACA, hypocomplementemia	Prednisone (5 mg/day), HCQ	None

Shaded subjects had anti-interferon α autoantibodies.
AA, African-American; ACA, anti-cardiolipin antibody; ANA, anti-nuclear antibody; anti-B2GP1, anti-beta2 glycoprotein; anti-dsDNA, anti-double stranded DNA; anti-RNP, anti-ribonucleoprotein; anti-SSA, anti-Sjögren's-syndrome-related antigen A; anti-SSB, anti-Sjögren's-syndrome-related antigen B; APLS, anti-phospholipid syndrome; BMI, Body mass index; C, Caucasian; DVT, deep vein thrombosis; F, female; H, Hispanic; HCQ, hydroxychloroquine; HTN, hypertension; ILD, interstitial lung disease; ITP, Idiopathic thrombocytopenic purpura; LA, lupus anticoagulant; LN, lupus nephritis; MMF, mycophenolate mofetil; N/A, not available; PE, pulmonary embolism; SLE, systemic lupus erythematosus; SOB, shortness of breath; SS, Sjögren's syndrome; URI, upper respiratory infection.

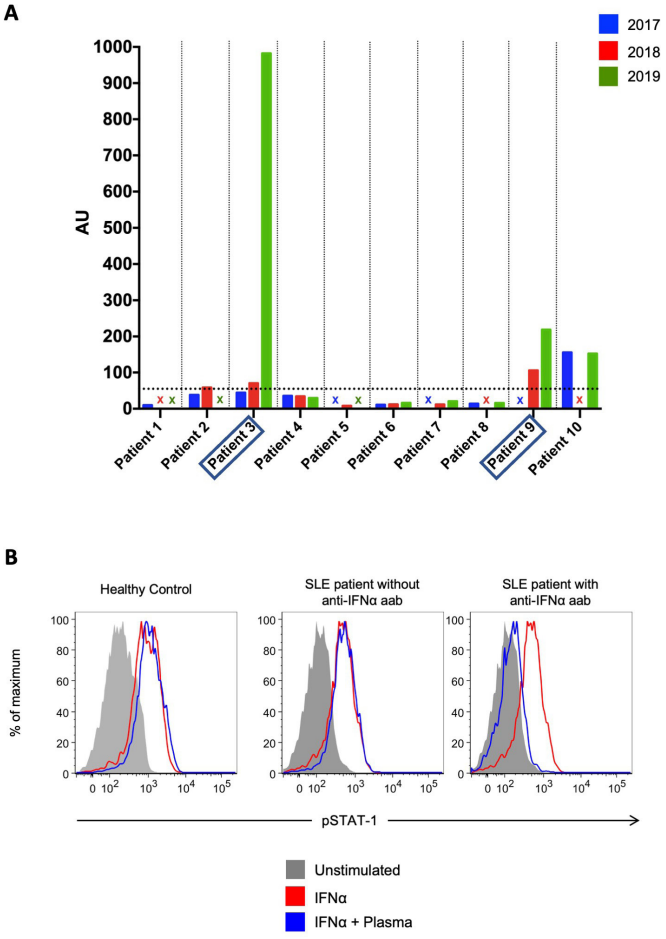

Figure 1 Presence of blocking autoantibodies to IFNα in SLE subjects. (A) Bar graph depicts arbitrary units (AU) of anti-IFNα measured by ELISA in 10 SLE subjects who developed RT-PCR confirmed COVID-19 between 1 April and 1 October 2020. Horizontal dotted line shows 2 SD above mean of 119 healthy controls (55 AU); individual subjects are separated by vertical dotted line, missing plasma samples are represented by X. Plasma samples from patients 3 and 9 (boxed) had blocking antibodies. (B) Representative example of detection of blocking anti-IFNα. Healthy control PBMCs were incubated with 10% plasma from healthy controls or from autoantibody-positive or negative SLE subjects with COVID-19, and then left unstimulated or stimulated with recombinant human IFNα. IFN-induced phosphorylation of STAT1 was measured by flow cytometry. SLE, systemic lupus erythematosus.

developed confirmed COVID-19 had anti-IFNα autoantibodies detected prior to the infection, suggesting a potential pathogenic role for these autoantibodies in increasing susceptibility to SARS-CoV-2 infection.

Our study is limited by the small sample size. Whether the presence of autoantibodies will contribute to modulating the severity and outcome of the SARS-CoV-2 infection in SLE requires systematic assessment in larger numbers of patients. The natural history of these autoantibodies should also be further evaluated in longitudinal studies.

This report highlights the key role that IFNα and autoantibodies against this cytokine may play in both SARS-CoV-2 infection and in SLE pathogenesis. The presence of anti-IFNα autoantibodies may prove a helpful prognostic marker to predict which SLE patients may develop COVID-19 and could inform preventive measures and management of this subset of patients.

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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*

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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-IFNα autoantibodies:

ELISA was performed as previously reported.[3] In brief, 96-well ELISA plates (Corning, catalog # 9018) were coated overnight at 4°C with 50µL of 2µg/mL recombinant human IFNα (rhIFNα) (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-IFNα autoantibodies from a prior study.[4] Values two standard deviations above mean in 119 healthy control samples were considered positive.

Functional evaluation of anti-IFNα autoantibodies:

The blocking activity of anti-IFNα 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 rhIFNα 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 Pharmigen). PBMCs (10^6/reaction) were then

incubated for 15minutes with rhIFNα (10ng/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-IFNα 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

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