Editorial

COVID-19: the new challenge for rheumatologists. First update

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In the previous issue of this journal we outlined why COVID-19 may be viewed as the new challenge for rheumatologists (1). At the same time, we tried to identify a number of questions that could be addressed in order to properly treat this new disease, on the one hand, with drugs commonly used in some rheumatic diseases (2), and on the other, how to continue to manage rheumatic patients in the COVID era. The dramatic impact of this pandemic viral disease has polarised the global attention of the scientific community, and we have therefore decided to regularly review the most recent advances, in order to try to give an answer to the previously identified questions.

What have we learned on the mechanisms involved in the development of COVID-19?

An aggressive inflammatory response observed in the severe COVID-19 was related initially to an excessive immune activity, without defining precisely the mechanisms that led to this event. In the last few weeks, the scientific world has clarified several aspects that regulate the tight interaction between virus and host immune system. Studies focusing on characterisation of SARS-CoV-2 structure and on identification of its target cells allowed the scientists to better define the mechanisms regulating the early phase of the infection. The destruction of lung cells by SARS-CoV-2 triggers a local immune response, leading to the recruitment into the tissue of macrophages, monocytes, lymphocytes, but not neutrophils (3). This seems to be a crucial aspect of the dysregulation of the host innate immunity. In fact, the persistence of neutrophils in the blood stream and their ability to form neutrophil extracellular traps (NETs) (4) may actively contribute to the development of different aspects of the disease such as inflammatory processes and cardiovascular manifestations (5). It is known that intravascular NETs play a vital role in initiating and accreting thrombosis in arteries and veins, and promoting the activation of coagulation. Therefore, we can hypothesise that high blood levels of NETs together with increased pro-inflammatory cytokines such as IL-6 and IL-17A may actively contribute to promote a pro-thrombotic state in severe COVID-19 (6). Moreover, the increase of pro-inflammatory cytokines produced by infiltrated monocytes and macrophages amplify neutrophil activities, leading to more NET formation. In parallel, other cellular components of the innate immune system, such as cytotoxic T cells (CTLs) and NK cells, both required to generate an effective immune response against viruses, have been proven to be dramatically compromised in SARS-CoV-2 infection. It seems that SARS-CoV-2 overrides the host innate immunity by over-expressing the inhibitory receptor NKG2A on CTLs and NK cells, resulting in the loss of their relevant cellular functions and their ability to produce CD107a, IFN-γ, IL-2, granzyme B, and TNF-α (7).

If the cellular components of the innate immune system are not able to exert an effective immune response against the virus, contributing to amplify the inflammatory process, the drastic reduction of lymphocytes, particularly CD4+ and CD8+ T cells, leads to a dysregulation of the adaptive immune system. The dynamic changes of lymphocyte subsets and an increase of a certain cytokine profile in COVID-19 seem to be crucial for the development of severe COVID-19. The virus has the power to reduce both T and B cell activities and cause a delay in T cell pathway activation during the first days of infection. However, after two weeks of symptoms SARS-CoV-2-specific memory T cells

against antigens of the virus, particularly central memory CD4 and effector memory CD8, are restored in the circulation, giving the possibility to the immune system to perform its protective function even in the absence of SARS-CoV-2 IgG (8). Whether SARS-CoV-2 IgG are protective and prevent re-infection or whether they have the ability to modulate virus activities is still a debated issue. Even if preliminary data support the efficacy of SARS-CoV-2 IgG in neutralising the virus, further studies, both *in-vitro* and *in-vivo*, are required in order to define the criteria necessary for their therapeutic use.

What have we learned about SARS-Cov-2 "window of opportunity" and on the rationale and timing of anti-cytokine therapies?

From the rheumatology perspective, COVID-19 closely resembles well-known models of viral-induced systemic vasculitis including HCV-cryoglobulinaemic vasculitis and HBV-polyarteritis nodosa: systemic disorders characterised by an initial viral infection that induces a dysregulation of the immune response which in turn is responsible for tissue damages largely independent from the initial viral insult. In accordance with this sequence of events, antiviral agents have been recommended in the initial phases of systemic vasculitis whereas the immune-mediated vasculitic manifestations generally require the employment of conventional and biological disease-modifying anti-rheumatic drug (DMARDs) (9).

Similarly, during the past two months, we have learned a great deal about the role of inflammatory cytokines in driving systemic and pulmonary SARS-CoV-2 manifestations and how crucial it is to early recognise those patients who present a rapid radiographic progression to take full advantage of the "window of opportunity" offered by anti-cytokines agents (2). We have previously highlighted in a possible therapeutic algorithm the crucial role of Tocilizumab (TCZ) (1) in these patients. Indeed, during the past two months several evidences have further clarified the rational and timing of anti-cytokine therapies.

Should we consider Tocilizumab for every COVID-19 patient?

Indeed, TCZ maintains a crucial role in the treatment of patients with rapidly progressive SARS-CoV-2 (2). Two months after our first editorial (1), we herein summarise preliminary data on the use of TCZ in SARS-CoV-2 patients, deriving from the analysis of case series in real-life pandemic setting. 187 cases of SARS-CoV-2 patients treated with TCZ have been reported so far, mainly from the Chinese, Italian and Spanish experiences; the largest cohorts of 63 and 57 patients are described in this issue by Sciascia *et al*. from Italy and by Campins *et al*. from Spain, respectively (10-17). Overall, these data highlight the good safety profile of anti-IL-6R treatment despite the underlying viral disease, even at high dosages and with repeated administration protocols, and in association with intravenous pulse steroid therapy as reported in the Spanish cohort (17). In two reports the therapeutic efficacy of this treatment has been proven in patients with systemic sclerosis (18) and multiple myeloma (19) with severe comorbidities.

In almost all the cases, TCZ was found to be effective in COVID-19 patients with lung involvement associated with biochemical alterations suggestive of cytokine release syndrome (CRS). In particular, responders experienced rapid resolution of persistent fever and subsequent improvement of respiratory parameters, especially when associated with progressive normalisation of inflammatory biomarkers (CRP, D-dimer, LDH and lymphopenia) (11-13). In a few cases, pulmonary improvement was also documented by a corresponding resolution of lung consolidations at CT scan (11, 15).

Moreover, recent data confirm that high IL-6 levels are associated with SARS-CoV-2 severe phenotype (10, 12), even if this parameter was not a useful biomarker to monitor the response to anti-IL-6R treatment. This is probably due to the fact that the cytokine levels temporarily increase a few days after TCZ administration (unlike the other inflammatory biomarkers) (12, 16) with known mechanisms already described

in rheumatoid arthritis (RA) and Castelman disease (20).

As previously reported (1), real-life data show that critical manifestations of SARS-CoV-2 infection often develop rapidly during the second week after viral disease onset (11, 13), confirming the importance of closely monitoring COVID-19 patients during this "hot zone". Further emphasising the importance of early treatment, Sciascia *et al*. reported an increased likelihood of survival (HR 2.2) in patients who received TCZ within 6 days of hospitalisation compared to patients treated later (16). Similarly, data from the large Spanish experiences suggest that early administration of TCZ could be effective in order to prevent cytokine storm and to reduce mortality (17). It is noteworthy that these patients were treated using TCZ according to "rheumatological" protocols, thus potentially limiting patient over-treatment. Nevertheless, these preliminary results need to be supported by ongoing clinical trials that will give information about the safety and efficacy profile, ideal patient phenotype and correct timing of TCZ administration in COVID-19.

Besides TCZ, what else regarding anti-cytokine strategies?

With respect to the previously proposed therapeutic algorithm (1), a major breakthrough in SARS-CoV-2 patients has been indeed represented by the use of Baricitinib (2). Recently, Dr Stebbing and colleagues reported the possibility of employing JAK1/2 inhibitors in COVID-19, focusing their attention on Baricitinib, Ruxolitinib and Fedratinib (21), due to the powerful anti-inflammatory action ascribed to these drugs, but with a caveat about their possible negative effects in the infection control. On April 22, 2020 a clinical trial aimed at assessing the effectiveness of Baricitinib in severe COVID-19 has been authorised by the Italian Drug Agency (AIFA) [https://www.aifa.gov.it/sperimentazioni-cliniche-covid-19] and 8 clinical trials with Ruxolitinib in COVID-19, with dosages ranging from 10 to 20 mg/day, have been until now registered in the "clinical trials.gov" website. Moreover, the

first 11 cases treated in Italy avoided the incoming intubation (Dr Capochiani's personal communication), thus confirming the anti-inflammatory efficacy of JAK1/2 inhibitors in real life. Baricitinib (anti-Janus Kinase therapy approved for RA) has been proposed as a potential treatment for SARS-CoV-2 considering its inhibitory activity both on cytokines production and on Coronavirus endocytosis (21, 22). Starting from this pathogenetic rationale, Cantini *et al.* have recently published the results of the first open-label clinical trial including 24 patients with moderate COVID-19 infection: 12 treated with Baricitinib as add-on therapy (administered for 2 weeks after a median of 6 days since disease onset) compared to 12 patients treated only with Standard of Care (SoC: HCQ and lopinavir-ritonavir). Remarkably, the Baricitinib-treated patients showed a significant improvement of clinical and laboratory parameters, with none of them requiring ICU support (*vs.* 4/12 in the SoC group), and most were discharged within 2 weeks of enrolment (7/12 *vs.* 1/12 in the SoC group). No adverse events were reported. These results, although from a small cohort of patients, demonstrate that Baricitinib could represent a safe and efficacious treatment strategy, especially in the early phase of COVID-19 infection (23). Hopefully, the ongoing five clinical trials will give important information in order to optimise the use of Baricitinib in this condition.

The encouraging results obtained with Baricitinib have opened up new options for other JAK1/2 inhibitors. Among JAK inhibitors, Ruxolitinib is currently used in myelofibrosis, a chronic myeloproliferative disease where the inflammatory status sustains the symptoms (fatigue, fever, flu-like syndrome). In these haematological conditions, by reducing secretion of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, and IFN-γ (24), this small molecule is able to rapidly improve the patients quality of life (25, 26). Moreover, the anti-inflammatory profile of Ruxolitinib has been well documented also in steroid-refractory acute graft-versus-host disease (aGVHD), where it offered 55% of overall rapid responses, espe-

cially in skin, gastrointestinal tract, and liver (27). Nevertheless, as observed by Dr Stebbing, the infective profile of JAK1/2 inhibitors might be not safe enough. In fact, it has been reported that CMV reactivation occurred in 15–39% of aGVHD patients receiving Ruxolitinib and in myelofibrosis and polycythemia vera, in cases of hepatitis B, herpes zoster and tuberculosis reactivation, in addition to pneumonia sustained by *Pneumocystis jiroveci* (28). Regarding the possible superiority of Baricitinib over other JAK1/2 inhibitors, these authors sustained that this small molecule is preferable for its ability to block the viral clathrin-mediated endocytosis. In an *in-vitro* elegant cellular model of SARS-CoV, Dr Wang and coworkers demonstrated that the old Coronavirus entered into cells via pH- and receptor-dependent endocytosis, but also in clathrin- and caveolae-independent way. Interestingly, the virus entry was inhibited by methyl-beta-cyclodextrin, an oligosaccharide used for depleting cholesterol from cell membranes (29). These observations could be taken into account also for deciding which kind of JAK inhibitors might be the best in COVID-19.

What should we expect from targeting IL-1?

Published data on the use of Anakinra (anti-IL1Ra) in COVID-19 patients have appeared less encouraging. Cavalli *et al.* (30) retrospectively evaluated two different dosages of Anakinra, as add-on therapy, compared with a control group (16 patients) treated with standard of care only (SoC: hydroxychloroquine, lopinavir/ritonavir), in moderate and severe acute respiratory distress syndrome (ARDS) requiring non-invasive ventilatory support. The "low dose" cohort (7 patients) received Anakinra 100 mg subcutaneously twice daily: treatment was interrupted early because of inefficacy on patients' biochemical and clinical status. The "high dose" cohort (29 patients) received Anakinra 5 mg/kg intravenously twice daily, resulting in reduction of CRP and progressive improvement of respiratory parameters. Nevertheless, despite a significant reduction in mortality on

the 21st day, no significant differences were found compared to SoC-only in terms of duration of hospitalisation and mechanical ventilation-free survival. Finally, Anakinra even at high dosages resulted quite safe in COVID-19 patients, confirming its potential role in selected conditions (compromised patients with superinfection or contraindications to other anti-rheumatic drugs), as hypothesised in our previous editorial (1). Eight ongoing clinical trials will clarify whether blocking IL-1 may have a significant role in the management of SARS-CoV-2 infection.

How should we treat critical patients in whom anti-cytokine therapies are contraindicated?

We previously proposed the use of immunoglobulins (IVIg) in selected cases of COVID-19 infection, starting from the pathogenetic rationale based on animal models and clinical evidence (1). In particular, the use of IVIg therapy in the early phase of SARS-CoV-2 infection might be an efficacious therapeutic approach due to its inhibitory effect on the FcR-mediated antibody-dependent enhancement (ADE) and macrophage hyper-activation mainly in early seroconverted patients (31-33). Confirming this hypothesis, in three COVID-19 cases IVIg at immunomodulatory dosage, added on therapy to anti-viral and/or antibiotics exerted clinical benefits. The treatment was administered at a mean of 11 days after the onset of viral symptoms, with sudden worsening of dyspnea, severe progression of CT abnormalities (multiple ground glass opacities and bilateral consolidations) and biochemical alterations suggestive of CRS. In these cases, a significant improvement of clinical and biological parameters, together with CT lung lesions has been observed (34).

As well known in the rheumatologic experience, IVIg represent a safe treatment even in infectious conditions and severe comorbid patients. In this regard, Xie *et al.* retrospectively described a fairly large cohort of 58 critical SARS-CoV-2 patients treated with IVIg in ICU. The administration of IVIg within 48h of admission to the ICU resulted in a reduction of the need

for ventilatory support, hospital stay and 28-day mortality (35). Therefore, these preliminary real-life data confirm that therapy with IVIg may represent a potential beneficial therapy, particularly in the early hyper-inflammatory phase of SARS-CoV-2 infection and also a safe option for critical patients with severe co-morbidities and contraindications to immunosuppressive drugs. Up to now, two clinical trials have been registered to investigate the use of IVIg in severe COVID-19 as adjunctive therapy and further studies are required in order to confirm the beneficial effects of this therapeutic option in different stages of COVID-19.

How can we use "old drugs"
such as colchicine to target
COVID-19 inflammatory cascade?
On the basis of the experience gained in the treatment of autoinflammatory disorders, colchicine, immediately appeared to be a promising drug to control innate immunity dysregulation and CRS in COVID-19 (1). Recently, colchicine has been used as an adjunctive therapy in infected patients due to its potential inhibitor effect on cytokine release, in particular on IL-1 and IL-6 axis by interfering with NLRP3 inflammasome. Moreover, the drug has broad anti-viral and anti-inflammatory activities by inhibition of polymorphonuclear cell chemotaxis, neutrophil recruitment and cell adhesion (36). Promising results come from the recent report of the successful employment of colchicine in a kidney transplant patient with COVID-19 pneumonia and in a patient with haemorrhagic pericardial effusion causing cardiac tamponade (37, 38). In the first case, colchicine was administered at onset of progressive respiratory failure, requiring non-invasive ventilation despite anti-viral therapy. Interestingly, colchicine clinical efficacy was associated with a rapid decrease of plasmatic IL-6 levels, thus confirming the potential use of colchicine in patients with signs of systemic inflammation before entering the critical stage of the disease (37). Actually, 10 randomised, controlled or open-label studies are ongoing to test the efficacy of colchicine

in COVID-19 patients (*www.clinicaltrial.gov*). In particular, an open-label, phase 2 study promoted by the Italian Society of Rheumatology (SIR), the Italian Society of Infectious and Tropical Diseases (SIMIT) and by the Italian Thoracic Society (AIPO) and approved by the Italian Drug Agency (AIFA), is currently enrolling hospitalised patients with COVID-19 pneumonia. The study aimed to evaluate the efficacy of colchicine in reducing the rate of progression to critical stage (mechanical ventilation, organ failure, Intensive Care Unit admission and death). Moreover, an interventional, multicentre, double-arm, randomised, open-label, phase 3 study promoted by SIR and the Italian Society of General Medicine and Primary Care (SIMG) aimed to evaluate the efficacy of colchicine in reducing the rate of hospitalisation in symptomatic non-hospitalised patients has been recently approved by AIFA (EudraCT number: 2020-001806-42).

**What have we learned about
the optimal doses of chloroquine
and antimalarial drugs?**
As previously discussed (1), since the early days of the COVID-19 global pandemic the use of antimalarial drugs has been indicated both in patients with minor symptoms and in more severe cases. More recently, chloroquine (CQ) and hydroxychloroquine (HCQ) have been extensively investigated in patients with COVID-19 pneumonia and moderate-to-severe disease due to their known *in-vitro* anti-viral promising effects. Both drugs are able to block viral replication by inhibition of cell entry of SARS-CoV-2 and to prevent virus-cell fusion by interfering with glycosylation of ACE2 receptor and its binding with spike protein. Moreover, HCQ is able to control the cytokine storm with a more potent effect than CQ as demonstrated by *in-vitro* experiments, and it has lower adverse cardiac effects (39). Overall, several trials have been conducted in order to evaluate the safety and efficacy of HCQ as both prophylactic and therapeutic approaches in subjects with COVID-19. Moreover, the majority of these are still in the recruitment phase and other trials are ongoing. Thus,

while the results from *in-vitro* experiments provide a rationale for the use of CQ and HCQ in these patients, the clinical efficacy is still very limited and mainly based on non-randomised clinical observational trials and single-arm and small-cohort protocols. The efficacy of different dosing regimens of HCQ, alone or in combination with azithromycin, has been tested both in hospitalised patients with COVID-19 severe pneumonia than in pauci-symptomatic or mild disease, and compared to standard treatment or no therapy (39-41). The outcomes of efficacy included both the virologic clearance, as proven by nasopharyngeal (NP) swab, and the improvement of different clinical and functional parameters, such as median time to symptom recovery, radiologic findings, haemodynamic stability, respiratory functional parameters, length of Intensive Care Unit (ICU) or hospital stay and mortality. However, up to now some results are controversial and other still unavailable, not allowing at moment to drawn firm and clear indications. In particular, the small sample size of cohorts included, the short follow-up, the lack of studies adequately powered to demonstrate a clinical or statistical difference in the results, and the consistent variability of outcome grade of severity hardly hamper data analysis and comparison (43). Moreover, a recent retrospective, multicentre, cohort study, including more than 1.400 hospitalised patients demonstrated no significant differences in in-hospital mortality between patients who received HCQ with or without azithromycin compared to neither treatment (44). The lack of observed benefit of HCQ on in-hospital mortality is consistent with reported data from other observational trials (42). Notably, cardiac arrest was more frequent in patients who received HCQ with azithromycin compared with patients who received neither drug, thus raising considerable concerns about drug safety in these patients (44). The risk of CQ- or HCQ-induced cardiomyopathy, QTc interval prolongation, which is synergic with azithromycin, and the possibility of cardiac arrhythmias have been widely reported in several anecdotal reports and cohort studies,

in particular in cases of high dose prescription or in the elderly COVID-19 population with multiple comorbidities or concurrent medications causing QTc prolongation (39-41). Indeed, HCQ has been extensively used in the rheumatology setting at doses ranging from 200 mg to 400 mg a day for the long-term treatment of chronic autoimmune diseases and has constantly exhibited an optimal safety profile even in the frailest patients, also providing a protective effect on the endothelium dysfunction (45, 46). Therefore, we may suggest that the reported toxicities should be mainly ascribed to the higher doses employed and to the combination with other drugs. In conclusion, according to available evidence, HCQ could be effective in combination with other drugs (*i.e.* azithromycin or other immunomodulatory or anti-viral agents) in mild or moderate cases but there is still a lack of clinical evidence to support their therapeutic use due to significant methodological flaws of published studies. Similarly, the concept of using HCQ as a preventive strategy in the general population is actually unsubstantiated. Indeed, the heterogeneity of the patients included, selection of the control population, the high rate of lost to follow-up and the handling of missing observations raise concerns about study results and actually limit HCQ administration as prevention of SARS-CoV-2 infection or post-exposure prophylaxis (47, 48).

To overcome all these issues, several randomised clinical trials are currently investigating pre-exposure or post-exposure prophylactic effect and therapeutic efficacy of HCQ in COVID-19 patients (*www.clinicaltrial.gov*). Waiting for the results of these high quality randomised clinical trials, scientific evidence of efficacy and safety of HCQ in patients with COVID-19 is still under debate.

What have we learned about the optimal doses and duration of corticosteroids?

The routine use of corticosteroids as adjuvant therapy in COVID-19 infection remains highly controversial (1). From this perspective, two meta-anal-

yses based on previous literature have recently been performed on this topic (49, 50) and the results of both studies suggested caution when using corticosteroids. In the first one, the authors made a literature search from January 1, 2002 to March 15, 2020, selecting 15 studies and a total of 5270 patients, and concluded that corticosteroid treatment might be associated with higher mortality, longer length of stay, a higher rate of bacterial infection, and hypokalaemia (49). From the second meta-analysis performed on 10 cohort studies and 1 randomised clinical trial involving 5249 subjects, the use of corticosteroids in SARS-CoV-2, SARS-CoV, and MERS-CoV infected patients was associated with a delay in the virus clearing. Furthermore, the treatment did not convincingly improve survival, hospitalisation duration or ICU admission rate and/or use of mechanical ventilation in these patients (50).

Despite evidence derived from retrospective analyses, during the last two months, corticosteroids have been frequently used in clinical practice, particularly in SARS or critical illness (51). Indeed, corticosteroids may control the inflammatory response related to the cytokine storm thus limiting lung damage progression. What we have learned from the clinical ground is that the timing of corticosteroid therapy may represent a crucial point that future studies should better clarify. At present, it has been widely recognised that some patients with COVID-19 present a biphasic disease evolution with an initial phase strongly correlated to viral infection and a subsequent cytokine-mediated respiratory deterioration, largely independent of the viral load. Therefore, the opinion of the experts (52) is to use corticosteroids earlier, in patients presenting a rapid radiographic progression, within 48 hours, associated or not with deterioration of arterial blood oxygen levels. Moreover, experts suggest using low-to-moderate dose, short-course glucocorticoids (*i.e.* prednisolone 40–80 mg/day rapidly tapered to 20 mg/day, with a total treatment period of less than 7 days). On the other hand, there is a general agreement on avoiding corticosteroids

in both milder patients who may benefit from a preserved and sustained immune response against the virus as well as in critically damaged patients when a "too late" employment of these drugs may not only be ineffective, but may also facilitate bacterial infections and metabolic complications. Ongoing trials and registries will provide more solid information regarding the effectiveness of glucocorticoids in subjects with SARS-CoV-2 infection in the near future. In this scenario, what about glucocorticoid therapy in patients with rheumatic diseases? From a rheumatology perspective, according to both ACR and EULAR guidance, low-dose glucocorticoids should be continued when necessary to maintain remission. If indicated, in newly diagnosed or active rheumatic disease low-dose glucocorticoids (<10 mg prednisone equivalent) may also be started (53).

How should we use heparine and anticoagulants in COVID-19?

During the last two months, it has been widely recognised that COVID19 patients may display an hypercoagulable state due to several factors including inflammation, vascular damage and microangiopathy associated with viral infection and immobilisation. Indeed, high D-dimer levels and high fibrinogen levels often observed in hospitalised COVID19 patients have appeared as common points of intersection between inflammation and hypercoagulability (54, 55). In this regard, anticoagulant treatment has been associated with a better prognosis and decreased mortality (56, 57). Therefore, both the International Society on Thrombosis and Haemostasis (ISTH) (58) and the American Society of Hematology (ASH) (59) have recommended the use of prophylactic-doses of LMWH or fondaparinux for hospitalised patients unless they have an increased risk of bleeding. It has also been suggested that unfractioned heparin and LMWH can act as potential inhibitors of tissue proteases and matrix metalloproteinases involved in virus infection, thus providing a potential further indication for their use in hospitalised patients (60). However, it has also been suggested that many pa-

Table I. Features and outcome of COVID-19 infection in patients with rheumatic disease.

Author Ref	Disease, pts (n), sex	Age, yrs	Disease activity at infection	Co-morbidities	Therapy at infection	Pneu-monia	Hospital-isation	ARDS	COVID-19 therapy	ICU	Oxygen therapy	Death	Discharge	Disease flare
Mathian (64)	SLE, 17, 76% F	53 (27-69) median	All SLEDAI 0	Yes	HCQ 100% GC 83% IS 41%	13 (76%)	14 (82%)	5 (29%)	TCZ 1 (6%) Antibiotics 9 (53%)	7 (41%)	11 (65%) 5/11 IMV	2 (14%)	5/14 (36%)	No
Han (65)	SLE, 1, F	47	No	No	GC	Yes	Yes	No	Antibiotics Antivirals	No	No	No	Yes	No
Mihai (18)	SSc, 1, F	57	No	Yes	IS (TCZ)	No	No	No	No	No	No	No	NA	No
Monti (66)	RA 3, SpA 1, 100% F	58±5 mean	NR	Yes	HCQ 25% GC 50% IS 75% b/tsDMARDs 100%	No	1 (25%)	No	Antibiotics Anti-virals HCQ	No	Yes	No	Yes	No
Song (67)	RA, 1, F	61	No	No	HCQ GC LEF	Yes	Yes	No	Anti-virals HCQ	No	No	No	Yes	No
Duret (68)	SpA, 1, M	60	No	No	MTX ETN	No	Yes	No	Paracetamol	No	No	No	Yes	No
Emmi (69)	SS, 1, F	68	NR	NR	HCQ GC	Yes	Yes	Yes	HCQ Antibiotics Anti-virals TCZ	Yes	Yes IMV	No	No at 14th April 2020	NR
Haberman (70)	RA, 17, F/M	NA	NA	Yes	c/b/ts DMARDs	6 (35%)	6 (35%)	No	HCQ Antibiotics Anti-virals TCZ	0%	6 (35%)	0%	6 (100%)	NR
Haberman (70)	PsA, 14, F/M	NA	NA	Yes	c/b/ts DMARDs	2 (14%)	3 (21%)	1 (7%)	HCQ Antibiotics Anti-virals	1 (7%)	3 (21%) 1 (7%) IMV	0%	2 (14%)	NR

SLE: systemic lupus erythematosus; SSc: systemic sclerosis; RA: rheumatoid arthritis; SpA: spondyloarthritis; SS: Sjögren's syndrome; SLEDAI: SLE disease activity index; ARDS: acute respiratory distress syndrome; HCQ: hydroxychloroquine; GC: glucocorticoid; IS: immunosuppressant; LEF: leflunomide; MTX: methotrexate ETN: etanercept; TCZ: tocilizumab; c/b/tsDMARD: conventional/biologic/targeted synthetic disease-modifying anti-rheumatic drug; F: female; M: male; RF: respiratory failure; ICU: Intensive Care Unit; IMV: invasive mechanical ventilation; NR: not reported; NA: not available.

tients may require higher doses of anticoagulation to contrast their risk of thrombotic complications (61). This latter point is currently a matter of important debate, being potentially associated with fatal major bleeding. Thus, to avoid fatal bleeding and prevent thrombotic events due to over/under treatment, it has also been suggested replacing oral anticoagulant therapies (VKA and DOAC) with LMWH. In support of this, it has been highlighted that patients on VKA/DOAC might be exposed to under/over treatment because of the variability of vitamin K metabolism, multiple pharmacological interactions and liver insufficiency (62). The ongoing clinical trials will help to clarify the efficacy and safety of these therapeutic approaches and their contribution to prevent thrombotic events in COVID patients.

What is the impact of SARS-CoV-2 infection in patients with systemic autoimmune and chronic inflammatory diseases?

Data on the occurrence of SARS-CoV-2 infection in patients with systemic autoimmune and chronic inflammatory diseases are actually limited. Despite the low level of current available evidence, the ACR has recently published guidance statements to promote optimal care of rheumatic patients during the current pandemic. This guidance is thought as a "living document" open to future updates (53). The International (EULAR COVD-19 Rheumatological Database *https://www.redcap.rss.mhs. man.ac.uk/surveys/?s=NEXNJFWX38* and COVID-19 Global Rheumatology Alliance *https://rheum-covid.org*) and the Italian Rheumatology Societies (Registro COVID-19-RMD SIR

https://redcap.reumatologia.it/surveys/ index.php?s=YLDERDE88W) created registries dedicated to the reporting and monitoring of COVID-19 occurring in adult and paediatric patients with rheumatic diseases, which will provide novel evidence regarding infection outcome in this group of patients and possible risk factors associated with worse outcome (63). In the meantime, a survey of single reports or case series published since the outbreak of COVID-19 highlights interesting considerations (Table I) (18, 64-70). Up to now, in almost all reported cases, patients with autoimmune diseases and SARS-CoV-2 infection were in remission state due to their concomitant treatment with non-biologic and biologic drugs. Most of them presented concomitant comorbidities, risk factors for poor outcomes of COVID-19. Interestingly,

although more than half of infected patients experienced SARS-CoV-2 related pneumonia, the clinical course of the infection was relatively favourable. Moreover, very few patients experienced ARDS, invasive mechanical ventilation or needed ICU admission, and the mortality rate was low. Surely, the multifaceted pathogenetic mechanisms and phenotypic features characterising systemic autoimmune diseases may exert a different impact on COVID-19 outcome. Indeed, patients with autoimmune disorders characterised by higher prevalence of interstitial lung disease, like systemic sclerosis and systemic lupus erythematosus (SLE), may be at increased risk of respiratory or life-threatening complications from SARS-CoV-2 compared with other rheumatic diseases (Table I). Nevertheless, the immunomodulatory effects induced by the concomitant treatment with immunosuppressive drugs may partly reduce the aggressive inflammatory response elicited by SARS-CoV-2 as well as the excessive host immune activity (71). Therefore, we can hypothesise that many drugs commonly used in patients with systemic autoimmune diseases may have a potential therapeutic role in the setting of COVID-19 (2). The heterogeneity of the patients included in the recent studies, selection of the control population, the high rate of lost to follow-up and the handling of missing observations raise concerns about study results. Waiting for more robust results from randomised controlled trials and data on the susceptibility, disease course and prognosis in COVID-19 patients with rheumatic diseases, the Rheumatology community encourages not to withdraw background non-biologic and biologic therapies in order to prevent disease flares that may be even more harmful in these patients (47, 48, 72-74). Temporary drug interruption should be highly recommended for infected patients. Moreover, close monitoring is recommended especially in patients with pre-existing disease-related pulmonary involvement. Following SARS-CoV-2 exposure, reintroduction of immunosuppressive drugs may be considered only after documentation of two negative NP swabs and after 2 weeks of symptom-free observation (74). The management and follow-up of these patients is highly critical due to the importance of guaranteeing care continuity in order to avoid patients having direct access to the hospital environment. The application of telemedicine in this context may represent an important tool to overcome all these issues and provide the best management and follow-up in patients with autoimmune systemic diseases.

How should we screen rheumatic patients prior to biologic therapy for active SARSCoV-2 infection and immunisation status?

The outbreak of COVID-19 still has an impact on the management of patients affected by rheumatic diseases and it will probably continue to influence our routine clinical activity for a long time. It has presented particular challenges in caring for and managing patients, suffice it to say the impossibility of performing periodic face-to-face outpatient evaluations or the consideration of the risk of infection when starting a new therapy (1).

Since the COVID-19 era began, one of the main issues for rheumatologists is to understand which screening tests to a patient with rheumatic disease should undergo before starting immunosuppressive therapy. Currently, no precise indications and protocols to follow have been given, but information that has come from the scientific world in recent months can help us choose.

Based on recent experiences, serological detection of antibodies directed towards different antigenic proteins of SARS-CoV-2 is required to determine the immune status of asymptomatic patients, but are unlikely to play any role in screening or for the diagnosis of early infections (75).

Up to now, lateral flow assays have been developed to detect not only SARS-CoV-2 virus antigens, but also IgM and IgG antibodies against S-proteins from the coronavirus spike (76), or against N-protein, one of the immunodominant antigens in the early diagnosis of COVID-19. These tests have the advantage of yielding results rapidly and a low cost method of detection and the disadvantage of having poor sensitivity. Both for the antigenic and for the antibody analysis there are some issues that limit the validity of these tests. For example, the analysis of viral antigens may be less reliable for the variability of viral loads due to low infectious burden or sampling variability. On the other hand, we have to take into account the non-specificity of IgM response to the virus and that the host immune system requires weeks to develop specific IgG responses. Therefore, the host immune system status and the timing of antibody analysis is essential to make the serological screening reliable. Serological assays are currently being developed for large-scale epidemiological investigations and for diagnosis/confirmation of late COVID-19 cases (77), but not for screening or diagnosis of early infections. Both antigenic and antibody serological testing currently available may help the rheumatologist for initial screening before prescribing immunosuppressive therapy, but we must consider all the limitations of these tests and we will need further validation studies.

So far, NP and/or oro-pharyngeal (OP) swabs are often recommended for screening or diagnosis of early infection (78-80). Swab analysis could be added to a hypothetical screening protocol for SARS-CoV-2 infection in rheumatologic patients who need to undergo immunosuppressive therapy to rule out a viral infection. Both swabs are necessary because SARS-CoV-2 RNA was detected only in 32% of OP swabs, which was significantly lower than that in NP swabs (63%) (80). Therefore, a correct execution of the swab allows a correct analysis of the viral load by means of real time RT-PCR, the assay recommended for molecular testing (78, 81-83). Its advantage is that amplification and analysis are done simultaneously in a closed system to minimise false-positive results associated with amplification product contamination. In fact, coronaviruses have a number of molecular targets within their positive-sense, single-standard RNA genome that can be used for PCR assays, including envelope glycoproteins spike (S), envelope (E), transmembrane (M),
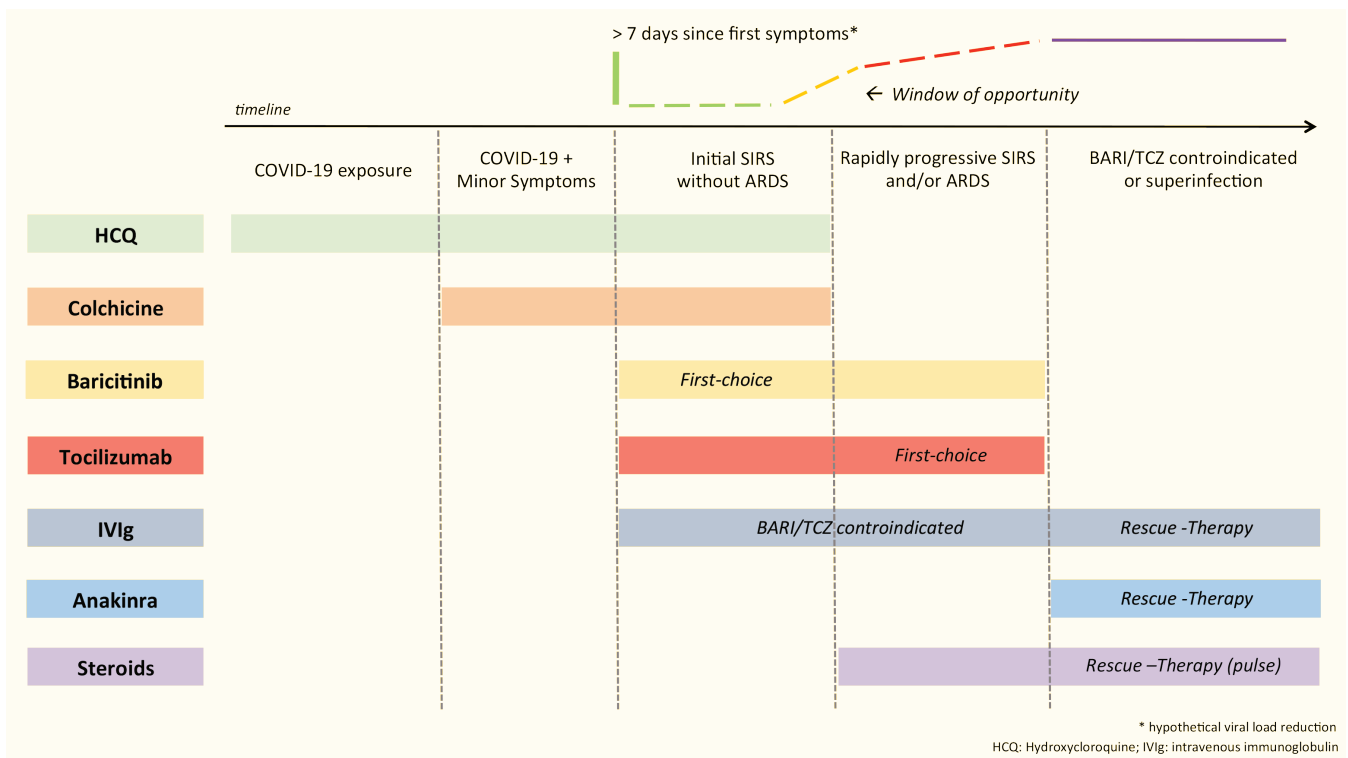
Fig. 1. Potential use and optimal timing of anti-rheumatic drugs in the management of COVID-19.
*These data derive from the critical analysis of the present literature. Drugs can be used alone or in combination, to the best of the clinician's knowledge.

heli case (Hel) and nucleocapsid (N). In addition, there are species-specific accessory genes that are required for viral replication such as (RdRp), haemoagglutinin-esterase (HE) and open reading frames ORF1a and ORF1b. Therefore, according to the recent international guidelines the WHO correctly advise screening with E gene assay followed by a confirmation assay with the RdRp gene (84), supporting the idea that the use of two molecular targets are required to avoid potential cross-reaction with other endemic coronaviruses. Real-time RT-PCR assay remains the molecular test of choice for the diagnosis of SARS-CoV-2 infection, useful for excluding latent infections in patients starting immunosuppressant drugs, while antibody-based techniques are being introduced as supplementary tools for investigating the immune status of asymptomatic patients. Therefore, in patients with rheumatic disease who need to start immunosuppressive therapy, the combination of viral and antibody serological analyses with detection of viral replication by RT-PCR could represent a potential useful screening approach.

Conclusions
As discussed previously, growing evidence indicates that the systemic manifestations of COVID-19 are mediated by an immune reaction to a virus. If so, this condition may resemble other diseases well known to rheumatologists, such as HBV-associated polyarteritis nodosa (85), or HCV-positive mixed cryoglobulinaemia (86-88).

In spite of their viral aetiology, the management of these conditions relies not only on the use of antiviral agents, but also on anti-inflammatory immunosuppressive therapies, especially to control the most severe manifestations of the disease (9).

In our previous editorial we discussed the rational basis for the use of some drugs currently employed in rheumatology as an adjunct therapy in the management of COVID-19, and their putative optimal timing. Based on the preliminary subsequent off-label experiences published in the last two months, we have tried to update the present hypothetical use of these medications (Fig. 1). Obviously, the real efficacy and safety of these drugs will be more definitely established when solid evidence deriving from the ongoing randomised prospective controlled trials will become available.

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