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Complete List of Authors:	Kralj-Hans, Ines; Ashford and Saint Peter's Hospitals NHS Trust, Li, Kuo; Imperial College London Wesek, Adrian; Imperial College London Lamorgese, Alexia; JB Medical Ltd Omar, Fatima; Ashford and Saint Peter's Hospitals NHS Trust Ranasinghe, Kapila; Ashford and Saint Peter's Hospitals NHS Trust McGee, Megan; Ashford and Saint Peter's Hospitals NHS Trust Brack, Kieran; Ashford and Saint Peter's Hospitals NHS Trust Li, Shiliang; East China University of Science and Technology Aggarwal, Ritesh; Max Hospital Bulle, Ajay; Meditrina institute of medical science Kodre, Aparna; Noble Hospital Sharma, Shashank; Ashford and Saint Peter's Hospitals NHS Trust, St Peters Hospital Emergency Department Fluck, David; Ashford and Saint Peter's Hospitals NHS Trust, Cardiology John, Isaac; Ashford and Saint Peter's Hospitals NHS Trust Sharma, Pankaj; Royal Holloway University of London Belsey, Jonathan; JB Medical Ltd Li, Ling; University of Kent Seshasai, Sreenivasa Rao Kondapally; St George's University Hospitals NHS Foundation Trust Li, HongLin; Shanghai Key Laboratory of New Drug Design Marczin, N; Imperial College London Chen, Zhong; Ashford and Saint Peter's Hospitals NHS Trust
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Leflunomide Treatment for Patients Hospitalised with COVID-19: DEFEAT-COVID Randomised Controlled Trial

Authors:

Ines Kralj-Hans[1], Kuo Li[2], Adrian Wesek[3], Alexia Lamorgese[4], Fatima Omar[1], Kapila Ranasinghe[1], Megan McGee[1], Kieran Brack[1], Shiliang Li[5], Ritesh Aggarwal[6], Ajay Bulle[7], Aparna Kodre[8], Shashank Sharma[1], David Fluck[1], Isaac John[1], Pankaj Sharma[9], Jonathan Belsey[4], Ling Li[3], Sreenivasa Rao Kondapally Seshasai[10], *HongLin Li[5], *Nandor Marczin[2], *Zhong Chen[1], on behalf of DEFEAT-COVID Investigators

*Joint senior authors

Affiliations:

1. Ashford and St Peter's Hospital NHS Foundation Trust, UK
2. Faculty of Medicine, Imperial College London, UK
3. University of Kent, UK
4. JB Medical, UK
5. Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, China
6. Max Hospital, New Delhi, India
7. Meditrina institute of medical science, Nagpur, India
8. Noble Hospital, Pune, India
9. Institute of cardiovascular research, Royal Holloway, University of London, UK
10. St George's University Hospitals NHS Foundation Trust, UK

Corresponding author: Dr Ines Kralj-Hans, i.kralj-hans@nhs.net

Ashford and St Peter's Hospital NHS Foundation Trust, Guildford Street, Chertsey, KT16 0PZ, UK

Abstract

Objective: To evaluate the clinical efficacy and safety of leflunomide (L) added to the standard of care (SOC) treatment in COVID-19 patients hospitalised with moderate/critical clinical symptoms.

Design: Prospective, open-label, multicentre, stratified, randomised clinical trial.

Setting: Five hospitals in United Kingdom and India, from September 2020 to May 2021.

Participants: Adults with polymerase-chain-reaction (PCR) confirmed COVID-19 infection with moderate/critical symptoms within 15-days of onset.

Intervention: Leflunomide 100mg/day (3-days) followed by 10-20mg/day (7-days) added to standard care.

Primary outcomes: The time to clinical improvement (TTCI) defined as two-point reduction on a clinical status scale or live discharge prior to 28 days; safety profile measured by the incidence of adverse events (AE) within 28 days.

Results: Eligible patients (n=214; age 56.3±14.9 years; 33% female) were randomised to SOC+L (n=104) and SOC group (n=110), stratified according to their clinical risk profile. TTCI was 7 vs. 8 days in SOC+L vs. SOC group (HR 1.317; CI 0.980, 1.768; p=0.070). Incidence of serious adverse events was similar between the groups and none was attributed to leflunomide. In sensitivity analyses, excluding 10 patients not fulfilling the inclusion criteria and 3 who withdrew consent before leflunomide treatment, TTCI was 7 vs. 8 days (HR 1.416, CI 1.041, 1.935; p=0.028), indicating a trend in favour of the intervention group. All-cause mortality rate was similar between groups, 9/104 vs. 10/110. Duration of oxygen dependence was shorter in the SOC+L group being a median 6-days (IQR 4-8) compared to 7-days (IQR 5-10) in SOC group (p=0.047).

Conclusion: Leflunomide, added to the SOC treatment for COVID-19, was safe and well tolerated but had no major impact on clinical outcomes. It may shorten the time of oxygen dependence by one day and thereby improve TTCI /hospital discharge in moderately affected COVID-19 patients.

Trial registration

EUDRACT: CTA 21517/0004/001-0001 2020-004994-27

ClinicalTrials.gov: NCT05007678

Strengths and limitations

- International, prospective, randomised controlled study
- Repurposing a marketed drug with established safety profile and promising dual antiviral and immunomodulating medication based on strong drug discovery data.
- Study participants had milder COVID-19 disease than originally intended, thus eroding the power of the study
- Evolving standard of care therapy possibly diminished measurable benefit of leflunomide

Introduction

COVID-19 pandemic caused unprecedented strain on health care services around the world. It has affected almost 16 million people globally and caused over 6 million deaths so far.[1] Symptoms include pneumonia, systemic inflammatory response and cardiovascular complications with high morbidity and mortality. Progressive deterioration is thought to be related to the kinetics of viral replication culminating in a surge of inflammatory mediator release, "cytokine storm".[2] Around 5-10% of infected patients experience severe or life-threatening symptoms with high mortality.[3]

Direct-acting and host-targeting antiviral treatments are the two approaches in treating viral infections. Host targeting antiviral treatments may have an advantage over direct antivirals as they enable the body to fight against a broad spectrum of viruses by simultaneously blocking viral replication and overcoming the potential of viral mutagenesis.[4] Anti-inflammatory medications have been shown to improve survival through dampening of the inappropriate immune response in susceptible patients.[5] This has led to the search for a drug with such therapeutic properties.

Leflunomide is a drug licenced to treat rheumatoid arthritis (RA).[6] It is widely available, cost-effective and can be easily administered both in the hospital and domestic settings. In preclinical models of cell and animal infection by SARS-CoV-2, leflunomide was shown to be a potent inhibitor of human dihydroorotate dehydrogenase (DHODH), an enzyme vital to viral replication in the host cell.[7,8,9] It has the potential advantage of not only targeting the virus infection but also suppressing the ensuing inflammatory response which may play a role in more progressive stages of infection leading to serious complications.

The DEFEAT-COVID study (Targeting **de** novo pyrimidine biosynthesis by le**f**lunomide for tr**eat**ment of **co**rona **vi**rus **d**isease 2019) tested whether leflunomide added to standard care was clinically effective and safe for COVID-19 moderate/severe symptoms.

Methods

Study design – This was a multicentre, international, open label, prospective, randomised controlled clinical trial set up at 5 hospitals (two in UK and three in India). The recruitment took place between September 2020 and May 2021, and was approved by all relevant ethics committees.

Participants - Patients aged 18 years and above presenting with moderate to critical symptoms of PCR-confirmed COVID-19 disease within 15 days of symptoms onset were recruited. Patients with respiratory compromise and blood oxygen saturation (SpO_2) <93% on room air detected on pulse oximeter were considered to fulfil the moderate infection criteria. Patients with respiratory failure, septic shock and/or multiple organ dysfunction/failure needing assisted ventilation were considered to be critically ill. Pregnant or breast-feeding women, individuals already receiving specific monoclonal antibody therapy or those with severe immunodeficiency syndrome and hypoalbuminaemia and patients with hypersensitivity to leflunomide or liver

enzymes aspartate transaminase (AST) / alanine transaminase (ALT) ≥ 2 x upper limits of normal (ULN) were excluded from the study. All participants gave written informed consent to a member of their clinical care team.

Randomisation – Consented participants were randomised by a member of the clinical care team to either the control arm (receiving standard of care treatment [SOC] alone) or the intervention arm (SOC treatment + leflunomide (SOC+L]) using a stratified block randomisation web-based algorithm. Patient admission data (age </≥ 70; co-morbidities; clinical status based on National Early Warning Score 2, NEWS2)[10] were used to stratify patients into 4 risk categories. Group 1: high/moderate comorbidity risk with NEWS2 score ≥5; Group 2: high/moderate comorbidity risk with NEWS2 score <5; Group 3: low comorbidity risk with NEWS2 score ≥5; and Group 4: low comorbidity risk with NEWS2 score <5.

Interventions - The definition of the SOC treatment for COVID-19 evolved nationally and internationally through the course of our study, with progressive evolution in the understanding of disease pathology and emerging treatment evidence. The SOC during the time of the study across all sites involved four main treatment domains: steroids, anticoagulation, antibiotics, and antiviral medications. The intervention group (SOC+L) received oral leflunomide at a loading dose of 100mg/day for three days and then 20mg/day for 7 days as a maintenance dose. The maintenance dose was reduced to 10mg/day if liver enzymes AST/ALT exceeded 2 x ULN. Leflunomide treatment was stopped early if AST/ATL exceeded 3 x ULN during the intervention. Study participants received additional COVID-19 therapies, including monoclonal antibodies, at the discretion of the direct care clinical team, even if leflunomide was initiated.

Study procedures - Patient related clinical/investigation data, treatment compliance, outcomes and adverse events (AE) were collected by the site investigators and recorded on the pre-specified daily electronic case report form (e-CRF) (see **Error! Reference source not found.**). Adverse events (AE) were graded according to the Common Terminology Criteria for Adverse Events.[11] Blood samples were collected and processed for quantifying viral load (on days 1, 7, 11, 15, 28 or day of discharge) and for future inflammatory profiling (on days 1, 3 and 11). Liver enzymes were measured at baseline, on day 3 after the leflunomide loading and on discharge. Patient questionnaire was administered at 28- and 90-days after randomisation to monitor the persistence of symptoms possibly associated with long COVID syndrome.[12]

Blinding - Site investigator teams and direct clinical care teams were not blinded to the randomisation outcomes, but neither were provided information about the aggregate patient outcomes.

Outcomes - The primary outcome is the time (days) from randomisation to clinical improvement (TTCI) of two points on a seven-category clinical status scale or live discharge from hospital prior to 28 days.[13] The clinical status ordinal scale consisted of the following: 1 not hospitalised, resumption of normal activities; 2 not hospitalized, but unable to resume normal activities; 3 hospitalised, not requiring supplemental oxygen; 4 hospitalised, requiring supplemental oxygen; 5 hospitalised, requiring nasal high-flow oxygen (HFNC) therapy, non-invasive mechanical ventilation (NIV), or both; 6 hospitalized, requiring extracorporeal membrane oxygenation (ECMO), invasive mechanical ventilation (IMV), or both; and 7 death.

Safety profile of leflunomide in this group of patients was assessed from incidence rates of AE deemed to be serious and/or severe (≥Grade 3). Grading guidelines suggest 5 categories: 1 mild, asymptomatic or mild symptoms, clinical or diagnostic observations only, intervention not indicated; 2 moderate, minimal, local or non-invasive intervention indicated, limiting age-appropriate instrumental activities of daily livings (ADL); 3 severe, medically significant but not immediately life-threatening, hospitalization or prolongation of hospitalization indicated, disabling; limiting self-care ADL; 4 life-threatening consequences, urgent intervention indicate; 5 death related to AE. In addition, the incidences, and levels of liver transaminitis (ALT, AST) were assessed.

The main secondary outcomes were focused on overall (all-cause) mortality, and oxygen dependence (duration in days) assessed by S/F ratio (i.e. oxygen saturation detected by pulse oximeter [SPO_2] / supplemental oxygen concentration [FiO_2]) and impact on viral replication (viral load). Additional secondary outcomes included inflammatory targets such as CRP, lymphocyte counts, and selected cytokines (initially focussing on IL2, IL6, TNF-α). The concept of long COVID emerged during the study, so we used the data from our questionnaires at 28 and 90 days to comment on long COVID symptoms

Statistical analyses

Sample size calculation - The primary outcome measure was a time-to-event analysis based on an assessment of TTCI. Since our study protocol was conceived and developed during the initial peak of the global pandemic, the precise hazard ratio for major clinical outcomes related to this infection was largely unknown and, therefore, sample size calculation was based on the proportion of patients expected to meet the outcome criteria by 28 days.[14] Assuming $\alpha = 0.05$, $\beta = 0.20$ and allocation ratio = 1:1, the number of patients per treatment arm was estimated to be 74. We expected a 20% attrition rate, so the total number of patients required in the study was calculated to be 178, 89 patients in each arm.

Analysis population - The full analysis set was defined according to the intention to treat principle (ITT). All subjects randomised were included in the ITT analysis set for the primary outcome, regardless of whether they received any dose of their allocated treatment. This analysis set was used to summarize baseline patient characteristics and to carry out all efficacy and safety assessments. Subjects were analysed according to their randomised treatment allocation. We also present a modified intention to treat analysis for the primary and secondary outcomes to account for study participants who were randomised in error and those who withdrew consent prior to the intervention.

Primary outcomes - The TTCI data was estimated using Kaplan-Meier survival curves. Hazard ratio and 95% confidence intervals were estimated using Cox proportional hazards regression models. The primary analysis was

stratified by the randomisation strata (baseline risk indicators and NEWS2 score). Log rank test was used for comparing the Kaplan-Meir curves, hazard ratios and their confidence intervals for the significance of the treatment effect.

Secondary outcomes - Continuous secondary outcomes were evaluated for within-groups differences using the Mann-Whitney U or Wilcoxon rank tests, respectively, depending on the data distribution identified: parametric or non-parametric. Statistical normality was assessed using the Shapiro-Wilk method. Categorical outcomes were assessed for between-group differences using the chi-square method and expressed as %. For all outcomes, statistical significance was accepted at a 2-sided α of 0.05.

Adverse events - AEs were coded using MedDRA and assigned grades based on National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03.[11]

Public and patient involvement

Patient volunteers were consulted regarding the study design and materials to be provided to the potential participants (patient information sheet, consent forms, questionnaires). Two lay members were appointed to the Trial Steering Committee and provided input on the conduct of the study.

Results

Recruitment, Randomisation, Assignment of Therapy and Follow-up

Between September 2020 and May 2021, 214 patients were recruited to the study from 2 UK Hospitals (n=66, 31%; Ashford and St Peters' NHS Trust, Surrey; Kingston Hospital NHS Trust, London) and 3 Hospitals in India; (n=148, 69%; Max Hospital, Delhi; Meditrina Institute, Nagpur; Noble Hospital, Pune). Due to the wavering new COVID-19 infections, the UK recruitment came to a halt in February 2021 and patients at the three Indian sites were recruited in the remaining period. Of the 214 participating patients, 104 were randomised to the intervention (SOC+L) group and 110 to the control (SOC) group. In the SOC+L group, 3 patients withdrew study consent after randomisation, and did not receive leflunomide therapy. During the data cleaning process, 10 patients were flagged as not meeting the inclusion criteria (6 in SOC+L; 4 in SOC), as they did not have moderate COVID-19 symptoms at the time of randomisation. Daily clinical data were collected for all patients during hospitalisation and the patients were asked to complete follow-up questionnaires at 28- and 90-days after randomisation, as shown in Figure 1 (CONSORT diagram).

Baseline patient characteristics were similar between the SOC+L and SOC groups, summarised in Table 1.

Characteristics	**SOC+L** n=104	**SOC** n=110	**p***
Age, yrs mean ± sd	55.2±14.7	56.4±15.2	NS
BMI, kg/m^2 mean ± sd	27.3±5.1	27.7±5.6	NS
Gender at birth, % Female	28.8	37.3	NS
Ethnicity; %			
South Asian	75.0	69.0	NS
White	24.0	30.0	
Arab	-	0.91	
Comorbidities, %			
BMI ≥ 40 kg/m^2	2.9	4.6	
Age ≥ 70 yrs	18.3	20.0	
Chronic respiratory disease	10.0	15.6	
Chronic cardiovascular disease	15.0	15.6	
Chronic renal disease	5.0	4.7	
Diabetes	39.9	36.0	NS
Immunosuppressive diseases	11.7	10.9	
Others			
Malignant neoplasm	6.7	4.7	
Chronic haematologic disease	1.7	1.6	
Chronic neurological disorder	18.3	6.3	
Malnutrition	1.67	1.6	
Smoking (present or past)	36.7	34.4	
Symptom duration at randomisation, day median (IQR)	6 (4-8)	6 (5-8)	NS
NEWS 2 score at randomisation median (IQR)	6 (4-8)	5 (4-8)	NS

CRP at randomisation median (IQR)	28 (9-77)	32 (13-64)	NS
Transaminase at randomisation >ULN, %			
ALT	44.7	31.7	NS
AST	35.4	28.4	
Stratification, %			
Group 1	12.5	14.5	
Group 2	14.4	16.4	NS
Group 3	48.1	46.4	
Group 4	25.0	22.7	

Table 1: Baseline patient characteristics at the time of randomization

**NS = non-significant, p ≥ 0.05 significant*
BMI: Body mass index; ULN = upper limits of normal; Group 1: High/Moderate comorbidity risk with NEWS2 score ≥5; Group 2: High/Moderate comorbidity risk with NEWS2 score <5; Group 3: Low comorbidity risk with NEWS2 score ≥5; and Group 4: Low comorbidity risk with NEWS2 score <5. Creatinine ULN: 104umol/L; ALT ULN: 49 U/L; AST ULN: 48 U/L; Immunosuppressive disease: asplenia, rheumatological disorder.

Treatment Assignment and Compliance

Full course of leflunomide therapy was completed by 81/104 patients (78%). Of the 19 patients (16 in UK, 3 in India) who did not complete treatment, 3 patients did not receive a single dose of leflunomide as they withdrew consent soon after randomization, 5 patients died prior to completion of the full course, 8 patients stopped leflunomide early when ALT/AST exceeded 3 x ULN laboratory reference range, 1 patient had tocilizumab introduced to replace leflunomide, 1 patient self-discharged early and 1 refused final two doses. Leflunomide treatment compliance appeared to be better in participants from Indian centres as 92% of them received the full dose of leflunomide compared to 52% of patients in the UK centres which was largely due to a higher incidence of liver enzyme transaminitis and mortality observed in the UK cohort.

There was no significant difference in the assignment of standard of care treatment between the SOC+L and SOC groups as shown in Figure 1. It included corticosteroids, anticoagulants, antibiotics, and antiviral therapies. There were some differences in the proportions of patients receiving additional adjunct therapies such as hydroxychloroquine and immunotherapy (Supplementary table 1). Overall, hydroxychloroquine was prescribed to similar proportion of patients in the intervention and the control group (47%) but the proportions of patients receiving it in the UK was much smaller, 3% compared to 67% in India. A small number of patients received immunomodulating drugs such as interferon alpha and beta (n = 20 in India), tocilizumab and bevacizumab (n = 5 in the UK, n = 2 in India).

Primary Outcomes

Time to clinical improvement of 2 points on a clinical status scale/discharge before 28 days

In the ITT analyses (n = 214), SOC+L group did not have a significantly shorter TTCI than the SOC group within 28 days of randomisation; the median was 7.0 (IQR 7.0 - 8.0) days vs. 8.0 (IQR 7.0 - 9.0) days, respectively; with a hazard ratio (HR) of 1.32 (CI 0.98 -1.77), p = 0.070 (Figure 2).

In modified ITT population (n = 201) where 3 patients who withdrew consent after being randomised to the SOC+L group but never received leflunomide treatment and 10 patients who did not fulfil moderate COVID-19 symptoms at randomisation were excluded from analysis, the median TTCI was significantly shorter in the SOC+L group than SOC group by 1.0 day, median 7.0 days (IQR 7.0 -8.0) vs. 8.0 (IQR 7.0 – 9.0), respectively, with a HR of 1.42 (CI 1.04 – 1.94); p = 0.028.

Safety

Incidences of AE of all grades are summarized in Table 2.

Patients	SOC+L (n = 104)	SOC (n = 110)
Adverse events (n) / Patients (n)	121 / 55	91/ 38
Grade 1 (Mild)	58 / 39	48 / 32
Grade 2 (Moderate)	23 / 13	17 / 9
Grade 3 (Severe)/ Grade 4 (Life threatening events)	31/15	16/9
Grade 5 (Deaths)	9	10

Table 2: Incidence of reported adverse events in both treatment arms.

At least one AE was reported in 99/214 participants, and most of them were mild in severity. AEs of moderate grade were reported in 13/104 patients in SOC+L group and 9/110 patients in SOC group. Serious AEs (n=47) were reported in 15/104 patients in SOC+L groups and 9/110 in SOC group and 19 patients died (9 in SOC+L group, 10 in the SOC group). There was no significant difference in the incidence of AE reported between the two groups. No Serious AEs were attributed to leflunomide.

Liver function

At baseline, more patients with greater than ULN levels of ALT and AST were randomized in the SOC+L group than the SOC group (ALT: 46 vs 33, p = 0.049; AST: 31 vs 24, p = 0.340). By Day 3/4, following the initial loading of leflunomide therapy in the SOC+L group, there was a significantly higher number of patients with greater than ULN level of ALT and AST in the SOC+L than the SOC group (64 vs 38, p <0.001; and 51 vs 24, p <0.001). By discharge, the difference in the number of patients with ALT and AST transaminitis between the SOC+L and SOC groups was no longer significant (28 vs 27, p = 0.633; and 20 vs 17, p = 0.318) (Supplementary table 2). Leflunomide therapy was terminated early if transaminase levels exceeded 3 x ULN. However, there were 5 patients in India who continued with leflunomide therapy at the discretion of the researcher and direct care team with close monitoring of their liver function. Interestingly, in this subset of patients, the transaminase levels improved despite continuation of therapy. There were no adverse events related to clinically significant liver injury due to leflunomide. AEs related to liver dysfunction were reported in 16/104 (15.4%) patients in SOC+L group, 7 were mild, 8 were moderate and 1 was severe. Of these, 10 were deemed possibly treatment related and leflunomide treatment was discontinued in 9 patients. Comparatively, in the control group, 6/110 (5.5%) patients had liver dysfunction related AE. Five of them were mild and 1 case was severe.

Secondary Outcomes

A modified intent to treat approach was used for data from 201 patients for all secondary outcomes. This included 95 patients in the SOC+L group and 106 patients in SOC group. For these analyses we excluded 3 patients in the SOC+L group who withdrew consent and never received leflunomide and 10 patients (6 SOC+L; 4 SOC) who did not fulfil moderate COVID symptoms inclusion criterion.

Mortality

There was no difference in all-cause mortality within 28 days of randomization between the treatment arms as 9/95 (9.47%) of patients died in SOC+L group compared to 10/106 (9.43%) in SOC groups. The survival curves diverge in favour of the SOC+L group after 10 days of hospital treatment, but the curves converged again after 3 weeks (when majority of the patients have been discharged). All deaths were attributed to complications related to Covid-19 (Figure 3**,** panel A)

Oxygenation and assisted ventilation

Oxygen independence is defined by maintenance of SpO_2/FiO_2 Air ratio > 4.43. There was a difference in the median time the participants required to be completely weaned off oxygen therapy between groups; 6.0 (IQR 4.0 – 8-0) days in the SOC + L group vs. 7.0 (5.0 – 10.0) days in the SOC group, p = 0.047 (Figure 3, panel B)

Non-invasive ventilation was required for 14.4% of patients in SOC+L group vs. 16.4% in the SOC group. The duration of non-invasive ventilation was 6.0 (IQR 2.0-9.0) days in the SOC+L group compared to 4.5 days (IQR 2.3-6.8) in the SOC group. The proportion of patients admitted to level-2 Intensive Care Unit (ICU) was 8.7 % in the SOC+L group and 8.2% in the SOC group. The median time spent at ICU was 8.0 (IQR 5.0-10.0) days vs. 9.0 (IQR 5.0-13.0) days, respectively**.** Invasive ventilation was required for 3.9% of patients in the SOC+L group and 5.5% in the SOC group with median duration of 6 (IQR 4.8, 6.0) days vs. 7.0 (IQR 5.3 - 11.8) days, respectively. None of the between group comparisons were statistically significant. Patients recruited in India were significantly less likely to require invasive or non-invasive ventilation or be admitted to ICU compared to patients recruited in the UK ($p<0.001$).

Viral load

Quantitative SARS-COV-2 PCR measurements from nasopharyngeal swabs at baseline showed no difference in median log viral loads between the two groups, SOC+L 4.68 (IQR 4.45-4.85) vs SOC 4.76 (IQR 4.48-4.92). We clustered the serial samples to reflect the crucial time intervals during the hospital stay: time coinciding with finishing leflunomide loading dose (by Day 4), time to 75% patients being discharged from hospital (by Day 7), time to finishing leflunomide maintenance dose (by Day 11) and beyond (Figure 4). Viral loads were significantly reduced in both treatment arms. There was no significant difference in the overall rate of the viral load clearance between the two groups by Day 11.

Cytokines, CRP and lymphocytes

Cytokine levels were assessed separately for UK and Indian sites as two laboratories using different assays processed the samples. The median baseline levels of IL 2, IL 6 and TNF-α levels (UK: IL2 0.43 [IQR 0.30-0.62] pg/ml; IL6 6.2 [IQR 2.9-9.7] pg/ml; TNF-α 10.1 [IQR 7.8 -13.5] pg/ml; India: IL2 4.3 [IQR 2.8-5.8] pg/ml; IL6 12.6 [IQR 6.5-43.1] pg/ml; TNF-α 6.1 [IQR 4.9-7.1] pg/ml) were not significantly raised from

normal reference ranges and were not different between treatment groups in both countries. The cytokine levels were reduced during hospitalisation, though the clinical significance of these changes within the normal range is uncertain. There was no significant difference in the trends observed between treatment arms.

The median baseline levels of CRP were similar in both groups, 28, (IQR 8-71) in SOC+L vs. 34 (14-71) mg/L in SOC. By one week of treatment, there was similar levels of reduction between groups.

The median baseline lymphocytes levels were lower than normal reference range in both groups (0.99 [IQR 0.6-1.6) x10^9/L in SOC+L vs 0.95 (IQR 0.6-1.6) x10^9/L in SOC. By 1 week of treatment, levels rose to normal range in both groups. There was no significant difference in the trends observed between groups.

28- and 90-days follow up

At 28 days, 59/81 patients (71.2%) in the SOC+L group and 60/91 patients (65.9%) in the SOC group experienced at least 1 of 9 common long-COVID symptoms (fatigue, cough, anxiety, chest pain, brain fog, breathlessness, disturbed sleep, palpitations, joint pain); with sleep quality (48.2% vs. 38.5%), breathlessness (40.7 vs 42.9%), joint pain (32.1 vs. 33%), fatigue (29.6 vs, 31.9%), and anxiety (24.7 vs. 19.8%) being the commonest symptoms experienced (Supplementary table 3). At 90 days, there was a reduction in overall prevalence of symptoms as 42/81 patients (51.2%) in the SOC+L group and 37/91 (40.7%) patients in the SOC group and any of the residual symptoms were of reduced severity. There was no significant difference in these outcomes between the treatment arms.

Myalgia symptoms were comparably reduced between the 2 groups at 90 days. Anosmia and loss of taste were still reported by 2 and 7 patients, respectively, in the SOC+L group, but none in the SOC group.

At 28 days, 41.5% patients in the SOC + L group and 52.8% in SOC group, reported being moderately to severely dyspnoeic (Grade 4: stops for breath after walking 100m; Grade 5: too breathless to leave the house or breathless when dressing). These proportions were further reduced at 90 days, to 22% in the SOC+L group compared to 19.8% in SOC group. These differences were not significant in between group comparisons.

Mental health issues were highlighted by reports of feeling depressed and losing interest in doing things. Comparable proportions of patients in the SOC+L group and SOC group reported those problems at 28 days (17.9% vs 16.0%; 11.6% vs 14.2%, respectively) which were further reduced in both groups at 90 days (11.6% vs 9.4%; 9.5% vs 6.6%, respectively).

At 28 days participants in the SOC+L group scored their current health as being 80±25% of the usual which increased to 89±17% at 90 days. In the SOC group the scores were similar, 82 ± 23% and 90 ± 17% at 28 and 90 days respectively.

Discussion

This study is the first prospective, multicentre, randomised, controlled clinical trial investigating the clinical efficacy and safety of leflunomide in treating acute COVID-19 infection. The study showed that a course of leflunomide (3 days of 100mg/day loading dose followed by 7 days of 20 mg/day maintenance dose) added to the standard care treatment (steroids, anticoagulants, antibiotics and antiviral therapy), did not influence the primary outcome of the trial and the acute clinical outcomes at 28 days, or the prevalence of long-COVID symptoms at 28 and 90 days. However, participants who received leflunomide as an adjunct therapy were weaned off oxygen earlier, which translated to reduced hospital stay by one day. The medication appeared to be safe and well tolerated with no severe adverse events attributable to it. A small proportion of patients in our study were still burdened by COVID-19 related symptoms 90 days after randomisation.

This multicentre trial advances the evidence base on the impact of leflunomide, a repurposed rheumatoid arthritis medication, on COVID-19 infection. Leflunomide was a potentially attractive therapeutic choice from early preclinical and clinical experience reported from hospitals in Wuhan, China. Dihydroorotate dihydrogenase (DHODH), located in the inner mitochondrial membrane is a rate-limiting enzyme in de novo pyrimidine biosynthesis. In virus-infected cells, a large intracellular nucleotide pool is consumed by rapid viral replication. RNA viruses need unique UMP but not TMP in their genomes. As UMP is the particular nucleoside produced by DHODH, RNA viruses are sensitive to reduced DHODH activity. Preclinical models of cell and animal infection by SARS-CoV-2 demonstrated that leflunomide attenuates viral genome replication, suppresses inflammatory response and the release of pro-inflammatory cytokines and chemokines.[7,8,9] Early reports from China advocated major clinical benefits in patients treated with leflunomide both in terms of less severe outcomes and duration of infection.[15,16,17] While the current study did not reproduce these overall benefits in the ITT analysis regarding the primary outcome, it confirmed some positive effects in those patients who received the trial intervention (in modified intent to treat analysis).

Our results are likely explained by the changing landscape and evolution of the routine COVID-19 treatment protocols in the standard arm of the study and the resultant severity of the COVID-19 outcomes in general. The initial phase of the COVID-19 pandemic was characterised by severe respiratory and systemic infections and poor outcomes due to the development of acute respiratory distress syndrome, multi-organ failure and eventual death.[18,19] Contrary to this early experience with COVID-19 management, the in-hospital mortality in the present study was much lower, less than 10% in both groups. The majority of patients in both treatment arms improved during hospitalisation and were discharged within a week of admission. Inclusion of prognostically significant COVID-19 therapies in both pharmacological and non-pharmacological standard of care treatments undoubtedly contributed to a reduction in severe complications and better overall outcomes. During patient recruitment of the current trial, various therapies have been introduced including more than 95% percent of the study population received steroids as standard of care.

Theoretical considerations suggest that leflunomide may effectively inhibit viral replication. The initial pilot study during the early outbreak of COVID-19 in Wuhan, China reported reduced viral shedding time following

leflunomide treatment during acute infection compared to the standard of care therapy.[15] Similarly, viral shedding duration was reduced in leflunomide treated patients who remained qPCR positive 1 month after the initial infection.[16] Our study addressed the viral load reduction at pre-specified time points. Values of viral load were reduced over time but there was no difference between the treatment arms. Both methodological considerations and the inclusion of comprehensive pharmacological treatment regimens in the SOC could explain these differences. For instance, corticosteroid therapy was absent in the early study from Wuhan, but the later study refers to the use of hydroxychloroquine, interferon-alpha and antiviral medications as part of acute standard of care therapy.[15,16] However, our results are in line with other reports from China which showed that duration of viral shedding was not affected by leflunomide added to nebulised interferon alpha therapy for treating long-term positive COVID-19 after 4 weeks of in-hospital treatment.[17] Interestingly a third of these patients received corticosteroid therapy during the initial acute treatment. [16,17]

Beyond the issue of therapeutic efficacy and viral load, our study confirms overall safety of leflunomide in COVID-19 infection. The safety profile of leflunomide is well established in the treatment of RA. [6] Leflunomide, repurposed for the COVID-19 treatment, was well tolerated since no serious adverse events were attributed to it. Similar findings were reported in other studies.[15,16,24] Mild transaminitis following long-term leflunomide use in the RA population is recognised, and usually resolves after medication is terminated. The mechanism is likely to be modulation of interleukins which may hinder the protection of hepatocytes from injury rather than direct toxicity.[25] There were comparable incidences of transaminitis in both treatment arms in our study. However, more patients in the UK cohort had raised liver function tests leading to modification or termination of leflunomide treatment. This may be accounted for by the difference in the severity of COVID-19 disease and spectrum of co-morbidities between UK and Indian participants rather than genetic polymorphism in drug metabolism. Overall, the proposed leflunomide regimen was well tolerated.

One of the motivations of the current trial employing leflunomide was to benefit from the anti-inflammatory effects of this drug. In this context, hydrocortisone has been demonstrated as an effective therapy in severe COVID-19 infections and recent trials also demonstrated the benefit of tocilizumab, a selective IL-6 inhibitor and a different disease modifying rheumatoid arthritis medication. However, such finding is not universal as the benefit of tocilizumab is mainly demonstrated in critically to moderately ill patients.[20,21] A recent meta-analysis showed that the benefit of IL-6 receptor antagonist was encountered only in patients who were also treated with glucocorticoids.[23] This is in keeping with observations that a broader spectrum of pro-inflammatory cytokines, macrophages and T cell response have all been documented in severely ill patients demonstrating the role of a more complex inflammatory response. It is exactly this broader inflammatory reaction that could be targeted by leflunomide as its effect on cytokines is not restricted to IL 6 and it may also have an impact on activated T cell response.[8,9,22] Such phenomena might contribute to the benefits of reduced oxygen dependence in patients who have received leflunomide treatment. However, it is conceivable that the full benefit of such anti-inflammatory effect may be more pronounced in severely ill patients, but this population was underrepresented in our trial and the (inadvertent) inclusion of patients with milder symptoms may have lead to some attrition of statistical power in our study. A more detailed analysis of the cytokine and metabolic profiles of our trial population is underway to clarify these important issues.

Another important consideration when discussing the potential benefits of leflunomide is the mutation ability of the SARS-CoV-2 virus.[26] So far, the mutations observed in different strains worldwide have largely been confined to the part of the spike protein affecting the virus's ability of cell entry as opposed to a region targeted by neutralising antibodies. However, the possibility of mutations in different regions cannot be excluded. Targeting the host's pyrimidine biosynthesis pathway by leflunomide, rather than using drugs with direct antiviral action, remains an advantage offering protection against a broader spectrum of viruses and potentially overcoming resistance. Indeed, DHODH inhibitors such as leflunomide has shown broad-spectrum antiviral effects against various RNA viruses in cell models.[7] Leflunomide may therefore be considered a viable pharmacological treatment for COVID-19 patients given it is well tolerated, safe, economical, and widely available. Its clinical effectiveness measured against recognised selective IL-6 inhibitors in the more severely/critically ill patients needs to be further explored as leflunomide may be the preferred option in countries where other immunomodulating agents, such as Talizumab, may not be practical or widely available.

Limitations

The present study has several limitations. The trial was not blinded, so the data collection and clinical management of the patients may have been affected. The study was set out to recruit more severely and critically affected patients in a single country. However, due to recruitment restrictions because of national prioritization of critically ill patients to only a few studies together with scarcity of NHS resources during the pandemic, the study was extended abroad, ultimately recruiting less affected patients with heterogeneous clinical profiles. Although patient characteristics and medications received as part of SOC did not differ between the randomised arms, the more heterogeneous population, milder COVID-19 disease, and more effective standard of care treatments most likely impacted on the hypothesised effect size and the ability of finding a difference in our recruited sample. Finally, the COVID-19 restrictions affected our protocolised laboratory investigations, such as the serial viral load and comprehensive inflammatory profiling. Nevertheless, studies focusing on the more severely affected participants are underway and will be the subject of a separate submission.

Conclusion

Leflunomide had no major impact on the clinical outcomes when administered together with the currently established but evolving therapies in moderately affected COVID-19 patients. It may shorten duration of oxygen dependence thereby affecting the TTCI and hospital discharge. Transaminitis associated with leflunomide therapy did not lead to excess adverse events compared to the control group and may have arisen in part due to the severity of clinical infection. Further studies are needed to investigate the potential benefits of leflunomide in the critically ill patients and the biological mechanisms involved.

Ethics statements

All patients taking part in the study signed written informed consent form once the study was ethically approved by relevant bodies in England (South Central - Berkshire Research Ethics Committee, Bristol REC Centre, reference number 20/SC/0264) and India (Max HealthCare Ethics Committee, reference number RS/MSSSH/GMHRCCMS/MHEC/CCM/20-23; Meditrina Institute Ethics Committee, reference number ECR/605/Inst/MH/2014/RR; Noble Hospital Institutional Ethics Committee, reference number NHIEC/FEB/2021/238).

Data availability statement

The anonymized data may be available upon request following approval from the Trial Management Group and the Sponsor.

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Trial Steering Committee: Brendan Madden (Chair), Patrick Yong, Sreenivasa Rao Kondapally Seshasai, Matt Mendes, Janice Rodrigues-Mendes, Rod Hughes, Sharanpal Jeetle, Subash Somalanka
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Meditrina Institute, Nagpur, India: Ajay Bulle
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Authors' contributions
All designated authors meet all four ICMJE criteria for authorship. The funders and the sponsor of the study had no role in the analyses or the interpretation of the results.

Competing interests
All authors have completed the ICMJE uniform disclosure form at www.icmje.org/disclosure-of-interest/ and declare no competing interests.

Dissemination

A summary of the results will be disseminated to the participants by principal investigators at each trial site via a newsletter.

Licencing Statement

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References

1. https://COVID19.who.int/
2. Chen YL, Quach TTT, COVID-19 cytokine storm syndrome: a threshold concept. Lancet Microbe. 2021 Feb;2(2):e49-e50.doi: 10.1016/S2666-5247(20)30223-8.Epub 2021 Feb 2
3. Gavriatopoulou M, Ntanasis-Stathopoulos I, Korompoki E, Fotiou D, Migkou M, Tzanninis IG, Psaltopoulou T, Kastritis E, Terpos E, Dimopoulos MA. Emerging treatment strategies for COVID-19 infection. Clin Exp Med. 2021 May;21(2):167-179. doi: 10.1007/s10238-020-00671-y. Epub 2020 Oct 30. PMID: 33128197; PMCID: PMC759894
4. Dwek, Raymond A et al. "Targeting glycosylation as a therapeutic approach." Nature reviews. Drug discovery vol. 1,1 (2002): 65-75. doi:10.1038/nrd708
5. Angus, Derek C et al. "The REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-acquired Pneumonia) Study. Rationale and Design." Annals of the American Thoracic Society vol. 17,7 (2020): 879-891. doi:10.1513/AnnalsATS.202003-192SD
6. Alfaro-Lara, et al. Systematic review and meta-analysis of the efficacy and safety of leflunomide and methotrexate in the treatment of rheumatoid arthritis. Reumatol Clin. 2019 May - Jun;15(3):133-1
7. Xiong R, Zhang L, Li S, Sun Y, Ding M, Wang Y, Zhao Y, Wu Y, Shang W, Jiang X, Shan J, Shen Z, Tong Y, Xu L, Chen Y, Liu Y, Zou G, Lavillete D, Zhao Z, Wang R, Zhu L, Xiao G, Lan K, Li H, Xu K. Novel and potent inhibitors targeting DHODH are broad-spectrum antivirals against RNA viruses including newly-emerged coronavirus SARS-CoV-2. Protein Cell. 2020 Oct;11(10):723-739. doi: 10.1007/s13238-020-00768-w. Epub 2020 Aug 4. Erratum in: Protein Cell. 2021 Jan;12(1):76-80. Erratum in: Protein Cell. 2020 Nov 9;: PMID: 32754890; PMCID: PMC7402641.
8. Yao, H. W.; Li, J.; Chen, J. Q.; Xu, S. Y. A 771726, the active metabolite of leflunomide, inhibits TNF-α and IL-1 from Kupffer cells. Inflammation 2004, 28 (2), 97-103.
9. Li, L. et al. The effects of teriflunomide on lymphocyte subpopulations in human peripheral blood mononuclear cells in vitro. J. Neuroimmunol. 2013, 265 (1-2), 82-90.
10. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2
11. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_60
12. Mandal S, Barnett J, Brill SE ARC Study Group, et al. 'Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax 2021;76:396-398.
13. http://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus/en/).
14. Machin D, Campbell M, Fayers, P, Pinol A (1997) Sample Size Tables for Clinical Studies. Second Ed. Blackwell Science IBSN 0-86542-870-0 p. 176-177
15. Hu, Ke et al. "A Small-Scale Medication of Leflunomide as a Treatment of COVID-19 in an Open-Label Blank-Controlled Clinical Trial." Virologica Sinica vol. 35,6 (2020): 725-733. doi:10.1007/s12250-020-00258-7
16. Wang, Qiang et al. "Efficacy and Safety of Leflunomide for Refractory COVID-19: A Pilot Study." Frontiers in pharmacology vol. 12 581833. 2 Jul. 2021, doi:10.3389/fphar.2021.581833

17. Mengmei Wang, Yang Zhao, Weihua Hu, Dong Zhao, Yunting Zhang, Tao Wang, Zhishui Zheng, Xiaochen Li, Shaolin Zeng, Zhenlian Liu. Treatment of Coronavirus Disease 2019 Patients With Prolonged Postsymptomatic Viral Shedding With Leflunomide: A Single-center Randomized Controlled Clinical Trial. Clinical Infectious Diseases, Volume 73, Issue 11, 1 December 2021, Pages e4012–e4019, https://doi.org/10.1093/cid/ciaa1417
18. Huang, Chaolin et al. "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China." Lancet (London, England) vol. 395,10223 (2020): 497-506. doi:10.1016/S0140-6736(20)30183-5
19. ISARIC Clinical Characterisation Group. "COVID-19 symptoms at hospital admission vary with age and sex: ISARIC multinational study." medRxiv : the preprint server for health sciences 2020.10.26.20219519. 19 Nov. 2020, doi:10.1101/2020.10.26.20219519. Preprint.
20. REMAP-CAP Investigators et al. "Interleukin-6 Receptor Antagonists in Critically Ill Patients with COVID-19." The New England journal of medicine vol. 384,16 (2021): 1491-1502. doi:10.1056/NEJMoa2100433
21. Abani, O., Abbas, A., Abbas, F., Abbas, M., Abbasi, S., Abbass, H., et al. (2021). Tocilizumab in Patients Admitted to Hospital with COVID-19 (RECOVERY): a Randomised, Controlled, Open-Label, Platform Trial. The Lancet 397, 1637–1645. doi:10.1016/S0140-6736(21)00676-0
22. Kraan MC, Smeets TJM, van Loon MJ, et al Differential effects of leflunomide and methotrexate on cytokine production in rheumatoid arthritis Annals of the Rheumatic Diseases 2004;63:1056-1061.
23. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group et al. "Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis." JAMA vol. 324,13 (2020): 1330-1341. doi:10.1001/jama.2020.17023
24. Chakravarty EF, Sanchez-Yamamoto D, Bush TM. The use of disease modifying antirheumatic drugs in women with rheumatoid arthritis of childbearing age: a survey of practice pattern and pregnancy outcomes. J Rheumatol. 2003;30:241–6.
25. Atsushi Ogata, Yasuhiro Kato, Shinji Higa, Kazuyuki Yoshizaki, IL-6 inhibitor for the treatment of rheumatoid arthritis: A comprehensive review, Modern Rheumatology, Volume 29, Issue 2, 4 March 2019, Pages 258–267, https://doi.org/10.1080/14397595.2018.1546357
26. Wang, Shihang et al. "Molecular evolutionary characteristics of SARS-CoV-2 emerging in the United States." Journal of medical virology vol. 94,1 (2022): 310-317. doi:10.1002/jmv.27331

Figure legends:

Figure 1: Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.

**= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.*

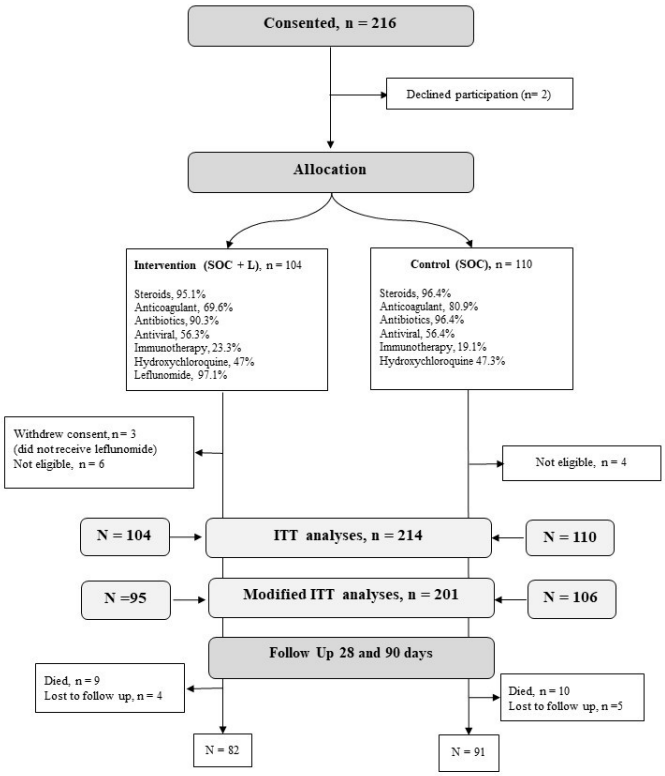
Figure 2: Time to clinical improvement of 2 points on a clinical status scale or discharge prior 28 day in a stratified ITT analysis (primary outcome).

Patients who died were censored at the time their death occurred, while all surviving patients who did not reach TTCI criteria by day 28 were right censored at that point. Most of the patients were discharged within the first 10 days of admission.

Figure 3: Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

Figure 4: Mean changes in log viral load from baseline.

Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.



Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.
*= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.

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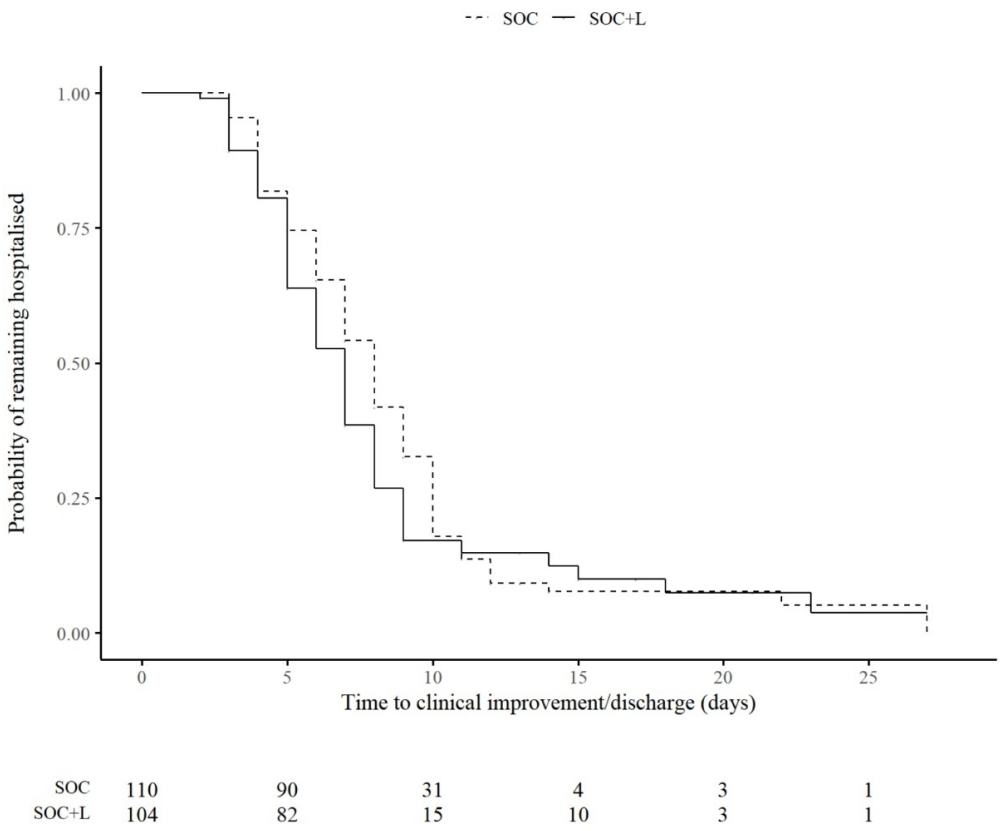
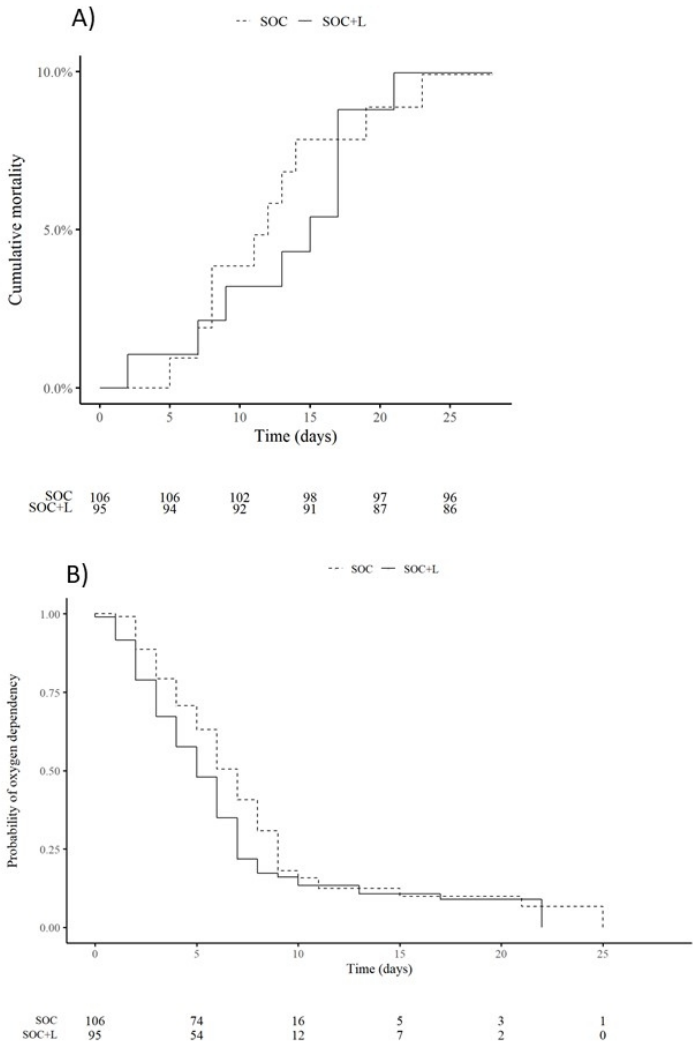


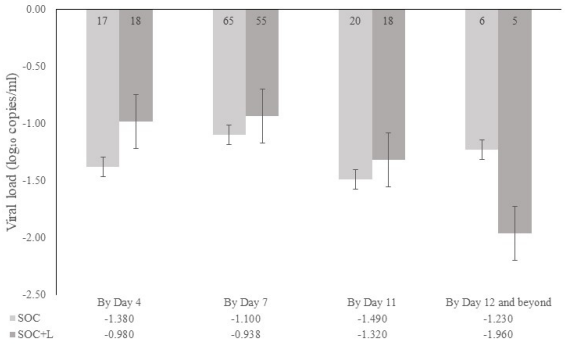
Time to clinical improvement of 2 points on a clinical status scale or discharge prior 28 day in a stratified ITT analysis (primary outcome).
Patients who died were censored at the time their death occurred, while all surviving patients who did not reach TTCI criteria by day 28 were right censored at that point. Most of the patients were discharged within the first 10 days of admission.

139x116mm (220 x 220 DPI)



Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

190x275mm (96 x 96 DPI)



Mean changes in log viral load from baseline.
Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.

338x190mm (96 x 96 DPI)

Centre	Group	N	Corticosteroid	Anticoagulant	Antiviral	Antibiotic	Immunotherapy*	Hydroxychloroquine
UK	Intervention	30	100%	97%	69%	100%	14%	3%
	Control	36	100%	100%	81%	100%	3%	3%
India	Intervention	74	87%	76%	70%	76%	67%	93%
	Control	74	89%	84%	70%	88%	50%	94%
All centres	Intervention	104	95%	79%	56%	90%	23%	47%
	Control	110	96%	80%	56%	96%	19%	47%

Supplementary Table 1: **Standard of care therapy in the UK and India**

**The "Immunotherapy" includes tocilizumab, bevacizumab, interferon alpha and beta*

		Baseline		Day 1 – 3/4		Day 4/5 – Discharge	
		UK	**India**	**UK**	**India**	**UK**	**India**
ALT							
SOC + L	**Median (IQR) U/L**	48 (30-60)		67 (36~87)		44 (36-71)	
		48 (32-71)	47 (29-59)	59 (37-94)	72 (34-86)	62(34-151)	42(35-56)
	1 - 2 x ULN (n)	44		50		17	
		12	32	9	41	4	13
	2 - 3 x ULN (n)	1		6		6	
		1	0	3	3	2	4
	> 3 x ULN (n)	1		8		5	
		1	0	3	5	4	1
	>ULN	14	32	15	49	10	18
SOC	**Median (IQR) U/L**	39 (26-56)		44 (34-65)		41 (35-52)	
		40 (27-59)	38 (25-54)	44 (31-63)	44 (36-67)	49 (33-63)	40 (35-52)
	1 - 2 x ULN (n)	33		29		24	
		13	20	8	21	7	17
	2 - 3 x ULN (n)	0		9		3	
		0	0	2	7	2	1
	>3 x ULN (n)	0		0		0	
		0	0	0	0	0	0
	>ULN	13	20	10	28	9	18
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.049**		**<0.001**		**0.633**	
AST							
SOC + L	**Median (IQR) U/L**	44 (30-54)		55(31-77)		41(27-50)	
		60 (42-102)	43(29-50)	58 (42-104)	53 (28-76)	54 (29-102)	40 (27-49)
	1 - 2 x ULN (n)	28		36		18	
		4	24	8	28	3	15
	2 - 3 x ULN (n)	2		9		1	
		2	0	2	7	1	0
	> 3 x ULN (n)	1		6		1	
		1	0	2	4	1	0
	>ULN	7	24	12	39	5	15
SOC	**Median (IQR) U/L**	39 (28-52)		39 (29-54)		37(27-47)	
		57 (34-75)	37(26-48)	45(38-55)	38(28-54)	45 (35-61)	37 (25-46)
	1 - 2 x ULN (n)	22		18		16	
		6	16	4	14	4	12
	2 - 3 x ULN (n)	2		5		0	
		2	0	0	5	0	0
	>3 x ULN (n)	0		1		1	
		0	0	0	1	1	0
	>ULN	8	16	4	20	5	12
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.3222**		**<0.001**		**0.318**	

Supplementary Table 2: Liver enzymes measurements

ULN: upper limits of normal (ALT: 10-49 U/L; AST 19 – 48U/L)). ALT: alanine transaminase. AST: aspartate transaminase; SOC + L: n = 30 UK, 74 India; SOC: n = 36 UK, 74 India

	Day 28					Day 90				
	SOC+L (n=81)		SOC (n=91)		p*	SOC+L (n=81)		SOC (n=91)		p*
	%	Median (IQR)	%	Median (IQR)		%	Median (IQR)	%	Median (IQR)	
Fatigue	29.6	5.00(2.75-8.00)	31.9	5.00(3.00-8.00)	0.751	22.2	5.00(3.25-7.00)	26.4	3.00(2.00-4.25)	NS
Cough	13.6	2.00(1.00-4.50)	18.7	2.00(1.00-4.00)	0.366	7.41	1.00(1.00-6.25)	8.79	2.50(1.00-3.50)	NS
Anxiety	24.7	3.50(2.00-7.25)	19.8	4.00(3.00-7.75)	0.438	21.0	3.00(1.00-5.00)	19.8	2.00(1.00-3.75)	NS
Chest pains	11.1	4.00(2.00-7.00)	8.79	4.00(2.50-5.00)	0.611	7.41	4.00(3.25-7.75)	6.59	3.00(1.25-7.75)	NS
Brain fog	14.8	5.00(3.75-7.25)	16.5	5.00(1.50-7.00)	0.764	14.8	3.50(1.75-5.00)	12.1	4.00(2.50-4.50)	NS
Breathlessness	40.7	2.00(1.00-6.00)	42.9	1.00(1.00-7.00)	0.779	22.2	5.00(1.00-7.00)	20.9	4.00(2.00-4.00)	NS
Sleep quality	48.2	2.00(1.00-5.00)	38.5	3.00(1.00-6.00)	0.200	34.6	2.00(1.00-4.25)	33.0	2.50(1.00-5.75)	NS
Palpitations	8.64	7.00(1.50-9.00)	5.49	1.00(1.00-7.00)	0.419	4.94	4.50(3.50-5.00)	3.30	1.00(1.00-4.00)	NS
Joint pain	32.1	3.00(1.00-4.75)	33.0	2.00(1.00-4.00)	0.903	22.2	3.50(2.00-7.00)	19.8	2.00(2.00-4.50)	NS
Myalgia	18.5	-	19.8		0.834	17.3		11.0		NS
Anosmia	6.17	-	11.0		0.264	2.47		-		-
Loss of taste	9.88	-	14.3		0.378	7.41		-		-
Depression	19.8	1.50(1.00-3.000	18.7	1.00(1.00-2.00)	0.859	11.1	1.00(1.00-1.00)	11.0	1.00(1.00-2.00)	NS
Loss of interest	12.4	2.50(1.25-3.00)	16.5	1.00(1.00-3.00)	0.442	9.88	1.00(1.00-1.25)	7.69	1.00(1.00-2.50)	NS
Dyspnoea; Mild (1) **Moderate (2-3)** **Severe (4-5)**	59.3 29.6 11.1	1.00(1.00-2.00)	47.3 39.6 13.2	2.00(1.00-2.00)	0.155 0.173 0.678	80.3 14.8 4.94	1.00(1.00-1.00)	81.3 17.6 1.10	1.00(1.00-1.00)	NS

Supplementary Table 3: Long COVID symptoms at 28 and 90 days after randomisation

p; alpha value. Statistical significance was assumed at 0.05 alpha value. To best summarise the data, symptom scales (e.g. 0-10) for all symptoms (except dyspnoea, which was measured in different terms) were binarized, accepting any score above 0 as prevalence (%) of experiencing that symptom. To reflect magnitude of symptom severity, median (IQR) of individual symptom scores (except for dyspnoea, myalgia, anosmia and loss of taste) were taken excluding scores of 0. For dyspnoea, prevalence (%) of the symptom was determined as the proportion of patients scoring any relevant category (e.g., mild, moderate, severe), and median (IQR) of symptom severity included all score values. Between-group differences at each point of follow-up (day 28 and day 90) for all symptoms were evaluated using patient proportions from the binarized symptom scales via the chi-square test of differences. The Shapiro-wilk test was used to assess statistical normality. No analysis was enabled for any day reporting n ≤ 3 datapoints per group for statistical reliability

Trial procedures	Screening	Day 0/1 (BL)	Daily	Day 3	Day 7	Day 11 +/- 1	Day 15 +/- 1	Day 28 +/-1	DC	Day 90 +/- 7
Confirmation of COVID Infection and severity	X									
Informed consent & Eligibility Assessment	X									
Demographics, Medical Hx, Cardiopulmonary Assessment (including ECG [1] & Echo [2])	X									
Concomitant medication		X	X					X [7]		X
Bloods – FBC, U&Es, LFT (AST [6] & ALT [6])		X	X [5]	X [6]					X	
– Clotting screen, Fibrinogen, D-Dimer, Ferritin		X	X [5]							
– Glucose		X	X [5]							
– Creatine Kinase, Troponin, BNP (NT-proBNP)		X	X [5]							
– Procalcitonin, CRP, LDH		X	X [5]	X		X				
– HIV		X								
– Cytokine profile		X		X		X			X [8]	
Pregnancy test (urine sample)		X								
Viral Load (nasopharyngeal swab)		X			X	X	X	X	X [8]	
Randomisation		X								
IMP dispense, loading (daily from Day 0/1 to 3) / maintenance dose (daily from Day 4 to 10) [3]		X								
Primary outcome assessment (TTCI)		X	X					X [7]		
Clinical Assessment, e.g. NEWS 2, body T°C*, vital signs, imaging**	X	X	X [5]							
*Blood and Urine cultures (in presence of fever)		X	X [5]							
**Urine for legionella and pneumococcal		X	X [5]							
Oxygenation assessment e.g. O_2 delivery method and level [SpO_2]		X	X [5]							
Arterial Blood Gas (ABG) – as available and where applicable		X	X [5]							
Serious Adverse Event(s) (SAE(s))/ Adverse Event(s) (AE(s)) [4]			X					X [7]		X [9]
Out-patient assessment (telephone call)								X [7]		X

Data collected and study time points

Key: BL – Baseline; IMP – Investigation Medicinal Product (trial treatment i.e. trial drug); SOC - Standard of Care; DC Discharge.

Notes: **1.** Check medical notes, if abnormal flag repeat imaging; **2.** Echo within 6 months to be used if no cardiac symptoms; **3.** Participant to take home IMP if DC'd; **4.** Participant to self-report events between DC to Day 90; **5.** Completed Daily, depending on clinical need and resources. If participant is DC'd early – record what is available as part of SOC; **6.** AST/ALT *must* be checked for treatment arm to determine maintenance dose; **7.** Participant DC'd called on Day 28 for Treatment Assessments, if not seen on-site; **8.** Cytokines/Viral Load to be collected if outside of scheduled collection. Day 11 is the last collection for Cytokines. on DC Medium (telephone call) and long term (by Sponsor) Treatment Assessments respectively.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5,6
	2b	Specific objectives or hypotheses	5,6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4,5,6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4,5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5,6,7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4,5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	NA

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	5,6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	5,6
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, page 7
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, page 7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	7
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	7, Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	5,6
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	5,6
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	5,6,7,8,9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2,13

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Leflunomide Treatment for Patients Hospitalised with COVID-19: DEFEAT-COVID Randomised Controlled Trial

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Leflunomide Treatment for Patients Hospitalised with COVID-19: DEFEAT-COVID Randomised Controlled Trial

Authors:

Ines Kralj-Hans[1], Kuo Li[2], Adrian Wesek[3], Alexia Lamorgese[4], Fatima Omar[1], Kapila Ranasinghe[1], Megan McGee[1], Kieran Brack[1], Shiliang Li[5], Ritesh Aggarwal[6], Ajay Bulle[7], Aparna Kodre[8], Shashank Sharma[1], David Fluck[1], Isaac John[1], Pankaj Sharma[9], Jonathan Belsey[4], Ling Li[3], Sreenivasa Rao Kondapally Seshasai[10], *HongLin Li[5], *Nandor Marczin[2], *Zhong Chen[1], on behalf of DEFEAT-COVID Investigators

*Joint senior authors

Affiliations:

1. Ashford and St Peter's Hospital NHS Foundation Trust, UK
2. Faculty of Medicine, Imperial College London, UK
3. University of Kent, UK
4. JB Medical, UK
5. Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, China
6. Max Hospital, New Delhi, India
7. Meditrina institute of medical science, Nagpur, India
8. Noble Hospital, Pune, India
9. Institute of cardiovascular research, Royal Holloway, University of London, UK
10. St George's University Hospitals NHS Foundation Trust, UK

Corresponding author: Dr Ines Kralj-Hans, i.kralj-hans@nhs.net

Ashford and St Peter's Hospital NHS Foundation Trust, Guildford Street, Chertsey, KT16 0PZ, UK

Abstract

Objective: To evaluate the clinical efficacy and safety of leflunomide (L) added to the standard of care (SOC) treatment in COVID-19 patients hospitalised with moderate/critical clinical symptoms.

Design: Prospective, open-label, multicentre, stratified, randomised clinical trial.

Setting: Five hospitals in United Kingdom and India, from September 2020 to May 2021.

Participants: Adults with polymerase-chain-reaction (PCR) confirmed COVID-19 infection with moderate/critical symptoms within 15-days of onset.

Intervention: Leflunomide 100 mg/day (3-days) followed by 10-20 mg/day (7-days) added to standard care.

Primary outcomes: The time to clinical improvement (TTCI) defined as two-point reduction on a clinical status scale or live discharge prior to 28 days; safety profile measured by the incidence of adverse events (AE) within 28 days.

Results: Eligible patients (n=214; age 56.3±14.9 years; 33% female) were randomised to SOC+L (n=104) and SOC group (n=110), stratified according to their clinical risk profile. TTCI was 7 vs. 8 days in SOC+L vs. SOC group (HR 1.317; CI 0.980, 1.768; p=0.070). Incidence of serious adverse events was similar between the groups and none was attributed to leflunomide. In sensitivity analyses, excluding 10 patients not fulfilling the inclusion criteria and 3 who withdrew consent before leflunomide treatment, TTCI was 7 vs. 8 days (HR 1.416, CI 1.041, 1.935; p=0.028), indicating a trend in favour of the intervention group. All-cause mortality rate was similar between groups, 9/104 vs. 10/110. Duration of oxygen dependence was shorter in the SOC+L group being a median 6-days (IQR 4-8) compared to 7-days (IQR 5-10) in SOC group (p=0.047).

Conclusion: Leflunomide, added to the SOC treatment for COVID-19, was safe and well tolerated but had no major impact on clinical outcomes. It may shorten the time of oxygen dependence by one day and thereby improve TTCI /hospital discharge in moderately affected COVID-19 patients.

Trial registration

EUDRACT: CTA 21517/0004/001-0001 2020-004994-27

ClinicalTrials.gov: NCT05007678

Strengths and limitations

- International, prospective, randomised controlled study
- Repurposing a marketed drug with established safety profile and promising dual antiviral and immunomodulating medication based on strong drug discovery data.
- Study participants had milder COVID-19 disease than originally intended, thus eroding the power of the study
- Evolving standard of care therapy possibly diminished measurable benefit of leflunomide

Introduction

COVID-19 pandemic caused unprecedented strain on health care services around the world. It has affected almost 16 million people globally and caused over 6 million deaths so far.[1] Associated clinical syndromes include pneumonia, systemic inflammatory response and cardiovascular complications with high morbidity and mortality. Progressive deterioration is thought to be related to the kinetics of viral replication culminating in a surge of inflammatory mediator release, "cytokine storm".[2] Around 5-10% of infected patients experience severe or life-threatening symptoms with high mortality.[3]

Direct-acting and host-targeting antiviral treatments are the two approaches in treating viral infections. Host targeting antiviral treatments may have an advantage over direct antivirals as they enable the body to fight against a broad spectrum of viruses by simultaneously blocking viral replication and overcoming the potential of viral mutagenesis.[4] Anti-inflammatory medications have been shown to improve survival through dampening of the inappropriate immune response in susceptible patients.[5] This has led to the search for a drug with such therapeutic properties.

Leflunomide is a drug licenced to treat rheumatoid arthritis (RA).[6] It is widely available, cost-effective and can be easily administered both in the hospital and domestic settings. In preclinical models of cell and animal infection by SARS-CoV-2, leflunomide was shown to be a potent inhibitor of human dihydroorotate dehydrogenase (DHODH), an enzyme vital to viral replication in the host cell.[7,8,9] It has the potential advantage of not only targeting the virus infection but also suppressing the ensuing inflammatory response which may play a role in more progressive stages of infection leading to serious complications.

The DEFEAT-COVID study (Targeting **de** novo pyrimidine biosynthesis by le**f**lunomide for tr**eat**ment of **co**rona **vi**rus **d**isease 2019) tested whether leflunomide added to standard care was clinically effective and safe for COVID-19 moderate/severe symptoms.

Methods

Study design – This was a multicentre, international, open label, prospective, randomised controlled clinical trial set up at 5 hospitals (two in UK and three in India). The recruitment took place between September 2020 and May 2021, and was approved by all relevant ethics committees.

Participants - Patients aged 18 years and above presenting with moderate to critical symptoms of PCR-confirmed COVID-19 disease within 15 days of symptoms onset were recruited. Patients with respiratory compromise and blood oxygen saturation (SpO_2) <93% on room air detected on pulse oximeter were considered to fulfil the moderate infection criteria. Patients with respiratory failure, septic shock and/or multiple organ dysfunction/failure needing assisted ventilation were considered to be critically ill. Pregnant or breast-feeding women, individuals already receiving specific monoclonal antibody therapy or those with severe immunodeficiency syndrome and hypoalbuminaemia and patients with hypersensitivity to leflunomide or liver

enzymes aspartate transaminase (AST) / alanine transaminase (ALT) ≥ 2 x upper limits of normal (ULN) were excluded from the study. All participants gave written informed consent to a member of their clinical care team.

Randomisation – Consented participants were randomised by a member of the clinical care team to either the control arm (receiving standard of care treatment [SOC] alone) or the intervention arm (SOC treatment + leflunomide (SOC+L]) using a stratified block randomisation web-based algorithm. Patient admission data (age </≥ 70; co-morbidities; clinical status based on National Early Warning Score 2, NEWS2)[10] were used to stratify patients into 4 risk categories. Group 1: high/moderate comorbidity risk with NEWS2 score ≥5; Group 2: high/moderate comorbidity risk with NEWS2 score <5; Group 3: low comorbidity risk with NEWS2 score ≥5; and Group 4: low comorbidity risk with NEWS2 score <5.

Interventions - The definition of the SOC treatment for COVID-19 evolved nationally and internationally through the course of our study, with progressive evolution in the understanding of disease pathology and emerging treatment evidence. The SOC during the time of the study across all sites involved four main treatment domains: steroids, anticoagulation, antibiotics, and antiviral medications. The intervention group (SOC+L) received oral leflunomide at a loading dose of 100mg/day for three days and then 20mg/day for 7 days as a maintenance dose. The maintenance dose was reduced to 10mg/day if liver enzymes AST/ALT exceeded 2 x ULN. Leflunomide treatment was stopped early if AST/ATL exceeded 3 x ULN during the intervention. Study participants received additional COVID-19 therapies, including monoclonal antibodies, at the discretion of the direct care clinical team, even if leflunomide was initiated.

Study procedures - Patient related clinical/investigation data, treatment compliance, outcomes and adverse events (AE) were collected by the site investigators and recorded on the pre-specified daily electronic case report form (e-CRF, see Appendix). Adverse events (AE) were graded according to the Common Terminology Criteria for Adverse Events.[11] Blood samples were collected and processed for quantifying viral load (on days 1, 7, 11, 15, 28 or day of discharge) and for future inflammatory profiling (on days 1, 3 and 11). Liver enzymes were measured at baseline, on day 3 after the leflunomide loading and on discharge. Patient questionnaire was administered at 28- and 90-days after randomisation to monitor the persistence of symptoms possibly associated with long COVID syndrome.[12] SpO_2/FiO_2 data were monitored daily. The frequency of SpO_2 monitoring varied with FiO_2 administration. It is standard clinical practice that SpO_2 is monitored every 4 hours in a clinically stable patient. The frequency increases to continuous SpO_2 monitoring in a patient with oxygen requirement or ventilation support. Where multiple daily values were recorded we selected the SpO_2/FiO_2 ratio reflecting increased oxygen demand.

Blinding - Site investigator teams and direct clinical care teams were not blinded to the randomisation outcomes, but neither were provided information about the aggregate patient outcomes.

Outcomes - The primary outcome is the time (days) from randomisation to clinical improvement (TTCI) of two points on a seven-category clinical status scale or live discharge from hospital prior to 28 days.[13] The clinical status ordinal scale consisted of the following: 1 not hospitalised, resumption of normal activities; 2 not hospitalized, but unable to resume normal activities; 3 hospitalised, not requiring supplemental oxygen; 4 hospitalised, requiring supplemental oxygen; 5 hospitalised, requiring nasal high-flow oxygen (HFNC) therapy, non-invasive mechanical ventilation (NIV), or both; 6 hospitalized, requiring extracorporeal membrane oxygenation (ECMO), invasive mechanical ventilation (IMV), or both; and 7 death.

Safety profile of leflunomide in this group of patients was assessed from incidence rates of AE deemed to be serious and/or severe (≥Grade 3). Grading guidelines suggest 5 categories: 1 mild, asymptomatic or mild symptoms, clinical or diagnostic observations only, intervention not indicated; 2 moderate, minimal, local or non-invasive intervention indicated, limiting age-appropriate instrumental activities of daily livings (ADL); 3 severe, medically significant but not immediately life-threatening, hospitalization or prolongation of hospitalization indicated, disabling; limiting self-care ADL; 4 life-threatening consequences, urgent intervention indicate; 5 death related to AE. In addition, the incidences, and levels of liver transaminitis (ALT, AST) were assessed.

The main secondary outcomes were focused on overall (all-cause) mortality, and oxygen dependence (duration in days) assessed by S/F ratio (i.e. oxygen saturation detected by pulse oximeter [SPO_2] / supplemental oxygen concentration [FiO_2]) and impact on viral replication (viral load). Additional secondary outcomes included inflammatory targets such as CRP, lymphocyte counts, and selected cytokines (initially focussing on IL2, IL6, TNF-α). The concept of long COVID emerged during the study, so we used the data from our questionnaires at 28 and 90 days to comment on long COVID symptoms

Statistical analyses

Sample size calculation - The primary outcome measure was a time-to-event analysis based on an assessment of TTCI. Since our study protocol was conceived and developed during the initial peak of the global pandemic, the precise hazard ratio for major clinical outcomes related to this infection was largely unknown and, therefore, sample size calculation was based on the proportion of patients expected to meet the outcome criteria by 28 days.[14] Assuming $\alpha = 0.05$, $\beta = 0.20$ and allocation ratio = 1:1, the number of patients per treatment arm was estimated to be 74. We expected a 20% attrition rate, so the total number of patients required in the study was calculated to be 178, 89 patients in each arm.

Analysis population - The full analysis set was defined according to the intention to treat principle (ITT). All subjects randomised were included in the ITT analysis set for the primary outcome, regardless of whether they received any dose of their allocated treatment. This analysis set was used to summarize baseline patient characteristics and to carry out all efficacy and safety assessments. Subjects were analysed according to their randomised treatment allocation. We also present a modified intention to treat analysis for the primary and secondary outcomes, as a sensitivity analysis, to account for study participants who were randomised in error and those who withdrew consent prior to the intervention.

Primary outcomes - The TTCI data was estimated using Kaplan-Meier survival curves. Hazard ratio and 95% confidence intervals were estimated using Cox proportional hazards regression models. The primary analysis was

stratified by the randomisation strata: baseline risk indicators (age </≥ 70 years, co-morbidities) and NEWS2 score. Log rank test was used for comparing the Kaplan-Meir curves, hazard ratios and their confidence intervals for the significance of the treatment effect.

Secondary outcomes - Continuous secondary outcomes were evaluated for within-groups differences using the Mann-Whitney U or Wilcoxon rank tests, respectively, depending on the data distribution identified: parametric or non-parametric. Statistical normality was assessed using the Shapiro-Wilk method. Categorical outcomes were assessed for between-group differences using the chi-square method and expressed as %. For all outcomes, statistical significance was accepted at a 2-sided α of 0.05.

Adverse events - AEs were coded using MedDRA and assigned grades based on National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03.[11]

Public and patient involvement

Patient volunteers were consulted regarding the study design and materials to be provided to the potential participants (patient information sheet, consent forms, questionnaires). Two lay members were appointed to the Trial Steering Committee and provided input on the conduct of the study.

Results

Recruitment, Randomisation, Assignment of Therapy and Follow-up

Between September 2020 and May 2021, 214 patients were recruited to the study from 2 UK Hospitals (n=66, 31%; Ashford and St Peters' NHS Trust, Surrey; Kingston Hospital NHS Trust, London) and 3 Hospitals in India; (n=148, 69%; Max Hospital, Delhi; Meditrina Institute, Nagpur; Noble Hospital, Pune). Due to the wavering new COVID-19 infections, the UK recruitment came to a halt in February 2021 and patients at the three Indian sites were recruited in the remaining period. Of the 214 participating patients, 104 were randomised to the intervention (SOC+L) group and 110 to the control (SOC) group. In the SOC+L group, 3 patients withdrew study consent after randomisation, and did not receive leflunomide therapy. During the data cleaning process, 10 patients were flagged as not meeting the inclusion criteria (6 in SOC+L; 4 in SOC), as they did not have moderate COVID-19 symptoms at the time of randomisation. Daily clinical data were collected for all patients during hospitalisation and the patients were asked to complete follow-up questionnaires at 28- and 90-days after randomisation, as shown in Figure 1 (CONSORT diagram).

Baseline patient characteristics were similar between the SOC+L and SOC groups, summarised in Table 1.

Characteristics	SOC+L n=104	SOC n=110
Age, yrs, mean ± sd	55.2±14.7	56.4±15.2
BMI, kg/m^2, mean ± sd	27.3±5.1	27.7±5.6
Female gender at birth, %	28.8	37.3
Ethnicity, %		
South Asian	75	69
White	24	30
Arab	-	0.91
Comorbidities, %		
BMI ≥ 40 kg/m^2	2.9	4.6
Age ≥ 70 yrs	18.3	20
Chronic respiratory disease	8.7	15.5
Chronic cardiovascular disease (including hypertension)	38.5	39.1
Chronic renal disease	2.9	2.7
Diabetes	23.1	20.9
Immunosuppressive diseases	6.7	6.4
Others		
Malignant neoplasm	3.9	2.7
Chronic haematological disease	1	0.9
Chronic neurological disorder	10.6	3.7
Malnutrition	1	0.9
Smoking (present or past)	21.1	20
Symptom duration, day, median (IQR)	6 (4-8)	6 (5-8)
Time from admission, day, median (IQR)	2 (1-4	2 (1-3)
Non-invasive ventilation, %	4.8	7.3
Invasive ventilation, %	1	1.8
NEWS 2 score median (IQR)	6 (4-8)	5 (4-8)
CRP, mg/L, median (IQR)	28 (9-77)	32 (13-64)
Transaminase, >ULN, %		
ALT	44.7	31.7
AST	35.4	28.4
Stratification, %		
Group 1	12.5	14.5
Group 2	14.4	16.4
Group 3	48.1	46.4
Group 4	25	22.7

Table 1: Baseline patient characteristics at the time of randomization

BMI: Body mass index; ULN = upper limits of normal; Group 1: High/Moderate comorbidity risk with NEWS2 score ≥5; Group 2: High/Moderate comorbidity risk with NEWS2 score <5; Group 3: Low comorbidity risk with NEWS2 score ≥5; and Group 4: Low comorbidity risk with NEWS2 score <5. Creatinine ULN: 104umol/L; ALT ULN: 49 U/L; AST ULN: 48 U/L; Immunosuppressive disease: asplenia, rheumatological disorder.

Baseline characteristics were similar in both arms but there were significantly more patients with chronic neurological disorders in the SOC+L group. None of the patients with this condition had contraindication to non-invasive ventilation.

Treatment Assignment and Compliance

Full course of leflunomide therapy was completed by 81/104 patients (78%). Of the 19 patients (16 in UK, 3 in India) who did not complete treatment, 3 patients did not receive a single dose of leflunomide as they withdrew consent soon after randomization, 5 patients died prior to completion of the full course, 8 patients stopped leflunomide early when ALT/AST exceeded 3 x ULN laboratory reference range, 1 patient had tocilizumab introduced to replace leflunomide, 1 patient self-discharged early and 1 refused final two doses. Leflunomide treatment compliance appeared to be better in participants from Indian centres as 92% of them received the full dose of leflunomide compared to 52% of patients in the UK centres which was largely due to a higher incidence of liver enzyme transaminitis and mortality observed in the UK cohort.

There was no significant difference in the assignment of standard of care treatment between the SOC+L and SOC groups as shown in Figure 1. It included corticosteroids, anticoagulants, antibiotics, and antiviral therapies. Overall, steroid uptake was >95% in both treatment arms with different protocols used at participating study centres: dexamethasone 4 mg/day for 3 days; dexamethasone 6 mg/day for 7-10 days; methylprednisolone 80 mg/day for 7 days and methylprednisolone 120 mg/day for 5 days. However, there was no difference in the steroid treatment assigned between the control and the treatment groups. There were some differences in the proportions of patients receiving additional adjunct therapies such as hydroxychloroquine and immunotherapy (Supplementary table 1). Overall, hydroxychloroquine was prescribed to similar proportion of patients in the intervention and the control group (47%) but the proportions of patients receiving it in the UK was much smaller, 3% compared to 67% in India. A small number of patients received immunomodulating drugs such as interferon alpha and beta (n = 20 in India), tocilizumab and bevacizumab (n = 5 in the UK, n = 2 in India).

Primary Outcomes

Time to clinical improvement of 2 points on a clinical status scale/discharge before 28 days

In the ITT analyses (n = 214), SOC+L group did not have a significantly shorter TTCI than the SOC group within 28 days of randomisation; the median was 7.0 (IQR 7.0 - 8.0) days vs. 8.0 (IQR 7.0 - 9.0) days, respectively; with a hazard ratio (HR) of 1.32 (CI 0.98 -1.77), p = 0.070 (Figure 2).

In modified ITT population (n = 201) where 3 patients who withdrew consent after being randomised to the SOC+L group but never received leflunomide treatment and 10 patients who did not fulfil moderate COVID-19 symptoms at randomisation were excluded from analysis, the median TTCI was significantly shorter in the SOC+L group than SOC group by 1.0 day, median 7.0 days (IQR 7.0 -8.0) vs. 8.0 (IQR 7.0 – 9.0), respectively, with a HR of 1.42 (CI 1.04 – 1.94); p = 0.028.

Safety

Incidences of AE of all grades are summarized in Table 2.

Adverse events	SOC+L (n= 104)	SOC (n=110)
Adverse events (n) / Patients (n)	121 / 56	91/ 42
Grade 1 (Mild)	58 / 39	48 / 32
Grade 2 (Moderate)	23 / 13	17/9
Grade 3 (Severe)/ Grade 4 (Life threatening events)	31/15	16/9
Grade 5 (Deaths)	9/9	10/10

Table 2: Incidence of reported adverse events in both treatment arms.
The table shows the number of adverse events recorded in the study and the number of patients affected by at least one adverse event.

At least one AE was reported in 98/214 participants, and most of them were mild in severity. AEs of moderate grade were reported in 13/104 patients in SOC+L group and 9/110 patients in SOC group. Serious AEs (n=47) were reported in 15/104 patients in SOC+L groups and 9/110 in SOC group and 19 patients died (9 in SOC+L group, 10 in the SOC group). There was no significant difference in the incidence of AE reported between the two groups. No Serious AEs were attributed to leflunomide. A supplementary Table 2 lists all adverse events recorded in the study according to MedDRA terms.

Liver function

At baseline, more patients with greater than ULN levels of ALT and AST were randomized in the SOC+L group than the SOC group (ALT: 46 vs 33, p = 0.049; AST: 31 vs 24, p = 0.340). By Day 3/4, following the initial loading of leflunomide therapy in the SOC+L group, there was a significantly higher number of patients with greater than ULN level of ALT and AST in the SOC+L than the SOC group (64 vs 38, p <0.001; and 51 vs 24, p <0.001). By discharge, the difference in the number of patients with ALT and AST transaminitis between the SOC+L and SOC groups was no longer significant (28 vs 27, p = 0.633; and 20 vs 17, p = 0.318) (Supplementary table 3). Leflunomide therapy was terminated early if transaminase levels exceeded 3 x ULN. However, there were 5 patients in India who continued with leflunomide therapy at the discretion of the researcher and direct care team with close monitoring of their liver function. Interestingly, in this subset of patients, the transaminase levels improved despite continuation of therapy. There were no adverse events related to clinically significant liver injury due to leflunomide. AEs related to liver dysfunction were reported in 16/104 (15.4%) patients in SOC+L group, 7 were mild, 8 were moderate and 1 was severe. Of these, 10 were deemed possibly treatment related and leflunomide treatment was discontinued in 9 patients. Comparatively, in the control group, 6/110 (5.5%) patients had liver dysfunction related AE. Five of them were mild and 1 case was severe.

Secondary Outcomes

A modified intent to treat approach was used for data from 201 patients for all secondary outcomes. This included 95 patients in the SOC+L group and 106 patients in SOC group. For these analyses we excluded 3 patients in the SOC+L group who withdrew consent and never received leflunomide and 10 patients (6 SOC+L; 4 SOC) who

did not fulfil moderate COVID symptoms inclusion criterion (did not show respiratory compromise and blood oxygen saturation (SpO_2) <93% on room air).

Mortality

There was no difference in all-cause mortality within 28 days of randomization between the treatment arms as 9/95 (9.47%) of patients died in SOC+L group compared to 10/106 (9.43%) in SOC groups. The survival curves diverge in favour of the SOC+L group after 10 days of hospital treatment, but the curves converged again after 3 weeks (when majority of the patients have been discharged). All deaths were attributed to complications related to Covid-19 (Figure 3**,** panel A).

Oxygenation and assisted ventilation

Oxygen independence is defined by maintenance of SpO_2/FiO_2 Air ratio > 4.43. There was a difference in the median time the participants required to be completely weaned off oxygen therapy between groups; 6.0 (IQR 4.0 – 8-0) days in the SOC + L group vs. 7.0 (5.0 – 10.0) days in the SOC group, p = 0.047 (Figure 3, panel B)

Non-invasive ventilation was required for 14.4% of patients in SOC+L group vs. 16.4% in the SOC group. The duration of non-invasive ventilation was 6.0 (IQR 2.0-9.0) days in the SOC+L group compared to 4.5 days (IQR 2.3-6.8) in the SOC group. Similar proportion of patients required non-invasive ventilation at the time of study enrolment (4.8% in SOC+L group vs. 7.3% in SOC group, p= 0.45).
The proportion of patients admitted to level-2 Intensive Care Unit (ICU) was 8.7 % in the SOC+L group and 8.2% in the SOC group. The median time spent at ICU was 8.0 (IQR 5.0-10.0) days vs. 9.0 (IQR 5.0-13.0) days, respectively**.** Invasive ventilation was required for 3.9% of patients in the SOC+L group and 5.5% in the SOC group with median duration of 6 (IQR 4.8, 6.0) days vs. 7.0 (IQR 5.3 - 11.8) days, respectively. None of the between group comparisons were statistically significant. Patients recruited in India were significantly less likely to require invasive or non-invasive ventilation or be admitted to ICU compared to patients recruited in the UK (p<0.001).

Viral load

Quantitative SARS-COV-2 PCR measurements from nasopharyngeal swabs at baseline showed no difference in median $\log^{10}$ viral loads (copies/ml) between the two groups, SOC+L 4.68 (IQR 4.45-4.85) vs SOC 4.76 (IQR 4.48-4.92), p =027.. We clustered the serial samples to reflect the crucial time intervals during the hospital stay: time coinciding with finishing leflunomide loading dose (by Day 4), time to 75% patients being discharged from hospital (by Day 7), time to finishing leflunomide maintenance dose (by Day 11) and beyond (Figure 4). Viral loads were significantly reduced in both treatment arms. There was no significant difference in the overall rate of the viral load clearance between the two groups by Day 11 and beyond. Viral loads were significantly reduced in both treatment arms by Day 7, p<0.001; and by Day 11, p <0.030. The rate of viral load reduction between groups by Day 11 appeared to be similar.

Cytokines, CRP and lymphocytes

Cytokine levels were assessed separately for UK and Indian sites as two laboratories using different assays processed the samples. The median baseline levels of IL 2, IL 6 and TNF-α levels (UK: IL2 0.43 [IQR 0.30-0.62] pg/ml; IL6 6.2 [IQR 2.9-9.7] pg/ml; TNF-α 10.1 [IQR 7.8 -13.5] pg/ml; India: IL2 4.3 [IQR 2.8-5.8] pg/ml; IL6 12.6 [IQR 6.5-43.1] pg/ml; TNF-α 6.1 [IQR 4.9-7.1] pg/ml) were not significantly raised from normal reference ranges and were not different between treatment groups in both countries. The cytokine levels were reduced during hospitalisation, though the clinical significance of these changes within the normal range is uncertain. There was no significant difference in the trends observed between treatment arms.

The median baseline levels of CRP were similar in both groups, 28, (IQR 8-71) in SOC+L vs. 34 (14-71) mg/L in SOC. By one week of treatment, there was similar levels of reduction between groups.

The median baseline lymphocytes levels were lower than normal reference range in both groups (0.99 [IQR 0.6-1.6) $x10^9$/L in SOC+L vs 0.95 (IQR 0.6-1.6) $x10^9$/L in SOC. By 1 week of treatment, levels rose to normal range in both groups. There was no significant difference in the trends observed between groups.

28- and 90-days follow up

At 28 days, 59/81 patients (71.2%) in the SOC+L group and 60/91 patients (65.9%) in the SOC group experienced at least 1 of 9 common long-COVID symptoms (fatigue, cough, anxiety, chest pain, brain fog, breathlessness, disturbed sleep, palpitations, joint pain); with sleep quality (48.2% vs. 38.5%), breathlessness (40.7 vs 42.9%), joint pain (32.1 vs. 33%), fatigue (29.6 vs, 31.9%), and anxiety (24.7 vs. 19.8%) being the commonest symptoms experienced (Supplementary table 4). At 90 days, there was a reduction in overall prevalence of symptoms as 42/81 patients (51.2%) in the SOC+L group and 37/91 (40.7%) patients in the SOC group and any of the residual symptoms were of reduced severity. There was no significant difference in these outcomes between the treatment arms.

Myalgia symptoms were comparably reduced between the 2 groups at 90 days. Anosmia and loss of taste were still reported by 2 and 7 patients, respectively, in the SOC+L group, but none in the SOC group.

At 28 days, 41.5% patients in the SOC + L group and 52.8% in SOC group, reported being moderately to severely dyspnoeic (Grade 4: stops for breath after walking 100m; Grade 5: too breathless to leave the house or breathless when dressing). These proportions were further reduced at 90 days, to 22% in the SOC+L group compared to 19.8% in SOC group. These differences were not significant in between group comparisons.

Mental health issues were highlighted by reports of feeling depressed and losing interest in doing things. Comparable proportions of patients in the SOC+L group and SOC group reported those problems at 28 days (17.9% vs 16.0%; 11.6% vs 14.2%, respectively) which were further reduced in both groups at 90 days (11.6% vs 9.4%; 9.5% vs 6.6%, respectively).

At 28 days participants in the SOC+L group scored their current health as being 80±25% of the usual which increased to 89±17% at 90 days. In the SOC group the scores were similar, 82 ± 23% and 90 ± 17% at 28 and 90 days respectively.

Discussion

This study is the first prospective, multicentre, randomised, controlled clinical trial investigating the clinical efficacy and safety of leflunomide in treating acute COVID-19 infection. The study showed that a course of leflunomide (3 days of 100 mg/day loading dose followed by 7 days of 20 mg/day maintenance dose) added to the standard care treatment (steroids, anticoagulants, antibiotics and antiviral therapy), did not influence the primary outcome of the trial and the acute clinical outcomes at 28 days, or the prevalence of long-COVID symptoms at 28 and 90 days. However, participants who received leflunomide as an adjunct therapy were weaned off oxygen earlier, which translated to reduced hospital stay by one day. The medication appeared to be safe and well tolerated with no severe adverse events attributable to it. A small proportion of patients in our study were still burdened by COVID-19 related symptoms 90 days after randomisation.

This multicentre trial advances the evidence base on the impact of leflunomide, a repurposed rheumatoid arthritis medication, on COVID-19 infection. Leflunomide was a potentially attractive therapeutic choice from early preclinical and clinical experience reported from hospitals in Wuhan, China. Dihydroorotate dihydrogenase (DHODH), located in the inner mitochondrial membrane is a rate-limiting enzyme in de novo pyrimidine biosynthesis. In virus-infected cells, a large intracellular nucleotide pool is consumed by rapid viral replication. RNA viruses need unique UMP but not TMP in their genomes. As UMP is the particular nucleoside produced by DHODH, RNA viruses are sensitive to reduced DHODH activity. Preclinical models of cell and animal infection by SARS-CoV-2 demonstrated that leflunomide attenuates viral genome replication, suppresses inflammatory response and the release of pro-inflammatory cytokines and chemokines.[7,8,9] Early reports from China advocated major clinical benefits in patients treated with leflunomide both in terms of less severe outcomes and duration of infection.[15,16,17] While the current study did not reproduce these overall benefits in the ITT analysis regarding the primary outcome, it confirmed some positive effects in those patients who received the trial intervention (in modified intent to treat analysis).

Our results are likely explained by the changing landscape and evolution of the routine COVID-19 treatment protocols in the standard arm of the study and the resultant severity of the COVID-19 outcomes in general. The initial phase of the COVID-19 pandemic was characterised by severe respiratory and systemic infections and poor outcomes due to the development of acute respiratory distress syndrome, multi-organ failure and eventual death.[18,19] Contrary to this early experience with COVID-19 management, the in-hospital mortality in the present study was much lower, less than 10% in both groups. The majority of patients in both treatment arms improved during hospitalisation and were discharged within a week of admission. Inclusion of prognostically significant

COVID-19 therapies in both pharmacological and non-pharmacological standard of care treatments undoubtedly contributed to a reduction in severe complications and better overall outcomes. During patient recruitment in our study, various therapies have been introduced based on the results of different trials.[20,21] For example, more than 95% percent of the study population received steroids as standard of care.

Theoretical considerations suggest that leflunomide may effectively inhibit viral replication. The initial pilot study during the early outbreak of COVID-19 in Wuhan, China reported reduced viral shedding time following leflunomide treatment during acute infection compared to the standard of care therapy.[15] Similarly, viral shedding duration was reduced in leflunomide treated patients who remained qPCR positive 1 month after the initial infection.[16] Our study addressed the viral load reduction at pre-specified time points. Values of viral load were reduced over time but there was no difference between the treatment arms. Both methodological considerations and the inclusion of comprehensive pharmacological treatment regimens in the SOC could explain these differences. For instance, corticosteroid therapy was absent in the early study from Wuhan, but the later study refers to the use of hydroxychloroquine, interferon-alpha and antiviral medications as part of acute standard of care therapy.[15,16] However, our results are in line with other reports from China which showed that duration of viral shedding was not affected by leflunomide added to nebulised interferon alpha therapy for treating long-term positive COVID-19 after 4 weeks of in-hospital treatment.[17] Interestingly a third of these patients received corticosteroid therapy during the initial acute treatment. [16,17]

Beyond the issue of therapeutic efficacy and viral load, our study confirms overall safety of leflunomide in COVID-19 infection. The safety profile of leflunomide is well established in the treatment of RA. [6] Leflunomide, repurposed for the COVID-19 treatment, was well tolerated since no serious adverse events were attributed to it. Similar findings were reported in other studies.[15,16,22] Mild transaminitis following long-term leflunomide use in the RA population is recognised, and usually resolves after medication is terminated. The mechanism is likely to be modulation of interleukins which may hinder the protection of hepatocytes from injury rather than direct toxicity.[23] There were comparable incidences of transaminitis in both treatment arms in our study. However, more patients in the UK cohort had raised liver function tests leading to modification or termination of leflunomide treatment. This may be accounted for by the difference in the severity of COVID-19 disease and spectrum of co-morbidities between UK and Indian participants rather than genetic polymorphism in drug metabolism. Overall, the proposed leflunomide regimen was well tolerated.

One of the motivations of the current trial employing leflunomide was to benefit from the anti-inflammatory effects of this drug. In this context, hydrocortisone has been demonstrated as an effective therapy in severe COVID-19 infections and recent trials also demonstrated the benefit of tocilizumab, a selective IL-6 inhibitor and a different disease modifying rheumatoid arthritis medication. However, such finding is not universal as the benefit of tocilizumab is mainly demonstrated in critically to moderately ill patients.[20,21] A recent meta-analysis showed that the benefit of IL-6 receptor antagonist was encountered only in patients who were also treated with glucocorticoids.[24] This is in keeping with observations that a broader spectrum of pro-inflammatory cytokines, macrophages and T cell response have all been documented in severely ill patients demonstrating the role of a more complex inflammatory response. It is exactly this broader inflammatory reaction that could be targeted by

leflunomide as its effect on cytokines is not restricted to IL 6 and it may also have an impact on activated T cell response.[8,9,25] Such phenomena might contribute to the benefits of reduced oxygen dependence in patients who have received leflunomide treatment. However, it is conceivable that the full benefit of such anti-inflammatory effect may be more pronounced in severely ill patients, but this population was underrepresented in our trial and the (inadvertent) inclusion of patients with milder symptoms may have led to some attrition of statistical power in our study. A more detailed analysis of the cytokine and metabolic profiles of our trial population is underway to clarify these important issues.

Another important consideration when discussing the potential benefits of leflunomide is the mutation ability of the SARS-CoV-2 virus.[26] So far, the mutations observed in different strains worldwide have largely been confined to the part of the spike protein affecting the virus's ability of cell entry as opposed to a region targeted by neutralising antibodies. However, the possibility of mutations in different regions cannot be excluded. Targeting the host's pyrimidine biosynthesis pathway by leflunomide, rather than using drugs with direct antiviral action, remains an advantage offering protection against a broader spectrum of viruses and potentially overcoming resistance. Indeed, DHODH inhibitors such as leflunomide has shown broad-spectrum antiviral effects against various RNA viruses in cell models.[7] Leflunomide may therefore be considered a viable pharmacological treatment for COVID-19 patients given it is well tolerated, safe, economical, and widely available. Its clinical effectiveness measured against recognised selective IL-6 inhibitors in the more severely/critically ill patients needs to be further explored as leflunomide may be the preferred option in countries where other immunomodulating agents, such as Talizumab, may not be practical or widely available.

Limitations

The present study has several limitations. In order to balance the needs of the trial with clinical care and to minimise disruption to already overstretched clinical resources during COVID-19 pandemic, we chose to adopt an open label study design. This design may have affected the data collection and clinical management of the patients and potentially introduced a bias. However, it also allowed early detection of significant adverse events and a potential outcome benefit. This was an important consideration when testing an off-label use of a medication in COVID-19, a disease with high morbidity and mortality.

The study was set out to recruit more severely and critically affected patients in a single country. However, due to recruitment restrictions because of national prioritization of critically ill patients to only a few studies together with scarcity of NHS resources during the pandemic, the study was extended abroad, ultimately recruiting less affected patients with heterogeneous clinical profiles. Although patient characteristics and medications received as part of SOC did not differ between the randomised arms, the more heterogeneous population, milder COVID-19 disease, and more effective standard of care treatments most likely impacted on the hypothesised effect size and the ability of finding a difference in our recruited sample. Finally, the COVID-19 restrictions affected our protocolised laboratory investigations, such as the serial viral load and comprehensive inflammatory profiling. Nevertheless, studies focusing on the more severely affected participants are underway and will be the subject of a separate submission.

Conclusion

Leflunomide had no major impact on the clinical outcomes when administered together with the currently established but evolving therapies in moderately affected COVID-19 patients. It may shorten duration of oxygen dependence thereby affecting the TTCI and hospital discharge. Transaminitis associated with leflunomide therapy did not lead to excess adverse events compared to the control group and may have arisen in part due to the severity of clinical infection. Further studies are needed to investigate the potential benefits of leflunomide in the critically ill patients and the biological mechanisms involved.

Ethics statements

All patients taking part in the study signed written informed consent form once the study was ethically approved by relevant bodies in England (South Central - Berkshire Research Ethics Committee, Bristol REC Centre, reference number 20/SC/0264) and India (Max HealthCare Ethics Committee, reference number RS/MSSSH/GMHRCCMS/MHEC/CCM/20-23; Meditrina Institute Ethics Committee, reference number ECR/605/Inst/MH/2014/RR; Noble Hospital Institutional Ethics Committee, reference number NHIEC/FEB/2021/238).

Data availability statement

The anonymized data may be available upon request following approval from the Trial Management Group and the Sponsor.

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Authors' contributions

All designated authors meet all four ICMJE criteria for authorship. The funders and the sponsor of the study had no role in the analyses or the interpretation of the results.

ZC, NM, HLL, SRKS, LL, JB, SL, IJ, DF and PS made substantial contribution to the conception and design of the study. SS, AK, AB, RA, KB, MM, KR, FO, KL, AL, SRKS, JB, HLL, NM, IKH and ZC made substantial contribution to the acquisition, analysis and interpretation of the data for the study. All authors contributed to the drafting, revision and final approval of the manuscript. ZC, NM and LL are responsible for the overall content.

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/disclosure-of-interest/ and declare no competing interests.

Dissemination

A summary of the results will be disseminated to the participants by principal investigators at each trial site via a newsletter.

Licencing Statement

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References

1. https://COVID19.who.int/
2. Chen YL, Quach TTT, COVID-19 cytokine storm syndrome: a threshold concept. Lancet Microbe. 2021 Feb;2(2):e49-e50.doi: 10.1016/S2666-5247(20)30223-8.Epub 2021 Feb 2
3. Gavriatopoulou M, Ntanasis-Stathopoulos I, Korompoki E, Fotiou D, Migkou M, Tzanninis IG, Psaltopoulou T, Kastritis E, Terpos E, Dimopoulos MA. Emerging treatment strategies for COVID-19 infection. Clin Exp Med. 2021 May;21(2):167-179. doi: 10.1007/s10238-020-00671-y. Epub 2020 Oct 30. PMID: 33128197; PMCID: PMC759894
4. Dwek, Raymond A et al. "Targeting glycosylation as a therapeutic approach." Nature reviews. Drug discovery vol. 1,1 (2002): 65-75. doi:10.1038/nrd708
5. Angus, Derek C et al. "The REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-acquired Pneumonia) Study. Rationale and Design." Annals of the American Thoracic Society vol. 17,7 (2020): 879-891. doi:10.1513/AnnalsATS.202003-192SD
6. Alfaro-Lara, et al. Systematic review and meta-analysis of the efficacy and safety of leflunomide and methotrexate in the treatment of rheumatoid arthritis. Reumatol Clin. 2019 May - Jun;15(3):133-1
7. Xiong R, Zhang L, Li S, Sun Y, Ding M, Wang Y, Zhao Y, Wu Y, Shang W, Jiang X, Shan J, Shen Z, Tong Y, Xu L, Chen Y, Liu Y, Zou G, Lavillete D, Zhao Z, Wang R, Zhu L, Xiao G, Lan K, Li H, Xu K. Novel and potent inhibitors targeting DHODH are broad-spectrum antivirals against RNA viruses including newly-emerged coronavirus SARS-CoV-2. Protein Cell. 2020 Oct;11(10):723-739. doi: 10.1007/s13238-020-00768-w. Epub 2020 Aug 4. Erratum in: Protein Cell. 2021 Jan;12(1):76-80. Erratum in: Protein Cell. 2020 Nov 9;: PMID: 32754890; PMCID: PMC7402641.
8. Yao, H. W.; Li, J.; Chen, J. Q.; Xu, S. Y. A 771726, the active metabolite of leflunomide, inhibits TNF-α and IL-1 from Kupffer cells. Inflammation 2004, 28 (2), 97-103.
9. Li, L. et al. The effects of teriflunomide on lymphocyte subpopulations in human peripheral blood mononuclear cells in vitro. J. Neuroimmunol. 2013, 265 (1-2), 82-90.
10. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2
11. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_60
12. Mandal S, Barnett J, Brill SE ARC Study Group, et al. 'Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax 2021;76:396-398.
13. http://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus/en/).
14. Machin D, Campbell M, Fayers, P, Pinol A (1997) Sample Size Tables for Clinical Studies. Second Ed. Blackwell Science IBSN 0-86542-870-0 p. 176-177
15. Hu, Ke et al. "A Small-Scale Medication of Leflunomide as a Treatment of COVID-19 in an Open-Label Blank-Controlled Clinical Trial." Virologica Sinica vol. 35,6 (2020): 725-733. doi:10.1007/s12250-020-00258-7
16. Wang, Qiang et al. "Efficacy and Safety of Leflunomide for Refractory COVID-19: A Pilot Study." Frontiers in pharmacology vol. 12 581833. 2 Jul. 2021, doi:10.3389/fphar.2021.581833

17. Mengmei Wang, Yang Zhao, Weihua Hu, Dong Zhao, Yunting Zhang, Tao Wang, Zhishui Zheng, Xiaochen Li, Shaolin Zeng, Zhenlian Liu. Treatment of Coronavirus Disease 2019 Patients With Prolonged Postsymptomatic Viral Shedding With Leflunomide: A Single-center Randomized Controlled Clinical Trial. Clinical Infectious Diseases, Volume 73, Issue 11, 1 December 2021, Pages e4012–e4019, https://doi.org/10.1093/cid/ciaa1417
18. Huang, Chaolin et al. "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China." Lancet (London, England) vol. 395,10223 (2020): 497-506. doi:10.1016/S0140-6736(20)30183-5
19. ISARIC Clinical Characterisation Group. "COVID-19 symptoms at hospital admission vary with age and sex: ISARIC multinational study." medRxiv : the preprint server for health sciences 2020.10.26.20219519. 19 Nov. 2020, doi:10.1101/2020.10.26.20219519. Preprint.
20. REMAP-CAP Investigators et al. "Interleukin-6 Receptor Antagonists in Critically Ill Patients with COVID-19." The New England journal of medicine vol. 384,16 (2021): 1491-1502. doi:10.1056/NEJMoa2100433
21. Abani, O., Abbas, A., Abbas, F., Abbas, M., Abbasi, S., Abbass, H., et al. (2021). Tocilizumab in Patients Admitted to Hospital with COVID-19 (RECOVERY): a Randomised, Controlled, Open-Label, Platform Trial. The Lancet 397, 1637–1645. doi:10.1016/S0140-6736(21)00676-0
22. Chakravarty EF, Sanchez-Yamamoto D, Bush TM. The use of disease modifying antirheumatic drugs in women with rheumatoid arthritis of childbearing age: a survey of practice pattern and pregnancy outcomes. J Rheumatol. 2003;30:241–6.
23. Atsushi Ogata, Yasuhiro Kato, Shinji Higa, Kazuyuki Yoshizaki, IL-6 inhibitor for the treatment of rheumatoid arthritis: A comprehensive review, Modern Rheumatology, Volume 29, Issue 2, 4 March 2019, Pages 258–267, https://doi.org/10.1080/14397595.2018.1546357
24. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group et al. "Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis." JAMA vol. 324,13 (2020): 1330-1341. doi:10.1001/jama.2020.17023
25. Kraan MC, Smeets TJM, van Loon MJ, et al Differential effects of leflunomide and methotrexate on cytokine production in rheumatoid arthritis Annals of the Rheumatic Diseases 2004;63:1056-1061.
26. Wang, Shihang et al. "Molecular evolutionary characteristics of SARS-CoV-2 emerging in the United States." Journal of medical virology vol. 94,1 (2022): 310-317. doi:10.1002/jmv.27331

Figure legends:

Figure 1: Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.

**= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.*

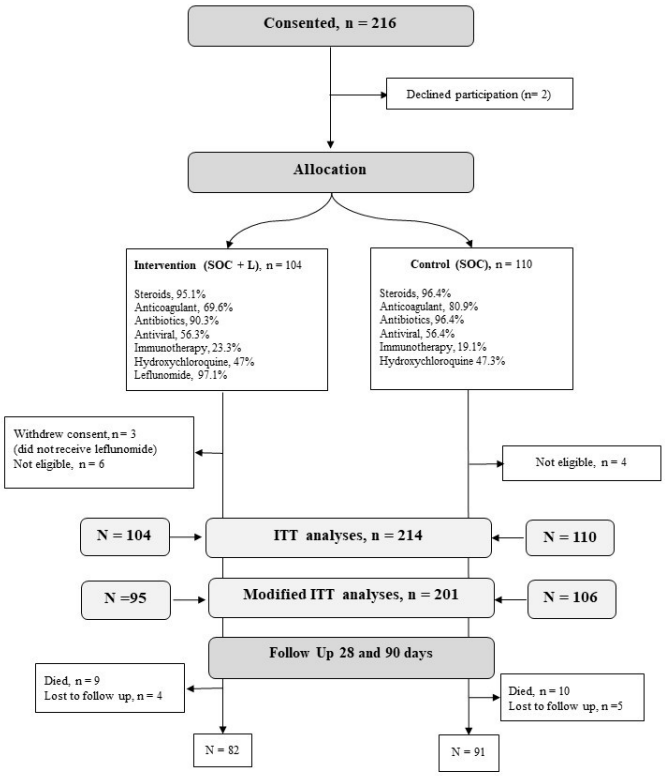
Figure 2: Time to clinical improvement of 2 points on a clinical status scale or discharge prior 28 day in a stratified ITT analysis (primary outcome).

Patients who died were censored at the time their death occurred, while all surviving patients who did not reach TTCI criteria by day 28 were right censored at that point. Most of the patients were discharged within the first 10 days of admission.

Figure 3: Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

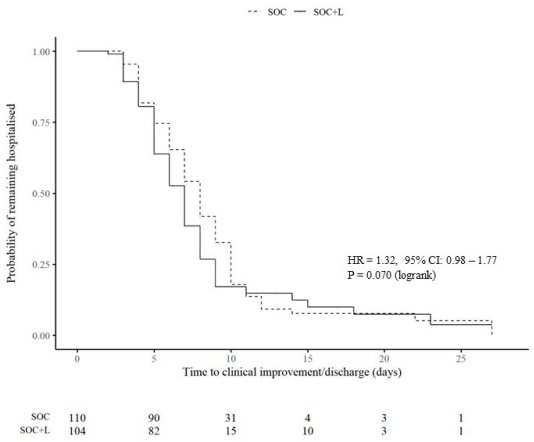
Figure 4: Mean changes in log^{10} viral load (copies/ml) from baseline.

Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.



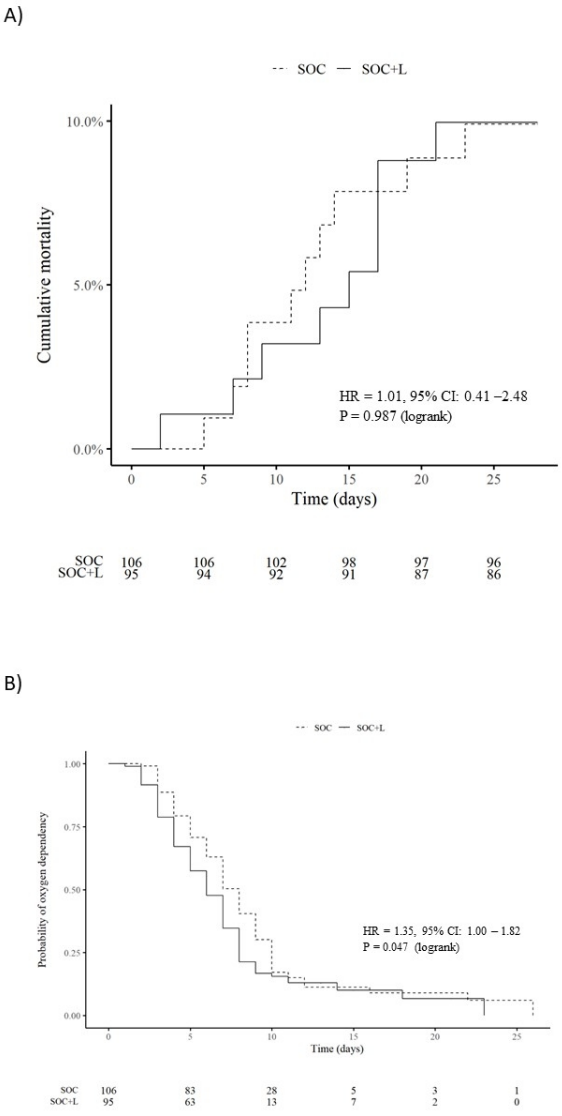
Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.
*= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.

190x338mm (96 x 96 DPI)



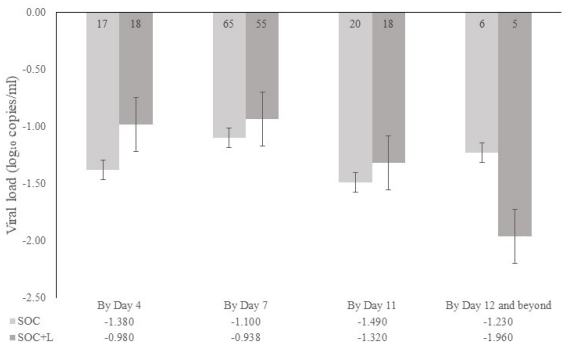
Patients who died were censored at the time their death occurred, while all surviving patients who did not reach TTCI criteria by day 28 were right censored at that point. Most of the patients were discharged within the first 10 days of admission.

338x190mm (96 x 96 DPI)



Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

190x338mm (96 x 96 DPI)



Mean changes in $\log_{10}$ viral load (copies/ml) from baseline. Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.

338x190mm (96 x 96 DPI)

Trial procedures	Screening	Day 0/1 (BL)	Daily	Day 3	Day 7	Day 11 +/- 1	Day 15 +/- 1	Day 28 +/-1	DC	Day 90 +/- 7
Confirmation of COVID Infection and severity	X									
Informed consent & Eligibility Assessment	X									
Demographics, Medical Hx, Cardiopulmonary Assessment (including ECG [1] & Echo [2])	X									
Concomitant medication		X	X					X [7]		X
Bloods – FBC, U&Es, LFT (AST [6] & ALT [6])		X	X [5]	X [6]					X	
– Clotting screen, Fibrinogen, D-Dimer, Ferritin		X	X [5]							
– Glucose		X	X [5]							
– Creatine Kinase, Troponin, BNP (NT-proBNP)		X	X [5]							
– Procalcitonin, CRP, LDH		X	X [5]	X		X				
– HIV		X								
– Cytokine profile		X		X		X			X [8]	
Pregnancy test (urine sample)		X								
Viral Load (nasopharyngeal swab)		X			X	X	X	X	X [8]	
Randomisation		X								
IMP dispense, loading (daily from Day 0/1 to 3) / maintenance dose (daily from Day 4 to 10) [3]		X								
Primary outcome assessment (TTCI)		X	X					X [7]		
Clinical Assessment, e.g. NEWS 2, body T°C*, vital signs, imaging**	X	X	X [5]							
*Blood and Urine cultures (in presence of fever)		X	X [5]							
**Urine for legionella and pneumococcal		X	X [5]							
Oxygenation assessment e.g. O_2 delivery method and level [SpO_2]		X	X [5]							
Arterial Blood Gas (ABG) – as available and where applicable		X	X [5]							
Serious Adverse Event(s) (SAE(s))/ Adverse Event(s) (AE(s)) [4]			X					X [7]		X [9]
Out-patient assessment (telephone call)								X [7]		X

Data collected and study time points

Key: BL – Baseline; IMP – Investigation Medicinal Product (trial treatment i.e. trial drug); SOC - Standard of Care; DC Discharge.

Notes: **1.** Check medical notes, if abnormal flag repeat imaging; **2.** Echo within 6 months to be used if no cardiac symptoms; **3.** Participant to take home IMP if DC'd; **4.** Participant to self-report events between DC to Day 90; **5.** Completed Daily, depending on clinical need and resources. If participant is DC'd early – record what is available as part of SOC; **6.** AST/ALT *must* be checked for treatment arm to determine maintenance dose; **7.** Participant DC'd called on Day 28 for Treatment Assessments, if not seen on-site; **8.** Cytokines/Viral Load to be collected if outside of scheduled collection. Day 11 is the last collection for Cytokines. on DC Medium (telephone call) and long term (by Sponsor) Treatment Assessments respectively.

Centre	Group	N	Corticosteroid	Anticoagulant	Antiviral	Antibiotic	Immunotherapy*	Hydroxychloroquine
UK	Intervention	30	100%	97%	69%	100%	14%	3%
	Control	36	100%	100%	81%	100%	3%	3%
India	Intervention	74	87%	76%	70%	76%	67%	93%
	Control	74	89%	84%	70%	88%	50%	94%
All centres	Intervention	104	95%	79%	56%	90%	23%	47%
	Control	110	96%	80%	56%	96%	19%	47%

Supplementary Table 1: **Standard of care therapy in the UK and India**

**The "Immunotherapy" includes tocilizumab, bevacizumab, interferon alpha and beta*

Adverse events categorised per MedDRA terminology		
	SOC+L (n = 104)	SOC (n = 110)
Patients with at least one reported AE, n (%)	56 (53.8%)	42 (38.2%)
All adverse events, n	121	91
Blood and lymphatic system disorder		
- DIC	1	4
- Lymph node enlargement	1	0
- Hilar lymphadenopathy	1	0
- Thrombocytopenia	4	4
- Neutropenia	0	1
- Splenomegaly	1	0
Cardiac Disorder		
- Acute coronary syndrome	2	1
- Aortic Valve disease	2	0
- Atrial fibrillation	2	5
- Chest pain	1	0
- Left ventricular systolic dysfunction	1	0
- Conduction disorder	0	1
- Infective endocarditis	1	0
- Tachycardia (sinus)	2	0
Endocrine disorder		
- Adrenal adenoma	1	0
Eye disorder		
- Dry eye	0	1
General disorders and administration site condition		
- Lethargy	1	0
Gastrointestinal disorders		
- Diarrhoea	1	1
- Gastritis	1	0
- Gastric haemorrhage	2	0

- Hiatus hernia	1	0
- Rectal haemorrhage	0	1
- Mucositis oral	1	0
- Dyspepsia	2	0
- Emesis	1	0
Hepatobiliary disorders		
- Acute liver dysfunction	1	1
- Cholelithiasis	2	0
- Hepatic granuloma	1	0
- Liver steatosis	1	0
Infection and infestations		
- Sepsis	0	1
Investigation		
- APTT prolonged	1	0
- ALP increased	1	0
- Bil increased	3	0
- Il-6 increased	1	0
- Leucocytosis	1	2
- ALT/AST increased	27	12
- Sgot increased	2	0
Metabolism and nutrition disorder		
- Hyperglycaemia	2	6
- Hyperkalaemia	0	1
- Hyponatraemia	1	1
- hypomagnesaemia	0	1
Musculoskeletal and connective tissue disorder		
- Discitis	1	0
Neoplasms		
- Lung cancer	1	0
Nervous system disorder		
- 6th nerve palsy	0	1

- Dysphasia	0	1
- Cognitive disturbances	1	0
- Cerebellar calcification	0	1
- Lower limb weakness	1	0
- Dysphagia	0	1
- Hemiparesis	0	1
- Headache	1	0
- Intracranial haemorrhage	0	1
Psychiatric disorders		
- Anxiety	2	1
- Delirium	0	2
Renal and urinary disorders		
- Acute kidney injury	5	5
- Haematuria	1	0
- Urinary urgency	1	0
Respiratory thoracic and mediastinal disorders		
- ARDS	1	0
- Wheezing	0	1
- Atelectasis	0	1
- Hypoxia	5	7
- Exacerbation of COPD	3	2
- Hoarseness	0	1
- Dyspnoea	4	3
- Pneumonitis	1	0
- Epistaxis	3	1
- Haemoptysis	0	1
- Respiratory failure	2	0
- Pneumothorax	0	1
- (subcutaneous emphysema)	0	1
Reproductive system and breast disorders		
- Endometrium thickening	1	0

Surgical and medical procedures		
- Aortic valve replacement	1	0
- Loop recorder implant	0	1
Vascular disorder		
- Aortic aneurysm	1	0
- Thromboembolic events		
o DVT	1	0
o Bilateral pedal vasculopathy	0	1
o Pulmonary embolism	1	1
- Hypertension	0	1
Death	9	10

Supplementary Table 2: All adverse events

		Baseline		Day 1 – 3/4		Day 4/5 – Discharge	
		UK	India	UK	India	UK	India
ALT							
SOC + L	**Median (IQR) U/L**	48 (30-60)		67 (36~87)		44 (36-71)	
		48 (32-71)	47 (29-59)	59 (37-94)	72 (34-86)	62(34-151)	42(35-56)
	1 - 2 x ULN (n)	44		50		17	
		12	32	9	41	4	13
	2 - 3 x ULN (n)	1		6		6	
		1	0	3	3	2	4
	> 3 x ULN (n)	1		8		5	
		1	0	3	5	4	1
	>ULN	14	32	15	49	10	18
SOC	**Median (IQR) U/L**	39 (26-56)		44 (34-65)		41 (35-52)	
		40 (27-59)	38 (25-54)	44 (31-63)	44 (36-67)	49 (33-63)	40 (35-52)
	1 - 2 x ULN (n)	33		29		24	
		13	20	8	21	7	17
	2 - 3 x ULN (n)	0		9		3	
		0	0	2	7	2	1
	>3 x ULN (n)	0		0		0	
		0	0	0	0	0	0
	>ULN	13	20	10	28	9	18
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.049**		**<0.001**		**0.633**	
AST							
SOC + L	**Median (IQR) U/L**	44 (30-54)		55(31-77)		41(27-50)	
		60 (42-102)	43(29-50)	58 (42-104)	53 (28-76)	54 (29-102)	40 (27-49)
	1 - 2 x ULN (n)	28		36		18	
		4	24	8	28	3	15
	2 - 3 x ULN (n)	2		9		1	
		2	0	2	7	1	0
	> 3 x ULN (n)	1		6		1	
		1	0	2	4	1	0
	>ULN	7	24	12	39	5	15
SOC	**Median (IQR) U/L**	39 (28-52)		39 (29-54)		37(27-47)	
		57 (34-75)	37(26-48)	45(38-55)	38(28-54)	45 (35-61)	37 (25-46)
	1 - 2 x ULN (n)	22		18		16	
		6	16	4	14	4	12
	2 - 3 x ULN (n)	2		5		0	
		2	0	0	5	0	0
	>3 x ULN (n)	0		1		1	
		0	0	0	1	1	0
	>ULN	8	16	4	20	5	12
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.3222**		**<0.001**		**0.318**	

Supplementary Table 3: Liver enzymes measurements

ULN: upper limits of normal (ALT: 10-49 U/L; AST 19 – 48U/L)). ALT: alanine transaminase. AST: aspartate transaminase; SOC + L: n = 30 UK, 74 India; SOC: n = 36 UK, 74 India

	Day 28					Day 90				
	SOC+L (n=81)		SOC (n=91)		p*	SOC+L (n=81)		SOC (n=91)		p*
	%	Median (IQR)	%	Median (IQR)		%	Median (IQR)	%	Median (IQR)	
Fatigue	29.6	5.00(2.75-8.00)	31.9	5.00(3.00-8.00)	0.751	22.2	5.00(3.25-7.00)	26.4	3.00(2.00-4.25)	NS
Cough	13.6	2.00(1.00-4.50)	18.7	2.00(1.00-4.00)	0.366	7.41	1.00(1.00-6.25)	8.79	2.50(1.00-3.50)	NS
Anxiety	24.7	3.50(2.00-7.25)	19.8	4.00(3.00-7.75)	0.438	21.0	3.00(1.00-5.00)	19.8	2.00(1.00-3.75)	NS
Chest pains	11.1	4.00(2.00-7.00)	8.79	4.00(2.50-5.00)	0.611	7.41	4.00(3.25-7.75)	6.59	3.00(1.25-7.75)	NS
Brain fog	14.8	5.00(3.75-7.25)	16.5	5.00(1.50-7.00)	0.764	14.8	3.50(1.75-5.00)	12.1	4.00(2.50-4.50)	NS
Breathlessness	40.7	2.00(1.00-6.00)	42.9	1.00(1.00-7.00)	0.779	22.2	5.00(1.00-7.00)	20.9	4.00(2.00-4.00)	NS
Sleep quality	48.2	2.00(1.00-5.00)	38.5	3.00(1.00-6.00)	0.200	34.6	2.00(1.00-4.25)	33.0	2.50(1.00-5.75)	NS
Palpitations	8.64	7.00(1.50-9.00)	5.49	1.00(1.00-7.00)	0.419	4.94	4.50(3.50-5.00)	3.30	1.00(1.00-4.00)	NS
Joint pain	32.1	3.00(1.00-4.75)	33.0	2.00(1.00-4.00)	0.903	22.2	3.50(2.00-7.00)	19.8	2.00(2.00-4.50)	NS
Myalgia	18.5	-	19.8		0.834	17.3		11.0		NS
Anosmia	6.17	-	11.0		0.264	2.47		-		-
Loss of taste	9.88	-	14.3		0.378	7.41		-		-
Depression	19.8	1.50(1.00-3.000	18.7	1.00(1.00-2.00)	0.859	11.1	1.00(1.00-1.00)	11.0	1.00(1.00-2.00)	NS
Loss of interest	12.4	2.50(1.25-3.00)	16.5	1.00(1.00-3.00)	0.442	9.88	1.00(1.00-1.25)	7.69	1.00(1.00-2.50)	NS
Dyspnoea; **Mild (1)** **Moderate (2-3)** **Severe (4-5)**	 59.3 29.6 11.1	1.00(1.00-2.00)	 47.3 39.6 13.2	2.00(1.00-2.00)	 0.155 0.173 0.678	 80.3 14.8 4.94	1.00(1.00-1.00)	 81.3 17.6 1.10	1.00(1.00-1.00)	NS

Supplementary Table 4: Long COVID symptoms at 28 and 90 days after randomisation

p; alpha value. Statistical significance was assumed at 0.05 alpha value. To best summarise the data, symptom scales (e.g. 0-10) for all symptoms (except dyspnoea, which was measured in different terms) were binarized, accepting any score above 0 as prevalence (%) of experiencing that symptom. To reflect magnitude of symptom severity, median (IQR) of individual symptom scores (except for dyspnoea, myalgia, anosmia and loss of taste) were taken excluding scores of 0. For dyspnoea, prevalence (%) of the symptom was determined as the proportion of patients scoring any relevant category (e.g., mild, moderate, severe), and median (IQR) of symptom severity included all score values. Between-group differences at each point of follow-up (day 28 and day 90) for all symptoms were evaluated using patient proportions from the binarized symptom scales via the chi-square test of differences. The Shapiro-wilk test was used to assess statistical normality. No analysis was enabled for any day reporting n ≤ 3 datapoints per group for statistical reliability

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5,6
	2b	Specific objectives or hypotheses	5,6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4,5,6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4,5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5,6,7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4,5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	NA

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	5,6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	5,6
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, page 7
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, page 7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	7
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	7, Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	5,6
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	5,6
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	5,6,7,8,9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2,13

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Leflunomide Treatment for Patients Hospitalised with COVID-19: DEFEAT-COVID Randomised Controlled Trial

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Keywords:	COVID-19, INFECTIOUS DISEASES, Respiratory infections < THORACIC MEDICINE

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Leflunomide Treatment for Patients Hospitalised with COVID-19: DEFEAT-COVID Randomised Controlled Trial

Authors:

Ines Kralj-Hans[1], Kuo Li[2], Adrian Wesek[2], Alexia Lamorgese[4], Fatima Omar[1], Kapila Ranasinghe[1], Megan McGee[1], Kieran Brack[1], Shiliang Li[5], Ritesh Aggarwal[6], Ajay Bulle[7], Aparna Kodre[8], Shashank Sharma[1], David Fluck[1], Isaac John[1], Pankaj Sharma[9], Jonathan Belsey[4], Ling Li[3], Sreenivasa Rao Kondapally Seshasai[10], *HongLin Li[5], *Nandor Marczin[2], *Zhong Chen[1], on behalf of DEFEAT-COVID Investigators

*Joint senior authors

Affiliations:

1. Ashford and St Peter's Hospital NHS Foundation Trust, UK
2. Faculty of Medicine, Imperial College London, UK
3. City University London, UK
4. JB Medical, UK
5. Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, China
6. Max Hospital, New Delhi, India
7. Meditrina institute of medical science, Nagpur, India
8. Noble Hospital, Pune, India
9. Institute of cardiovascular research, Royal Holloway, University of London, UK
10. St George's University Hospitals NHS Foundation Trust, UK

Corresponding author: Dr Ines Kralj-Hans, i.kralj-hans@nhs.net

Ashford and St Peter's Hospital NHS Foundation Trust, Guildford Street, Chertsey, KT16 0PZ, UK

Abstract

Objective: To evaluate the clinical efficacy and safety of leflunomide (L) added to the standard of care (SOC) treatment in COVID-19 patients hospitalised with moderate/critical clinical symptoms.

Design: Prospective, open-label, multicentre, stratified, randomised clinical trial.

Setting: Five hospitals in United Kingdom and India, from September 2020 to May 2021.

Participants: Adults with polymerase-chain-reaction (PCR) confirmed COVID-19 infection with moderate/critical symptoms within 15-days of onset.

Intervention: Leflunomide 100 mg/day (3-days) followed by 10-20 mg/day (7-days) added to standard care.

Primary outcomes: The time to clinical improvement (TTCI) defined as two-point reduction on a clinical status scale or live discharge prior to 28 days; safety profile measured by the incidence of adverse events (AE) within 28 days.

Results: Eligible patients (n=214; age 56.3±14.9 years; 33% female) were randomised to SOC+L (n=104) and SOC group (n=110), stratified according to their clinical risk profile. TTCI was 7 vs. 8 days in SOC+L vs. SOC group (HR 1.317; CI 0.980, 1.768; p=0.070). Incidence of serious adverse events was similar between the groups and none was attributed to leflunomide. In sensitivity analyses, excluding 10 patients not fulfilling the inclusion criteria and 3 who withdrew consent before leflunomide treatment, TTCI was 7 vs. 8 days (HR 1.416, CI 1.041, 1.935; p=0.028), indicating a trend in favour of the intervention group. All-cause mortality rate was similar between groups, 9/104 vs. 10/110. Duration of oxygen dependence was shorter in the SOC+L group being a median 6-days (IQR 4-8) compared to 7-days (IQR 5-10) in SOC group (p=0.047).

Conclusion: Leflunomide, added to the SOC treatment for COVID-19, was safe and well tolerated but had no major impact on clinical outcomes. It may shorten the time of oxygen dependence by one day and thereby improve TTCI /hospital discharge in moderately affected COVID-19 patients.

Trial registration

EUDRACT: CTA 21517/0004/001-0001 2020-004994-27

ClinicalTrials.gov: NCT05007678

Strengths and limitations

- International, prospective, randomised controlled study
- Repurposing a marketed drug with established safety profile and promising dual antiviral and immunomodulating medication based on strong drug discovery data.
- Study participants had milder COVID-19 disease than originally intended, thus eroding the power of the study
- Evolving standard of care therapy possibly diminished measurable benefit of leflunomide

Introduction

COVID-19 pandemic caused unprecedented strain on health care services around the world. It has affected almost 16 million people globally and caused over 6 million deaths so far.[1] Associated clinical syndromes include pneumonia, systemic inflammatory response and cardiovascular complications with high morbidity and mortality. Progressive deterioration is thought to be related to the kinetics of viral replication culminating in a surge of inflammatory mediator release, "cytokine storm".[2] Around 5-10% of infected patients experience severe or life-threatening symptoms with high mortality.[3]

Direct-acting and host-targeting antiviral treatments are the two approaches in treating viral infections. Host targeting antiviral treatments may have an advantage over direct antivirals as they enable the body to fight against a broad spectrum of viruses by simultaneously blocking viral replication and overcoming the potential of viral mutagenesis.[4] Anti-inflammatory medications have been shown to improve survival through dampening of the inappropriate immune response in susceptible patients.[5] This has led to the search for a drug with such therapeutic properties.

Leflunomide is a drug licenced to treat rheumatoid arthritis (RA).[6] It is widely available, cost-effective and can be easily administered both in the hospital and domestic settings. In preclinical models of cell and animal infection by SARS-CoV-2, leflunomide was shown to be a potent inhibitor of human dihydroorotate dehydrogenase (DHODH), an enzyme vital to viral replication in the host cell.[7,8,9] It has the potential advantage of not only targeting the virus infection but also suppressing the ensuing inflammatory response which may play a role in more progressive stages of infection leading to serious complications.

The DEFEAT-COVID study (Targeting **de** novo pyrimidine biosynthesis by le**f**lunomide for tr**eat**ment of **co**rona **vi**rus **d**isease 2019) tested whether leflunomide added to standard care was clinically effective and safe for COVID-19 moderate/severe symptoms.

Methods

Study design – This was a multicentre, international, open label, prospective, randomised controlled clinical trial set up at 5 hospitals (two in UK and three in India). The recruitment took place between September 2020 and May 2021, and was approved by all relevant ethics committees.

Participants - Patients aged 18 years and above presenting with moderate to critical symptoms of PCR-confirmed COVID-19 disease within 15 days of symptoms onset were recruited. Patients with respiratory compromise and blood oxygen saturation (SpO_2) <93% on room air detected on pulse oximeter were considered to fulfil the moderate infection criteria. Patients with respiratory failure, septic shock and/or multiple organ dysfunction/failure needing assisted ventilation were considered to be critically ill. Pregnant or breast-feeding women, individuals already receiving specific monoclonal antibody therapy or those with severe immunodeficiency syndrome and hypoalbuminaemia and patients with hypersensitivity to leflunomide or liver

enzymes aspartate transaminase (AST) / alanine transaminase (ALT) ≥ 2 x upper limits of normal (ULN) were excluded from the study. All participants gave written informed consent to a member of their clinical care team.

Randomisation – Consented participants were randomised by a member of the clinical care team to either the control arm (receiving standard of care treatment [SOC] alone) or the intervention arm (SOC treatment + leflunomide (SOC+L]) using a stratified block randomisation web-based algorithm. Patient admission data (age </≥ 70; co-morbidities; clinical status based on National Early Warning Score 2, NEWS2)[10] were used to stratify patients into 4 risk categories. Group 1: high/moderate comorbidity risk with NEWS2 score ≥5; Group 2: high/moderate comorbidity risk with NEWS2 score <5; Group 3: low comorbidity risk with NEWS2 score ≥5; and Group 4: low comorbidity risk with NEWS2 score <5.

Interventions - The definition of the SOC treatment for COVID-19 evolved nationally and internationally through the course of our study, with progressive evolution in the understanding of disease pathology and emerging treatment evidence. The SOC during the time of the study across all sites involved four main treatment domains: steroids, anticoagulation, antibiotics, and antiviral medications. The intervention group (SOC+L) received oral leflunomide at a loading dose of 100mg/day for three days and then 20mg/day for 7 days as a maintenance dose. The maintenance dose was reduced to 10mg/day if liver enzymes AST/ALT exceeded 2 x ULN. Leflunomide treatment was stopped early if AST/ATL exceeded 3 x ULN during the intervention. Study participants received additional COVID-19 therapies, including monoclonal antibodies, at the discretion of the direct care clinical team, even if leflunomide was initiated.

Study procedures - Patient related clinical/investigation data, treatment compliance, outcomes and adverse events (AE) were collected by the site investigators and recorded on the pre-specified daily electronic case report form (e-CRF, see Appendix). Adverse events (AE) were graded according to the Common Terminology Criteria for Adverse Events.[11] Blood samples were collected and processed for quantifying viral load (on days 1, 7, 11, 15, 28 or day of discharge) and for future inflammatory profiling (on days 1, 3 and 11). Liver enzymes were measured at baseline, on day 3 after the leflunomide loading and on discharge. Patient questionnaire was administered at 28- and 90-days after randomisation to monitor the persistence of symptoms possibly associated with long COVID syndrome.[12] SpO_2/FiO_2 data were monitored daily. The frequency of SpO_2 monitoring varied with FiO_2 administration. It is standard clinical practice that SpO_2 is monitored every 4 hours in a clinically stable patient. The frequency increases to continuous SpO_2 monitoring in a patient with oxygen requirement or ventilation support. Where multiple daily values were recorded we selected the SpO_2/FiO_2 ratio reflecting increased oxygen demand.

Blinding - Site investigator teams and direct clinical care teams were not blinded to the randomisation outcomes, but neither were provided information about the aggregate patient outcomes.

Outcomes - The primary outcome is the time (days) from randomisation to clinical improvement (TTCI) of two points on a seven-category clinical status scale or live discharge from hospital prior to 28 days.[13] The clinical status ordinal scale consisted of the following: 1 not hospitalised, resumption of normal activities; 2 not hospitalized, but unable to resume normal activities; 3 hospitalised, not requiring supplemental oxygen; 4 hospitalised, requiring supplemental oxygen; 5 hospitalised, requiring nasal high-flow oxygen (HFNC) therapy, non-invasive mechanical ventilation (NIV), or both; 6 hospitalized, requiring extracorporeal membrane oxygenation (ECMO), invasive mechanical ventilation (IMV), or both; and 7 death.

Safety profile of leflunomide in this group of patients was assessed from incidence rates of AE deemed to be serious and/or severe (≥Grade 3). Grading guidelines suggest 5 categories: 1 mild, asymptomatic or mild symptoms, clinical or diagnostic observations only, intervention not indicated; 2 moderate, minimal, local or non-invasive intervention indicated, limiting age-appropriate instrumental activities of daily livings (ADL); 3 severe, medically significant but not immediately life-threatening, hospitalization or prolongation of hospitalization indicated, disabling; limiting self-care ADL; 4 life-threatening consequences, urgent intervention indicate; 5 death related to AE. In addition, the incidences, and levels of liver transaminitis (ALT, AST) were assessed.

The main secondary outcomes were focused on overall (all-cause) mortality, and oxygen dependence (duration in days) assessed by S/F ratio (i.e. oxygen saturation detected by pulse oximeter [SPO_2] / supplemental oxygen concentration [FiO_2]) and impact on viral replication (viral load). Additional secondary outcomes included inflammatory targets such as CRP, lymphocyte counts, and selected cytokines (initially focussing on IL2, IL6, TNF-α). The concept of long COVID emerged during the study, so we used the data from our questionnaires at 28 and 90 days to comment on long COVID symptoms

Statistical analyses

Sample size calculation - The primary outcome measure was a time-to-event analysis based on an assessment of TTCI. Since our study protocol was conceived and developed during the initial peak of the global pandemic, the precise hazard ratio for major clinical outcomes related to this infection was largely unknown and, therefore, sample size calculation was based on the proportion of patients expected to meet the outcome criteria by 28 days.[14] Assuming $\alpha = 0.05$, $\beta = 0.20$ and allocation ratio = 1:1, the number of patients per treatment arm was estimated to be 74. We expected a 20% attrition rate, so the total number of patients required in the study was calculated to be 178, 89 patients in each arm.

Analysis population - The full analysis set was defined according to the intention to treat principle (ITT). All subjects randomised were included in the ITT analysis set for the primary outcome, regardless of whether they received any dose of their allocated treatment. This analysis set was used to summarize baseline patient characteristics and to carry out all efficacy and safety assessments. Subjects were analysed according to their randomised treatment allocation. We also present a modified intention to treat analysis for the primary and secondary outcomes, as a sensitivity analysis, to account for study participants who were randomised in error and those who withdrew consent prior to the intervention.

Primary outcomes - The TTCI data was estimated using Kaplan-Meier survival curves. Hazard ratio and 95% confidence intervals were estimated using Cox proportional hazards regression models. The primary analysis was

stratified by the randomisation strata: baseline risk indicators (age </≥ 70 years, co-morbidities) and NEWS2 score. Log rank test was used for comparing the Kaplan-Meir curves, hazard ratios and their confidence intervals for the significance of the treatment effect.

Secondary outcomes - Continuous secondary outcomes were evaluated for within-groups differences using the Mann-Whitney U or Wilcoxon rank tests, respectively, depending on the data distribution identified: parametric or non-parametric. Statistical normality was assessed using the Shapiro-Wilk method. Categorical outcomes were assessed for between-group differences using the chi-square method and expressed as %. For all outcomes, statistical significance was accepted at a 2-sided α of 0.05.

Adverse events - AEs were coded using MedDRA and assigned grades based on National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03.[11]

Public and patient involvement

Patient volunteers were consulted regarding the study design and materials to be provided to the potential participants (patient information sheet, consent forms, questionnaires). Two lay members were appointed to the Trial Steering Committee and provided input on the conduct of the study.

Results

Recruitment, Randomisation, Assignment of Therapy and Follow-up

Between September 2020 and May 2021, 214 patients were recruited to the study from 2 UK Hospitals (n=66, 31%; Ashford and St Peters' NHS Trust, Surrey; Kingston Hospital NHS Trust, London) and 3 Hospitals in India; (n=148, 69%; Max Hospital, Delhi; Meditrina Institute, Nagpur; Noble Hospital, Pune). Due to the wavering new COVID-19 infections, the UK recruitment came to a halt in February 2021 and patients at the three Indian sites were recruited in the remaining period. Of the 214 participating patients, 104 were randomised to the intervention (SOC+L) group and 110 to the control (SOC) group. In the SOC+L group, 3 patients withdrew study consent after randomisation, and did not receive leflunomide therapy. During the data cleaning process, 10 patients were flagged as not meeting the inclusion criteria (6 in SOC+L; 4 in SOC), as they did not have moderate COVID-19 symptoms at the time of randomisation. Daily clinical data were collected for all patients during hospitalisation and the patients were asked to complete follow-up questionnaires at 28- and 90-days after randomisation, as shown in Figure 1 (CONSORT diagram).

Baseline patient characteristics were similar between the SOC+L and SOC groups, summarised in Table 1.

Characteristics	SOC+L n=104	SOC n=110
Age, yrs, mean ± sd	55.2±14.7	56.4±15.2
BMI, kg/m^2, mean ± sd	27.3±5.1	27.7±5.6
Female gender at birth, %	28.8	37.3
Ethnicity, %		
South Asian	75	69
White	24	30
Arab	-	0.91
Comorbidities, %		
BMI ≥ 40 kg/m^2	2.9	4.6
Age ≥ 70 yrs	18.3	20
Chronic respiratory disease	8.7	15.5
Chronic cardiovascular disease (including hypertension)	38.5	39.1
Chronic renal disease	2.9	2.7
Diabetes	23.1	20.9
Immunosuppressive diseases	6.7	6.4
Others		
Malignant neoplasm	3.9	2.7
Chronic haematological disease	1	0.9
Chronic neurological disorder	10.6	3.7
Malnutrition	1	0.9
Smoking (present or past)	21.1	20
Symptom duration, day, median (IQR)	6 (4-8)	6 (5-8)
Time from admission, day, median (IQR)	2 (1-4	2 (1-3)
Non-invasive ventilation, %	4.8	7.3
Invasive ventilation, %	1	1.8
NEWS 2 score median (IQR)	6 (4-8)	5 (4-8)
CRP, mg/L, median (IQR)	28 (9-77)	32 (13-64)
Transaminase, >ULN, %		
ALT	44.7	31.7
AST	35.4	28.4
Stratification, %		
Group 1	12.5	14.5
Group 2	14.4	16.4
Group 3	48.1	46.4
Group 4	25	22.7

Table 1: Baseline patient characteristics at the time of randomization

BMI: Body mass index; ULN = upper limits of normal; Group 1: High/Moderate comorbidity risk with NEWS2 score ≥5; Group 2: High/Moderate comorbidity risk with NEWS2 score <5; Group 3: Low comorbidity risk with NEWS2 score ≥5; and Group 4: Low comorbidity risk with NEWS2 score <5. Creatinine ULN: 104umol/L; ALT ULN: 49 U/L; AST ULN: 48 U/L; Immunosuppressive disease: asplenia, rheumatological disorder.

Baseline characteristics were similar in both arms but there were significantly more patients with chronic neurological disorders in the SOC+L group. None of the patients with this condition had contraindication to non-invasive ventilation.

Treatment Assignment and Compliance

Full course of leflunomide therapy was completed by 81/104 patients (78%). Of the 19 patients (16 in UK, 3 in India) who did not complete treatment, 3 patients did not receive a single dose of leflunomide as they withdrew consent soon after randomization, 5 patients died prior to completion of the full course, 8 patients stopped leflunomide early when ALT/AST exceeded 3 x ULN laboratory reference range, 1 patient had tocilizumab introduced to replace leflunomide, 1 patient self-discharged early and 1 refused final two doses. Leflunomide treatment compliance appeared to be better in participants from Indian centres as 92% of them received the full dose of leflunomide compared to 52% of patients in the UK centres which was largely due to a higher incidence of liver enzyme transaminitis and mortality observed in the UK cohort.

There was no significant difference in the assignment of standard of care treatment between the SOC+L and SOC groups as shown in Figure 1. It included corticosteroids, anticoagulants, antibiotics, and antiviral therapies. Overall, steroid uptake was >95% in both treatment arms with different protocols used at participating study centres: dexamethasone 4 mg/day for 3 days; dexamethasone 6 mg/day for 7-10 days; methylprednisolone 80 mg/day for 7 days and methylprednisolone 120 mg/day for 5 days. However, there was no difference in the steroid treatment assigned between the control and the treatment groups. There were some differences in the proportions of patients receiving additional adjunct therapies such as hydroxychloroquine and immunotherapy (Supplementary table 1). Overall, hydroxychloroquine was prescribed to similar proportion of patients in the intervention and the control group (47%) but the proportions of patients receiving it in the UK was much smaller, 3% compared to 67% in India. A small number of patients received immunomodulating drugs such as interferon alpha and beta (n = 20 in India), tocilizumab and bevacizumab (n = 5 in the UK, n = 2 in India).

Primary Outcomes

Time to clinical improvement of 2 points on a clinical status scale/discharge before 28 days

In the ITT analyses (n = 214), SOC+L group did not have a significantly shorter TTCI than the SOC group within 28 days of randomisation; the median was 7.0 (IQR 7.0 - 8.0) days vs. 8.0 (IQR 7.0 - 9.0) days, respectively; with a hazard ratio (HR) of 1.32 (CI 0.98 -1.77), p = 0.070 (Figure 2).

In modified ITT population (n = 201) where 3 patients who withdrew consent after being randomised to the SOC+L group but never received leflunomide treatment and 10 patients who did not fulfil moderate COVID-19 symptoms at randomisation were excluded from analysis, the median TTCI was significantly shorter in the SOC+L group than SOC group by 1.0 day, median 7.0 days (IQR 7.0 -8.0) vs. 8.0 (IQR 7.0 – 9.0), respectively, with a HR of 1.42 (CI 1.04 – 1.94); p = 0.028.

Safety

Incidences of AE of all grades are summarized in Table 2.

Adverse events	SOC+L (n= 104)	SOC (n=110)
Adverse events (n) / Patients (n)	121 / 56	91/ 42
Grade 1 (Mild)	58 / 39	48 / 32
Grade 2 (Moderate)	23 / 13	17/9
Grade 3 (Severe)/ Grade 4 (Life threatening events)	31/15	16/9
Grade 5 (Deaths)	9/9	10/10

Table 2: Incidence of reported adverse events in both treatment arms.
The table shows the number of adverse events recorded in the study and the number of patients affected by at least one adverse event.

At least one AE was reported in 98/214 participants, and most of them were mild in severity. AEs of moderate grade were reported in 13/104 patients in SOC+L group and 9/110 patients in SOC group. Serious AEs (n=47) were reported in 15/104 patients in SOC+L groups and 9/110 in SOC group and 19 patients died (9 in SOC+L group, 10 in the SOC group). There was no significant difference in the incidence of AE reported between the two groups. No Serious AEs were attributed to leflunomide. A supplementary Table 2 lists all adverse events recorded in the study according to MedDRA terms.

Liver function

At baseline, more patients with greater than ULN levels of ALT and AST were randomized in the SOC+L group than the SOC group (ALT: 46 vs 33, p = 0.049; AST: 31 vs 24, p = 0.340). By Day 3/4, following the initial loading of leflunomide therapy in the SOC+L group, there was a significantly higher number of patients with greater than ULN level of ALT and AST in the SOC+L than the SOC group (64 vs 38, p <0.001; and 51 vs 24, p <0.001). By discharge, the difference in the number of patients with ALT and AST transaminitis between the SOC+L and SOC groups was no longer significant (28 vs 27, p = 0.633; and 20 vs 17, p = 0.318) (Supplementary table 3). Leflunomide therapy was terminated early if transaminase levels exceeded 3 x ULN. However, there were 5 patients in India who continued with leflunomide therapy at the discretion of the researcher and direct care team with close monitoring of their liver function. Interestingly, in this subset of patients, the transaminase levels improved despite continuation of therapy. There were no adverse events related to clinically significant liver injury due to leflunomide. AEs related to liver dysfunction were reported in 16/104 (15.4%) patients in SOC+L group, 7 were mild, 8 were moderate and 1 was severe. Of these, 10 were deemed possibly treatment related and leflunomide treatment was discontinued in 9 patients. Comparatively, in the control group, 6/110 (5.5%) patients had liver dysfunction related AE. Five of them were mild and 1 case was severe.

Secondary Outcomes

A modified intent to treat approach was used for data from 201 patients for all secondary outcomes. This included 95 patients in the SOC+L group and 106 patients in SOC group. For these analyses we excluded 3 patients in the SOC+L group who withdrew consent and never received leflunomide and 10 patients (6 SOC+L; 4 SOC) who

did not fulfil moderate COVID symptoms inclusion criterion (did not show respiratory compromise and blood oxygen saturation (SpO_2) <93% on room air).

Mortality

There was no difference in all-cause mortality within 28 days of randomization between the treatment arms as 9/95 (9.47%) of patients died in SOC+L group compared to 10/106 (9.43%) in SOC groups. The survival curves diverge in favour of the SOC+L group after 10 days of hospital treatment, but the curves converged again after 3 weeks (when majority of the patients have been discharged). All deaths were attributed to complications related to Covid-19 (Figure 3**,** panel A)

Oxygenation and assisted ventilation

Oxygen independence is defined by maintenance of SpO_2/FiO_2 Air ratio > 4.43. There was a difference in the median time the participants required to be completely weaned off oxygen therapy between groups; 6.0 (IQR 4.0 – 8-0) days in the SOC + L group vs. 7.0 (5.0 – 10.0) days in the SOC group, p = 0.047 (Figure 3, panel B)

Non-invasive ventilation was required for 14.4% of patients in SOC+L group vs. 16.4% in the SOC group. The duration of non-invasive ventilation was 6.0 (IQR 2.0-9.0) days in the SOC+L group compared to 4.5 days (IQR 2.3-6.8) in the SOC group. Similar proportion of patients required non-invasive ventilation at the time of study enrolment (4.8% in SOC+L group vs. 7.3% in SOC group, p= 0.45).
The proportion of patients admitted to level-2 Intensive Care Unit (ICU) was 8.7 % in the SOC+L group and 8.2% in the SOC group. The median time spent at ICU was 8.0 (IQR 5.0-10.0) days vs. 9.0 (IQR 5.0-13.0) days, respectively**.** Invasive ventilation was required for 3.9% of patients in the SOC+L group and 5.5% in the SOC group with median duration of 6 (IQR 4.8, 6.0) days vs. 7.0 (IQR 5.3 - 11.8) days, respectively. None of the between group comparisons were statistically significant. Patients recruited in India were significantly less likely to require invasive or non-invasive ventilation or be admitted to ICU compared to patients recruited in the UK (p<0.001).

Viral load

Quantitative SARS-COV-2 PCR measurements from nasopharyngeal swabs at baseline showed no difference in median $\log^{10}$ viral loads (copies/ml) between the two groups, SOC+L 4.68 (IQR 4.45-4.85) vs SOC 4.76 (IQR 4.48-4.92), p =027.. We clustered the serial samples to reflect the crucial time intervals during the hospital stay: time coinciding with finishing leflunomide loading dose (by Day 4), time to 75% patients being discharged from hospital (by Day 7), time to finishing leflunomide maintenance dose (by Day 11) and beyond (Figure 4). Viral loads were significantly reduced in both treatment arms. There was no significant difference in the overall rate of the viral load clearance between the two groups by Day 11 and beyond. Viral loads were significantly reduced in both treatment arms by Day 7, p<0.001; and by Day 11, p <0.030. The rate of viral load reduction between groups by Day 11 appeared to be similar.

Cytokines, CRP and lymphocytes

Cytokine levels were assessed separately for UK and Indian sites as two laboratories using different assays processed the samples. The median baseline levels of IL 2, IL 6 and TNF-α levels (UK: IL2 0.43 [IQR 0.30-0.62] pg/ml; IL6 6.2 [IQR 2.9-9.7] pg/ml; TNF-α 10.1 [IQR 7.8 -13.5] pg/ml; India: IL2 4.3 [IQR 2.8-5.8] pg/ml; IL6 12.6 [IQR 6.5-43.1] pg/ml; TNF-α 6.1 [IQR 4.9-7.1] pg/ml) were not significantly raised from normal reference ranges and were not different between treatment groups in both countries. The cytokine levels were reduced during hospitalisation, though the clinical significance of these changes within the normal range is uncertain. There was no significant difference in the trends observed between treatment arms.

The median baseline levels of CRP were similar in both groups, 28, (IQR 8-71) in SOC+L vs. 34 (14-71) mg/L in SOC. By one week of treatment, there was similar levels of reduction between groups.

The median baseline lymphocytes levels were lower than normal reference range in both groups (0.99 [IQR 0.6-1.6) $x10^9$/L in SOC+L vs 0.95 (IQR 0.6-1.6) $x10^9$/L in SOC. By 1 week of treatment, levels rose to normal range in both groups. There was no significant difference in the trends observed between groups.

28- and 90-days follow up

At 28 days, 59/81 patients (71.2%) in the SOC+L group and 60/91 patients (65.9%) in the SOC group experienced at least 1 of 9 common long-COVID symptoms (fatigue, cough, anxiety, chest pain, brain fog, breathlessness, disturbed sleep, palpitations, joint pain); with sleep quality (48.2% vs. 38.5%), breathlessness (40.7 vs 42.9%), joint pain (32.1 vs. 33%), fatigue (29.6 vs, 31.9%), and anxiety (24.7 vs. 19.8%) being the commonest symptoms experienced (Supplementary table 4). At 90 days, there was a reduction in overall prevalence of symptoms as 42/81 patients (51.2%) in the SOC+L group and 37/91 (40.7%) patients in the SOC group and any of the residual symptoms were of reduced severity. There was no significant difference in these outcomes between the treatment arms.

Myalgia symptoms were comparably reduced between the 2 groups at 90 days. Anosmia and loss of taste were still reported by 2 and 7 patients, respectively, in the SOC+L group, but none in the SOC group.

At 28 days, 41.5% patients in the SOC + L group and 52.8% in SOC group, reported being moderately to severely dyspnoeic (Grade 4: stops for breath after walking 100m; Grade 5: too breathless to leave the house or breathless when dressing). These proportions were further reduced at 90 days, to 22% in the SOC+L group compared to 19.8% in SOC group. These differences were not significant in between group comparisons.

Mental health issues were highlighted by reports of feeling depressed and losing interest in doing things. Comparable proportions of patients in the SOC+L group and SOC group reported those problems at 28 days (17.9% vs 16.0%; 11.6% vs 14.2%, respectively) which were further reduced in both groups at 90 days (11.6% vs 9.4%; 9.5% vs 6.6%, respectively).

At 28 days participants in the SOC+L group scored their current health as being 80±25% of the usual which increased to 89±17% at 90 days. In the SOC group the scores were similar, 82 ± 23% and 90 ± 17% at 28 and 90 days respectively.

Discussion

This study is the first prospective, multicentre, randomised, controlled clinical trial investigating the clinical efficacy and safety of leflunomide in treating acute COVID-19 infection. The study showed that a course of leflunomide (3 days of 100 mg/day loading dose followed by 7 days of 20 mg/day maintenance dose) added to the standard care treatment (steroids, anticoagulants, antibiotics and antiviral therapy), did not influence the primary outcome of the trial and the acute clinical outcomes at 28 days, or the prevalence of long-COVID symptoms at 28 and 90 days. However, participants who received leflunomide as an adjunct therapy were weaned off oxygen earlier, which translated to reduced hospital stay by one day. The medication appeared to be safe and well tolerated with no severe adverse events attributable to it. A small proportion of patients in our study were still burdened by COVID-19 related symptoms 90 days after randomisation.

This multicentre trial advances the evidence base on the impact of leflunomide, a repurposed rheumatoid arthritis medication, on COVID-19 infection. Leflunomide was a potentially attractive therapeutic choice from early preclinical and clinical experience reported from hospitals in Wuhan, China. Dihydroorotate dihydrogenase (DHODH), located in the inner mitochondrial membrane is a rate-limiting enzyme in de novo pyrimidine biosynthesis. In virus-infected cells, a large intracellular nucleotide pool is consumed by rapid viral replication. RNA viruses need unique UMP but not TMP in their genomes. As UMP is the particular nucleoside produced by DHODH, RNA viruses are sensitive to reduced DHODH activity. Preclinical models of cell and animal infection by SARS-CoV-2 demonstrated that leflunomide attenuates viral genome replication, suppresses inflammatory response and the release of pro-inflammatory cytokines and chemokines.[7,8,9] Early reports from China advocated major clinical benefits in patients treated with leflunomide both in terms of less severe outcomes and duration of infection.[15,16,17] While the current study did not reproduce these overall benefits in the ITT analysis regarding the primary outcome, it confirmed some positive effects in those patients who received the trial intervention (in modified intent to treat analysis).

Our results are likely explained by the changing landscape and evolution of the routine COVID-19 treatment protocols in the standard arm of the study and the resultant severity of the COVID-19 outcomes in general. The initial phase of the COVID-19 pandemic was characterised by severe respiratory and systemic infections and poor outcomes due to the development of acute respiratory distress syndrome, multi-organ failure and eventual death.[18,19] Contrary to this early experience with COVID-19 management, the in-hospital mortality in the present study was much lower, less than 10% in both groups. The majority of patients in both treatment arms improved during hospitalisation and were discharged within a week of admission. Inclusion of prognostically significant COVID-19 therapies in both pharmacological and non-pharmacological standard of care treatments undoubtedly contributed to a reduction in severe complications and better overall outcomes. During patient recruitment of the

current trial, various therapies have been introduced including more than 95% percent of the study population received steroids as standard of care.

Theoretical considerations suggest that leflunomide may effectively inhibit viral replication. The initial pilot study during the early outbreak of COVID-19 in Wuhan, China reported reduced viral shedding time following leflunomide treatment during acute infection compared to the standard of care therapy.[15] Similarly, viral shedding duration was reduced in leflunomide treated patients who remained qPCR positive 1 month after the initial infection.[16] Our study addressed the viral load reduction at pre-specified time points. Values of viral load were reduced over time but there was no difference between the treatment arms. Both methodological considerations and the inclusion of comprehensive pharmacological treatment regimens in the SOC could explain these differences. For instance, corticosteroid therapy was absent in the early study from Wuhan, but the later study refers to the use of hydroxychloroquine, interferon-alpha and antiviral medications as part of acute standard of care therapy.[15,16] However, our results are in line with other reports from China which showed that duration of viral shedding was not affected by leflunomide added to nebulised interferon alpha therapy for treating long-term positive COVID-19 after 4 weeks of in-hospital treatment.[17] Interestingly a third of these patients received corticosteroid therapy during the initial acute treatment. [16,17]

Beyond the issue of therapeutic efficacy and viral load, our study confirms overall safety of leflunomide in COVID-19 infection. The safety profile of leflunomide is well established in the treatment of RA. [6] Leflunomide, repurposed for the COVID-19 treatment, was well tolerated since no serious adverse events were attributed to it. Similar findings were reported in other studies.[15,16,20] Mild transaminitis following long-term leflunomide use in the RA population is recognised, and usually resolves after medication is terminated. The mechanism is likely to be modulation of interleukins which may hinder the protection of hepatocytes from injury rather than direct toxicity.[21] There were comparable incidences of transaminitis in both treatment arms in our study. However, more patients in the UK cohort had raised liver function tests leading to modification or termination of leflunomide treatment. This may be accounted for by the difference in the severity of COVID-19 disease and spectrum of co-morbidities between UK and Indian participants rather than genetic polymorphism in drug metabolism. Overall, the proposed leflunomide regimen was well tolerated.

One of the motivations of the current trial employing leflunomide was to benefit from the anti-inflammatory effects of this drug. In this context, hydrocortisone has been demonstrated as an effective therapy in severe COVID-19 infections and recent trials also demonstrated the benefit of tocilizumab, a selective IL-6 inhibitor and a different disease modifying rheumatoid arthritis medication. However, such finding is not universal as the benefit of tocilizumab is mainly demonstrated in critically to moderately ill patients.[22,23] A recent meta-analysis showed that the benefit of IL-6 receptor antagonist was encountered only in patients who were also treated with glucocorticoids.[24] This is in keeping with observations that a broader spectrum of pro-inflammatory cytokines, macrophages and T cell response have all been documented in severely ill patients demonstrating the role of a more complex inflammatory response. It is exactly this broader inflammatory reaction that could be targeted by leflunomide as its effect on cytokines is not restricted to IL 6 and it may also have an impact on activated T cell response.[8,9,25] Such phenomena might contribute to the benefits of reduced oxygen dependence in patients who

have received leflunomide treatment. However, it is conceivable that the full benefit of such anti-inflammatory effect may be more pronounced in severely ill patients, but this population was underrepresented in our trial and the (inadvertent) inclusion of patients with milder symptoms may have led to some attrition of statistical power in our study. A more detailed analysis of the cytokine and metabolic profiles of our trial population is underway to clarify these important issues.

Another important consideration when discussing the potential benefits of leflunomide is the mutation ability of the SARS-CoV-2 virus.[26] So far, the mutations observed in different strains worldwide have largely been confined to the part of the spike protein affecting the virus's ability of cell entry as opposed to a region targeted by neutralising antibodies. However, the possibility of mutations in different regions cannot be excluded. Targeting the host's pyrimidine biosynthesis pathway by leflunomide, rather than using drugs with direct antiviral action, remains an advantage offering protection against a broader spectrum of viruses and potentially overcoming resistance. Indeed, DHODH inhibitors such as leflunomide has shown broad-spectrum antiviral effects against various RNA viruses in cell models.[7] Leflunomide may therefore be considered a viable pharmacological treatment for COVID-19 patients given it is well tolerated, safe, economical, and widely available. Its clinical effectiveness measured against recognised selective IL-6 inhibitors in the more severely/critically ill patients needs to be further explored as leflunomide may be the preferred option in countries where other immunomodulating agents, such as Talizumab, may not be practical or widely available.

Limitations

The present study has several limitations. In order to balance the needs of the trial with clinical care and to minimise disruption to already overstretched clinical resources during COVID-19 pandemic, we chose to adopt an open label study design. This design may have affected the data collection and clinical management of the patients and potentially introduced a bias. However, it also allowed early detection of significant adverse events and a potential outcome benefit. This was an important consideration when testing an off-label use of a medication in COVID-19, a disease with high morbidity and mortality.

The study was set out to recruit more severely and critically affected patients in a single country. However, due to recruitment restrictions because of national prioritization of critically ill patients to only a few studies together with scarcity of NHS resources during the pandemic, the study was extended abroad, ultimately recruiting less affected patients with heterogeneous clinical profiles. Although patient characteristics and medications received as part of SOC did not differ between the randomised arms, the more heterogeneous population, milder COVID-19 disease, and more effective standard of care treatments most likely impacted on the hypothesised effect size and the ability of finding a difference in our recruited sample. Finally, the COVID-19 restrictions affected our protocolised laboratory investigations, such as the serial viral load and comprehensive inflammatory profiling. Nevertheless, studies focusing on the more severely affected participants are underway and will be the subject of a separate submission.

Conclusion

Leflunomide had no major impact on the clinical outcomes when administered together with the currently established but evolving therapies in moderately affected COVID-19 patients. It may shorten duration of oxygen

dependence thereby affecting the TTCI and hospital discharge. Transaminitis associated with leflunomide therapy did not lead to excess adverse events compared to the control group and may have arisen in part due to the severity of clinical infection. Further studies are needed to investigate the potential benefits of leflunomide in the critically ill patients and the biological mechanisms involved.

Ethics statements

All patients taking part in the study signed written informed consent form once the study was ethically approved by relevant bodies in England (South Central - Berkshire Research Ethics Committee, Bristol REC Centre, reference number 20/SC/0264) and India (Max HealthCare Ethics Committee, reference number RS/MSSSH/GMHRCCMS/MHEC/CCM/20-23; Meditrina Institute Ethics Committee, reference number ECR/605/Inst/MH/2014/RR; Noble Hospital Institutional Ethics Committee, reference number NHIEC/FEB/2021/238).

Data availability statement

The anonymized data may be available upon request following approval from the Trial Management Group and the Sponsor.

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Data Monitoring Committee: David Morgan (Chair), Lajos Szentgyorgyi, Yee Cheah

Trial Steering Committee: Brendan Madden (Chair), Patrick Yong, Sreenivasa Rao Kondapally Seshasai, Matt Mendes, Janice Rodrigues-Mendes, Rod Hughes, Sharanpal Jeetle, Subash Somalanka
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Meditrina Institute, Nagpur, India: Ajay Bulle
Noble Hospital, Pune, India: Aparna Kodre

Authors' contributions

All designated authors meet all four ICMJE criteria for authorship. The funders and the sponsor of the study had no role in the analyses or the interpretation of the results.

ZC, NM, HLL, SRKS, LL, JB, SL, IJ, DF and PS made substantial contribution to the conception and design of the study. SS, AK, AB, RA, KB, MM, KR, FO, KL, AL, SRKS, JB, HLL, NM, IKH and ZC made substantial contribution to the acquisition, analysis and interpretation of the data for the study. All authors contributed to the drafting, revision and final approval of the manuscript. ZC, NM and LL are responsible for the overall content.

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/disclosure-of-interest/ and declare no competing interests.

Dissemination

A summary of the results will be disseminated to the participants by principal investigators at each trial site via a newsletter.

Licencing Statement

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References

1. https://COVID19.who.int/
2. Chen YL, Quach TTT, COVID-19 cytokine storm syndrome: a threshold concept. Lancet Microbe. 2021 Feb;2(2):e49-e50.doi: 10.1016/S2666-5247(20)30223-8.Epub 2021 Feb 2
3. Gavriatopoulou M, Ntanasis-Stathopoulos I, Korompoki E, Fotiou D, Migkou M, Tzanninis IG, Psaltopoulou T, Kastritis E, Terpos E, Dimopoulos MA. Emerging treatment strategies for COVID-19 infection. Clin Exp Med. 2021 May;21(2):167-179. doi: 10.1007/s10238-020-00671-y. Epub 2020 Oct 30. PMID: 33128197; PMCID: PMC759894
4. Dwek, Raymond A et al. "Targeting glycosylation as a therapeutic approach." Nature reviews. Drug discovery vol. 1,1 (2002): 65-75. doi:10.1038/nrd708
5. Angus, Derek C et al. "The REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-acquired Pneumonia) Study. Rationale and Design." Annals of the American Thoracic Society vol. 17,7 (2020): 879-891. doi:10.1513/AnnalsATS.202003-192SD
6. Alfaro-Lara, et al. Systematic review and meta-analysis of the efficacy and safety of leflunomide and methotrexate in the treatment of rheumatoid arthritis. Reumatol Clin. 2019 May - Jun;15(3):133-1
7. Xiong R, Zhang L, Li S, Sun Y, Ding M, Wang Y, Zhao Y, Wu Y, Shang W, Jiang X, Shan J, Shen Z, Tong Y, Xu L, Chen Y, Liu Y, Zou G, Lavillete D, Zhao Z, Wang R, Zhu L, Xiao G, Lan K, Li H, Xu K. Novel and potent inhibitors targeting DHODH are broad-spectrum antivirals against RNA viruses including newly-emerged coronavirus SARS-CoV-2. Protein Cell. 2020 Oct;11(10):723-739. doi: 10.1007/s13238-020-00768-w. Epub 2020 Aug 4. Erratum in: Protein Cell. 2021 Jan;12(1):76-80. Erratum in: Protein Cell. 2020 Nov 9;: PMID: 32754890; PMCID: PMC7402641.
8. Yao, H. W.; Li, J.; Chen, J. Q.; Xu, S. Y. A 771726, the active metabolite of leflunomide, inhibits TNF-α and IL-1 from Kupffer cells. Inflammation 2004, 28 (2), 97-103.
9. Li, L. et al. The effects of teriflunomide on lymphocyte subpopulations in human peripheral blood mononuclear cells in vitro. J. Neuroimmunol. 2013, 265 (1-2), 82-90.
10. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2
11. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_60
12. Mandal S, Barnett J, Brill SE ARC Study Group, et al. 'Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax 2021;76:396-398.
13. http://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus/en/).
14. Machin D, Campbell M, Fayers, P, Pinol A (1997) Sample Size Tables for Clinical Studies. Second Ed. Blackwell Science IBSN 0-86542-870-0 p. 176-177
15. Hu, Ke et al. "A Small-Scale Medication of Leflunomide as a Treatment of COVID-19 in an Open-Label Blank-Controlled Clinical Trial." Virologica Sinica vol. 35,6 (2020): 725-733. doi:10.1007/s12250-020-00258-7
16. Wang, Qiang et al. "Efficacy and Safety of Leflunomide for Refractory COVID-19: A Pilot Study." Frontiers in pharmacology vol. 12 581833. 2 Jul. 2021, doi:10.3389/fphar.2021.581833

17. Mengmei Wang, Yang Zhao, Weihua Hu, Dong Zhao, Yunting Zhang, Tao Wang, Zhishui Zheng, Xiaochen Li, Shaolin Zeng, Zhenlian Liu. Treatment of Coronavirus Disease 2019 Patients With Prolonged Postsymptomatic Viral Shedding With Leflunomide: A Single-center Randomized Controlled Clinical Trial. Clinical Infectious Diseases, Volume 73, Issue 11, 1 December 2021, Pages e4012–e4019, https://doi.org/10.1093/cid/ciaa1417
18. Huang, Chaolin et al. "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China." Lancet (London, England) vol. 395,10223 (2020): 497-506. doi:10.1016/S0140-6736(20)30183-5
19. ISARIC Clinical Characterisation Group. "COVID-19 symptoms at hospital admission vary with age and sex: ISARIC multinational study." medRxiv : the preprint server for health sciences 2020.10.26.20219519. 19 Nov. 2020, doi:10.1101/2020.10.26.20219519. Preprint.
20. Chakravarty EF, Sanchez-Yamamoto D, Bush TM. The use of disease modifying antirheumatic drugs in women with rheumatoid arthritis of childbearing age: a survey of practice pattern and pregnancy outcomes. J Rheumatol. 2003;30:241–6.
21. Atsushi Ogata, Yasuhiro Kato, Shinji Higa, Kazuyuki Yoshizaki, IL-6 inhibitor for the treatment of rheumatoid arthritis: A comprehensive review, Modern Rheumatology, Volume 29, Issue 2, 4 March 2019, Pages 258–267, https://doi.org/10.1080/14397595.2018.1546357
22. REMAP-CAP Investigators et al. "Interleukin-6 Receptor Antagonists in Critically Ill Patients with COVID-19." The New England journal of medicine vol. 384,16 (2021): 1491-1502. doi:10.1056/NEJMoa2100433
23. Abani, O., Abbas, A., Abbas, F., Abbas, M., Abbasi, S., Abbass, H., et al. (2021). Tocilizumab in Patients Admitted to Hospital with COVID-19 (RECOVERY): a Randomised, Controlled, Open-Label, Platform Trial. The Lancet 397, 1637–1645. doi:10.1016/S0140-6736(21)00676-0
24. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group et al. "Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis." JAMA vol. 324,13 (2020): 1330-1341. doi:10.1001/jama.2020.17023
25. Kraan MC, Smeets TJM, van Loon MJ, et al Differential effects of leflunomide and methotrexate on cytokine production in rheumatoid arthritis Annals of the Rheumatic Diseases 2004;63:1056-1061.
26. Wang, Shihang et al. "Molecular evolutionary characteristics of SARS-CoV-2 emerging in the United States." Journal of medical virology vol. 94,1 (2022): 310-317. doi:10.1002/jmv.27331

Figure legends:

Figure 1: Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.

**= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.*

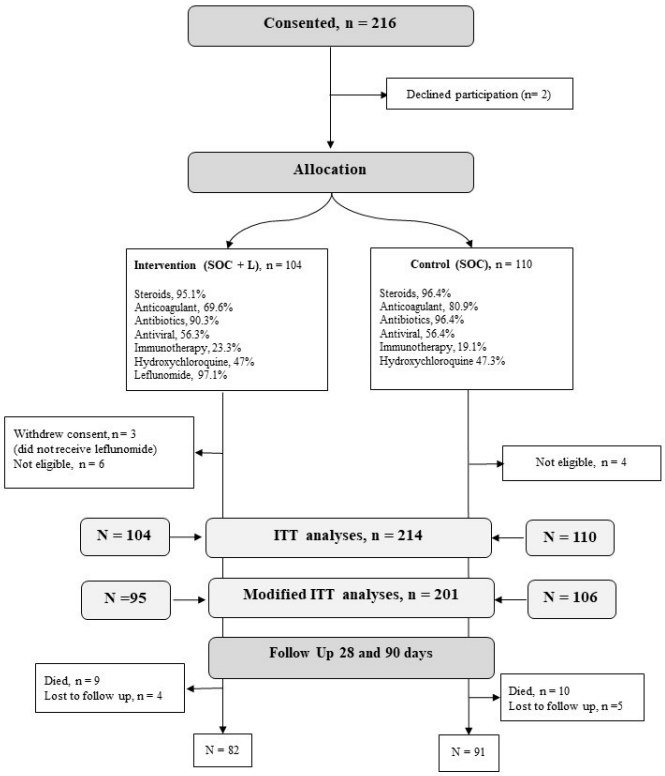
Figure 2: Time to clinical improvement of 2 points on a clinical status scale or discharge prior 28 day in a stratified ITT analysis (primary outcome).

Patients who died were censored at the time their death occurred, while all surviving patients who did not reach TTCI criteria by day 28 were right censored at that point. Most of the patients were discharged within the first 10 days of admission.

Figure 3: Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

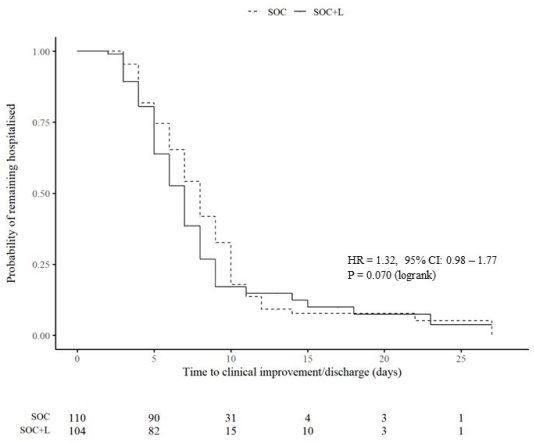
Figure 4: Mean changes in log^{10} viral load (copies/ml) from baseline.

Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.



Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.
*= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.

190x338mm (96 x 96 DPI)



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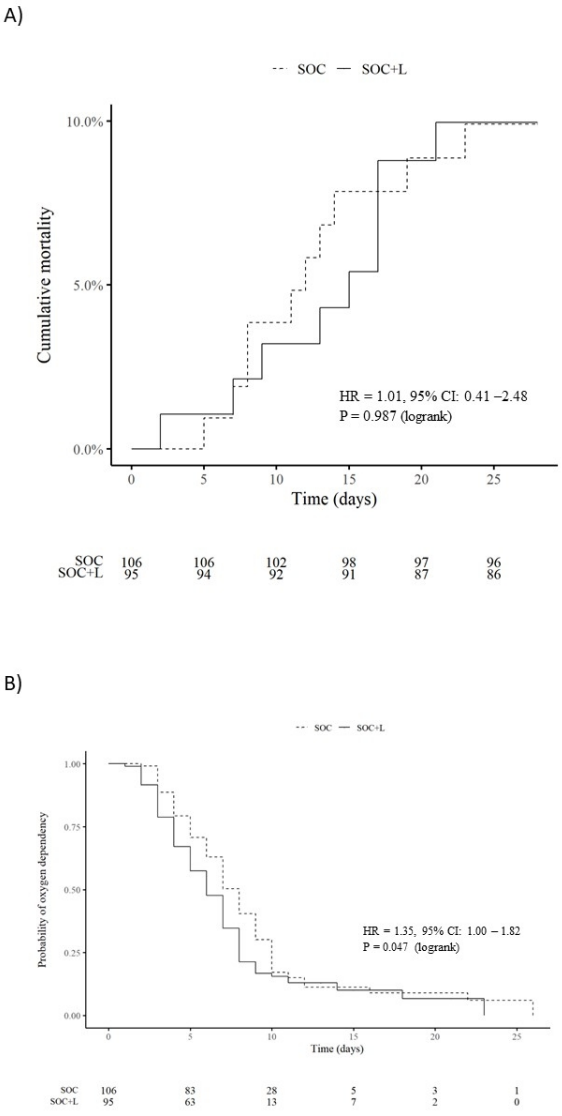
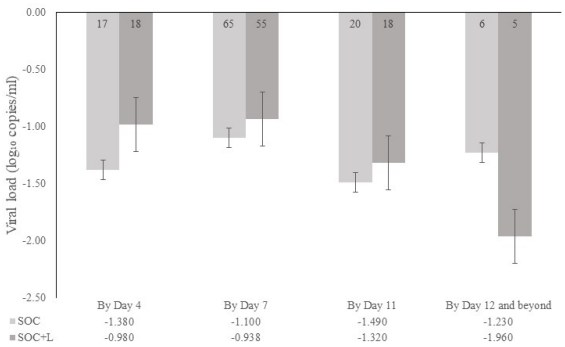
338x190mm (96 x 96 DPI)



Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

190x338mm (96 x 96 DPI)



Mean changes in $\log_{10}$ viral load (copies/ml) from baseline. Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.

338x190mm (96 x 96 DPI)

Trial procedures	Screening	Day 0/1 (BL)	Daily	Day 3	Day 7	Day 11 +/- 1	Day 15 +/- 1	Day 28 +/-1	DC	Day 90 +/- 7
Confirmation of COVID Infection and severity	X									
Informed consent & Eligibility Assessment	X									
Demographics, Medical Hx, Cardiopulmonary Assessment (including ECG [1] & Echo [2])	X									
Concomitant medication		X	X					X [7]		X
Bloods – FBC, U&Es, LFT (AST [6] & ALT [6])		X	X [5]	X [6]					X	
– Clotting screen, Fibrinogen, D-Dimer, Ferritin		X	X [5]							
– Glucose		X	X [5]							
– Creatine Kinase, Troponin, BNP (NT-proBNP)		X	X [5]							
– Procalcitonin, CRP, LDH		X	X [5]	X		X				
– HIV		X								
– Cytokine profile		X		X		X			X [8]	
Pregnancy test (urine sample)		X								
Viral Load (nasopharyngeal swab)		X			X	X	X	X	X [8]	
Randomisation		X								
IMP dispense, loading (daily from Day 0/1 to 3) / maintenance dose (daily from Day 4 to 10) [3]		X								
Primary outcome assessment (TTCI)		X	X					X [7]		
Clinical Assessment, e.g. NEWS 2, body T°C*, vital signs, imaging**	X	X	X [5]							
*Blood and Urine cultures (in presence of fever)		X	X [5]							
**Urine for legionella and pneumococcal		X	X [5]							
Oxygenation assessment e.g. O_2 delivery method and level [SpO_2]		X	X [5]							
Arterial Blood Gas (ABG) – as available and where applicable		X	X [5]							
Serious Adverse Event(s) (SAE(s))/ Adverse Event(s) (AE(s)) [4]			X					X [7]		X [9]
Out-patient assessment (telephone call)								X [7]		X

Data collected and study time points

Key: BL – Baseline; IMP – Investigation Medicinal Product (trial treatment i.e. trial drug); SOC - Standard of Care; DC Discharge.

Notes: **1.** Check medical notes, if abnormal flag repeat imaging; **2.** Echo within 6 months to be used if no cardiac symptoms; **3.** Participant to take home IMP if DC'd; **4.** Participant to self-report events between DC to Day 90; **5.** Completed Daily, depending on clinical need and resources. If participant is DC'd early – record what is available as part of SOC; **6.** AST/ALT *must* be checked for treatment arm to determine maintenance dose; **7.** Participant DC'd called on Day 28 for Treatment Assessments, if not seen on-site; **8.** Cytokines/Viral Load to be collected if outside of scheduled collection. Day 11 is the last collection for Cytokines. on DC Medium (telephone call) and long term (by Sponsor) Treatment Assessments respectively.

Centre	Group	N	Corticosteroid	Anticoagulant	Antiviral	Antibiotic	Immunotherapy*	Hydroxychloroquine
UK	Intervention	30	100%	97%	69%	100%	14%	3%
	Control	36	100%	100%	81%	100%	3%	3%
India	Intervention	74	87%	76%	70%	76%	67%	93%
	Control	74	89%	84%	70%	88%	50%	94%
All centres	Intervention	104	95%	79%	56%	90%	23%	47%
	Control	110	96%	80%	56%	96%	19%	47%

Supplementary Table 1: **Standard of care therapy in the UK and India**

**The "Immunotherapy" includes tocilizumab, bevacizumab, interferon alpha and beta*

Adverse events categorised per MedDRA terminology		
	SOC+L (n = 104)	SOC (n = 110)
Patients with at least one reported AE, n (%)	56 (53.8%)	42 (38.2%)
All adverse events, n	121	91
Blood and lymphatic system disorder		
- DIC	1	4
- Lymph node enlargement	1	0
- Hilar lymphadenopathy	1	0
- Thrombocytopenia	4	4
- Neutropenia	0	1
- Splenomegaly	1	0
Cardiac Disorder		
- Acute coronary syndrome	2	1
- Aortic Valve disease	2	0
- Atrial fibrillation	2	5
- Chest pain	1	0
- Left ventricular systolic dysfunction	1	0
- Conduction disorder	0	1
- Infective endocarditis	1	0
- Tachycardia (sinus)	2	0
Endocrine disorder		
- Adrenal adenoma	1	0
Eye disorder		
- Dry eye	0	1
General disorders and administration site condition		
- Lethargy	1	0
Gastrointestinal disorders		
- Diarrhoea	1	1
- Gastritis	1	0
- Gastric haemorrhage	2	0

- Hiatus hernia	1	0
- Rectal haemorrhage	0	1
- Mucositis oral	1	0
- Dyspepsia	2	0
- Emesis	1	0
Hepatobiliary disorders		
- Acute liver dysfunction	1	1
- Cholelithiasis	2	0
- Hepatic granuloma	1	0
- Liver steatosis	1	0
Infection and infestations		
- Sepsis	0	1
Investigation		
- APTT prolonged	1	0
- ALP increased	1	0
- Bil increased	3	0
- Il-6 increased	1	0
- Leucocytosis	1	2
- ALT/AST increased	27	12
- Sgot increased	2	0
Metabolism and nutrition disorder		
- Hyperglycaemia	2	6
- Hyperkalaemia	0	1
- Hyponatraemia	1	1
- hypomagnesaemia	0	1
Musculoskeletal and connective tissue disorder		
- Discitis	1	0
Neoplasms		
- Lung cancer	1	0
Nervous system disorder		
- 6th nerve palsy	0	1

- Dysphasia	0	1
- Cognitive disturbances	1	0
- Cerebellar calcification	0	1
- Lower limb weakness	1	0
- Dysphagia	0	1
- Hemiparesis	0	1
- Headache	1	0
- Intracranial haemorrhage	0	1
Psychiatric disorders		
- Anxiety	2	1
- Delirium	0	2
Renal and urinary disorders		
- Acute kidney injury	5	5
- Haematuria	1	0
- Urinary urgency	1	0
Respiratory thoracic and mediastinal disorders		
- ARDS	1	0
- Wheezing	0	1
- Atelectasis	0	1
- Hypoxia	5	7
- Exacerbation of COPD	3	2
- Hoarseness	0	1
- Dyspnoea	4	3
- Pneumonitis	1	0
- Epistaxis	3	1
- Haemoptysis	0	1
- Respiratory failure	2	0
- Pneumothorax	0	1
- (subcutaneous emphysema)	0	1
Reproductive system and breast disorders		
- Endometrium thickening	1	0

Surgical and medical procedures		
- Aortic valve replacement	1	0
- Loop recorder implant	0	1
Vascular disorder		
- Aortic aneurysm	1	0
- Thromboembolic events		
o DVT	1	0
o Bilateral pedal vasculopathy	0	1
o Pulmonary embolism	1	1
- Hypertension	0	1
Death	9	10

Supplementary Table 2: All adverse events

		Baseline		Day 1 – 3/4		Day 4/5 – Discharge	
		UK	India	UK	India	UK	India
ALT							
SOC + L	**Median (IQR) U/L**	48 (30-60)		67 (36~87)		44 (36-71)	
		48 (32-71)	47 (29-59)	59 (37-94)	72 (34-86)	62(34-151)	42(35-56)
	1 - 2 x ULN (n)	44		50		17	
		12	32	9	41	4	13
	2 - 3 x ULN (n)	1		6		6	
		1	0	3	3	2	4
	> 3 x ULN (n)	1		8		5	
		1	0	3	5	4	1
	>ULN	14	32	15	49	10	18
SOC	**Median (IQR) U/L**	39 (26-56)		44 (34-65)		41 (35-52)	
		40 (27-59)	38 (25-54)	44 (31-63)	44 (36-67)	49 (33-63)	40 (35-52)
	1 - 2 x ULN (n)	33		29		24	
		13	20	8	21	7	17
	2 - 3 x ULN (n)	0		9		3	
		0	0	2	7	2	1
	>3 x ULN (n)	0		0		0	
		0	0	0	0	0	0
	>ULN	13	20	10	28	9	18
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.049**		**<0.001**		**0.633**	
AST							
SOC + L	**Median (IQR) U/L**	44 (30-54)		55(31-77)		41(27-50)	
		60 (42-102)	43(29-50)	58 (42-104)	53 (28-76)	54 (29-102)	40 (27-49)
	1 - 2 x ULN (n)	28		36		18	
		4	24	8	28	3	15
	2 - 3 x ULN (n)	2		9		1	
		2	0	2	7	1	0
	> 3 x ULN (n)	1		6		1	
		1	0	2	4	1	0
	>ULN	7	24	12	39	5	15
SOC	**Median (IQR) U/L**	39 (28-52)		39 (29-54)		37(27-47)	
		57 (34-75)	37(26-48)	45(38-55)	38(28-54)	45 (35-61)	37 (25-46)
	1 - 2 x ULN (n)	22		18		16	
		6	16	4	14	4	12
	2 - 3 x ULN (n)	2		5		0	
		2	0	0	5	0	0
	>3 x ULN (n)	0		1		1	
		0	0	0	1	1	0
	>ULN	8	16	4	20	5	12
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.3222**		**<0.001**		**0.318**	

Supplementary Table 3: Liver enzymes measurements

ULN: upper limits of normal (ALT: 10-49 U/L; AST 19 – 48U/L)). ALT: alanine transaminase. AST: aspartate transaminase; SOC + L: n = 30 UK, 74 India; SOC: n = 36 UK, 74 India

	Day 28					Day 90				
	SOC+L (n=81)		SOC (n=91)		p*	SOC+L (n=81)		SOC (n=91)		p*
	%	Median (IQR)	%	Median (IQR)		%	Median (IQR)	%	Median (IQR)	
Fatigue	29.6	5.00(2.75-8.00)	31.9	5.00(3.00-8.00)	0.751	22.2	5.00(3.25-7.00)	26.4	3.00(2.00-4.25)	NS
Cough	13.6	2.00(1.00-4.50)	18.7	2.00(1.00-4.00)	0.366	7.41	1.00(1.00-6.25)	8.79	2.50(1.00-3.50)	NS
Anxiety	24.7	3.50(2.00-7.25)	19.8	4.00(3.00-7.75)	0.438	21.0	3.00(1.00-5.00)	19.8	2.00(1.00-3.75)	NS
Chest pains	11.1	4.00(2.00-7.00)	8.79	4.00(2.50-5.00)	0.611	7.41	4.00(3.25-7.75)	6.59	3.00(1.25-7.75)	NS
Brain fog	14.8	5.00(3.75-7.25)	16.5	5.00(1.50-7.00)	0.764	14.8	3.50(1.75-5.00)	12.1	4.00(2.50-4.50)	NS
Breathlessness	40.7	2.00(1.00-6.00)	42.9	1.00(1.00-7.00)	0.779	22.2	5.00(1.00-7.00)	20.9	4.00(2.00-4.00)	NS
Sleep quality	48.2	2.00(1.00-5.00)	38.5	3.00(1.00-6.00)	0.200	34.6	2.00(1.00-4.25)	33.0	2.50(1.00-5.75)	NS
Palpitations	8.64	7.00(1.50-9.00)	5.49	1.00(1.00-7.00)	0.419	4.94	4.50(3.50-5.00)	3.30	1.00(1.00-4.00)	NS
Joint pain	32.1	3.00(1.00-4.75)	33.0	2.00(1.00-4.00)	0.903	22.2	3.50(2.00-7.00)	19.8	2.00(2.00-4.50)	NS
Myalgia	18.5	-	19.8		0.834	17.3		11.0		NS
Anosmia	6.17	-	11.0		0.264	2.47		-		-
Loss of taste	9.88	-	14.3		0.378	7.41		-		-
Depression	19.8	1.50(1.00-3.000	18.7	1.00(1.00-2.00)	0.859	11.1	1.00(1.00-1.00)	11.0	1.00(1.00-2.00)	NS
Loss of interest	12.4	2.50(1.25-3.00)	16.5	1.00(1.00-3.00)	0.442	9.88	1.00(1.00-1.25)	7.69	1.00(1.00-2.50)	NS
Dyspnoea; **Mild (1)** **Moderate (2-3)** **Severe (4-5)**	 59.3 29.6 11.1	1.00(1.00-2.00)	 47.3 39.6 13.2	2.00(1.00-2.00)	 0.155 0.173 0.678	 80.3 14.8 4.94	1.00(1.00-1.00)	 81.3 17.6 1.10	1.00(1.00-1.00)	NS

Supplementary Table 4: Long COVID symptoms at 28 and 90 days after randomisation

p; alpha value. Statistical significance was assumed at 0.05 alpha value. To best summarise the data, symptom scales (e.g. 0-10) for all symptoms (except dyspnoea, which was measured in different terms) were binarized, accepting any score above 0 as prevalence (%) of experiencing that symptom. To reflect magnitude of symptom severity, median (IQR) of individual symptom scores (except for dyspnoea, myalgia, anosmia and loss of taste) were taken excluding scores of 0. For dyspnoea, prevalence (%) of the symptom was determined as the proportion of patients scoring any relevant category (e.g., mild, moderate, severe), and median (IQR) of symptom severity included all score values. Between-group differences at each point of follow-up (day 28 and day 90) for all symptoms were evaluated using patient proportions from the binarized symptom scales via the chi-square test of differences. The Shapiro-wilk test was used to assess statistical normality. No analysis was enabled for any day reporting n ≤ 3 datapoints per group for statistical reliability

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5,6
	2b	Specific objectives or hypotheses	5,6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4,5,6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4,5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5,6,7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4,5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	NA

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	5,6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	5,6
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, page 7
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, page 7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	7
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	7, Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	5,6
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	5,6
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	5,6,7,8,9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2,13

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Leflunomide Treatment for Patients Hospitalised with COVID-19: DEFEAT-COVID Randomised Controlled Trial

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Leflunomide Treatment for Patients Hospitalised with COVID-19: DEFEAT-COVID Randomised Controlled Trial

Authors:

Ines Kralj-Hans[1], Kuo Li[2], Adrian Wesek[2], Alexia Lamorgese[4], Fatima Omar[1], Kapila Ranasinghe[1], Megan McGee[1], Kieran Brack[1], Shiliang Li[5], Ritesh Aggarwal[6], Ajay Bulle[7], Aparna Kodre[8], Shashank Sharma[1], David Fluck[1], Isaac John[1], Pankaj Sharma[9], Jonathan Belsey[4], Ling Li[3], Sreenivasa Rao Kondapally Seshasai[10], *HongLin Li[5], *Nandor Marczin[2], *Zhong Chen[1], on behalf of DEFEAT-COVID Investigators

*Joint senior authors

Affiliations:

1. Ashford and St Peter's Hospital NHS Foundation Trust, UK
2. Faculty of Medicine, Imperial College London, UK
3. City University London, UK
4. JB Medical, UK
5. Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, China
6. Max Hospital, New Delhi, India
7. Meditrina institute of medical science, Nagpur, India
8. Noble Hospital, Pune, India
9. Institute of cardiovascular research, Royal Holloway, University of London, UK
10. St George's University Hospitals NHS Foundation Trust, UK

Corresponding author: Dr Ines Kralj-Hans, i.kralj-hans@nhs.net

Ashford and St Peter's Hospital NHS Foundation Trust, Guildford Street, Chertsey, KT16 0PZ, UK

Abstract

Objective: To evaluate the clinical efficacy and safety of leflunomide (L) added to the standard of care (SOC) treatment in COVID-19 patients hospitalised with moderate/critical clinical symptoms.

Design: Prospective, open-label, multicentre, stratified, randomised clinical trial.

Setting: Five hospitals in United Kingdom and India, from September 2020 to May 2021.

Participants: Adults with polymerase-chain-reaction (PCR) confirmed COVID-19 infection with moderate/critical symptoms within 15-days of onset.

Intervention: Leflunomide 100 mg/day (3-days) followed by 10-20 mg/day (7-days) added to standard care.

Primary outcomes: The time to clinical improvement (TTCI) defined as two-point reduction on a clinical status scale or live discharge prior to 28 days; safety profile measured by the incidence of adverse events (AE) within 28 days.

Results: Eligible patients (n=214; age 56.3±14.9 years; 33% female) were randomised to SOC+L (n=104) and SOC group (n=110), stratified according to their clinical risk profile. TTCI was 7 vs. 8 days in SOC+L vs. SOC group (HR 1.317; CI 0.980, 1.768; p=0.070). Incidence of serious adverse events was similar between the groups and none was attributed to leflunomide. In sensitivity analyses, excluding 10 patients not fulfilling the inclusion criteria and 3 who withdrew consent before leflunomide treatment, TTCI was 7 vs. 8 days (HR 1.416, CI 1.041, 1.935; p=0.028), indicating a trend in favour of the intervention group. All-cause mortality rate was similar between groups, 9/104 vs. 10/110. Duration of oxygen dependence was shorter in the SOC+L group being a median 6-days (IQR 4-8) compared to 7-days (IQR 5-10) in SOC group (p=0.047).

Conclusion: Leflunomide, added to the SOC treatment for COVID-19, was safe and well tolerated but had no major impact on clinical outcomes. It may shorten the time of oxygen dependence by one day and thereby improve TTCI /hospital discharge in moderately affected COVID-19 patients.

Trial registration

EUDRACT: CTA 21517/0004/001-0001 2020-004994-27

ClinicalTrials.gov: NCT05007678

Strengths and limitations

- International, prospective, randomised controlled study
- Repurposing a marketed drug with established safety profile and promising dual antiviral and immunomodulating medication based on strong drug discovery data.
- Study participants had milder COVID-19 disease than originally intended, thus eroding the power of the study
- Evolving standard of care therapy possibly diminished measurable benefit of leflunomide

Introduction

COVID-19 pandemic caused unprecedented strain on health care services around the world. It has affected almost 16 million people globally and caused over 6 million deaths so far.[1] Associated clinical syndromes include pneumonia, systemic inflammatory response and cardiovascular complications with high morbidity and mortality. Progressive deterioration is thought to be related to the kinetics of viral replication culminating in a surge of inflammatory mediator release, "cytokine storm".[2] Around 5-10% of infected patients experience severe or life-threatening symptoms with high mortality.[3]

Direct-acting and host-targeting antiviral treatments are the two approaches in treating viral infections. Host targeting antiviral treatments may have an advantage over direct antivirals as they enable the body to fight against a broad spectrum of viruses by simultaneously blocking viral replication and overcoming the potential of viral mutagenesis.[4] Anti-inflammatory medications have been shown to improve survival through dampening of the inappropriate immune response in susceptible patients.[5] This has led to the search for a drug with such therapeutic properties.

Leflunomide is a drug licenced to treat rheumatoid arthritis (RA).[6] It is widely available, cost-effective and can be easily administered both in the hospital and domestic settings. In preclinical models of cell and animal infection by SARS-CoV-2, leflunomide was shown to be a potent inhibitor of human dihydroorotate dehydrogenase (DHODH), an enzyme vital to viral replication in the host cell.[7,8,9] It has the potential advantage of not only targeting the virus infection but also suppressing the ensuing inflammatory response which may play a role in more progressive stages of infection leading to serious complications.

The DEFEAT-COVID study (Targeting **de** novo pyrimidine biosynthesis by le**f**lunomide for tr**eat**ment of **co**rona **vi**rus **d**isease 2019) tested whether leflunomide added to standard care was clinically effective and safe for COVID-19 moderate/severe symptoms.

Methods

Study design – This was a multicentre, international, open label, prospective, randomised controlled clinical trial set up at 5 hospitals (two in UK and three in India). The recruitment took place between September 2020 and May 2021, and was approved by all relevant ethics committees.

Participants - Patients aged 18 years and above presenting with moderate to critical symptoms of PCR-confirmed COVID-19 disease within 15 days of symptoms onset were recruited. Patients with respiratory compromise and blood oxygen saturation (SpO_2) <93% on room air detected on pulse oximeter were considered to fulfil the moderate infection criteria. Patients with respiratory failure, septic shock and/or multiple organ dysfunction/failure needing assisted ventilation were considered to be critically ill. Pregnant or breast-feeding women, individuals already receiving specific monoclonal antibody therapy or those with severe immunodeficiency syndrome and hypoalbuminaemia and patients with hypersensitivity to leflunomide or liver

enzymes aspartate transaminase (AST) / alanine transaminase (ALT) ≥ 2 x upper limits of normal (ULN) were excluded from the study. All participants gave written informed consent to a member of their clinical care team.

Randomisation – Consented participants were randomised by a member of the clinical care team to either the control arm (receiving standard of care treatment [SOC] alone) or the intervention arm (SOC treatment + leflunomide (SOC+L]) using a stratified block randomisation web-based algorithm. Patient admission data (age </≥ 70; co-morbidities; clinical status based on National Early Warning Score 2, NEWS2)[10] were used to stratify patients into 4 risk categories. Group 1: high/moderate comorbidity risk with NEWS2 score ≥5; Group 2: high/moderate comorbidity risk with NEWS2 score <5; Group 3: low comorbidity risk with NEWS2 score ≥5; and Group 4: low comorbidity risk with NEWS2 score <5.

Interventions - The definition of the SOC treatment for COVID-19 evolved nationally and internationally through the course of our study, with progressive evolution in the understanding of disease pathology and emerging treatment evidence. The SOC during the time of the study across all sites involved four main treatment domains: steroids, anticoagulation, antibiotics, and antiviral medications. The intervention group (SOC+L) received oral leflunomide at a loading dose of 100mg/day for three days and then 20mg/day for 7 days as a maintenance dose. The maintenance dose was reduced to 10mg/day if liver enzymes AST/ALT exceeded 2 x ULN. Leflunomide treatment was stopped early if AST/ATL exceeded 3 x ULN during the intervention. Study participants received additional COVID-19 therapies, including monoclonal antibodies, at the discretion of the direct care clinical team, even if leflunomide was initiated.

Study procedures - Patient related clinical/investigation data, treatment compliance, outcomes and adverse events (AE) were collected by the site investigators and recorded on the pre-specified daily electronic case report form (e-CRF, see Appendix). Adverse events (AE) were graded according to the Common Terminology Criteria for Adverse Events.[11] Blood samples were collected and processed for quantifying viral load (on days 1, 7, 11, 15, 28 or day of discharge) and for future inflammatory profiling (on days 1, 3 and 11). Liver enzymes were measured at baseline, on day 3 after the leflunomide loading and on discharge. Patient questionnaire was administered at 28- and 90-days after randomisation to monitor the persistence of symptoms possibly associated with long COVID syndrome.[12] SpO_2/FiO_2 data were monitored daily. The frequency of SpO_2 monitoring varied with FiO_2 administration. It is standard clinical practice that SpO_2 is monitored every 4 hours in a clinically stable patient. The frequency increases to continuous SpO_2 monitoring in a patient with oxygen requirement or ventilation support. Where multiple daily values were recorded we selected the SpO_2/FiO_2 ratio reflecting increased oxygen demand.

Blinding - Site investigator teams and direct clinical care teams were not blinded to the randomisation outcomes, but neither were provided information about the aggregate patient outcomes.

Outcomes - The primary outcome is the time (days) from randomisation to clinical improvement (TTCI) of two points on a seven-category clinical status scale or live discharge from hospital prior to 28 days.[13] The clinical status ordinal scale consisted of the following: 1 not hospitalised, resumption of normal activities; 2 not hospitalized, but unable to resume normal activities; 3 hospitalised, not requiring supplemental oxygen; 4 hospitalised, requiring supplemental oxygen; 5 hospitalised, requiring nasal high-flow oxygen (HFNC) therapy, non-invasive mechanical ventilation (NIV), or both; 6 hospitalized, requiring extracorporeal membrane oxygenation (ECMO), invasive mechanical ventilation (IMV), or both; and 7 death.

Safety profile of leflunomide in this group of patients was assessed from incidence rates of AE deemed to be serious and/or severe (≥Grade 3). Grading guidelines suggest 5 categories: 1 mild, asymptomatic or mild symptoms, clinical or diagnostic observations only, intervention not indicated; 2 moderate, minimal, local or non-invasive intervention indicated, limiting age-appropriate instrumental activities of daily livings (ADL); 3 severe, medically significant but not immediately life-threatening, hospitalization or prolongation of hospitalization indicated, disabling; limiting self-care ADL; 4 life-threatening consequences, urgent intervention indicate; 5 death related to AE. In addition, the incidences, and levels of liver transaminitis (ALT, AST) were assessed.

The main secondary outcomes were focused on overall (all-cause) mortality, and oxygen dependence (duration in days) assessed by S/F ratio (i.e. oxygen saturation detected by pulse oximeter [SPO_2] / supplemental oxygen concentration [FiO_2]) and impact on viral replication (viral load). Additional secondary outcomes included inflammatory targets such as CRP, lymphocyte counts, and selected cytokines (initially focussing on IL2, IL6, TNF-α). The concept of long COVID emerged during the study, so we used the data from our questionnaires at 28 and 90 days to comment on long COVID symptoms

Statistical analyses

Sample size calculation - The primary outcome measure was a time-to-event analysis based on an assessment of TTCI. Since our study protocol was conceived and developed during the initial peak of the global pandemic, the precise hazard ratio for major clinical outcomes related to this infection was largely unknown and, therefore, sample size calculation was based on the proportion of patients expected to meet the outcome criteria by 28 days.[14] Assuming $\alpha = 0.05$, $\beta = 0.20$ and allocation ratio = 1:1, the number of patients per treatment arm was estimated to be 74. We expected a 20% attrition rate, so the total number of patients required in the study was calculated to be 178, 89 patients in each arm.

Analysis population - The full analysis set was defined according to the intention to treat principle (ITT). All subjects randomised were included in the ITT analysis set for the primary outcome, regardless of whether they received any dose of their allocated treatment. This analysis set was used to summarize baseline patient characteristics and to carry out all efficacy and safety assessments. Subjects were analysed according to their randomised treatment allocation. We also present a modified intention to treat analysis for the primary and secondary outcomes, as a sensitivity analysis, to account for study participants who were randomised in error and those who withdrew consent prior to the intervention.

Primary outcomes - The TTCI data was estimated using Kaplan-Meier survival curves. Hazard ratio and 95% confidence intervals were estimated using Cox proportional hazards regression models. The primary analysis was

stratified by the randomisation strata: baseline risk indicators (age </≥ 70 years, co-morbidities) and NEWS2 score. Log rank test was used for comparing the Kaplan-Meir curves, hazard ratios and their confidence intervals for the significance of the treatment effect.

Secondary outcomes - Continuous secondary outcomes were evaluated for within-groups differences using the Mann-Whitney U or Wilcoxon rank tests, respectively, depending on the data distribution identified: parametric or non-parametric. Statistical normality was assessed using the Shapiro-Wilk method. Categorical outcomes were assessed for between-group differences using the chi-square method and expressed as %. For all outcomes, statistical significance was accepted at a 2-sided α of 0.05.

Adverse events - AEs were coded using MedDRA and assigned grades based on National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03.[11]

Public and patient involvement

Patient volunteers were consulted regarding the study design and materials to be provided to the potential participants (patient information sheet, consent forms, questionnaires). Two lay members were appointed to the Trial Steering Committee and provided input on the conduct of the study.

Results

Recruitment, Randomisation, Assignment of Therapy and Follow-up

Between September 2020 and May 2021, 214 patients were recruited to the study from 2 UK Hospitals (n=66, 31%; Ashford and St Peters' NHS Trust, Surrey; Kingston Hospital NHS Trust, London) and 3 Hospitals in India; (n=148, 69%; Max Hospital, Delhi; Meditrina Institute, Nagpur; Noble Hospital, Pune). Due to the wavering new COVID-19 infections, the UK recruitment came to a halt in February 2021 and patients at the three Indian sites were recruited in the remaining period. Of the 214 participating patients, 104 were randomised to the intervention (SOC+L) group and 110 to the control (SOC) group. In the SOC+L group, 3 patients withdrew study consent after randomisation, and did not receive leflunomide therapy. During the data cleaning process, 10 patients were flagged as not meeting the inclusion criteria (6 in SOC+L; 4 in SOC), as they did not have moderate COVID-19 symptoms at the time of randomisation. Daily clinical data were collected for all patients during hospitalisation and the patients were asked to complete follow-up questionnaires at 28- and 90-days after randomisation, as shown in Figure 1 (CONSORT diagram).

Baseline patient characteristics were similar between the SOC+L and SOC groups, summarised in Table 1.

Characteristics	SOC+L n=104	SOC n=110
Age, yrs, mean ± sd	55.2±14.7	56.4±15.2
BMI, kg/m^2, mean ± sd	27.3±5.1	27.7±5.6
Female gender at birth, %	28.8	37.3
Ethnicity, %		
South Asian	75	69
White	24	30
Arab	-	0.91
Comorbidities, %		
BMI ≥ 40 kg/m^2	2.9	4.6
Age ≥ 70 yrs	18.3	20
Chronic respiratory disease	8.7	15.5
Chronic cardiovascular disease (including hypertension)	38.5	39.1
Chronic renal disease	2.9	2.7
Diabetes	23.1	20.9
Immunosuppressive diseases	6.7	6.4
Others		
Malignant neoplasm	3.9	2.7
Chronic haematological disease	1	0.9
Chronic neurological disorder	10.6	3.7
Malnutrition	1	0.9
Smoking (present or past)	21.1	20
Symptom duration, day, median (IQR)	6 (4-8)	6 (5-8)
Time from admission, day, median (IQR)	2 (1-4	2 (1-3)
Non-invasive ventilation, %	4.8	7.3
Invasive ventilation, %	1	1.8
NEWS 2 score median (IQR)	6 (4-8)	5 (4-8)
CRP, mg/L, median (IQR)	28 (9-77)	32 (13-64)
Transaminase, >ULN, %		
ALT	44.7	31.7
AST	35.4	28.4
Stratification, %		
Group 1	12.5	14.5
Group 2	14.4	16.4
Group 3	48.1	46.4
Group 4	25	22.7

Table 1: Baseline patient characteristics at the time of randomization

BMI: Body mass index; ULN = upper limits of normal; Group 1: High/Moderate comorbidity risk with NEWS2 score ≥5; Group 2: High/Moderate comorbidity risk with NEWS2 score <5; Group 3: Low comorbidity risk with NEWS2 score ≥5; and Group 4: Low comorbidity risk with NEWS2 score <5. Creatinine ULN: 104umol/L; ALT ULN: 49 U/L; AST ULN: 48 U/L; Immunosuppressive disease: asplenia, rheumatological disorder.

Baseline characteristics were similar in both arms but there were significantly more patients with chronic neurological disorders in the SOC+L group. None of the patients with this condition had contraindication to non-invasive ventilation.

Treatment Assignment and Compliance

Full course of leflunomide therapy was completed by 81/104 patients (78%). Of the 19 patients (16 in UK, 3 in India) who did not complete treatment, 3 patients did not receive a single dose of leflunomide as they withdrew consent soon after randomization, 5 patients died prior to completion of the full course, 8 patients stopped leflunomide early when ALT/AST exceeded 3 x ULN laboratory reference range, 1 patient had tocilizumab introduced to replace leflunomide, 1 patient self-discharged early and 1 refused final two doses. Leflunomide treatment compliance appeared to be better in participants from Indian centres as 92% of them received the full dose of leflunomide compared to 52% of patients in the UK centres which was largely due to a higher incidence of liver enzyme transaminitis and mortality observed in the UK cohort.

There was no significant difference in the assignment of standard of care treatment between the SOC+L and SOC groups as shown in Figure 1. It included corticosteroids, anticoagulants, antibiotics, and antiviral therapies. Overall, steroid uptake was >95% in both treatment arms with different protocols used at participating study centres: dexamethasone 4 mg/day for 3 days; dexamethasone 6 mg/day for 7-10 days; methylprednisolone 80 mg/day for 7 days and methylprednisolone 120 mg/day for 5 days. However, there was no difference in the steroid treatment assigned between the control and the treatment groups. There were some differences in the proportions of patients receiving additional adjunct therapies such as hydroxychloroquine and immunotherapy (Supplementary table 1). Overall, hydroxychloroquine was prescribed to similar proportion of patients in the intervention and the control group (47%) but the proportions of patients receiving it in the UK was much smaller, 3% compared to 67% in India. A small number of patients received immunomodulating drugs such as interferon alpha and beta (n = 20 in India), tocilizumab and bevacizumab (n = 5 in the UK, n = 2 in India).

Primary Outcomes

Time to clinical improvement of 2 points on a clinical status scale/discharge before 28 days

In the ITT analyses (n = 214), SOC+L group did not have a significantly shorter TTCI than the SOC group within 28 days of randomisation; the median was 7.0 (IQR 7.0 - 8.0) days vs. 8.0 (IQR 7.0 - 9.0) days, respectively; with a hazard ratio (HR) of 1.32 (CI 0.98 -1.77), p = 0.070 (Figure 2).

In modified ITT population (n = 201) where 3 patients who withdrew consent after being randomised to the SOC+L group but never received leflunomide treatment and 10 patients who did not fulfil moderate COVID-19 symptoms at randomisation were excluded from analysis, the median TTCI was significantly shorter in the SOC+L group than SOC group by 1.0 day, median 7.0 days (IQR 7.0 -8.0) vs. 8.0 (IQR 7.0 – 9.0), respectively, with a HR of 1.42 (CI 1.04 – 1.94); p = 0.028.

Safety

Incidences of AE of all grades are summarized in Table 2.

Adverse events	SOC+L (n= 104)	SOC (n=110)
Adverse events (n) / Patients (n)	121 / 56	91/ 42
Grade 1 (Mild)	58 / 39	48 / 32
Grade 2 (Moderate)	23 / 13	17/9
Grade 3 (Severe)/ Grade 4 (Life threatening events)	31/15	16/9
Grade 5 (Deaths)	9/9	10/10

Table 2: Incidence of reported adverse events in both treatment arms.
The table shows the number of adverse events recorded in the study and the number of patients affected by at least one adverse event.

At least one AE was reported in 98/214 participants, and most of them were mild in severity. AEs of moderate grade were reported in 13/104 patients in SOC+L group and 9/110 patients in SOC group. Serious AEs (n=47) were reported in 15/104 patients in SOC+L groups and 9/110 in SOC group and 19 patients died (9 in SOC+L group, 10 in the SOC group). There was no significant difference in the incidence of AE reported between the two groups. No Serious AEs were attributed to leflunomide. A supplementary Table 2 lists all adverse events recorded in the study according to MedDRA terms.

Liver function

At baseline, more patients with greater than ULN levels of ALT and AST were randomized in the SOC+L group than the SOC group (ALT: 46 vs 33, p = 0.049; AST: 31 vs 24, p = 0.340). By Day 3/4, following the initial loading of leflunomide therapy in the SOC+L group, there was a significantly higher number of patients with greater than ULN level of ALT and AST in the SOC+L than the SOC group (64 vs 38, p <0.001; and 51 vs 24, p <0.001). By discharge, the difference in the number of patients with ALT and AST transaminitis between the SOC+L and SOC groups was no longer significant (28 vs 27, p = 0.633; and 20 vs 17, p = 0.318) (Supplementary table 3). Leflunomide therapy was terminated early if transaminase levels exceeded 3 x ULN. However, there were 5 patients in India who continued with leflunomide therapy at the discretion of the researcher and direct care team with close monitoring of their liver function. Interestingly, in this subset of patients, the transaminase levels improved despite continuation of therapy. There were no adverse events related to clinically significant liver injury due to leflunomide. AEs related to liver dysfunction were reported in 16/104 (15.4%) patients in SOC+L group, 7 were mild, 8 were moderate and 1 was severe. Of these, 10 were deemed possibly treatment related and leflunomide treatment was discontinued in 9 patients. Comparatively, in the control group, 6/110 (5.5%) patients had liver dysfunction related AE. Five of them were mild and 1 case was severe.

Secondary Outcomes

A modified intent to treat approach was used for data from 201 patients for all secondary outcomes. This included 95 patients in the SOC+L group and 106 patients in SOC group. For these analyses we excluded 3 patients in the SOC+L group who withdrew consent and never received leflunomide and 10 patients (6 SOC+L; 4 SOC) who

did not fulfil moderate COVID symptoms inclusion criterion (did not show respiratory compromise and blood oxygen saturation (SpO_2) <93% on room air).

Mortality

There was no difference in all-cause mortality within 28 days of randomization between the treatment arms as 9/95 (9.47%) of patients died in SOC+L group compared to 10/106 (9.43%) in SOC groups. The survival curves diverge in favour of the SOC+L group after 10 days of hospital treatment, but the curves converged again after 3 weeks (when majority of the patients have been discharged). All deaths were attributed to complications related to Covid-19 (Figure 3**,** panel A)

Oxygenation and assisted ventilation

Oxygen independence is defined by maintenance of SpO_2/FiO_2 Air ratio > 4.43. There was a difference in the median time the participants required to be completely weaned off oxygen therapy between groups; 6.0 (IQR 4.0 – 8-0) days in the SOC + L group vs. 7.0 (5.0 – 10.0) days in the SOC group, p = 0.047 (Figure 3, panel B)

Non-invasive ventilation was required for 14.4% of patients in SOC+L group vs. 16.4% in the SOC group. The duration of non-invasive ventilation was 6.0 (IQR 2.0-9.0) days in the SOC+L group compared to 4.5 days (IQR 2.3-6.8) in the SOC group. Similar proportion of patients required non-invasive ventilation at the time of study enrolment (4.8% in SOC+L group vs. 7.3% in SOC group, p= 0.45).
The proportion of patients admitted to level-2 Intensive Care Unit (ICU) was 8.7 % in the SOC+L group and 8.2% in the SOC group. The median time spent at ICU was 8.0 (IQR 5.0-10.0) days vs. 9.0 (IQR 5.0-13.0) days, respectively**.** Invasive ventilation was required for 3.9% of patients in the SOC+L group and 5.5% in the SOC group with median duration of 6 (IQR 4.8, 6.0) days vs. 7.0 (IQR 5.3 - 11.8) days, respectively. None of the between group comparisons were statistically significant. Patients recruited in India were significantly less likely to require invasive or non-invasive ventilation or be admitted to ICU compared to patients recruited in the UK (p<0.001).

Viral load

Quantitative SARS-COV-2 PCR measurements from nasopharyngeal swabs at baseline showed no difference in median $\log^{10}$ viral loads (copies/ml) between the two groups, SOC+L 4.68 (IQR 4.45-4.85) vs SOC 4.76 (IQR 4.48-4.92), p =027.. We clustered the serial samples to reflect the crucial time intervals during the hospital stay: time coinciding with finishing leflunomide loading dose (by Day 4), time to 75% patients being discharged from hospital (by Day 7), time to finishing leflunomide maintenance dose (by Day 11) and beyond (Figure 4). Viral loads were significantly reduced in both treatment arms. There was no significant difference in the overall rate of the viral load clearance between the two groups by Day 11 and beyond. Viral loads were significantly reduced in both treatment arms by Day 7, p<0.001; and by Day 11, p <0.030. The rate of viral load reduction between groups by Day 11 appeared to be similar.

Cytokines, CRP and lymphocytes

Cytokine levels were assessed separately for UK and Indian sites as two laboratories using different assays processed the samples. The median baseline levels of IL 2, IL 6 and TNF-α levels (UK: IL2 0.43 [IQR 0.30-0.62] pg/ml; IL6 6.2 [IQR 2.9-9.7] pg/ml; TNF-α 10.1 [IQR 7.8 -13.5] pg/ml; India: IL2 4.3 [IQR 2.8-5.8] pg/ml; IL6 12.6 [IQR 6.5-43.1] pg/ml; TNF-α 6.1 [IQR 4.9-7.1] pg/ml) were not significantly raised from normal reference ranges and were not different between treatment groups in both countries. The cytokine levels were reduced during hospitalisation, though the clinical significance of these changes within the normal range is uncertain. There was no significant difference in the trends observed between treatment arms.

The median baseline levels of CRP were similar in both groups, 28, (IQR 8-71) in SOC+L vs. 34 (14-71) mg/L in SOC. By one week of treatment, there was similar levels of reduction between groups.

The median baseline lymphocytes levels were lower than normal reference range in both groups (0.99 [IQR 0.6-1.6) $x10^9$/L in SOC+L vs 0.95 (IQR 0.6-1.6) $x10^9$/L in SOC. By 1 week of treatment, levels rose to normal range in both groups. There was no significant difference in the trends observed between groups.

28- and 90-days follow up

At 28 days, 59/81 patients (71.2%) in the SOC+L group and 60/91 patients (65.9%) in the SOC group experienced at least 1 of 9 common long-COVID symptoms (fatigue, cough, anxiety, chest pain, brain fog, breathlessness, disturbed sleep, palpitations, joint pain); with sleep quality (48.2% vs. 38.5%), breathlessness (40.7 vs 42.9%), joint pain (32.1 vs. 33%), fatigue (29.6 vs, 31.9%), and anxiety (24.7 vs. 19.8%) being the commonest symptoms experienced (Supplementary table 4). At 90 days, there was a reduction in overall prevalence of symptoms as 42/81 patients (51.2%) in the SOC+L group and 37/91 (40.7%) patients in the SOC group and any of the residual symptoms were of reduced severity. There was no significant difference in these outcomes between the treatment arms.

Myalgia symptoms were comparably reduced between the 2 groups at 90 days. Anosmia and loss of taste were still reported by 2 and 7 patients, respectively, in the SOC+L group, but none in the SOC group.

At 28 days, 41.5% patients in the SOC + L group and 52.8% in SOC group, reported being moderately to severely dyspnoeic (Grade 4: stops for breath after walking 100m; Grade 5: too breathless to leave the house or breathless when dressing). These proportions were further reduced at 90 days, to 22% in the SOC+L group compared to 19.8% in SOC group. These differences were not significant in between group comparisons.

Mental health issues were highlighted by reports of feeling depressed and losing interest in doing things. Comparable proportions of patients in the SOC+L group and SOC group reported those problems at 28 days (17.9% vs 16.0%; 11.6% vs 14.2%, respectively) which were further reduced in both groups at 90 days (11.6% vs 9.4%; 9.5% vs 6.6%, respectively).

At 28 days participants in the SOC+L group scored their current health as being 80±25% of the usual which increased to 89±17% at 90 days. In the SOC group the scores were similar, 82 ± 23% and 90 ± 17% at 28 and 90 days respectively.

Discussion

This study is the first prospective, multicentre, randomised, controlled clinical trial investigating the clinical efficacy and safety of leflunomide in treating acute COVID-19 infection. The study showed that a course of leflunomide (3 days of 100 mg/day loading dose followed by 7 days of 20 mg/day maintenance dose) added to the standard care treatment (steroids, anticoagulants, antibiotics and antiviral therapy), did not influence the primary outcome of the trial and the acute clinical outcomes at 28 days, or the prevalence of long-COVID symptoms at 28 and 90 days. However, participants who received leflunomide as an adjunct therapy were weaned off oxygen earlier, which translated to reduced hospital stay by one day. The medication appeared to be safe and well tolerated with no severe adverse events attributable to it. A small proportion of patients in our study were still burdened by COVID-19 related symptoms 90 days after randomisation.

This multicentre trial advances the evidence base on the impact of leflunomide, a repurposed rheumatoid arthritis medication, on COVID-19 infection. Leflunomide was a potentially attractive therapeutic choice from early preclinical and clinical experience reported from hospitals in Wuhan, China. Dihydroorotate dihydrogenase (DHODH), located in the inner mitochondrial membrane is a rate-limiting enzyme in de novo pyrimidine biosynthesis. In virus-infected cells, a large intracellular nucleotide pool is consumed by rapid viral replication. RNA viruses need unique UMP but not TMP in their genomes. As UMP is the particular nucleoside produced by DHODH, RNA viruses are sensitive to reduced DHODH activity. Preclinical models of cell and animal infection by SARS-CoV-2 demonstrated that leflunomide attenuates viral genome replication, suppresses inflammatory response and the release of pro-inflammatory cytokines and chemokines.[7,8,9] Early reports from China advocated major clinical benefits in patients treated with leflunomide both in terms of less severe outcomes and duration of infection.[15,16,17] While the current study did not reproduce these overall benefits in the ITT analysis regarding the primary outcome, it confirmed some positive effects in those patients who received the trial intervention (in modified intent to treat analysis).

Our results are likely explained by the changing landscape and evolution of the routine COVID-19 treatment protocols in the standard arm of the study and the resultant severity of the COVID-19 outcomes in general. The initial phase of the COVID-19 pandemic was characterised by severe respiratory and systemic infections and poor outcomes due to the development of acute respiratory distress syndrome, multi-organ failure and eventual death.[18,19] Contrary to this early experience with COVID-19 management, the in-hospital mortality in the present study was much lower, less than 10% in both groups. The majority of patients in both treatment arms improved during hospitalisation and were discharged within a week of admission. Inclusion of prognostically significant COVID-19 therapies in both pharmacological and non-pharmacological standard of care treatments undoubtedly contributed to a reduction in severe complications and better overall outcomes. During patient recruitment of the

current trial, various therapies have been introduced including more than 95% percent of the study population received steroids as standard of care.

Theoretical considerations suggest that leflunomide may effectively inhibit viral replication. The initial pilot study during the early outbreak of COVID-19 in Wuhan, China reported reduced viral shedding time following leflunomide treatment during acute infection compared to the standard of care therapy.[15] Similarly, viral shedding duration was reduced in leflunomide treated patients who remained qPCR positive 1 month after the initial infection.[16] Our study addressed the viral load reduction at pre-specified time points. Values of viral load were reduced over time but there was no difference between the treatment arms. Both methodological considerations and the inclusion of comprehensive pharmacological treatment regimens in the SOC could explain these differences. For instance, corticosteroid therapy was absent in the early study from Wuhan, but the later study refers to the use of hydroxychloroquine, interferon-alpha and antiviral medications as part of acute standard of care therapy.[15,16] However, our results are in line with other reports from China which showed that duration of viral shedding was not affected by leflunomide added to nebulised interferon alpha therapy for treating long-term positive COVID-19 after 4 weeks of in-hospital treatment.[17] Interestingly a third of these patients received corticosteroid therapy during the initial acute treatment. [16,17]

Beyond the issue of therapeutic efficacy and viral load, our study confirms overall safety of leflunomide in COVID-19 infection. The safety profile of leflunomide is well established in the treatment of RA. [6] Leflunomide, repurposed for the COVID-19 treatment, was well tolerated since no serious adverse events were attributed to it. Similar findings were reported in other studies.[15,16,20] Mild transaminitis following long-term leflunomide use in the RA population is recognised, and usually resolves after medication is terminated. The mechanism is likely to be modulation of interleukins which may hinder the protection of hepatocytes from injury rather than direct toxicity.[21] There were comparable incidences of transaminitis in both treatment arms in our study. However, more patients in the UK cohort had raised liver function tests leading to modification or termination of leflunomide treatment. This may be accounted for by the difference in the severity of COVID-19 disease and spectrum of co-morbidities between UK and Indian participants rather than genetic polymorphism in drug metabolism. Overall, the proposed leflunomide regimen was well tolerated.

One of the motivations of the current trial employing leflunomide was to benefit from the anti-inflammatory effects of this drug. In this context, hydrocortisone has been demonstrated as an effective therapy in severe COVID-19 infections and recent trials also demonstrated the benefit of tocilizumab, a selective IL-6 inhibitor and a different disease modifying rheumatoid arthritis medication. However, such finding is not universal as the benefit of tocilizumab is mainly demonstrated in critically to moderately ill patients.[22,23] A recent meta-analysis showed that the benefit of IL-6 receptor antagonist was encountered only in patients who were also treated with glucocorticoids.[24] This is in keeping with observations that a broader spectrum of pro-inflammatory cytokines, macrophages and T cell response have all been documented in severely ill patients demonstrating the role of a more complex inflammatory response. It is exactly this broader inflammatory reaction that could be targeted by leflunomide as its effect on cytokines is not restricted to IL 6 and it may also have an impact on activated T cell response.[8,9,25] Such phenomena might contribute to the benefits of reduced oxygen dependence in patients who

have received leflunomide treatment. However, it is conceivable that the full benefit of such anti-inflammatory effect may be more pronounced in severely ill patients, but this population was underrepresented in our trial and the (inadvertent) inclusion of patients with milder symptoms may have led to some attrition of statistical power in our study. A more detailed analysis of the cytokine and metabolic profiles of our trial population is underway to clarify these important issues.

Another important consideration when discussing the potential benefits of leflunomide is the mutation ability of the SARS-CoV-2 virus.[26] So far, the mutations observed in different strains worldwide have largely been confined to the part of the spike protein affecting the virus's ability of cell entry as opposed to a region targeted by neutralising antibodies. However, the possibility of mutations in different regions cannot be excluded. Targeting the host's pyrimidine biosynthesis pathway by leflunomide, rather than using drugs with direct antiviral action, remains an advantage offering protection against a broader spectrum of viruses and potentially overcoming resistance. Indeed, DHODH inhibitors such as leflunomide has shown broad-spectrum antiviral effects against various RNA viruses in cell models.[7] Leflunomide may therefore be considered a viable pharmacological treatment for COVID-19 patients given it is well tolerated, safe, economical, and widely available. Its clinical effectiveness measured against recognised selective IL-6 inhibitors in the more severely/critically ill patients needs to be further explored as leflunomide may be the preferred option in countries where other immunomodulating agents, such as Talizumab, may not be practical or widely available.

Limitations

The present study has several limitations. In order to balance the needs of the trial with clinical care and to minimise disruption to already overstretched clinical resources during COVID-19 pandemic, we chose to adopt an open label study design. This design may have affected the data collection and clinical management of the patients and potentially introduced a bias. However, it also allowed early detection of significant adverse events and a potential outcome benefit. This was an important consideration when testing an off-label use of a medication in COVID-19, a disease with high morbidity and mortality.

The study was set out to recruit more severely and critically affected patients in a single country. However, due to recruitment restrictions because of national prioritization of critically ill patients to only a few studies together with scarcity of NHS resources during the pandemic, the study was extended abroad, ultimately recruiting less affected patients with heterogeneous clinical profiles. Although patient characteristics and medications received as part of SOC did not differ between the randomised arms, the more heterogeneous population, milder COVID-19 disease, and more effective standard of care treatments most likely impacted on the hypothesised effect size and the ability of finding a difference in our recruited sample. Finally, the COVID-19 restrictions affected our protocolised laboratory investigations, such as the serial viral load and comprehensive inflammatory profiling. Nevertheless, studies focusing on the more severely affected participants are underway and will be the subject of a separate submission.

Secondary outcomes assessing organ and multi-organ endpoints set out in the original protocol were not reported because the data are incomplete for meaningful analyses. **Conclusion**

Leflunomide had no major impact on the clinical outcomes when administered together with the currently established but evolving therapies in moderately affected COVID-19 patients. It may shorten duration of oxygen

dependence thereby affecting the TTCI and hospital discharge. Transaminitis associated with leflunomide therapy did not lead to excess adverse events compared to the control group and may have arisen in part due to the severity of clinical infection. Further studies are needed to investigate the potential benefits of leflunomide in the critically ill patients and the biological mechanisms involved.

Ethics statements

All patients taking part in the study signed written informed consent form once the study was ethically approved by relevant bodies in England (South Central - Berkshire Research Ethics Committee, Bristol REC Centre, reference number 20/SC/0264) and India (Max HealthCare Ethics Committee, reference number RS/MSSSH/GMHRCCMS/MHEC/CCM/20-23; Meditrina Institute Ethics Committee, reference number ECR/605/Inst/MH/2014/RR; Noble Hospital Institutional Ethics Committee, reference number NHIEC/FEB/2021/238).

Data availability statement

The anonymized data may be available upon request following approval from the Trial Management Group and the Sponsor.

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Trial Steering Committee: Brendan Madden (Chair), Patrick Yong, Sreenivasa Rao Kondapally Seshasai, Matt Mendes, Janice Rodrigues-Mendes, Rod Hughes, Sharanpal Jeetle, Subash Somalanka
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Authors' contributions

All designated authors meet all four ICMJE criteria for authorship. The funders and the sponsor of the study had no role in the analyses or the interpretation of the results.

ZC, NM, HLL, SRKS, LL, JB, SL, IJ, DF and PS made substantial contribution to the conception and design of the study. SS, AK, AB, RA, KB, MM, KR, FO, KL, AW, AL, SRKS, JB, HLL, NM, IKH and ZC made substantial contribution to the acquisition, analysis and interpretation of the data for the study. All authors contributed to the drafting, revision and final approval of the manuscript. ZC, NM and LL are responsible for the overall content.

Competing interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/disclosure-of-interest/ and declare no competing interests.

Dissemination

A summary of the results will be disseminated to the participants by principal investigators at each trial site via a newsletter.

Licencing Statement

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References

1. https://COVID19.who.int/
2. Chen YL, Quach TTT, COVID-19 cytokine storm syndrome: a threshold concept. Lancet Microbe. 2021 Feb;2(2):e49-e50.doi: 10.1016/S2666-5247(20)30223-8.Epub 2021 Feb 2
3. Gavriatopoulou M, Ntanasis-Stathopoulos I, Korompoki E, Fotiou D, Migkou M, Tzanninis IG, Psaltopoulou T, Kastritis E, Terpos E, Dimopoulos MA. Emerging treatment strategies for COVID-19 infection. Clin Exp Med. 2021 May;21(2):167-179. doi: 10.1007/s10238-020-00671-y. Epub 2020 Oct 30. PMID: 33128197; PMCID: PMC759894
4. Dwek, Raymond A et al. "Targeting glycosylation as a therapeutic approach." Nature reviews. Drug discovery vol. 1,1 (2002): 65-75. doi:10.1038/nrd708
5. Angus, Derek C et al. "The REMAP-CAP (Randomized Embedded Multifactorial Adaptive Platform for Community-acquired Pneumonia) Study. Rationale and Design." Annals of the American Thoracic Society vol. 17,7 (2020): 879-891. doi:10.1513/AnnalsATS.202003-192SD
6. Alfaro-Lara, et al. Systematic review and meta-analysis of the efficacy and safety of leflunomide and methotrexate in the treatment of rheumatoid arthritis. Reumatol Clin. 2019 May - Jun;15(3):133-1
7. Xiong R, Zhang L, Li S, Sun Y, Ding M, Wang Y, Zhao Y, Wu Y, Shang W, Jiang X, Shan J, Shen Z, Tong Y, Xu L, Chen Y, Liu Y, Zou G, Lavillete D, Zhao Z, Wang R, Zhu L, Xiao G, Lan K, Li H, Xu K. Novel and potent inhibitors targeting DHODH are broad-spectrum antivirals against RNA viruses including newly-emerged coronavirus SARS-CoV-2. Protein Cell. 2020 Oct;11(10):723-739. doi: 10.1007/s13238-020-00768-w. Epub 2020 Aug 4. Erratum in: Protein Cell. 2021 Jan;12(1):76-80. Erratum in: Protein Cell. 2020 Nov 9;: PMID: 32754890; PMCID: PMC7402641.
8. Yao, H. W.; Li, J.; Chen, J. Q.; Xu, S. Y. A 771726, the active metabolite of leflunomide, inhibits TNF-α and IL-1 from Kupffer cells. Inflammation 2004, 28 (2), 97-103.
9. Li, L. et al. The effects of teriflunomide on lymphocyte subpopulations in human peripheral blood mononuclear cells in vitro. J. Neuroimmunol. 2013, 265 (1-2), 82-90.
10. https://www.rcplondon.ac.uk/projects/outputs/national-early-warning-score-news-2
11. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_60
12. Mandal S, Barnett J, Brill SE ARC Study Group, et al. 'Long-COVID': a cross-sectional study of persisting symptoms, biomarker and imaging abnormalities following hospitalisation for COVID-19. Thorax 2021;76:396-398.
13. http://www.who.int/blueprint/priority-diseases/key-action/novel-coronavirus/en/).
14. Machin D, Campbell M, Fayers, P, Pinol A (1997) Sample Size Tables for Clinical Studies. Second Ed. Blackwell Science IBSN 0-86542-870-0 p. 176-177
15. Hu, Ke et al. "A Small-Scale Medication of Leflunomide as a Treatment of COVID-19 in an Open-Label Blank-Controlled Clinical Trial." Virologica Sinica vol. 35,6 (2020): 725-733. doi:10.1007/s12250-020-00258-7
16. Wang, Qiang et al. "Efficacy and Safety of Leflunomide for Refractory COVID-19: A Pilot Study." Frontiers in pharmacology vol. 12 581833. 2 Jul. 2021, doi:10.3389/fphar.2021.581833

17. Mengmei Wang, Yang Zhao, Weihua Hu, Dong Zhao, Yunting Zhang, Tao Wang, Zhishui Zheng, Xiaochen Li, Shaolin Zeng, Zhenlian Liu. Treatment of Coronavirus Disease 2019 Patients With Prolonged Postsymptomatic Viral Shedding With Leflunomide: A Single-center Randomized Controlled Clinical Trial. Clinical Infectious Diseases, Volume 73, Issue 11, 1 December 2021, Pages e4012–e4019, https://doi.org/10.1093/cid/ciaa1417
18. Huang, Chaolin et al. "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China." Lancet (London, England) vol. 395,10223 (2020): 497-506. doi:10.1016/S0140-6736(20)30183-5
19. ISARIC Clinical Characterisation Group. "COVID-19 symptoms at hospital admission vary with age and sex: ISARIC multinational study." medRxiv : the preprint server for health sciences 2020.10.26.20219519. 19 Nov. 2020, doi:10.1101/2020.10.26.20219519. Preprint.
20. Chakravarty EF, Sanchez-Yamamoto D, Bush TM. The use of disease modifying antirheumatic drugs in women with rheumatoid arthritis of childbearing age: a survey of practice pattern and pregnancy outcomes. J Rheumatol. 2003;30:241–6.
21. Atsushi Ogata, Yasuhiro Kato, Shinji Higa, Kazuyuki Yoshizaki, IL-6 inhibitor for the treatment of rheumatoid arthritis: A comprehensive review, Modern Rheumatology, Volume 29, Issue 2, 4 March 2019, Pages 258–267, https://doi.org/10.1080/14397595.2018.1546357
22. REMAP-CAP Investigators et al. "Interleukin-6 Receptor Antagonists in Critically Ill Patients with COVID-19." The New England journal of medicine vol. 384,16 (2021): 1491-1502. doi:10.1056/NEJMoa2100433
23. Abani, O., Abbas, A., Abbas, F., Abbas, M., Abbasi, S., Abbass, H., et al. (2021). Tocilizumab in Patients Admitted to Hospital with COVID-19 (RECOVERY): a Randomised, Controlled, Open-Label, Platform Trial. The Lancet 397, 1637–1645. doi:10.1016/S0140-6736(21)00676-0
24. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group et al. "Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis." JAMA vol. 324,13 (2020): 1330-1341. doi:10.1001/jama.2020.17023
25. Kraan MC, Smeets TJM, van Loon MJ, et al Differential effects of leflunomide and methotrexate on cytokine production in rheumatoid arthritis Annals of the Rheumatic Diseases 2004;63:1056-1061.
26. Wang, Shihang et al. "Molecular evolutionary characteristics of SARS-CoV-2 emerging in the United States." Journal of medical virology vol. 94,1 (2022): 310-317. doi:10.1002/jmv.27331

Figure legends:

Figure 1: Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.

**= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.*

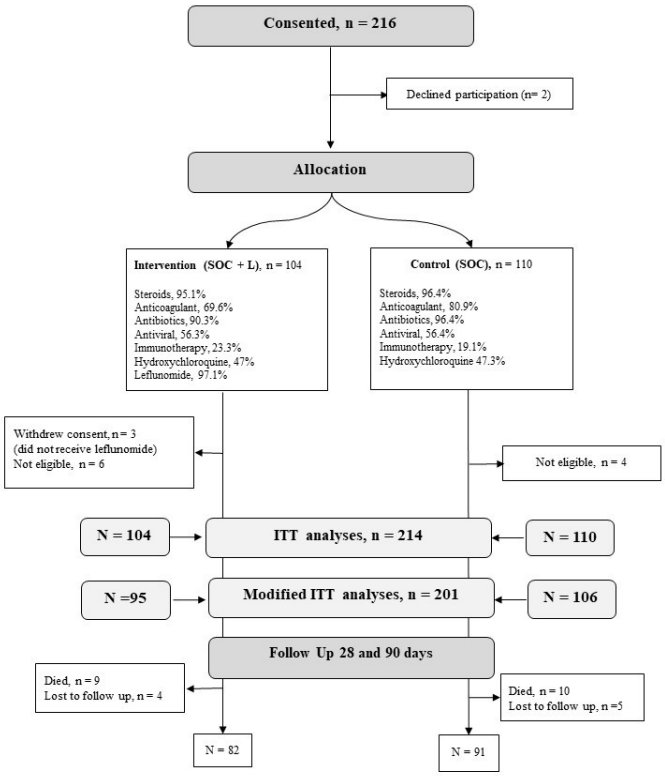
Figure 2: Time to clinical improvement of 2 points on a clinical status scale or discharge prior 28 day in a stratified ITT analysis (primary outcome).

Patients who died were censored at the time their death occurred, while all surviving patients who did not reach TTCI criteria by day 28 were right censored at that point. Most of the patients were discharged within the first 10 days of admission.

Figure 3: Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

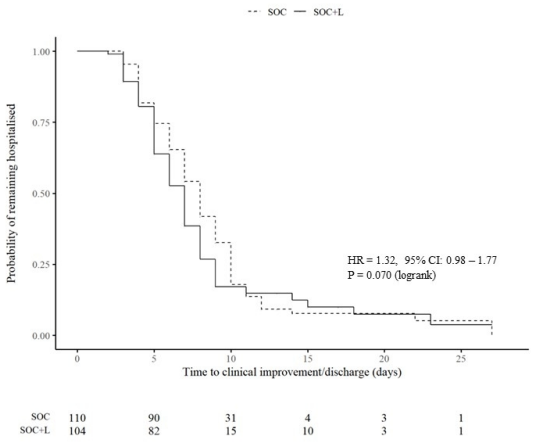
Figure 4: Mean changes in log^{10} viral load (copies/ml) from baseline.

Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.



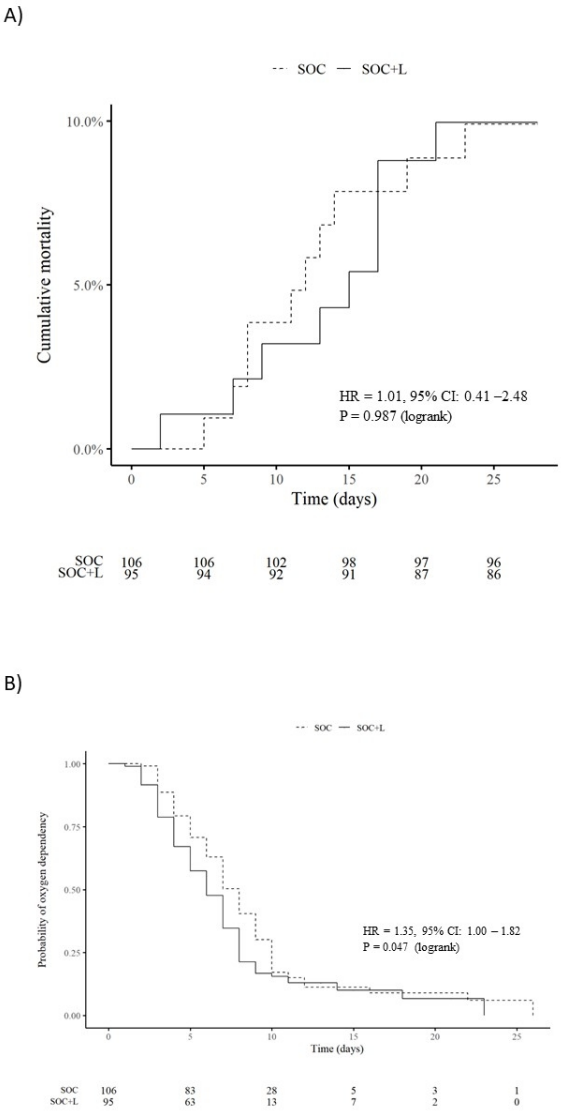
Randomisation, treatment assignment and follow up of DEFEAT-COVID study participant.
*= immunotherapy included Tocilizumab, Bevacizumab and Interferon alpha and beta.

190x338mm (96 x 96 DPI)



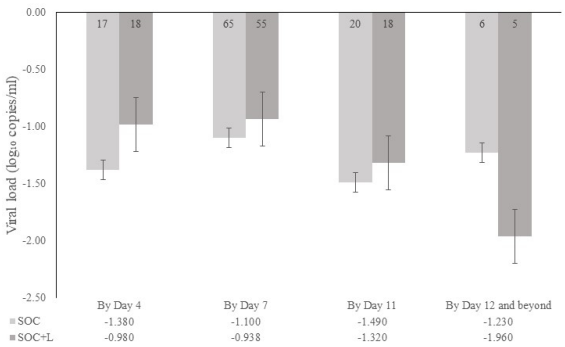
Patients who died were censored at the time their death occurred, while all surviving patients who did not reach TTCI criteria by day 28 were right censored at that point. Most of the patients were discharged within the first 10 days of admission.

338x190mm (96 x 96 DPI)



Cumulative all-cause mortality (A), oxygen dependence (B) by 28 days

190x338mm (96 x 96 DPI)



Mean changes in $\log_{10}$ viral load (copies/ml) from baseline. Error bars represent standard error. Numbers in the bars represent the number of samples available for measurements.

338x190mm (96 x 96 DPI)

Trial procedures	Screening	Day 0/1 (BL)	Daily	Day 3	Day 7	Day 11 +/- 1	Day 15 +/- 1	Day 28 +/-1	DC	Day 90 +/- 7
Confirmation of COVID Infection and severity	X									
Informed consent & Eligibility Assessment	X									
Demographics, Medical Hx, Cardiopulmonary Assessment (including ECG [1] & Echo [2])	X									
Concomitant medication		X	X					X [7]		X
Bloods – FBC, U&Es, LFT (AST [6] & ALT [6])		X	X [5]	X [6]					X	
– Clotting screen, Fibrinogen, D-Dimer, Ferritin		X	X [5]							
– Glucose		X	X [5]							
– Creatine Kinase, Troponin, BNP (NT-proBNP)		X	X [5]							
– Procalcitonin, CRP, LDH		X	X [5]	X		X				
– HIV		X								
– Cytokine profile		X		X		X			X [8]	
Pregnancy test (urine sample)		X								
Viral Load (nasopharyngeal swab)		X			X	X	X	X	X [8]	
Randomisation		X								
IMP dispense, loading (daily from Day 0/1 to 3) / maintenance dose (daily from Day 4 to 10) [3]		X								
Primary outcome assessment (TTCI)		X	X					X [7]		
Clinical Assessment, e.g. NEWS 2, body T°C*, vital signs, imaging**	X	X	X [5]							
*Blood and Urine cultures (in presence of fever)		X	X [5]							
**Urine for legionella and pneumococcal		X	X [5]							
Oxygenation assessment e.g. O_2 delivery method and level [SpO_2]		X	X [5]							
Arterial Blood Gas (ABG) – as available and where applicable		X	X [5]							
Serious Adverse Event(s) (SAE(s))/ Adverse Event(s) (AE(s)) [4]			X					X [7]		X [9]
Out-patient assessment (telephone call)								X [7]		X

Data collected and study time points

Key: BL – Baseline; IMP – Investigation Medicinal Product (trial treatment i.e. trial drug); SOC - Standard of Care; DC Discharge.

Notes: **1.** Check medical notes, if abnormal flag repeat imaging; **2.** Echo within 6 months to be used if no cardiac symptoms; **3.** Participant to take home IMP if DC'd; **4.** Participant to self-report events between DC to Day 90; **5.** Completed Daily, depending on clinical need and resources. If participant is DC'd early – record what is available as part of SOC; **6.** AST/ALT *must* be checked for treatment arm to determine maintenance dose; **7.** Participant DC'd called on Day 28 for Treatment Assessments, if not seen on-site; **8.** Cytokines/Viral Load to be collected if outside of scheduled collection. Day 11 is the last collection for Cytokines. on DC Medium (telephone call) and long term (by Sponsor) Treatment Assessments respectively.

Centre	Group	N	Corticosteroid	Anticoagulant	Antiviral	Antibiotic	Immunotherapy*	Hydroxychloroquine
UK	Intervention	30	100%	97%	69%	100%	14%	3%
	Control	36	100%	100%	81%	100%	3%	3%
India	Intervention	74	87%	76%	70%	76%	67%	93%
	Control	74	89%	84%	70%	88%	50%	94%
All centres	Intervention	104	95%	79%	56%	90%	23%	47%
	Control	110	96%	80%	56%	96%	19%	47%

Supplementary Table 1: **Standard of care therapy in the UK and India**

**The "Immunotherapy" includes tocilizumab, bevacizumab, interferon alpha and beta*

Adverse events categorised per MedDRA terminology		
	SOC+L (n = 104)	SOC (n = 110)
Patients with at least one reported AE, n (%)	56 (53.8%)	42 (38.2%)
All adverse events, n	121	91
Blood and lymphatic system disorder		
- DIC	1	4
- Lymph node enlargement	1	0
- Hilar lymphadenopathy	1	0
- Thrombocytopenia	4	4
- Neutropenia	0	1
- Splenomegaly	1	0
Cardiac Disorder		
- Acute coronary syndrome	2	1
- Aortic Valve disease	2	0
- Atrial fibrillation	2	5
- Chest pain	1	0
- Left ventricular systolic dysfunction	1	0
- Conduction disorder	0	1
- Infective endocarditis	1	0
- Tachycardia (sinus)	2	0
Endocrine disorder		
- Adrenal adenoma	1	0
Eye disorder		
- Dry eye	0	1
General disorders and administration site condition		
- Lethargy	1	0
Gastrointestinal disorders		
- Diarrhoea	1	1
- Gastritis	1	0
- Gastric haemorrhage	2	0

- Hiatus hernia	1	0
- Rectal haemorrhage	0	1
- Mucositis oral	1	0
- Dyspepsia	2	0
- Emesis	1	0
Hepatobiliary disorders		
- Acute liver dysfunction	1	1
- Cholelithiasis	2	0
- Hepatic granuloma	1	0
- Liver steatosis	1	0
Infection and infestations		
- Sepsis	0	1
Investigation		
- APTT prolonged	1	0
- ALP increased	1	0
- Bil increased	3	0
- Il-6 increased	1	0
- Leucocytosis	1	2
- ALT/AST increased	27	12
- Sgot increased	2	0
Metabolism and nutrition disorder		
- Hyperglycaemia	2	6
- Hyperkalaemia	0	1
- Hyponatraemia	1	1
- hypomagnesaemia	0	1
Musculoskeletal and connective tissue disorder		
- Discitis	1	0
Neoplasms		
- Lung cancer	1	0
Nervous system disorder		
- 6th nerve palsy	0	1

- Dysphasia	0	1
- Cognitive disturbances	1	0
- Cerebellar calcification	0	1
- Lower limb weakness	1	0
- Dysphagia	0	1
- Hemiparesis	0	1
- Headache	1	0
- Intracranial haemorrhage	0	1
Psychiatric disorders		
- Anxiety	2	1
- Delirium	0	2
Renal and urinary disorders		
- Acute kidney injury	5	5
- Haematuria	1	0
- Urinary urgency	1	0
Respiratory thoracic and mediastinal disorders		
- ARDS	1	0
- Wheezing	0	1
- Atelectasis	0	1
- Hypoxia	5	7
- Exacerbation of COPD	3	2
- Hoarseness	0	1
- Dyspnoea	4	3
- Pneumonitis	1	0
- Epistaxis	3	1
- Haemoptysis	0	1
- Respiratory failure	2	0
- Pneumothorax	0	1
- (subcutaneous emphysema)	0	1
Reproductive system and breast disorders		
- Endometrium thickening	1	0

Surgical and medical procedures		
- Aortic valve replacement	1	0
- Loop recorder implant	0	1
Vascular disorder		
- Aortic aneurysm	1	0
- Thromboembolic events		
o DVT	1	0
o Bilateral pedal vasculopathy	0	1
o Pulmonary embolism	1	1
- Hypertension	0	1
Death	9	10

Supplementary Table 2: All adverse events

		Baseline		Day 1 – 3/4		Day 4/5 – Discharge	
		UK	India	UK	India	UK	India
ALT							
SOC + L	**Median (IQR) U/L**	48 (30-60)		67 (36~87)		44 (36-71)	
		48 (32-71)	47 (29-59)	59 (37-94)	72 (34-86)	62(34-151)	42(35-56)
	1 - 2 x ULN (n)	44		50		17	
		12	32	9	41	4	13
	2 - 3 x ULN (n)	1		6		6	
		1	0	3	3	2	4
	> 3 x ULN (n)	1		8		5	
		1	0	3	5	4	1
	>ULN	14	32	15	49	10	18
SOC	**Median (IQR) U/L**	39 (26-56)		44 (34-65)		41 (35-52)	
		40 (27-59)	38 (25-54)	44 (31-63)	44 (36-67)	49 (33-63)	40 (35-52)
	1 - 2 x ULN (n)	33		29		24	
		13	20	8	21	7	17
	2 - 3 x ULN (n)	0		9		3	
		0	0	2	7	2	1
	>3 x ULN (n)	0		0		0	
		0	0	0	0	0	0
	>ULN	13	20	10	28	9	18
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.049**		**<0.001**		**0.633**	
AST							
SOC + L	**Median (IQR) U/L**	44 (30-54)		55(31-77)		41(27-50)	
		60 (42-102)	43(29-50)	58 (42-104)	53 (28-76)	54 (29-102)	40 (27-49)
	1 - 2 x ULN (n)	28		36		18	
		4	24	8	28	3	15
	2 - 3 x ULN (n)	2		9		1	
		2	0	2	7	1	0
	> 3 x ULN (n)	1		6		1	
		1	0	2	4	1	0
	>ULN	7	24	12	39	5	15
SOC	**Median (IQR) U/L**	39 (28-52)		39 (29-54)		37(27-47)	
		57 (34-75)	37(26-48)	45(38-55)	38(28-54)	45 (35-61)	37 (25-46)
	1 - 2 x ULN (n)	22		18		16	
		6	16	4	14	4	12
	2 - 3 x ULN (n)	2		5		0	
		2	0	0	5	0	0
	>3 x ULN (n)	0		1		1	
		0	0	0	1	1	0
	>ULN	8	16	4	20	5	12
***p* value of abnormal ALT counts between SOC+L and SOC**		**0.3222**		**<0.001**		**0.318**	

Supplementary Table 3: Liver enzymes measurements

ULN: upper limits of normal (ALT: 10-49 U/L; AST 19 – 48U/L)). ALT: alanine transaminase. AST: aspartate transaminase; SOC + L: n = 30 UK, 74 India; SOC: n = 36 UK, 74 India

	Day 28					Day 90				
	SOC+L (n=81)		SOC (n=91)		p*	SOC+L (n=81)		SOC (n=91)		p*
	%	Median (IQR)	%	Median (IQR)		%	Median (IQR)	%	Median (IQR)	
Fatigue	29.6	5.00(2.75-8.00)	31.9	5.00(3.00-8.00)	0.751	22.2	5.00(3.25-7.00)	26.4	3.00(2.00-4.25)	NS
Cough	13.6	2.00(1.00-4.50)	18.7	2.00(1.00-4.00)	0.366	7.41	1.00(1.00-6.25)	8.79	2.50(1.00-3.50)	NS
Anxiety	24.7	3.50(2.00-7.25)	19.8	4.00(3.00-7.75)	0.438	21.0	3.00(1.00-5.00)	19.8	2.00(1.00-3.75)	NS
Chest pains	11.1	4.00(2.00-7.00)	8.79	4.00(2.50-5.00)	0.611	7.41	4.00(3.25-7.75)	6.59	3.00(1.25-7.75)	NS
Brain fog	14.8	5.00(3.75-7.25)	16.5	5.00(1.50-7.00)	0.764	14.8	3.50(1.75-5.00)	12.1	4.00(2.50-4.50)	NS
Breathlessness	40.7	2.00(1.00-6.00)	42.9	1.00(1.00-7.00)	0.779	22.2	5.00(1.00-7.00)	20.9	4.00(2.00-4.00)	NS
Sleep quality	48.2	2.00(1.00-5.00)	38.5	3.00(1.00-6.00)	0.200	34.6	2.00(1.00-4.25)	33.0	2.50(1.00-5.75)	NS
Palpitations	8.64	7.00(1.50-9.00)	5.49	1.00(1.00-7.00)	0.419	4.94	4.50(3.50-5.00)	3.30	1.00(1.00-4.00)	NS
Joint pain	32.1	3.00(1.00-4.75)	33.0	2.00(1.00-4.00)	0.903	22.2	3.50(2.00-7.00)	19.8	2.00(2.00-4.50)	NS
Myalgia	18.5	-	19.8		0.834	17.3		11.0		NS
Anosmia	6.17	-	11.0		0.264	2.47		-		-
Loss of taste	9.88	-	14.3		0.378	7.41		-		-
Depression	19.8	1.50(1.00-3.000	18.7	1.00(1.00-2.00)	0.859	11.1	1.00(1.00-1.00)	11.0	1.00(1.00-2.00)	NS
Loss of interest	12.4	2.50(1.25-3.00)	16.5	1.00(1.00-3.00)	0.442	9.88	1.00(1.00-1.25)	7.69	1.00(1.00-2.50)	NS
Dyspnoea; **Mild (1)** **Moderate (2-3)** **Severe (4-5)**	 59.3 29.6 11.1	1.00(1.00-2.00)	 47.3 39.6 13.2	2.00(1.00-2.00)	 0.155 0.173 0.678	 80.3 14.8 4.94	1.00(1.00-1.00)	 81.3 17.6 1.10	1.00(1.00-1.00)	NS

Supplementary Table 4: Long COVID symptoms at 28 and 90 days after randomisation

p; alpha value. Statistical significance was assumed at 0.05 alpha value. To best summarise the data, symptom scales (e.g. 0-10) for all symptoms (except dyspnoea, which was measured in different terms) were binarized, accepting any score above 0 as prevalence (%) of experiencing that symptom. To reflect magnitude of symptom severity, median (IQR) of individual symptom scores (except for dyspnoea, myalgia, anosmia and loss of taste) were taken excluding scores of 0. For dyspnoea, prevalence (%) of the symptom was determined as the proportion of patients scoring any relevant category (e.g., mild, moderate, severe), and median (IQR) of symptom severity included all score values. Between-group differences at each point of follow-up (day 28 and day 90) for all symptoms were evaluated using patient proportions from the binarized symptom scales via the chi-square test of differences. The Shapiro-wilk test was used to assess statistical normality. No analysis was enabled for any day reporting n ≤ 3 datapoints per group for statistical reliability

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5,6
	2b	Specific objectives or hypotheses	5,6
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4,5,6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4,5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5,6,7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	6
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4,5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	NA

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	5,6
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	5,6
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, page 7
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1, page 7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	7
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	7, Figure 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	5,6
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	5,6
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	5,6,7,8,9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13
Other information			
Registration	23	Registration number and name of trial registry	2
Protocol	24	Where the full trial protocol can be accessed, if available	NA
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2,13

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.