



Efficacy Outcomes on morbidity and mortality of Intravenous Bolus Artesunate Therapy among Rapid Antigen Test and RT-PCR Negative Hospitalized Moderate to Severe Clinically Proven COVID-19 Patients: A Breakthrough large case series

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Abstract

COVID-19 infection is not restricted to respiratory system and is a multisystem inflammatory disease, involving complex interplay of immunological, inflammatory, coagulation cascades, age and co-morbidities manifested by elevated inflammatory markers, coagulation disorders and immunological dysfunction with variable clinical manifestations. The diagnostic gold standard RT-PCR test has frequent false negative results. Diagnosis of clinical COVID-19 infection among highly suspected RT-PCR negative moderate to severe cases were done by measurement of panel of diagnostic criteria from baseline routine blood test, inflammatory blood biomarkers, coagulation dysfunction and imaging studies. Further confirmation of diagnosis was made by testing of COVID-19 specific antibodies at appropriate follow up time. Artesunate have antiviral effects against SARS-CoV-2 in vitro studies. Empirical IV bolus Artesunate therapy of clinical COVID-19 associate with faster resolution of symptoms and appears to be very effective with excellent safety profile; prevents disease progression, thereby reduces morbidity and mortality among clinically diagnosed moderate to severe COVID-19 patients.

Keywords: COVID-19, IV Artesunate, Inflammatory blood biomarkers, diagnosis, COVID-19 antibodies.

Introduction

COVID-19 is not a localized respiratory infection, but a multisystem disease caused by a diffuse systemic process involving a complex interplay of immunological, inflammatory and coagulation cascades. Genetic and acquired differences in the host immune system further complicate the host repertoire leading to wide heterogeneity in the clinical picture, course and outcome. COVID-19 is a heterogeneous disease spectrum with manifestations varying with age and co-morbidities. Blood biomarkers play crucial role in early diagnosis in suspicion, monitoring, and recognition of complications and management of

patients. Testing panels of blood biomarkers, rather than single biomarker may provide more reliable information.¹

Strategies for Diagnosis of Clinical COVID-19 and IV Artesunate Therapy

Patients having RAT and RT-PCR test negative reports directly admitted in the indoor department of general medicine or shifted from COVID-19 isolation wards, clinically still suspected to have COVID-19 infection were diagnosed as clinical COVID-19, if they met ≥ 1 one of the following diagnostic criteria i.e. elevated inflammatory markers i.e. serum LDH, CRP, Ferritin and coagulation dysfunction i.e. D-dimer levels and

decreased lymphocyte, eosinophils and platelet counts, and/or with X-ray and HRCT chest findings. Severity of infection was determined clinically and after obtaining informed consent from patient or his/her relatives, empirical Artesunate 4mg/kg IV bolus (in 2-10 minute) was

administered twice daily for five days. Morbidity and mortality outcome and adverse reactions, if any were noted during the hospital stay. Further COVID-19 specific serologic antibodies tests for TAb, IgM, and IgG were done at appropriate time to confirm the final diagnosis.

Table 1 Clinical profiles of Rapid Antigen and RT-PCR test negative moderate to severe hospitalized Clinically Proven COVID-19 patients and their outcome with IV Bolus Artesunate Therapy

Sr.No	Age	Sex	Co-morbidity	Symptoms & signs on presentation	Ferritin ng/ml	LDH IU/L	CRP mg/L	D-dimer mg/L	X-Ray/HRCT	Antibody OD ratio >1 +ve	Outcome
1	48	M	Cirrhosis of liver & CKD	Jaundice-1 month. Fever-4 days, Abdomen distension & pain 1 day	>2000 & >1000	656 & 513	70.7	>5 & 4.2	L/L Pneumonia	RT-PCR +ve, IgG+ve-23.9	Improved & survived
2	64	M	T2DMCKD, HTN	SOB, Oedema, CHF-7 days	>1000	1239	24	20	B/L GGO & Cardiomegally	IgG +ve	Improved & survived
3	60	F	T2DM HTN, CCF, CKD	Cough, fever, SOB, Upper GI-bleed, LL DVT-5 days	463 & >1000	867 & 688	82 & 10.1	9.3	B/L-GGO, Pulmonary Oedema	IgG +ve -9.31, IgM+ve-2.98	Improved & survived
4	54	M	DM HTN	Fever, SOB-4 days	>1000 & >1000	403 & 1855	182 & 200	0.1 & 2.8	Rt.LLP, Multiple B/L GGO of lungs	IgG+VE-6.61, TAb-5.34+VE	improved & survived
5	52	M	T2DM	Fever -5 days, SOB-3days	1092 & 748	233 & 787	58 & 26.3	228 & 2.0	B/L patchy Pneumonia	IgG+ve-2.3 & TAb+ve-2.88	survived & improved
6	30	M	SCD	G.Weakness-5 days, SOB-1 day	>1000 & >100	1449 & 2878	47 & 85	13 & 8.5	CHF	IgG +ve	survived & improved
7	28	M	Cirrhosis of liver	Abdominal pain & Distension-7 days	640 & on 5 th day 523	560 & on 5 th day 316	3.9 & on 5 th day 10	3.0 & on 5 th day 12.1	B/L Pleural Effusion	IgG +ve	survived & improved
8	50	M	Cirrhosis of liver	Fever & Distension of Abdomen-5 days	869 & 728	831 & 916	32 & 30	8.5 & 9.0	Ascites & PHTN	IgG +ve-2.74	survived & improved
9	54	M		Fever-3 & Cough 7 days	884 & >1000	1205 & 1058	104.1 & 62.4	0.5 & 1.7	Ascites & PHTN	TAbs +ve-5.87 & IgG +ve-3.84	survived & improved
10	40	M	Gout?	Gen. Arthritis -25 days	540 & 1000	290 & 628	117 & 120	0.5 & 1.5	Hyperuricemia	TAbs +ve >10 & IgG 0.12	improved & survived
11	45	M	Nil	Fever, chest pain, SOB -7 days, Left Hemiparesis 1 day	414 & 5 th day 771	538 & 5 th day 749	5.9	10 & 5 th day 2121	Ischemic stroke Rt. MCA & HRCT-GGO Rt. lung, mild Rt. PE	IgG+VE-5.92, TAb+ve	improved & survived
12	40	M	Cirrhosis of liver	Abdominal Distension -2 months, Epistaxis -15 days,	519	279		20	Ascites & PHTN	IgG +VE-16.65, TAb+ve	Improved & Survived
13	62	F	DM HTN	Breathlessness-2 months, cough -2 month	70 & 3 rd day 74	431 & 3 rd day 440	9.4 & 3 rd day <5	0.2 & 3 rd day 0.8	L/L lung Patchy opacities	IgG +VE 7.2, TAb+ve	improved & survived
14	65	M	NIL	Fever-1 day, Cough & SOB-5days, PaO2 89%(RA)	181	698	116	0.2	B/L Lower zone GGO	IgG +VE	SOB decreased on 3 rd day, PaO2 94%(RA) & survived
15	53	M	Cirrhosis Alco	Increase Abdominal distension & Hiccough-8 days	697	471	70	11.7	Ascites-Exudative	IgG+VE-3.24, TAb-5.26	Stable & survived

16	70	M	HTN,DCM	SOB , Abdominal distension, puffy face-3days	143.9	299	46.93	4043.96	Cardiomegal ly, mild PE & pericardial effusion. EF- 62% , good LVF		Improved & stable
17	50	M	NIL	SOB-2 months, Fever, pain abdomen -4 days	245	495	< 5	1.7	Cardiomegal ly, mild B/L PE, Ascites, pericardial effusion, hepatosplen omegally	IgG-1.78, TAB-3.74 +ve	Stable & survived
18	38	M	NIL	Chest pain, palpitation, sweating-3 days.				40000 & 3 rd day 13693	Ant. Wall AMI	IgG-1.70 +ve	Stable & survived & discharged
19	33	M	NIL	Diarrhoea 4 episode 20 days back, weakness of extremities-10 days. (GBS?)	817	555	<5	6.1	Baseline weakness-3/5, after 5 days-4/5	IgG+ve -3.25	Improved & discharged
20	56	M	Alcoholic Liver Disease	Abdominal Distension 10 days, hiccough-7 days. Ascites, thickened Omentum -TB peritonitis?	657	417	70	11.7	Ascites & PHTN	IgG +ve-3.24, TAB+ve-5.26 on 14 th day from symptoms onset	Improved
21	70	M	DCM	Swelling abdomen & pedal edema-15 days, SOB-10 days.CCF, Rt. Side exudative pleural effusion.	463	799	<5	1.9	DCM with features of CHF	IgG+ve-1.27, TAB +ve-1.33 on 18 th day from onset of symptoms	Improved
22	33	F	ESRD.HTN	H/O Fever 5 days 1 month back. Vomiting altered sensorium, decreased urination-4 days, & dyselectrolytemia.	678		5	1.3	Conscious with increased urination	IgG +ve-3.33	Improved & on haemodialysis.
23	57	M	HTN,COPD NASH	Diarrhoea-8 days, vomiting & SOB-2 days. PaO2-70% (RA)	178.3	583	75		Hypokalemi -a	IgG +ve-17.5, TAB +ve-2.88 on 10 th day	Improved
24	65	F	CKD, COPD	Fever 3 days 1 moth back, Generalized anasarca 1 month, Oliguria 10 days & SOB 7 days SpaO2 89% (RA).	273	406	32	0.2	PaO2 98% after 5 days with adequate urination.	IgG-0.8 & TAB+ve 3.77 on 10 th day test	Markedly improved & on dialysis.
25	69	F	CKD,HTN,C CF	SOB-5 days, cough-2 days, edema both leg- 5 days. PaO2-82%.B/L PE.	270	492	80	0.04	PaO2-98% after 4 days	IgG-2.57 +ve & TAB-5.52+ve on 10 th day.	Improved.
26	22	F		fever with chills for 5 days 12 days back ,SOB for 6 days, vomiting and fever for 1 day	413	299	64.32	1306l	Fever , vomiting subsided after 48hr	TAB-1.66 and IgG-10.25 OD	Improved.
28	72	M	T2DM	Fever, loss of appetite 15 days,SOB,PaO2-88%,& CHF	260	291 IU/L	10	0.1mg/ L	X-ray & CT +ve GGO	IgM +ve 4.33 & IgG +ve -7.54	Improved
29	53	M	Br. Asthma	Fever, cough 15 days, SOB 3 days,PaO2-94%	445	698	84	<0.1	PaO2-96% 3 rd day	IgG +ve 20 Index & TAB +ve 7.01 OD ratio	Improved & discharged on 5 th day
30	42	F		Fever 7 days, altered sensorium 1 day with h/o pf +ve.	466 & 360 5 th day	500	28.25 & 5.2 on 5 th day	5.65 & 1.93 on 5 th day	X-ray chest B/L peripheral GGO , HRCT-CORAD- 4	Follow up 14 th day IgM +ve 1.5	Improved & survived
31	19	M	NIL	Fever-21days & Gen – weakness- 7 days	852	879	45.3	0.13	Pallor+++ ,pf -ICT +ve, MP-QBC-ve	IgM-1.32 +ve	Improved & survived

Cases Presentation

Case 1 A 48 years old male alcoholic cirrhosis of liver and CKD with COVID-19 RT-PCR positive history 3 months back presented with yellow coloration of urine and jaundice for 2 months, fever for 4 days and abdominal distension 1 day. On investigation haemoglobin was 8.7 gm%, TLC $14.14 \times 10^3/\mu\text{L}$, neutrophils 84%, lymphocyte-8.4%, serum ferritin was $>2000\text{ng/ml}$, LDH 656 IU/L, CRP-70mg/L, D-dimer 5 mg/L, serum urea 93mg/dl, creatinine -3.7mg/dl, serum albumin 2.7mg/dl, total serum bilirubin was raised 12.32mg/dl with mildly elevated transaminases levels. X-ray chest showed left lower lobe pneumonia and HRCT showed ground glass opacities (GGO). Bolus IV Artesunate 4mg/kg administered twice daily for 5 days and received 3 blood transfusions. Fever subsided after 3 days and patient felt better. Follow up serum ferritin after 5th day was 1000ng/ml, LDH - 513 IU/L and D-dimer 4.25ng/ μL . COVID-19 specific antibodies (ELISA) IgG -23.90 and IgM 0.67 OD ratios positive. This was a case of severe long COVID-19 syndrome with cirrhosis of liver with CKD.

Case 2 A 64 years old male presented with shortness of breaths (SOB) and swelling of both feet for 7 days. On examination pulse rate 100/min, PaO₂ was 86% with room air (RA), Respiratory Rate (RR)- 28/min. On investigation TLC was $1200/\mu\text{L}$, serum Na⁺ -123mEq/L, K⁺-3.2mEq/L, Ferritin $>1000\text{ng/ml}$, LDH-1239 IU/L, CRP-26mg/L, D-dimer-20mg/L. X-ray chest revealed peripheral lesions characteristic of COVID-19 with cardiomegally. Treated with IV Artesunate for 5 days along with standard treatment of heart failure. All symptoms improved after 5 days and covid-19 specific IgM and IgG antibody were positive (qualitative). This was a case of underlying cardiomyopathy and severe acute COVID-19 infection with hypokalaemia and CHF.

Case 3 A 60 years old female with h/o T2DM, hypertension, CKD presented with history of cough, fever, SOB and upper GI bleeding for 5

days. On examination BP-130/70mmHg, pulse-100/min, RR-28/min, PaO₂-94% (RA). On investigation TLC- $13,15 \times 10^3/\text{L}$, N-70.8%, L-22.9%, Serum ferritin-463ng/ml, LDH-867 IU/L, CRP-82 mg/L, D-dimer-9.3 mg/L, serum urea-80mg/dl and creatinine -2.4mg/dl and on 3rd day ferritin level 1000ng/ml, LDH 688IU/L and CRP-101 mg/L. HRCT of thorax showed bilateral GGO with pulmonary oedema. Treated with IV Artesunate for 5 days and symptoms improved markedly. Follow up COVID-19 antibody tests positive for IgG-9.31 and IgM-2.98 OD ratio. This was a case of moderately severe acute COVID-19 infection.

Case 4 A 54 years old T2DM and HTN male presented with fever and SOB for 4 days. On presentation, BP-140/90mmHg, pulse-96/min. RR-36/min, PaO₂-94%(RA), x-ray chest showed right lower lobe GGO and HRCT- multiple GGO bilaterally, CT severity score of 10/25. Baseline serum ferritin level was $>1000\text{ng/ml}$, LDH-403 IU/L, CRP-182 mg/L, D-dimer 0.1 mg/L. Patient treated with IV Artesunate and symptoms improved within 5 days. Follow up Ferritin on 3rd day was $>1000\text{ng/ml}$, LDH -1855 IU/L, CRP-100 mg/L, D-dimer-2.8 mg/L. Follow up COVID-19 antibodies tests positive for IgG-6.61 and TAb-5.34 OD ratio. This was a case of moderately severe acute COVID-19 infection.

Case 5 A 52 years old male presented with fever for 5 days and SOB for 3 days with h/o T2DM. On examination BP-110/70mmHg, pulse-92/min, RR-30/min, PaO₂-98 % (RA). On investigations TLC- $7.9 \times 10^3/\text{L}$, TPC-2.3/ μL , Hb-12gm%. Serum ferritin-1092ng/ml, LDH-233 IU/L, CRP-58 mg/L, D-dimer-228 mg/L. Chest x-ray- bilateral patchy GGO. Treated with IV Artesunate along with other standard treatment and symptoms improved and follow up ferritin level on 5th day decreased to 748ng/ml, LDH-increased to 787IU/L, CRP-decreased to 26.3 mg/L and D-dimer-to 2.0 mg/L. Follow up antibodies were positive for IgG-2.3 and TAb-2.8 OD ratio. This was a moderately severe acute covid-19 infection with T2DM.

Case 6 A 30 years old male known sickle cell disease(SS), presented with general weakness for 5 days, polyarthralgia and SOB for 1 day .On examination BP-130/80mmHg, pulse-98/min, PaO₂-90%(RA). On investigations serum ferritin was >1000ng/ml, LDH-1949 IU/L, CRP-47 mg/L, D-dimer-13 mg/L and IV Artesunate administered for 5 days. Patient's oxygen saturation increased with symptoms improvement. Follow up serum ferritin on 5th day was to 100ng/ml, LDH-2878 IU/L, CRP-85 mg/L ,D-dimer-8.5 mg/L. Follow up antibody was positive for IgM and IgG. This was a case of severe acute **COVID-19** infection precipitated Vaso-occlusive Crisis in sickle cell disease.

Case.7. A 28 years old male, known alcoholic cirrhosis of liver presented with distension and pain abdomen for 7 days. On examinations BP-110/70 mmHg, pulse-80/min, PaO₂-97%. TLC-4000/ μ L. TPC-66000, Hb-9.7gm%,Na+-138mEq/L,K+-3.4mEq/L. Serum ferritin level was 640ng/ml, LDH-560 IU/L, CRP-3.9 mg/L , D-dimer-3.0 mg/L , x-ray chest-B/L pleural effusion. COVID-19 IgG antibody was positive. Follow up ferritin after 5 days was 523ng/ml, LDH-316 IU/L, CRP-10 mg/L and D- dimer -12.1 mg/L . Patient's symptoms improved with 5 days of Artesunate therapy. This was a case of moderately severe acute COVID-19 infection with cirrhosis of liver.

Case 8 A 50 years old known male alcoholic cirrhosis of liver presented with fever and distension of abdomen for 5 days. On investigation serum ferritin was 867ng/ml, LDH-831 IU/L, CRP-32 mg/L, and D-dimer was 8.9 mg/L . After 5 days of Artesunate therapy along with standard therapy, patient's symptoms improved and serum ferritin was 728ng/ml, LDH- was 916 IU/L, CRP-30 mg/L and D-dimer was 9 mg/L. Follow up IgG antibody test done was positive (2.74 OD ratio) .This was a moderately severe acute COVID-19 infection with cirrhosis of liver.

Case 9 A 54 years old male chronic alcoholic presented with h/o fever for 3 days and cough for

7 days with alcohol withdrawal syndrome. On examination BP-80/50mmHg, pulse-128/min, PaO₂-94% (RA), RR-19/min. On routine blood test TLC-9980/ μ L,L-7.3%.E-0%,L-0.72 X 10³ / μ L, TPC-171000/ μ L, Hb-11.8gm%, Na+-143mEq/L,K+-2.6mEq/L. Baseline serum ferritin was 884ng/ml,LDH-1205 IU/L,CRP-104 mg/L ,D-dimer-0.5mg/L . Follow up serum ferritin was >1000ng/L, LDH-1058 IU/L,CRP-62.4 mg/L, D-dimer-1.7 mg/L. Patient improved with IV Artesunate for 5 days and follow up antibodies were positive (TAb-5.87 and IgG-3.84 OD ratio). This was a case of moderately severe acute COVID-19 infection.

Case 10 A 40 years old male presented with severe generalized arthritis for 25 days. On examination BP-110/80mmHg,pulse-86/min,RR-15/min On routine blood test TLC-10.49X10³ / μ L,E-2%, L-28%, TPC-460X10³/ μ L,Hb-10.3gm%, Na+-139mEq/L, K+-4.0mEq/L. Baseline serum ferritin was 540ng/ml,LDH-290 IU/L, CRP-117mg/L ,D-dimer-0.5 mg/L. After 3rd day of Artesunate therapy repeat ferritin was 1000ng/ml, LDH-628 IU/L, CRP-120 mg/L , D-dimer-1 mg/L and serum uric acid was 10mg/dl. Patient improved with IV Artesunate therapy. Follow up antibody positive for TAb >10 and IgG-0.12 OD ratio. This was a case of moderately severe acute COVID-19 infection with hyperuricemia.

Case 11 A 45 years old male presented with h/o intermittent fever, chest pain, SOB for 7 days and weakness of left upper and lower limb for one day with altered sensorium .CT scan of brain showed Rt. Side MCA territory ischemic infarction. On examination BP-140/90mmHg, pulse-84/min, PaO₂-88% (RA), and 98% with 2L/min O₂ therapies put on artificial ventilation for >5 days. On routine test TLC-11.8X10³/ μ L,N-84%,L-7.5%, E-0.3%,M-3.3%, TPC-179X10³/ μ L, Hb-11.79gm%, K+-3.8mEq/L,Na+-140mEq// μ L. Baseline Serum ferritin was 414ng/ml,LDH-538 IU/L,CRP-5.9 mg/L,D-dimer >10,000 mg/L. After 5 days of Artesunate therapy repeat D-dimer test was 2121 mg/L, Troponin -I was 116 ng/ml and

CPK-MB was 15 IU. After seven days ,repeat ferritin level was 771ng/ml,LDH-749 IU/L,CRP <5 mg/L ,D-dimer-3.2 mg/L.HRCT of thorax done after 15th day showed B/L upper and superior segment of lower lobe fibrocalcification suggestive of old PTB and diffused GGO in Rt. lung field with cardiomegally and mild pleural effusion and fissural collection of fluid. Ultimately patient recovered. Follow up antibodies test for COVID-19 was positive for IgG-5.92 OD ratio after 21th day. This was a case of critically severe acute COVID-19 infection with acute ischemic stroke in MCA territory.

Case 12 A 40 years old with known alcoholic cirrhosis presented with epistaxis and increasing abdominal distension for 15 days and h/o fever with chill for 1 day 4 months back. On examination BP-110/70mmHg, PR- 80/min, PaO₂-99%. On Routine blood test TLC-13940/ μ L, N-73%,L-10.9%, E-0%,B-0.9%, TPC-61000/ μ L, Hb-9.3gm%. Baseline serum ferritin-519ng/ml, LDH-279 IU/L, D-dimer-20 mg/L . Follow up repeat ferritin was 638ng/ml, LDH-354 IU/L. Patients improved with Artesunate therapy with resolution of symptoms and signs. Follow up IgG antibody for COVID-19 was positive (16.65 OD ratio) at the time of discharge. This may be a case of moderately severe prolonged COVID-19 infection in cirrhosis of liver with portal hypertension.

Case 13 A 62 years old female with h/o T2DM and HTN presented with cough and SOB for 2 months. On examination her BP was 120/70mmHg, pulse-84/min, PaO₂-94 % (RA) and RR-32/min. On routine blood test TLC-29340/ μ L, lymphocyte - 1.5×10^3 / μ L, TPC-308000/ μ L,Hb-12gm%, serum K⁺3.3mEq/L, Na⁺140mEq/L. Chest x-ray showed Left/LL patchy opacities. Serum ferritin at baseline was 70ng/ml, LDH-431 IU/L.CRP-9.4 mg/L, D-dimer-0.2mg/L. Repeat test for ferritin was 70ng/ml, LDH-440 IU/L, CRP was < 5 mg/L . PaO₂ increased to 98% (RA) on 3rd day after Artesunate therapy along with decrease of breathlessness and cough and discharged on 7th day. Follow-up COVID-19

antibody TAb and IgG was positive (16.6 and 20 OD ratio), suggested a case of moderately severe ongoing symptomatic COVID-19 infection.

Case 14 A 65 years old male presented with **fever for 1 day, cough & SOB for 5 days** .On examination BP-120/74mmHg, pulse-86/min, PaO₂-89% (RA), and 96% with O₂ 4L/min & 94% on day 3 (RA).Baseline routine blood test TLC- 6.9×10^3 / μ L,N-74.8%,L-12.6%,M-9.1%,E-3.2%, B-0.3%,TPC-203 $\times 10^3$ / μ L,Hb-11.19gm%, Na⁺-140mEq/L, K⁺-3.8mEq/L. Baseline serum ferritin -181ng/ml, LDH-698 IU/L,CRP-116 mg/L, D-dimer-0.2 mg/L , Chest x-ray-B/L Lower zone GGO. Follow up COVID-19 only antibody test done was positive for IgG +ve- 5.29 OD ratios. This was a moderate severe case of acute COVID-19 infection.

Case 15 A 53 years old male with chronic liver disease (alcoholic cirrhosis), with h/o repeated abdominal paracentesis for ascites presented with increased abdominal distension and hiccough for 8 days. On examination BP-118/80mmHg, pulse-80/min, PaO₂-98%, RR-14/min. On routine blood tests TLC- 5.17×10^3 / μ L,N- 3.5×10^3 / μ L,L- 0.50×10^3 / μ L,TPC-256 $\times 10^3$ / μ L,Hb-13.6gm%, Na⁺-130mEq/L, K⁺-4.3mEq/L. Serum ferritin was 657ng/ml, LDH-477 IU/L,CRP-70 mg/L,D-dimer-11 mg/L. Ascitic fluid analysis showed exudative in nature with TLC-3000/ μ L with lymphocytes predominance, total protein - 4.15gm%, albumin-2.01gm% and ADA postive 122 OD ratio. Patient improved with Artesunate therapy. Follow up antibody test positive for IgM and IgG (qualitative). This was a case of moderately severe acute COVID-19 infection reactivation of TB peritonitis.

Case 16 A 70 years old known Hypertensive male on Amlodipine 5 mg daily, presented with h/o exertion dyspnoea and abdominal distension and puffy face for 3 days. On examination his BP was 140/80mmHg, pulse-100.min, PaO₂ was 95 % (RA). Routine blood test showed TLC- 7.5×10^3 / μ L, N- 4.76×10^3 / μ L, L- 1.92×10^3 / μ L, TPC-220 $\times 10^3$ / μ L,Hb-10.9gm%.Na⁺-135mEq/L, K⁺-3.6mEq/L. Baseline serum ferritin was

143.9ng/ml, LDH-299 IU/L,CRP-46.93 mg/L ,D-dimer-5043.96 mg/L. Chest x-ray showed gross cardiomegally. Ultrasonography of abdomen showed dilated IVC and dilatation of hepatic vein and right ventricle with mild PE.Follow up ECHO study of heart showed moderate pericardial effusion with ejection fraction 62% with good LV function. COVID-19 Antibody test was positive for IgM. Patient symptoms and signs improved with Artesunate therapy for 5 days. This was a case of moderately severe acute COVID-19 infection with CHF.

Case 17 A 50 years old male presented with h/o SOB-2 moths, fever and pain abdomen for 4 days. On examination BP-100/70mmHg, pulse-80/min, PaO₂-96 % (RA). Routine blood test TLC-15.46 X10³/μL,N-72.5 X10³/μL ,L-22.45,E-0.6%,M-4.5%, B-0.1%,TPC-17.8 X10³/μL. Baseline serum ferritin was 245ng/ml, LDH-495 IU/L,D-dimer <5.0 mg/L . X-ray chest showed Rt. sided mild loculated pleural effusion. CT abdomen showed hepatosplenomegally with minimal ascites. On ECHO of heart there was severe concentric LVH with global hypokinesia, moderate LV dysfunction, mild MR, mild pericardial effusion and mild AR and TR. Treated with IV Artesunate 5 days. Follow up COVID-19 antibody was positive for IgG-1.78 and TAb-3.14 OD ratio. This was a case of moderate severe ongoing symptomatic COVID-19 infection with CHF.

Case 18 A 38 years old male presented with h/o chest pain, palpitation and sweating for 3 days. On examination BP-100/60mmHg, pulse-88/min, PaO₂-98% (RA) ,RR-20/min. Routine blood test revealed TLC-6.70 X10³/μL, N-3.6 X10³/μL ,L-2.17 X10³/μL ,TPC-229 X10³/μL ,Hb-15gm%, ESR-35mm, Na⁺-138mEq/L, K⁺-2.8mEq/L. Ferritin-560ng/ml, LDH-500IU/L. lipid profile – TC-105mg%, HDL-24mg%, TG-70mg%,LDL-47%, ECG showed acute anterior wall myocardial infarction. Baseline Serum troponin-I was 40,000 ng/ml and after 2 days it was 13693ng/ml. Patient responded to IV Artesunate very well. Follow up COVID -19 antibodies IgG was positive 1.70 OD ratio on 10th day after onset of symptoms. This

was a case of moderately severe acute COVID-19 infection precipitated AMI.

Case 19 A 33 years old male presented with quadraparesis for 10 days with h/o bout of loose motion of 4-5 episodes 20 days back. On examinations BP-110/70mmHg, pulse-84.min, PaO₂-98 % (RA), RR-16/min. On routine blood test TLC-4.91 X10³/μL, N-60.3 X10³/μL, L-16.9 X10³/μL,E-2.3 X10³/μL,M-19.5 X10³/μL,B-1%. Serum Na⁺ was 134mEq/l, K⁺ 4.4mEq/L, Serum ferritin was 817ng/ml, LDH-555 IU/L, CRP < 5 mg/L, D-dimer-6.1 mg/L. Treated with IV Artesunate for 5 days. Follow up COVID-19 IgG done only was positive 3.25 OD ratios. Diagnosed to be a case of GBS by nerve conduction study. Patient improved and discharged after able to walk on 14th day. This was a case of moderately severe acute COVID-19 infection presented like GBS.

Case 20 A 56 years old male chronic alcoholic presented with increased distension of abdomen for 10 days and hiccough for 7 days. On examination BP-114/80mmHg, pulse-100/min. On routine blood test TLC-5.17X10³ /μL,N-68%,L-9.7%,M-20.8%, E-0.7%,B-0.8%,TPC-256X10³ /μL. Serum ferritin was 657ng/ml, LDH-477 IU/L, D-dimer-11.7 mg/L. USG study showed gross ascites with thickened peritoneum.COVID-19 antibody positive for IgG-5.26 and TAb -3.24 OD ratios. Patient improved with IV Artesunate therapy. Ascites fluid was exudative in nature with lymphocyte predominance and ADA positive This was a case of moderately severe acute COVID-19 infection with TB Peritonitis.

Case 21 A 70 years old male on treatment for DCM & CHF stable for 1 year, presented with swelling of abdomen and both legs for 15 days and SOB for 10 days. On routine blood test TLC-10.5X10³ /μL,N-85.8%,L-6.8%,M-7.2%,E-0.2%, TPC-13.79X10³ /μL. serum Na⁺129mEq/L,K⁺-3.3mEq/L. Serum ferritin-463ng/ml, LDH-799 IU/L, CRP- 5 mg/L, D-dimer-1.9 mg/L, troponin-I -49 IU/L. X-ray chest showed right sided pleural effusion (exudative).USG abdomen revealed mild ascites, moderate right sided pleural effusion and

features of CKD. ECHO of heart showed mild MR with severe LV dysfunction with EF-26%. COVID-19 antibody positive for IgG-1.27 and TAb- 1.33 OD ratios on 20th day from symptom onset. Patient improved with Artesunate therapy and discharged in good conditions. This was a case of moderately severe acute COVID-19 infection precipitated CHF.

Case 22 A 33 year old known CKD and hypertensive female presented with intractable vomiting and altered sensorium and decreased urination for 4 days, with h/o fever for 5 days 1 month back. On examination BP-100/70 mmHg, pulse-80/min, PaO₂-94 % (RA), RR-18/min. On routine blood test TLC-18.10X10³/μL, N-96.4%, L-1.5%, M-2.1%, TPC-147 X10³/μL, Hb-8.7%, Na+-104 mEq/L, K+-4.4mEq/L and serum ferritin 678ng/ml, CRP-5.0 mg/L, total CPK-371μg/L, serum urea-128mg/dl, serum creatinine-7.1mg/dl. Follow up IgG COVID-19 antibody positive 3.33 OD ratios. After correction of serum sodium and Artesunate therapy, patient conscious improved and was on maintenance dialysis. This was a case of moderately severe ongoing symptomatic COVID-19 with acute AKI on CKD with hyponatraemia.

Case 23 A 57 years old known male hypertensive, COPD and NASH patient presented with loose motion, vomiting, pain abdomen and SOB for 2 days in low conditions. On examinations his BP was 80/50mmHg, pulse-90/min, PaO₂-70%(RA), and 92% with 5 LO₂/min, RR-21/min. On routine blood test TLC-13.2 X10³/μL, N-12.1 X10³/μL, L-1.8 X10³/μL, TPC-12.2 X10³/μL, Hb-12.2gm/dl, Na+-142mEq/L, K+-3.1mEq/L. Serum ferritin was 178.30ng/ml, LDH-583 IU/L, CRP-75 mg/L, Blood urea-81mg/dl, serum creatinine-2.9mg/dl. Patient treated with IV Artesunate, nasal oxygen therapy and replacement of fluid with potassium and markedly improved within 48 hours. Follow up antibody was positive for IgG -17.5 and TAb-2.88 OD ratios. This was a case of severe acute COVID-19 presented with gastrointestinal manifestation.

Case 24 A 65 years old known CKD and COPD female presented with history of fever for 3 days 1 month back and generalized anasarca for 1 month, decreased urination for 10 days and SOB for 7 days with SpO₂ of 89%(RA), BP-120/70mmHg, pulse-80/min, RR-21/min. Baseline ferritin was 173ng/ml, LDH-402 IU/ml, CRP-32mg/L, D-dimer-0.2 mg/L, blood urea-92mg/dl, creatinine-4.0mg/dl chest x-ray showed features of COPD. Patient on nasal oxygen for 7 days and SpO₂ became 98 % (RA) on 7th day. COVID-19 antibody IgG -0.8 and TAb 3.77 OD ratios was positive on 10th day from symptom onset. Patient markedly improved and discharged. This was a case of severe ongoing symptomatic COVID-19 case precipitated AKI on CKD.

Case 25 A 68 years old known HTN and CKD female patient presented with SOB and swelling of both leg for 5 days and cough for 2 days with features of CHF. On examination her BP was 160/100mmHg, pulse-78/min, SpO₂-82 % (RA), RR-28/min. On routine blood test TLC-7.15X10³/μL, N-74%, L-13%, E-0.9%, L-6%, TPC-250,000 /μL, Hb-8.4 gm%, Na+-133mEq/L, K+-4.2mEq/L. Serum ferritin was 270ng/ml, LDH-492 IU/L, CRP-80mg/L, D-dimer-0.04 mg/L, urea-53mg/dl, creatinine-3.8mg/dl. Chest x-ray showed B/L pleural effusion. USG study showed B/L PE and moderate pericardial effusion. ECHO study of heart showed mild MS, sclerotic aortic valve with calcification, pericardial effusion, Grade I diastolic dysfunction with normal LV systolic function. She was improved with IV Artesunate therapy and COVID-19 specific antibodies on 10th tested positive for IgG-2.57 and TAb-5.52 OD ratios. This was a case of severe acute COVID-19 with CKD with anemia.

Case 26 A 22 years old female presented with fever with chills for 5 days 12 days back and SOB for 6 days and again fever and vomiting for 1 day. On examination her BP was 110/70 mmHg, pulse 96/min, temperature 102⁰ F, RR-22/min, PaO₂-96% (RA). On routine blood test, TLC-16500/μL, N-87.2%, L-8.8%, M-2.5%, E-1.5%, TPC-577000/μL, Hb-9.2gm/dl, Na+-136mEq/L,

K⁺-3.6mEq/L. Baseline serum ferritin was 413ng/ml, LDH-299 IU/L,CRP-64 mg/L and after 5 days 32 mg/L, ESR-109mm, D-dimer-130 mg/L. Fever and vomiting subsided within 48 hours after IV Artesunate therapy and serum ferritin was increased to 719ng/ml and LDH-708 IU/L, D-dimer decreased to 1.2 mg/L on 4th day. COVID-19 antibodies tested on 14th day were positive for TAb-1.66 and IgG-10.25 OD ratios. It was a case of moderately severe acute COVID-19 infection.

Case 27 A 55 years old known male diabetic presented with h/o intermittent fever since 5 months and yellow coloration of urine since 20 days and jaundice for 15 days. On examination his BP was 110/70mmHg, PR-84/min, RR-15/min, severely pallor, PaO₂-90 % (RA), FBS->500mg/dl, managed with insulin, pioglitazone, Metformin and Teneligliptin. On routine blood test TLC-2.29X10³ /μL, TPC-148X10³ /μL, Hb-3.1gm/dl with biocytopenia. Blood biomarker ferritin was >1000ng/ml, LDH-370 IU/L, D-dimer-82 mg/L, Blood urea-196mg/dl,creatinine-9.0mg/dl. USG study showed hepatosplenomegally. Fever subsided after 3 days of Artesunate therapy and Hemoglobin level remained stable at 6gm/dl after three blood transfusions. After 5 days of Artesunate therapy repeat serum ferritin was >1000ng/ml, LDH-301 IU/L, D-dimer-7.1 mg/L. COVID-19 antibody test was done after 7 days of hospitalization tested positive for TAb-6.16 and IgG was >20 OD ratios. It was a case of severe ongoing symptomatic COVID-19 or a long COVID-19 syndrome.

Case 28 A 72 years old known T2DM, CAD male presented with cough with expectoration, chest pain and SOB for 3 days. On examination his BP was 100/80mmHg, pulse rate 130/min, RR-42/min, PaO₂ was 88 % (RA). On routine blood test TLC -20.7x10³ /μL,N-74%, L-17%,E-9%, Na+126mEq/L, K+-4.0mEq/L. Blood biomarker-ferritin was 260ng/ml, LDH-291 IU/L, D-dimer-0.1 mg/L, Troponin-I-45.9ng/dl, chest x-ray showed GGO of right middle and lower lobe of lung. HRCT of thorax showed patchy

consolidation of right middle and multifocal GGO in bilateral upper lobe.2D ECHO of heart showed RWMA in LAD, LCX territory with mild MR with severe LV dysfunction. Patient treated with IV Artesunate and his oxygen saturation increased to >94% (RA) on 3rd day with marked improvement with PaO₂ >98 % (RA) on 5th day. COVID-19 antibody test for IgM was positive 4.33 and IgG-7.54 OD ratios. This was a case of severe acute COVID-19 infection.

Case 29 A 53 years old male of known bronchial asthma on maintenance Formonide inhalation 200 μg twice daily presented with intermittent fever and loss of appetite for 15 days, general weakness for 12 days , cough with expectoration and SOB for 5 days with PaO₂ 93% (RA).On routine blood test TLC-9.18X10³/μL,N-81%,L-11.8%,M-6.4%, E-0.7%, Hb-13.4g/dl.TPC-302X10³/μL. Serum K⁺-3.2mmol/L, Na⁺-130mmol/L. Serum LDH was 698 IU/ml,CRP-84 mg/L , D-dimer was <0.01 mg/Land serum ferritin was 445ng/ml. Repeated serum LDH on 4th day was 514 IU/L, CRP-65.7 mg/L, ferritin-993ng/ml, D-dimer- 0.1 mg/L. HRCT of thorax done on 1st day of admission was characteristic of COVID-19 infection with CT score of 12/25 (moderate severity). His fever subsided after one day of Artesunate therapy and oxygen saturation on 2nd days 98% (RA) and discharged on 5th day. Follow up COVID-19 antibody test positive for IgG positive >20 index (CLIA) and TAb- 7.01 OD ratios (ELISA) on 20th day. This was a case of moderately severe acute COVID-19 infection.

Case 30 A 42 years old female presented with intermittent fever with chills for 7 days and altered sensorium with irritability (GCS-8/15) from 6th day and primary referral report with ICT pf. Malaria positive .O/E BP-110/70 mmHg, PR-100/min, RR-20/min,Temp-100.1⁰ F ,SpO₂ 94%(RA), Pallor present. Baseline TLC-3.8X10³/μL, N-44%,L-38.4%,E-0.3,M-16.5%, Hb-6.0gm/dl, TPC-91X10³/ μL,Urea-53mg/dl, Creatinine-1.3gm/dl, CRP-28.25 mg/L, D-dimer-5.65 mg/L, Ferritin 466ng/ml.MP-QBC and ICT for malaria was negative .Chest x-ray showed B/L

peripheral lung infiltrates with GGO. She was treated with IV Artesunate for 5 days and markedly improved within 48 hrs and repeated biomarkers tests on 5th day showed TLC- $5.72 \times 10^3/\mu\text{L}$, N-89%, L-4.6%, M-6.4%, E-0.0%, Hb-6.2gm%, TPC- $284 \times 10^3/\mu\text{L}$, Creatinine-1.0mg/dl, Urea-31mg/dl, CRP-5.2 mg/L, D-dimer-1.93 mg/L, serum Ferritin-360ng/ml. SARS-CoV-2 antibody (ELISA)- IgM was positive (1.50 OD ratio) on 14th day of illness and HRCT thorax showed CORAD-4(severity Index-3/25). This was a case of cerebral malaria with moderately severe acute COVID-19 infection.

Case 31 A 19 old male presented with intermittent fever with chills and rigor for 21 days and profound weakness for 7 days. On examination there was severe pallor, BP-80/60mmHg with vasopressor, PaO₂-98%(RA), PR-136/min, RR-30/min. On routine blood test -TLC- $11.23 \times 10^3/\mu\text{L}$, N- $6.73 \times 10^3/\mu\text{L}$, L- $3.53 \times 10^3/\mu\text{L}$, N-59.9%, I-31%, M-7.6%, E-0.6%, B-0.6%.Hb-3.7gm%,CV-71fl,MCH-23.2pg.Dengue Ns1 and IgM-ve, Scrubtyphus IgM and IgG - ve, MP-QBC- ve, but ICT +ve for pf. Malaria, CRP-45.7 mg/L,Ferritin-852ng/ml,D-dimer-0.13 mg/L, ESR-78mm. Follow up antibodies for COVID-19 positive for IgM-1.32 OD ratio. Patient improved with IV Artesunate and two blood transfusions. This was a case of pf. Malaria with moderately severe acute COVID-19 infection responded both to IV Artesunate.

Discussion

1.1: RT-PCR Test for COVID-19 Infection

The diagnostic performance of gold standard RT-PCR test depends on many factors such as the sample types, different stages of infection, the skill of sample collection and the quality and consistency of the PCR assays being used.² These problems lead to a noteworthy delay of early diagnosis, following management and propose serious challenges.³ In a study RT-PCR test done in 213 COVID-19 patients by Yang et al,⁴ collected 205 throat, 490 nasal swab and 142 sputum samples from day 1 to 7 days of illness, of

whom 11% sputum ,27% nasal ,40% throat swabs were falsely negative. In another study by Zhao et al,⁵ in 107 hospitalized patients with acute symptoms and chest-x-ray typical of COVID-19 infection, with at least one sample positive for SARS-CoV-2 antibodies seroconversion were in 93% and RT-PCR test was positive from day 1 to 7 was in 67%. In a systemic review of five studies among 957 patients by Steven et al,⁶ false negative results reported from 2-94%. Thus, there are frequent false negative RT-PCR results and the clinicians should assume a negative result as 'false negative' in a person with typical symptoms and signs.

In-vitro analysis suggest that RT-PCR test is highly specific and sensitive for SARS-Cov-2 viral RNA detection in nasopharyngeal swab, but in clinical setting of 87 Chinese patients RT-PCR nasopharyngeal swab test sensitivity and specificity was 78.3% and 98.8% respectively and sensitivity was 62.5% in mild cases. In another study of 205 confirmed COVID-19 patients with 398 pharyngeal swab, only 126 (32%) were positive, but with BALF test RT-PCR was positive in 93% and with sputum 72% (Wang et al).⁷ In a study of 51 patients with HRCT characteristic of COVID-19 in 50 (98%) initial RT-PCR was positive only in 30 (71%). Thus, RT-PCR test has high specificity but the sensitivity is between 63-78%. For patients with moderately severe cases, HRCT imaging of the chest may be more sensitivity than RT-PCR. Thus, negative RT-PCR test does not rule out the disease.⁸

1.2: Symptoms of COVID-19 Infection

Fever, cough and fatigue are the most common symptoms, whereas nasal congestion, runny and diarrhoea are less common. It has been noted that some of COVID-19 patients had mild atypical symptoms initially, even with severe and critical illness.⁹ Severe cases might rapidly progress to acute respiratory distress syndrome (ARDS), septic shock and metabolic acidosis and bleeding disorders.¹⁰ Fever and symptoms screening misses many coronavirus cases¹¹ New study adds to

COVID-19 Symptoms are weakness, poor blood sugar control and gastrointestinal complaints as warning signs of COVID-19. Among 12000 Patients 57.1% attended New York City hospital complained of weakness, falls or altered mental status, 55.5% had GIT problem and aged ≥ 65 years persons mostly complain atypical symptoms such as diarrhoea, fatigue, and weakness.¹²

1.3: Definitions of COVID-19 Infection according to Duration of Illness

Guideline has been developed jointly by NICE, SIGN and RCGP and recommended the used of the following clinical definitions for the initial illness and long COVID-19: Acute COVID-19: Signs and symptoms of COVID-19 lasted for up to 4 weeks. Ongoing symptomatic COVID-19: Signs and symptoms continue from 4 to 12 weeks. Post-COVID-19 syndrome: Signs and symptoms that develop during or after an infection consistent with COVID-19 continue for more than 12 weeks and are not explained by an alternative diagnosis. Long COVID: The term 'long COVID' is commonly used to describe signs and symptoms those continue or develop after acute COVID-19. It includes both ongoing symptomatic COVID-19 (from 4 to 12 weeks) and post-COVID-19 syndrome (≥ 12 weeks or more).¹³

1.4: Diagnostic and Prognostic Blood Biomarkers Criteria of COVID-19 Infection

In a study of 203 patients with typical clinical features of fever, cough and/or dyspnoea with chest x-ray and HRCT of chest findings, RT-PCR tests were positive in 46% patients and negative in 54% patients. As COVID-19 infection is an inflammatory disease. Diagnostic criteria for suspected Clinical COVID-19 were done with blood inflammatory biomarkers to determine sensitivity and specificity by analyzing ROC curve, calculating the AUC and the cutoff value with a specificity of 89% was considered for following blood biomarkers to met the diagnosis of clinical COVID-19 infection: If Serum ferritin levels $>125\%$ (1.25 times) of URL 300 $\mu\text{g/L}$ -(M) and 200 $\mu\text{g/L}$ -(F) had sensitivity of 66% (56.8-76.4) and specificity of 85% (77.3-91.4) was the

most accurate biomarker. Eosinophils counts $\leq 0.02 \times 10^9/\text{L}$ with sensitivity of 64 % (54.4-74.5) and specificity of 79 % (71.1-86.9). Serum LDH levels $>125\%$ of URL-(290 IU/L(F) and 325 U/L for (M) with sensitivity of 62 % (51.1-71.5) and specificity of 77 % (67-83.8), hsCRP level $>80\text{mg/L}$ with sensitivity of 46 % (36.4-57.4) and specificity of 81 % (72.1- 87.7).Lymphocyte count ≤ 1000 cells/ μL with sensitivity of 43 % (32.4-33.2) and specificity of 81 % (72.1-87.7).Serum D-dimer levels $>1.2\text{mg/L}$ with sensitivity of 33 % (23.6-43.4) and specificity of 79% (71.1-86.9). Over all, if \geq one criteria present in clinically suspected COVID-19 patients then diagnosis of clinical COVID-19 was made with sensitivity of 91% (83.9-96.3) and specificity was 47% (38.1-57.5). Thus, these criteria can be used as diagnostic tools to differentiate patients with and without COVID-19 and among suspected clinical COVID-19 with RT-PCR negative patients as complementary along with other tests.¹⁴ A recent systematic review highlighted the most important laboratory tests alterations were increased values of CRP (95.8%), LDH (76.9%), ferritin (60.7%), D-dimer (54.8%), neutrophil to lymphocyte ratio [NLR] (50.1%) and aspartate aminotransferase [AST] (48.9%), as well as lymphocytopenia (49.8%) within the first 24 hr at ED visit.¹⁵ If some or all of these parameters raised and there was lymphocytopenia or eosinopenia suggested diagnosis in RT-PCR negative cases and considered as severe disease.^{16,17}Lymphocyte count <1000 cells/ μL correlate with increased severity and development of AKI during hospitalization in 68% patients with >7 days ICU stay and increased mortality.¹⁸

Lactate dehydrogenase (LDH) is an enzyme involved in conversion of lactate to pyruvate and interconversion of NADH and NAD⁺. It is present in almost all body cells increased following tissue breakdown and decreased oxygenation, considered as an inflammatory marker. Severe infections may cause cytokine-mediated tissue damage and LDH release. LDH enzymes are five separate isoenzymes and LDH-3 is present in

pneumocytes. C-reactive protein (CRP) is a reliable marker of acute inflammation, synthesized in the liver regulated by the cytokine IL-6 and IL-1. LDH and CRP could be useful marker for the early identification of COVID-19 patients.^{19,20} Elevated LDH levels were associated with a ~6-fold increase in odds of developing severe disease and a ~16-fold increase in odds of mortality.^{21, 22} These findings could be related to the cytokine storm in patients with severe to critical disease.²³ Trends on serial laboratory measurements with progressive lymphopenia, thrombocytopenia and elevated CRP, ferritin, LDH, increased liver enzymes, decreased renal function, and coagulation derangements were more common among critically ill patient.²⁴ Studies demonstrated that routine blood and biomarkers tests had detection rates similar to those of RT-PCR with a positive predictive value and negative predictive value of 83.3% and 90.6% respectively.²⁵

1.5. HRCT Thorax and RT-PCR Sensitivity for Diagnosis of COVID-19 Infection

In a series of 81 patients HRCT of thorax detected COVID-19 infection greater than RT-PCR testing (98% versus 71%), suggest screening of HRCT chest of patients with typical clinical features particularly when RT-PCR is negative.²⁶ In a study of chest HRCT of 1014 patients, RT-PCR was positive in 59% and HRCT was positive in 88% and HRCT sensitivity was 97%.²⁷ Combination of RT-PCR and chest CT had higher sensitivity (91.9%) and CT alone have sensitivity of 66.7% versus RT-PCR alone 78.2% for detection of COVID-19 infection.²⁸

1.6: Testing of COVID-19 Specific Antibodies

The cumulative seroconversion curve showed that the rate for total antibody (TAb) and IgM reached 100% around 1-month after onset of disease. The seroconversions were sequentially appeared for TAb, IgM and then IgG. The median time to TAb, IgM and IgG seroconversion were 11, 12 and 14-day respectively. Overall, the seroconversion of TAb was significantly quicker than that of IgM and IgG. In the early phase of illness within 7 days, RT-PCR had the highest sensitivity of 66.7%,

whereas the antibody positivity rate of 38.3%. However, the sensitivity of TAb overtook that of RNA test since day 8 and reached over 90% across day 12 after onset. In serum samples from patients during day 8-14, the sensitivities of TAb was (89.6%), IgM (73.3%) and IgG (54.1%) were higher than the RT-PCR test (54.0%). Among patient's samples collected during day 15-39, sensitivities of TAb, IgM and IgG were 100.0%, 94.3% and 79.8%, respectively; in contrast RT-PCR test positive in 45.5%. Respiratory tract samples collected among patients during day 1-3, 4-7, 8-14 and 15-39, there were 28.6%, 53.6%, 98.2%, and 100% respectively had detectable TAb. Detection of TAb was more sensitive than IgM and IgG for detecting SARS-CoV-2 infection in early stages and similar to IgM.²⁹

1.7: Scientific Rationale of IV Bolus Artesunate Therapy for COVID-19 Infection

Ruiyuan Cao, et al in their study found EC_{50} (50% effective concentration) of Artesunate was $12.8 \pm 5.30 \mu\text{M}$ and Dihydroartemisinin (DHA) had $13.31 \pm 1.24 \mu\text{M}$ against SARS-CoV-2 in-vitro, indicating Artesunate as a potential countermeasure against COVID-19 infection. Artesunate could inhibit SARS-CoV-2 replication in a dose-dependent manner, might function at the post-entry stage and SARS-CoV-2 inhibited by Artesunate and DHA.³⁰ Bae JY et al, in their study in Vero cells, shown that Artesunate inhibited SARS-CoV-2 replication with IC_{50} of $53.06 \mu\text{M}$, CC_{50} of $> 100 \mu\text{M}$. Interestingly, in Calu-3 cells, (which are derived from human airway epithelial cells was more representative of susceptible cells in actual human airway infection), Artesunate inhibitory effect had IC_{50} of $1.76 \mu\text{M}$, $CC_{50} > 100 \mu\text{M}$, better than in Vero cells and reduced viral replication in a dose-dependent manner.³¹ After 120mg of IV AS produce C_{max} of 11, 343ng/ml ($42 \mu\text{M}$) with $t_{1/2}$ of 0.05 hrs and C_{max} of DHA was 2,646ng/ml with $t_{1/2}$ of 0.67 hrs (total 13,987ng/ml),³² which were greater than EC_{50} of Artesunate and DHA against SARS-CoV-2. In another study 120mg IV bolus Artesunate produced C_{max} of $29.5 \mu\text{M}$ with elimination $t_{1/2}$

of 2.7 min and C_{max} for DHA was 9.3 μM with t_{1/2} of 40 min and 100mg oral AS produce DHA C_{max} of 2.6 μM, t_{1/2} of 39 min.³³ Gilmore K et al **in Vero E6 cells study:** Artesunate EC₅₀ had 7-12 μg/ml or (0.7-1.2 μM) was more potent than Artemisinin. C_{max} of Artesunate exceeding EC₅₀ can be achievable clinically in plasma and tissue concentrations of 15μg/ml and the typical doses of 2 - 2.4 mg/kg IV bolus produces C_{max} of AS between 19.4 and 29.7μg/ml. In animal studies tissue concentrations including lung, kidney, intestine, and spleen were several-fold higher than plasma concentrations.³⁴ Artemisinins administration leads to autoinduction of hepatic drug metabolism and reduces its own bioavailability. The plasma concentrations of same daily dose of AS were 1/3rd less on day 3 onwards than on day 1.³⁵ The PK variability following 120mg IV AS with high C_{max} occurs with first exposure time and the C_{max} variability ranges from 735-1890ng/ml(AS+DHA) and this variability was 25 fold among different clinical trials. There is large inter-individual PK/PD variability and such low drug concentrations in some patients may explain treatment failure. Thus, low dose regimen of AS to be avoided.³⁶ High dose IV bolus Artesunate preferred to achieve higher free peak plasma levels of Artesunate and DHA and have higher bioavailability to enter the human cells in comparison to Artemether and Arteether.³⁷ Patients with severe COVID-19 may have many critical and variable conditions and comorbidities with variable severity scores that may determine the drug's PK/PD characteristics and prognosis. The current dose of 120mg IV AS produce variable C_{max} ranging from 735-1890ng/ml with greater inter individual variability have lower C_{max}. The 4-8mg/kg loading dose is safe and in phase I-II study IV AS 4-8mg/kg loading doses were extremely well tolerated in humane volunteers and malaria patients.³⁸ Artesunate C_{max} is more important than AUC in producing improved efficacy and IV bolus Artesunate provide sufficient high C_{max} in patients and avoid inter-individual variability in

PK/PD. Artesunate IV bolus injection following 4mg/kg produce C_{max} of 36,100ng/ml and following 8mg/kg C_{max} of 89,340ng/ml.³⁹ Optimal doses and dose intervals for Artesunate and DHA have not been determined. Therefore, in the absence of well controlled dose-ranging studies and valid pharmacodynamic relationships, widely used empirical regimens remain unchallenged.³³

1.9: Coronavirus replication cycle is around 8-10 hrs.^{40,41} Artesunate has C_{max} dependent effects and IV bolus infusion (in 2-10 minutes) initiated with 4-8mg/kg at interval of 8-10 hr or even 12hly can achieve higher plasma C_{max} for the first exposure given for a short course of ≥ 3 to 5 days to cover 9 to 15 replication cycles in early stage of robust viral replication can prevent disease progression as well as avoided auto-induction of its own metabolism and low C_{max}. Besides its antiviral effects, Artesunate have anti-inflammatory, immunomodulatory, antioxidant, anticytokine, anti-fibrotic and organs protective effects in hypoxia and can be beneficial even in later stages or in the cytokine storm to reduce morbidity and mortality.⁴²

1.10: Artesunate effectiveness in clinical trial of COVID-19 patients: In a prospective study of 43 cases of COVID-19 patients divided into routine treatment group (n = 25) and Artesunate group 60 mg IV twice daily along with routine treatment for 10 days (n = 18). Among Artesunate group, time for significant improvement of symptoms was (days: 3.33±1.91 vs. 4.84±2.19), RT-PCR negative conversion time was (days: 4.72±2.16 vs. 6.68±3.76), lung lesion absorption starting time (days: 5.39±2.36 vs. 7.48±3.78), lung lesion absorption > 70% time (days: 14.11±4.16 vs. 17.04±4.42) and length of hospital stay (days: 16.56±3.71 vs. 18.04±3.97) were significantly shorter, than those in routine treatment group with fewer adverse reactions.⁴³

Conclusions

COVID-19 infection is not localized to respiratory tract but is a "multisystem disease" and affects

any organs of the body or in combinations. There have been variable clinical presentations of COVID-19 infection in different co-morbidities. Rapid antigen and RT-PCR test have frequent false negative results. Routine blood, inflammatory and coagulation biomarkers along with imaging studies and COVID-19 specific antibodies tests are very sensitive indicators of diagnosis of COVID-19 infection. HRCT of thorax can detect COVID-19 infection better than RT-PCR test. Combinations of these tests have high sensitivity and specificity for the diagnosis than RT-PCR test alone. Artesunate have anti-SARS-CoV-2 effects in vitro studies, having very short half-life and dose dependent effects with excellent safety profiles during treatment of patients. High dose IV bolus Artesunate produces high Cmax and exceeds many times EC₅₀ of Artesunate and its metabolite DHA against SARS-CoV-2. Thus, high dose IV bolus Artesunate producing high Cmax to be given for 3-5 days to cover 10 to 15 replication cycle of (8-10hour) coronavirus in the early stage of robust viral multiplication to prevent progression of disease, complications and deaths. Beside its antimalarial and anti-viral effect it have anti-inflammatory, anti-cytokine, immunomodulatory, anti-oxidant, anti-fibrotic, hypoxic organ protective effects, may effective even in later stages of the disease. High dose IV bolus Artesunate therapy for 5 days appears to be very effective with excellent safety profile with faster resolution of symptoms, improves oxygen saturation rapidly in hypoxic patients and decreased morbidity and mortality among COVID-19 infected patients. However, large multicenter placebo control, double blind, dose ranges, frequency and duration of therapy studies are required among RT-PCR/ RAT positive COVID-19 patients for its recommendation.

Conflicts of Interest: Nil

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