Original Article

Study of Effectiveness of Convalescent Plasma-therapy in Moderate to Severely ill COVID-19 Patients

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Background : Convalescent Plasma-therapy, a classic adaptive immunotherapy used in the treatment of SARS, MERS and 2009 H1N1 pandemic with acceptable efficacy and safety in the past. Convalescent Plasma-therapy was taken into consideration in management of COVID-19 disease during the initial days of pandemic but was withdrawn later due to its doubtful beneficial role. This study aims to explore the beneficial role of Convalescent plasma and to determine whether Convalescent Plasma-therapy holds a second chance in treating SARS-CoV-2.

Methods : This cross-sectional observational study includes 82 cases of moderate to severely ill COVID-19 patients who received Convalescent Plasma-therapy and 41 controls who didn't. regular monitoring of Total Leukocyte Count (TLC), PaO2/FiO2 (PaO2 is partial pressure of Oxygen in arterial blood, fractional inspired oxygen (P/F ratio), Neutrophil to Lymphocyte Ratio (N/L ratio) inflammatory markers, respiratory rate, oxygen saturation, ABG and Radiological Imaging was done for comparative analysis.

Results : In case group 39 patients (47.56%) were on oxygen mask, 17 patients (20.73%) on Non-invasive Ventilation (NIV), 9 Patients on Non-rebrether Mask (NRM) (10.97%), 16 patients (19.51%) on room air, 1(1.21%) on High Flow Nasal Cannula (HFNC) initially. After 7th day of Convalescent Plasma-therapy 49 patients (59.75%) were on room air which suggests significant improvement in mode of ventilation in case group as compared to Control Group. Mean respiratory rate in case group was 30.46 Cycles Per Minute (CPM) initially and 24.7 CPM on day 7th of Plasma-therapy which is statically significant.

Conclusion : Plasma-therapy is effective if given in early stage of disease and Convalescent Plasma donors having adequate antibody titre.

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***Key words* : Convalescent plasma-therapy, COVID-19, P/F ratio, Mode of ventilation.**

An epidemic of Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) emerged in Wuhan, China. It was named as Coronavirus Disease 2019 (COVID-19) by World Health Organization (WHO). This epidemic spread Globally at great pace and within 3 months it was declared a pandemic by WHO on March 11, 2020. As of now on August, 2021, 21.9 crore cases with 45 lakh deaths have been recorded worldwide. India also has its fair share with 3.3 crore cases and 4.4 lakh deaths owing to this pandemic[1-3]. SARS-CoV-2 transmits through inhalation or direct contact with droplets of infected people with an incubation period ranging from 2 to ≥14 days.

Convalescent Plasma (CP) therapy, a classic adaptive immunotherapy, is used in prevention and treatment of many infectious diseases. Convalescent plasma delivers passive immunity in form of neutralizing antibodies.

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Editor's Comment :

- In the present times when mutated variant strains are emerging at a phenomenal pace, mRNA vaccine based on specific protein antigens may not conquer immunity against newer variants whereas convalescent plasma with its natural antibodies carries potential to offer broad immunity against all variants.
- Plasmatherapy instead of being outrightly excluded from therapeutic armamentarium against Corona Virus, needs a re-evaluation as afresh so that its therapeutic potential may be exploited for the benefit of Corona Virus victims.

Convalescent Plasma is donated by recovered cases of COVID-19. It is the acellular component of blood that contains antibodies which specifically recognizes SARS-CoV-2. These antibodies are thought to exert an antiviral effect by suppressing virus replication. Virus-specific antibodies from recovered persons are often the first available therapy for an emerging infectious disease, till new antivirals and vaccines are being developed[4-6].

Convalescent Plasma is relatively safe, with comparable risk to that of non-immune plasma. Known general risks of Plasma-therapy includes allergic reactions, Transfusion-Associated Circulatory Overload (TACO), and Transfusion-Associated Acute Lung Injury (TRALI). On August 23, 2020, the US FDA granted Emergency Use Authorization (EUA) of CP in

hospitalized individuals with COVID-19[7-11].

However, some recent studies show no benefit of Plasma-therapy in COVID. In may 2020, ICMR had started a study regarding the efficacy of Plasma-therapy in COVID patients known as PLACID trial[12]. This study showed no role of Convalescent Plasma-therapy in disease progression and mortality. There are some limitations made out from this study. Most of the Plasma donors had only mild disease and around 2/3rd of these donors had median titre value of 1:40 which is way lesser than FDA recommended 1:160 neutralising titre. In some donor's antibody titres were not measured due to unavailability of antibody titre kit or faulty kits. From several studies it is observed that there is a positive correlation between magnitude of neutralising antibody response and disease severity in recovered COVID-19 patients[13-14]. Convalescent Plasma-therapy used in the treatment of SARS, MERS, and 2009 H1N1 pandemic with acceptable efficacy and safety in the past.

Materials and Methods

This study is a cross-sectional observational study performed during a period of July, 2020 to Jan, 2021 at Government Medical College, Kota and attached hospitals. Subjects falling in inclusion criteria were lab-confirmed RT-PCR positive for nasopharyngeal swab according to CDC criteria. Moderate to severely ill admitted patients were included.

This study includes 82 cases of moderate to severely ill COVID-19 patients who received convalescent Plasma-therapy and 41 controls of moderate to severely ill COVID-19 patients who did not received Convalescent Plasma-therapy. Moderately ill COVID-19 patients were those who had Respiratory Rate between 24-30 per minute and spo2 of 90-94% on room air. Severely ill defined as respiratory rate >30 and SpO_2 of <90% on room air.

Patients who were asymptomatic or with mild symptoms, pregnant & lactating women, having known hypersensitivity to blood products and recipients of immunoglobulins in past 30 days were excluded. Patients who were critically ill PaO_2/ FiO_2 <100 or in shock requiring vasopressors to maintain a Mean Arterial Pressure (MAP) of ≥65 mm Hg or MAP of <65 mm Hg were also excluded.

After admission, each patient was monitored till the end of hospital stay/ demise by a multispecialty team. Temporal assessment of the patient's profile was ensured by regular monitoring of vitals, daily assessment of the patients, and serial blood biochemistry, inflammatory markers, ABG and radiological imaging. Baseline parameters were taken before giving Convalescent Plasma-therapy (Day 0) and data was collected after giving Plasma-therapy on day 3, day 7 and comparative analysis was done.

Convalescent Plasma Donors :

Potential donors must have had documented SARS-CoV-2 infection (either nasopharyngeal swab positivity or serologic positivity), be symptom-free for at least 14 days and meet standard blood donor eligibility requirements. Currently, individuals who themselves were treated with Convalescent Plasma for their own COVID-19 illness are not allowed to donate blood products, including Convalescent Plasma, for 3 months. Donations can occur as frequently as weekly for several months following clearance of infection before antibody titres begin decreasing[15].

Statistical Methodology :

Statistical analysis was performed using Statistical Package for Social Science (SPSS) Version 22.0. Quantitative Continuous variables data were expressed as Mean ± Standard Deviation whereas Quantitative discrete variables data were expressed as frequencies are expressed as number (%). The Qualitative data were expressed in Medians with interquartile ranges. The student's t-test and χ2-test were used to compare the difference for means between two or more than two groups or to compare categorical variables, while continuous variables were compared using the Mann-Whitney U test. All statistical tests were two-tailed. Statistical significance was taken as $p<0.05$.

Results

This study included 82 participants who received Plasma-therapy. Patients who were critically ill were excluded. There were 41 patients in the Control Group which received the same treatment as of case group except Convalescent Plasma. Out of 82 patients of case group, 60 was male and 22 were females with mean age of 55.6±14.6 years (age range 26-85). 75 patients (91.46%) got discharged and 7 patients (8.54%) died. Co-morbidities (pre-existing illness) were present in 45 patients (54.88%).

Table 1 shows no statistical difference among the case group and Control Group patient with respect to gender and age. Further the both Case and Control group found statistically similar on the basis of outcome and co-morbidity.

It is observed form Table 2 that there is no statistical difference among the case group and control group patient with respect to Total Leukocyte Count (TLC), Oxygen saturation, duration of hospitalisation and inflammatory markers measured on Day 0, Day 3, Day 7.

In case group N : L value increases on day 3 from the baseline and significantly decreases on day 7. However, in Control Group it decreases on day 3 &

Table 1 — *Comparison between COVID-19 Patients with CP treatment (case group) and COVID-19 patients without CP treatment (control group)*

Demographic Factors	Case Group (N=82)	Control Group (N=41)	T test	Chi square test	P value
Gender :					
Male	60 (73.17%)	29 (70.73%)		0.08	0.77
Female	22 (26.83%)	12(29.27%)			
Age, year :					
Mean ± SD	55.6±14.6	56.8± 16.7	0.399	0.689	
Outcome :					
Discharged	75(91.46%)	36(87.80%)		0.416	0.51
Expired	7(8.54%)	5(12.20%)			
Comorbidity :					
COPD	1(1.21%)	3(7.31%)		3.23	0.07
CVA	0 (0%)	2(4.87%)		0.158	0.69
HTN	29(35.36%)	16(39.02%)		0.676	0.41
T2DM	24(29.27%))	15(36.58%)		0.051	0.47
IHD	3(3.63%)	2(4.87%)		0.504	0.477
CKD	1(1.21%)	2(4.87%)		2.446	0.117
Hypothyroidism	5(6.05%)	4(9.75%)		0.051	0.47
Asthma	1(1.21%)	0(0%)		1.537	0.215
Obesity	2 (2.42%)	0(0%)		0.051	0.47
Post Renal Transplant	1(1.21%)	0(0%)		1.537	0.215
None	37(45.12%)	18(43.90%)		0.016	0.899

Values are presented as number (%) or Mean ± SD

day 7 respectively. There is significant improvement in P: F value in both the groups.

Further, both Case and Control Group found statistically similar on the basis of saturation % and inflammatory markers.

Case group shows significant improvement in mode of ventilation and respiratory rate as compared to controls.

Table 3 shows comparison among variables day 0, day 3 and day 7 in case group before and after giving Convalescent Plasma-therapy.

Table 4 shows comparisons among variables in control group.

Fig 1 shows no significant difference in mean PF value post Plasma-therapy in cases when compared to control group.

Fig 2 shows significant difference in change of mode of ventilation post Plasma-therapy in cases as compared to controls.

Discussion

Our study explores the effectiveness of Convalescent Plasma-therapy in moderate to severely ill COVID-19 patients. We have taken 82 cases of COVID-19 and each of these were given 200-400ml of CP and effect of this was noted in different variables at 3rd and 7th day. 41 controls were taken which includes the COVID-19 patients who received all standard care except Convalescent Plasma.

The variables included were as follows : -

1. TLC count [effect noted as decrease or increase in TLC count], 2. N/L ratio (effect noted as whether there is decrease or increase in this ratio), 3. P/F ratio (with the help of ABG we have calculated Pao2 and then we calculated Pao2/Fio2 ratio), 4. Oxygen Saturation (SpO2), 5. Mode of ventilation, 6. Respiratory rate and 7. Duration of hospital stay.

Previous studies have reported the use of Convalescent Plasma transfusion in treatment of various infections. Convalescent Plasma obtained from COVID-19 recovered patients who had established humoral immunity against the virus, contains high neutralizing antibodies. These antibodies are capable of neutralizing SARS-CoV-2 and eradicating the pathogen from blood circulation and Lung tissues. In our study we have included the donors who were recently recovered from COVID-19 and had high titres of neutralizing antibodies.

We have found from our study that in cases there is significant improvement in all these variables in form of decreased TLC count, decrease in N/L ratio, increased P/F ratio, decrease in Respiratory Rate, improvement in Saturation and change in Mode of ventilation (patients wean off from oxygen support) on day 3 and day 7 after giving Convalescent Plasma-therapy when no comparison was made to Control group.

To increase the validity of our study we have also included 41 controls who received all standard care except Convalescent Plasma. We have compared the

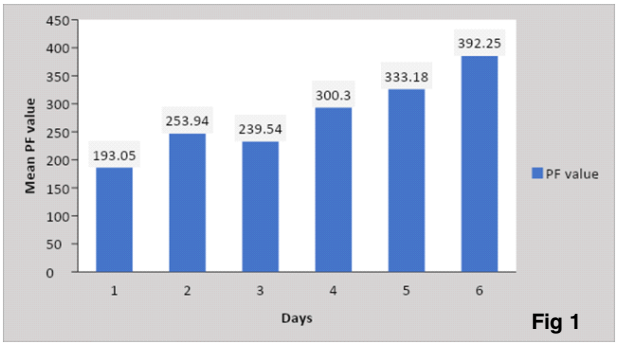

Fig 1

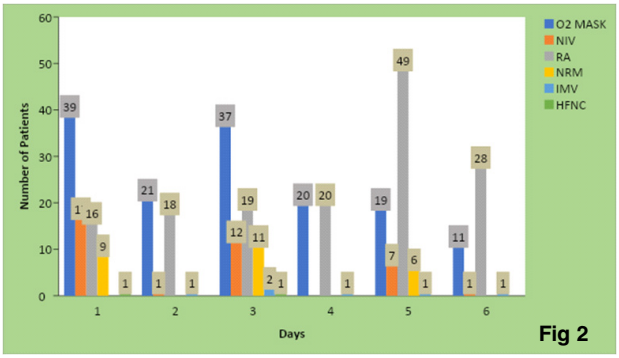

Fig 2

Table 2 — *Comparison between COVID 19 Patients with CP treatment (case group) and COVID 19 patients without CP treatment (control group)*

Clinicopathologic Factors		Case Group (N=82)	Control Group (N=41)	T test	Chi square test	P value
TLC value on Day 0	Mean ± SD	9.09 ± 4.17	10.05±6.10	1.026		0.30
TLC value on Day 3	Mean ± SD	10.78 ± 5.43	9.50±4.35	1.313		0.192
TLC value on Day 7	Mean ± SD	9.32 ± 4.26	9.32±4.95	0		1.0
NL Ratio value on Day 0	Mean ± SD	11.97 ± 10.43	10.65±12.30	0.352		0.725
NL Ratio value on Day 3	Mean ± SD	17.27 ± 19.2	10.18±13.87	2.104		0.03
NL Ratio value on Day 7	Mean ±SD	12.91 ± 11.93	7.95±10.78	2.243		0.02
PF value on Day 0	Mean ± SD	193.05 ± 92.64	253.94±90.89	3.458		0.00
PF value on Day 3	Mean ± SD	239.54±117.64	300.39±103.76	2.809		0.00
PF value on Day 7	Mean ± SD	333.18±149.09	392.25±144.21	2.094		0.00
Mode of Ventilation on Day 0	O2 MASK	39(47.56%)	21(51.41%)		19.207	0.00
	NIV	17(20.73%)	1(2.44%)			
	RA	16(19.51%)	18(43.90%)			
	NRM	9(10.97%)	0 (0%)			
	IMV	0(0%)	1(2.44%)			
	HFNC	1(1.21%)	0 (0%)			
Mode of Ventilation on Day 3	O2 MASK	37(45.12%)	20(48.78%)		17.733	0.01
	NIV	12(14.63%)	0 (0%)			
	RA	19(23.17%)	20(48.78%)			
	NRM	11(13.41%)	0 (0%)			
	IMV	2(2.42%)	1(2.44%)			
	HFNC	1(1.21%)	0 (0%)			
Mode of Ventilation on Day 7	O2 MASK	19(23.17%)	11(26.83%)		5.281	0.25
	NIV	7(8.53%)	1(2.44%)			
	RA	49(59.75%)	28(68.29%)			
	NRM	6(7.31%)	0 (0%)			
	IMV	1(1.21%)	1(2.44%)			
	HFNC	0 (0%)	0 (0%)			
Respiratory Rate (CPM) value on Day 0	Mean ± SD	30.46± 3.36	27.32±2.81	5.148		0.00
Respiratory Rate (CPM) value on Day 3	Mean ± SD	27.68 ± 4.07	24.73±3.24	4.042		0.00
Respiratory Rate (CPM) value on Day 7	Mean ± SD	24.70 ± 4.82	23.07±5.27	1.714		0.08
Saturation% on Day 0	Mean ± SD	93.72 ± 3.96	93.07±3.38	0.899		0.37
Saturation% on Day 3	Mean ± SD	94.87 ± 3.18	94.53±2.14	0.618		0.53
Saturation% on Day 7	Mean ± SD	95.29 ± 2.35	95.68±2.42	0.859		0.39
Duration of Hospitalization	Mean ± SD	10.162±6.19	9.27±3.84	0.793		0.43
Inflammatory Markers	Normal	2(2.42%)	0(0%)		1.017	0.06
	Raised	80(97.58%	41(100%)			

Values are presented as number (% or Mean ± SD

Table 3 — *Correlation between clinicopathologic factors of case group before & after convalescent plasma-therapy using one way ANOVA*

Clinicopathologic factors	Day 0 Pre-Plasma	Day 3 Post Plasma	Day 7 Post Plasma	F Test	P value
TLC value	9.09 ± 4.17	10.78 ± 5.43	9.32 ± 4.26	3.186	0.043
NL Ratio	11.97 ± 10.43	17.27 ± 19.2	12.91 ± 11.93	3.172	0.043
PF value	193.05 ± 92.64	239.54±117.64	333.18±149.09	28.066	0.000
Respiratory Rate	30.46± 3.36	27.68 ± 4.07	24.70 ± 4.82	39.833	0.000
Saturation%	93.72 ± 3.96	94.87 ± 3.18	95.29 ± 2.35	5.2149	0.006

Values are presented as number (%) or Mean ± SD

baseline characteristics of both case and control group. Both groups are almost similar in age and sex characteristics. These controls were selected from those COVID-19 patients who had not received Convalescent Plasma due to unavailability of donor or cross matched Plasma or who didn't give the consent.

While on comparing with Control Group (41 in number) the difference is significant only in improvement in Respiratory Rate and change in Mode of Ventilation. Otherwise, there is no significant difference in TLC count, N/L ratio, P/F ratio, and saturation between Case and Control group.

In Case group 39 patients (47.56%) were on Oxygen mask, 17 patients (20.73%) on NIV, 9 Patients on NRM (10.97%), 16 patients (19.51%) on room air, 1(1.21%) on HFNC initially. After 7th day of convalescent Plasma-therapy 49 patients (59.75%) were on room air which suggests significant improvement in mode of ventilation in case group as compared to control group.

Similarly mean Respiratory Rate in Case group was 30.46 CPM initially while in Control group it was 27.32 CPM and this difference was statistically significant. On day 7 mean respiratory rate was 24.7 CPM in case group while it was 23.07CPM in control group and the difference was statistically insignificant. It implies that there is significant improvement in respiratory rate in case group.

Comparison with other studies :

A large observational study finds the usefulness of Convalescent Plasma for treatment of COVID-19 patients. It shows that 7-day mortality and 30-day mortality were lower in those patients who received Convalescent Plasma within 3 days of onset of symptoms. The conceived trial from Netherlands was terminated early because they could not find any effect on mortality at 60 days, hospital stay or severity at 15 days. A randomised control trial of 103 patients

with severe COVID-19 in China shows no effect of Convalescent Plasma on time to clinical improvement. However, in that trial a subgroup of 45 patients with severe disease showed clinical improvement. One retrospective observational study conducted in South-West China explored the potential efficacy and safety of Convalescent Plasma treatment in 8 critically and severely ill patients which suggest early administration of Convalescent plasma may beneficial in improvement of clinical features. In a study conducted by ICMR (placid trial) did not show any benefit of giving Convalescent Plasma transfusion in disease progression and mortality. By using proper Convalescent Plasma collection with high neutralising antibody titre and timing of giving Plasma-therapy might hasten it being a more potential COVID-19 treatment.

Table 4 — *Correlation between clinicopathologic factors of control patients at different days using one way ANOVA*

Clinicopathologic factors	Day 0	Day 3	Day 7	F Test	P value
TLC value	10.05±4.35	9.50±4.35	9.32±4.95	0.216	0.805
NL Ratio	10.65±12.30	10.18±13.87	7.95±10.78	0.558	0.573
PF value	253.94±90.89	300.39±103.76	392.25±144.21	15.30	0.000
Respiratory Rate	27.32±2.81	24.73±3.24	23.07±5.27	12.153	0.000
Saturation%	93.07±3.38	94.53±2.14	95.68±2.42	9.589	0.000

Values are presented as number (%) or Mean ± SD.

Limitations of our study :

There are some limitations of this study. First, except for Convalescent Plasma, patients also received other standard care like antiviral treatment despite the uncertainty of the efficacy of the drug used. These antivirals might contribute to the recovery of patients or synergize with the therapeutic effects of Convalescent Plasma. Most of the patients received glucocorticoids which might interfere with Immune System and can cause delay in viral clearance[16,17]. Second is small sample size of the study group.

Despite of these limitations our study shows Convalescent Plasma might be a beneficial option for treating moderate to severely ill COVID-19 patients.

Conclusion

It is observed from this study that there is improvement in Lung Function (respiratory rate and mode of ventilation) whereas no significant effect on duration of hospital stays, laboratory parameters and mortality benefit. As there is development of variant SARS-CoV-2 strains, Convalescent Plasma donated by variant strain affected population may prove beneficial. Furthermore, evaluation and studies are required to see the long-term benefits like prevention of restrictive pattern and Fibrosis of lung by Convalescent Plasma-therapy. Plasma-therapy still holds a chance if given in early stage of disease and Convalescent Plasma donors having adequate antibody titre.

References

1 Zhou P — A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; **579:** 270-3. https://doi.org/10.1038/s41586-020-2012-7

2 Chen N — Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet* 2020; 395: 507-13. doi: 10.1016/S0140-6736(20)30211-7. Epub 2020 Jan 30. PMID: 32007143; PMCID: PMC7135076.

3 World Health Organization, Coronavirus disease (COVID-19) Pandemic. https://www. who.int/emergencies/diseases/novel-coronavirus-2019. Accessed 11 March 2020.

4 Casadevall A, Pirofski L — The convalescent sera option for containing COVID-19. *J Clin Invest*, 10.1172/JCI138003.

5 Convalescent plasma antibody levels and the risk of death from COVID-19, *New Eng J Med* 2021; 10.1056/NEJMoa2031893.

6 Agarwal A — Convalescent plasma in the management of moderate COVID-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID trial), *Brit Med J* 2020; 371:m3939 http://dx.doi.org/10.1136/bmj.m3939.

7 Simonovich VA — A randomized trial of convalescent plasma in severe COVID-19 pneumonia. *New Eng J Med* 2020; 10.1056/NEJMoa2031304.

8 Libster R — Early high titer plasma therapy to prevent severe COVID-19 in older adults. *New Eng J Med* 2021; 10.1056/NEJMoa2033700.

9 Joyner MJ — Early safety indicators of COVID-19 convalescent plasma in 5000 patients. *J Clin Invest* 10.1172/JCI140200.

10 Chen — SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with Covid-19. *N Engl J Med* 2020: 10.1056/NEJMoa2029849.

11 Bandyopadhyay, Mondal T, Mondal S — A Brief Outline of COVID 19 Specific Therapy in Hospitalised Patients In India Syamasis. *J Indian Med Assoc* 2020; **118(7):** 28-33] https://onlinejima.com/read_journals.php?article=447

12 BMJ 2020; 371 doi: https://doi.org/10.1136/bmj.m3939 (Published 22 October 2020) Cite this as: BMJ 2020;371:m3939

13 Gontu A, Srinivasan S, Salazar E — Limited window for donation of convalescent plasma with high live-virus neutralizing antibody titers for COVID-19 immunotherapy. Commun Biol 2021; 4: 267. https://doi.org/10.1038/s42003-021-01813-y

14 Legros V, Denolly S, Vogrig M — A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19 severity. Cell Mol Immunol 2021; **18:** 318-27 (2021). https://doi.org/10.1038/s41423-020-00588-2

15 Joyner MJ, Carter RE, Senefeld JW, Klassen SA, Mills JR, Johnson PW, *et al* — Convalescent Plasma Antibody Levels and the Risk of Death from Covid-19. *N Engl J Med* 2021; **384(11):** 1015-27. doi: 10.1056/NEJMoa2031893. Epub 2021 Jan

16 Raju R, Prajith V, Biatris PS — Therapeutic role of corticosteroids in COVID-19: a systematic review of registered clinical trials. *Futur J Pharm Sci* 2021; **7:** 67. https://doi.org/10.1186/s43094-021-00217-3

17 Beigel JH — What is the role of remdesivir in patients with COVID-19? *Curr Opin Crit Care* 2021; **27(5):** 487-92. doi: 10.1097/MCC.0000000000000866. PMID: 34353998; PMCID: PMC8416929.