

Supplementary documents

Title: An approach of scoring early host immune response from oro-/ nasopharyngeal swabs predicts COVID-19 outcome providing possibility of early therapeutic intervention

Table S1. Study subject groups and distribution of age and sex

Category Covid-19 +/- by RT-PCR		Group (#)		Asymptomatic (A) or symptomatic (S)	Required Oxygen support (Yes /No)	ICU / Death outside ICU (Yes /No)	comorbidity (Yes/No) 1.	No of samples used		Age (M±SD)		Sex ratio %M, %F	
Case	RdRP +ve E gene+ve	1 * asymptomatic		A	No	No	No	16		37.25±19.25		37%, 63%	
		2 * mild		S	No	No	No	20 (total)	18	47.57±16.32	52%, 48%		
							Yes					2	
		3 Moderate	3a	S	Yes	No	No	9 (total)	4	58.8±14.7	51±18.6	33.3, 66.6%	75%, 25%
			3b	S	Yes	No	Yes						5
		4 Severe		S	Yes	Yes	No	12		42.5±17.9		83%, 17%	
		5 Severe		S	Yes	Yes	Yes	13		51±15.7		38%, 62%	
II.	RdRP -ve E gene+ve	6 Non COVID-19 Como cold		S	No	No	No	15		37.3±11.7		46%, 53.8%	

*As the groups recover without complication at home quarantine or in general facility we assumed this patient groups should have effective immune response correlated with the good clinical outcome irrespective of present or absent of any comorbidity.

Table S2. Potential innate immunity and T cell response markers for RT-PCR evaluation

Potential marker's code		Gene name	Ref Seq ID	Rational for inclusion	Primer Sequence
Innate immunity response markers	I1	ACTB	NM_001101.5	Standard housekeeping gene which is used as internal reference	ACTBF: GCCGAGACCGCGTCC ACTBR: ATCCATGGTGAGCTGGCG
	I2	OAS3	NM_006187.4	Represent interferon responsive genes (16)	OAS3F: TCAGAAGCCCAGGCCTATCAT OAS3R: CTTACACAGCAGCCTTCACT
	I3	IL10	NM_000572.3	Key mediator of anti-inflammatory response (17)	IL10F: GGCACCCAGTCTGAGAACAG IL10R: CAACCCAGGTAACCCTTAAAGTC
	I4	IL6	NM_001371096.1	Key mediator of inflammation (30)	IL6F: TACATCCTCGACGGCATCTC IL6R: CACCAGGCAAGTCTCCTCATTG
T cell response markers	T1	FOXP3	NM_014009.4	Regulation of regulatory T (Treg) cell gene expression which are responsible for suppression of immune response (31)	T1F: ACATTTTCATGCACCAGCTCTC T1R: ATTCCAGGCTGGCCACGTTG
	T2	ZEP36	NM_003407.5	The gene keep T cell in inactive stage by its expression of ZFP RBP in resting phase and its down regulation upon acute viral infection bring T cell to activated state (32)	T2F: GACTGCCATCTACGAGAGCCT T2R: CACTAGGCTGGTGGAGCG
	T3	IL17A	NM_002190.3	Th17 marker, responsible for Inflammation and immune driven tissue injury (33), associated with ARDS (34)	T3F: AACCGATCCACCTCACCTTG T3R: TCTCTTGCTGGATGGGGACA
	T4	CD69	NM_001781.2	Earliest activation marker of T cells (35,36)	T4F: GAGAACAGCTCTTTGCATCCG T4R: GCACACAGGACAGGAACCTG
	T5	CD25	NM_000417.3	IL-2 receptor, an early activation marker, activated following stimulation of the TCR/CD3 complex (37,38)	T5F: CAGGGCTCTACACAGAGGTC T5R: GTGACGAGGCAGGAAGTCTC
	T6	CD62L	NM_000655.5	Naive T cells receptor L-selectin to recognize and extravasate through specialized high endothelial venules of lymphoid tissue (39).	T6F: CCACCTGTGGACCATTTGGA T6R: CCAGGGGATGGCTACAGTTC
	T7	CCR7	NM_001301714.2	Express in naive T cells, a receptor for the constitutive secondary lymphoid tissue chemokine (SLC), down-regulated in effector T cells (40,41)	T7F: TGTGTGGTTTTACCGCCCAG T7R: CACTGTGGTGTGTCTCCGA
	T8	GZMB	NM_004131.6	Activation marker of T cells, which gives a killer phenotype to activated T cells. GzmB a protease which cleaves target proteins upon activation and exert the strongest proapoptotic function (42).	T8F: ATGACAGTGCAGGAAGATCG T8R: GGCCTCCAGAGTCCCCCTTAA

ACTB: β -actin, OAS3 Gene : 2'-5'-Oligoadenylate Synthetase 3, IL10: Interleukin 10, IL6: Interleukin 6, FOXP3: forkhead box P3 protein, ZEP36: zinc finger protein 36 homolog, Interleukin-17A, CD69: human transmembrane C-Type lectin protein, CD25: Interleukin-2 Receptor Alpha Chain, CD62L: L-selectin, CCR7: C-C chemokine receptor type 7, GZMB: Granzyme B.

Table S3. Profile of illness duration from time of sampling of COVID-19 symptomatic groups

Days of illness before Sample collection			Days of hospital admission from date of sample collection (group 3+4+5) Mean $\pm$ SD (range)	Time of death following sample collection (group 4+5) Mean $\pm$ SD (range)
(group 2+3+4+5) Mean $\pm$ SD (range)	(group 2) Mean $\pm$ SD (range)	(group 3+4+5) Mean $\pm$ SD (range)		
4.1 $\pm$ 3.2 (0-12)	4 $\pm$ 2.9 (0-7)	4.09 $\pm$ 3.4 (0-12)	0.26 $\pm$ 1.34 (-1 – 4)	5.1 $\pm$ 5.7 (0-18)

Table S4. Days of hospital stay among Covid-19 survived group

Group 3+4+5	Days hospital stay From date of sample collection	
All Survived (16)	16.6 $\pm$ 8.5(6-39)	
Treated in ICU (10)	18.6 $\pm$ 9.5(7-39)	<i>P</i> = 0.18
Treated in general facility (6)	13.5 $\pm$ 4.7(6-20)	

Table S5. Comparison of normalized Ct values of IL10, OAS3 and IL6, in as symptomatic /mild and moderate/severe groups and modelling for detecting severe group.

Mode of analysis	Potential biomarkers of innate immunity response	Group 1+2(36) Mean $\pm$ SD	Group 4+5+3(31) Mean $\pm$ SD	T-TEST (P)	Cocktail of 15 sample of group 6S
Δ Ct values normalized with ACTB	IL-10-ACTB	11.77 $\pm$ 2.8	12.75 $\pm$ 1.9	0.1	12.3 $\pm$ 0.4
	OAS3-ACTB	7.35 $\pm$ 2.35	7.57 $\pm$ 1.9	0.7	9 $\pm$ 0.6
	IL6-ACTB	11 $\pm$ 3.2	11 $\pm$ 2.8	1.0	12.2 $\pm$ 0.75
		% no expression/ Δ Ct >12.5	% no expression/ Δ Ct >12.5		
% no expression or Δ Ct >12.5	IL10-ACTIN	44.4% (16/36)	64.5% (20/31)	0.1	
	OAS3-ACTIN	0%(0/36)	0%(0/31)	1.0	
	IL6-ACTIN	42%(15/36)	45% (14/31)	0.80	
Composite model: low IL10 in the background of high OAS3 expression	Δ Ct IL10 >12.5, Δ Ct OAS3 <9	19.4% (7/36)	51.6% (16/31)	0.006*	

ACTB: β -actin , Δ Ct : Ct difference between target gene – β -actin, * *P* statistically significance at *P*<0.05

Table S6. Gender and age specific association of Impaired IL-10 response in COVID-19 patients groups

Group	Gender	% Impaired IL10 response : %(n/N)		High risk category	Age Mean $\pm$ SD	
1+2	Male (17)	18%(3/17)	<i>P</i> =0.8	Impaired IL10 response (7)	49.6 $\pm$ 19.4	<i>P</i> =0.31
	Female (19)	21%(4/19)		No Impaired IL10 response (29)	40.9 $\pm$ 17.8	
3+4+5	Male (16)	50% (8/16)	<i>P</i> =0.8	Impaired IL10 response(16)	54.75 $\pm$	<i>P</i> =0.08
	Female (15)	53% (8/15)		No Impaired IL10 response(15)	44 $\pm$ 17.2	

Table S7. Ct profile of the 8 potential T cell markers following performing RT-PCR.

Potential marker's primer code	Non-severe Group (n=10)		Severe Group (n=9)	
	% of samples failed to detect amplification	Mean Ct	% of samples failed to detect amplification	Mean Ct
T1	40%	37.46	67%	38.8
T2	0%	28.52	0%	28.9
T3	70%	38.72	78%	39
T4	0%	31.32	22%	35.3
T5	50%	37.23	67%	38.46
T6	0%	31.68	0%	32.16
T7	20%	34.29	22%	35.76
T8	0%	32.74	22%	35.67
ACTB	0%	24.8	0%	25.8

ACTB: β -actin, Ct: cycle threshold of the individual target genes in RT-PCR, n: Number of samples

Table S8. Test of normality of the samples (using the Kolmogorov-Smirnov Test of Normality).

	Covid-19 +		Non Covid-19 corona virus + (11)
	Group 1+2 (25)	Group3+4+5 (30)	
K-S test statistic (D)	0.152	0.111	0.198
<i>P</i>	0.560	0.818	0.575
Normality	Yes	Yes	Yes

Table S9. Coefficient of variation (CV) and Repeatability of the ‘severity score’

	Sample1 Severity score	Sample 2 Severity score:	Sample 3 Severity score	Sample 4 Severity score:
Intraday CV: T4+T8-2*T2	4%	17%	7%	16%
Interday CV T4+T8-2*T2	12%	21%	4%	
Agreement of repeated independent test in reference to Severity score cut off				
Independent Experiment	Sample1 Severity score cut off : >12/<12	Sample 2 Severity score cut off : >12/<12	Sample 3 Severity score cut off : >12/<12	Sample 4 Severity score cut off : >12/<12
1	>12	<12	>12	<12
2	>12	<12	>12	<12
3	>12	<12	>12	<12
4	>12	<12	>12	<12
5	>12	<12	>12	<12

Table S10a. Correlation of severity score with days of illness before sampling among COVID-19 symptomatic groups

Groups (outcome)	Correlation: days of illness before sampling vs Severity score R(P)
Group 2 (mild)	-0.24, P=0.6
Group 3 (moderate)	-0.04, P=0.9
Group 4+5 (severe without and with co- morbidity)	0.31, P=0.16
Group 3+4+5	0.29, P = 0.13
Group 2+3+4+5	0.19, P =0.25

Table S10b. Correlation of severity score with days of hospital stay among Covid-19 survived group

Group 3+4+5	Correlation: days of hospital stay vs Severity score R(P)
All Survived (14)	0.55 <i>P</i> =0.041*
Treated in ICU (8)	0.64 <i>P</i> =0.087
Treated in general facility (6)	-0.111

Table S11. Gender and age specific association of severity score

Group		Severity score	T-TEST p	Correlation Age vs Severity score: R(P)
1+2	Male (12)	6.66±2.6	<i>P</i> =0.25	-0.149
	Female (13)	8.16±3.7		0.262842
3+4+5	Male (16)	12.18±4.5	<i>P</i> =0.56	-0.012
	Female (14)	11.29±5		-0.082

Table S12. Comorbidity profile along with severity score and COVID-19 outcome

Comorbidity	No of cases	High severity score (>12)	Covid19 Outcome	
			Death	Survived
DM	5	0	0	5
DM+HTN	3	2	1	2
DM+CKD	1	0	1	0
CKD	1	0	1	0
HTN	4	2	1	3
Cancer	1	1	1	0
Anaemia	1	1	1	0
TB	1	1	1	0
Thyroid	1	0	0	1
Total	18	7	7	11

DM: Diabetes mellitus, HTN: Hypertension , CKD: Chronic kidney disease, TB

Individual Case Example 1

A 42 years male with history of diabetes and hypertension admitted to hospital following 9 days after appearance of symptoms and underwent Covid-19 test by RT-PCR. The tests showed SARS-CoV-2 positive. The leftover RNA sample which was extracted from the oro-/nasopharyngeal sample in VTM was used for further downstream RT-PCR analysis for T cell response ‘severity score’

Table 13. ‘Severity score’ in sample of case example 1

Sample	ZFP36 (primer: T2F/T2R)		CD69 (primer: T4F/T4R)	GZMB (primer: T8F/T8R)	T cell response Score	>Score 12	Predicted outcome
ID	Ct	Acceptable range (Ct<33) Yes/No	Ct	Ct	T4+T8-2T2	Yes/No	Severe/ No Severe
26	24.04	Yes	27.74	34.76	14.4	yes	Severe

Outcome: The patient was transferred to ICU following 4 days of the SARS-COV2 diagnosis and subsequently required ventilation support. After 39 days following the sampling for Covid-19 diagnosis the patient was recovered and discharged from hospital.

Individual Case Example 2

A 20 years female with no co-morbidity, 3 days after appearance of covid-19 symptoms underwent Covid-19 test by RT-PCR. The tests showed SARS-CoV-2 positive. The leftover RNA sample which was extracted from the oro-/nasopharyngeal sample in VTM was used for further downstream RT-PCR analysis for T cell response 'severity score'.

Table S14. 'Severity score' in sample of case example 2

Sample	ZFP36 (primer:T2F/T2R)		CD69 (primer:T4F/T4R)	GZMB (primer:T8F/T8R)	T cell response Score	>Score 12	Predicted Outcome
ID	Ct	Acceptable range (Ct<33) Yes/No	Ct	Ct	T4+T8- 2T2	Yes/No	Severe/ No Severe
	30.58	Yes	34.02	35.78	8.63	No	No severe

As the T cell score was <12, 2nd downstream test for impaired IL10 response was performed to predict chance of requirement of Oxygen.

Table S15. 'Impaired IL10 response' in sample of case example 2

Sample	ACTB (primer: T2F/T2R)		OAS3 (primer: T4F/T4R)		IL-10 (primer: T8F/T8R)		IL-10 response		Impaired IL-10 response	Predicted outcome
ID	Ct	Acceptabl e range (Ct<27.5) Yes/No	Ct	ΔCt OAS3	Ct	ΔCt IL- 10	ΔOAS3<9 Yes/no	ΔIL- 10>12.5 Yes/no	Yes/No	Asymptoma tic or mild/ requirement of oxygen
	25.1	Yes	34.41	9.3	40	14.9	No	Yes	No	Asymptoma tic or Mild

Outcome: Patients had mild Covid-19 outcome and recovered completely at home quarantine.

Individual Case Example 3

A 20 years female with no comorbidity, 3 days after appearance of COVID-19 symptoms underwent COVID-19 test by RT-PCR. The tests showed SARS-CoV-2 positive. The leftover RNA sample which was extracted from the oro-/nasopharyngeal sample in VTM was used for further downstream RT-PCR analysis for T cell response ‘severity score’.

Table S16. ‘Severity score’ in sample of case example 3

sample	ZFP36 (primer:T2F/T2R)		CD69 (primer:T4F/T4R)	GZMB (primer:T8F/T8R)	T cell response Score	>Score 12	Predicted Outcome
ID	Ct	Acceptable range (Ct<33) Yes/No	Ct	Ct	T4+T8-2T2	Yes/No	Severe/ No Severe
26	30.58	Yes	34.02	35.78	8.63	No	No severe

As the T cell score was <12, 2nd downstream test for impaired IL-10 response was performed to predict chance of requirement of Oxygen.

Table S17. ‘Impaired IL10 response’ in sample of case example 3

Sample	ACTB (primer: T2F/T2R)		OAS3 (primer: T4F/T4R)		IL-10 (primer: T8F/T8R)		IL-10 response		Impaired IL-10 response	Predicted outcome
ID	Ct	Acceptable range (Ct<27.5) Yes/No	Ct	ΔCt OAS3	Ct	ΔCt IL-10	ΔOAS3<9 Yes/no	ΔIL- 10>12.5 Yes/no	Yes/No	Asymptomatic or mild/ requirement of oxygen
23	26.93	Yes	35.01	8.08	40	13.07	Yes	Yes	Yes	requirement of oxygen

Outcome: Patients had moderate COVID-19 outcome having requirement of oxygen and recovered completely at home quarantine.

Figure S1

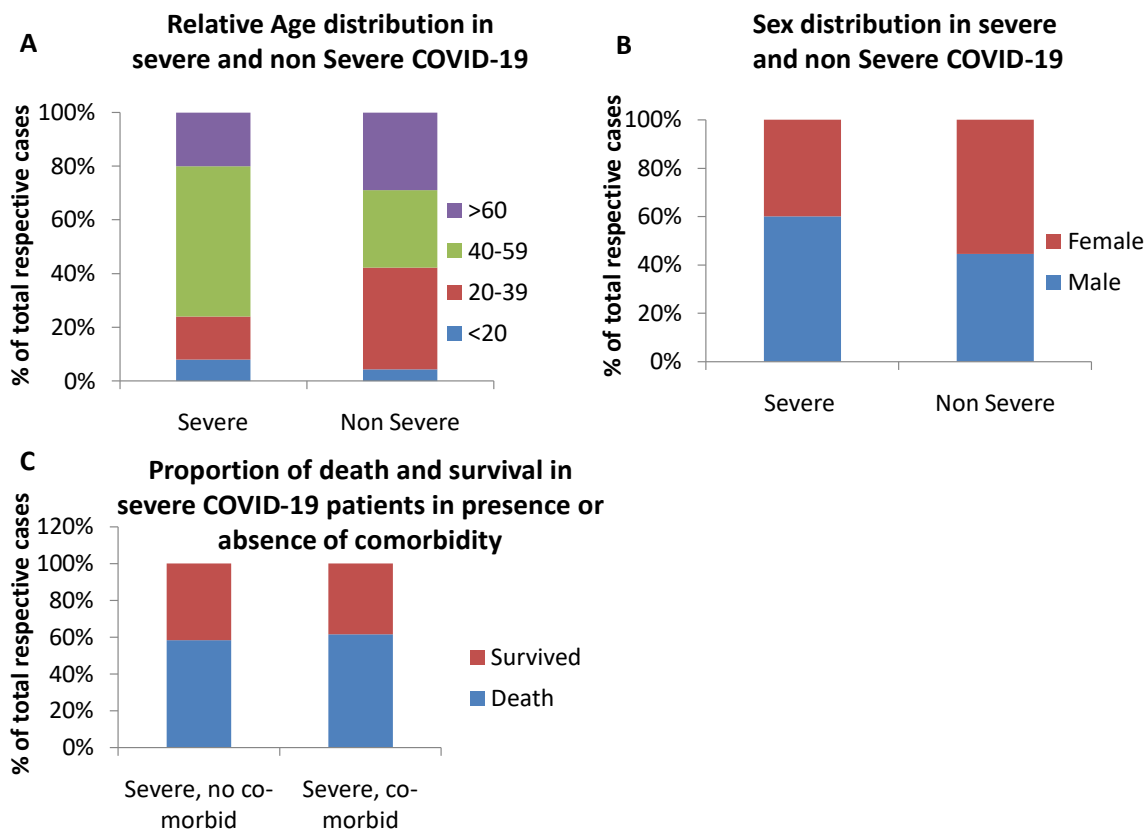


Figure S1A: Distribution of age, in severe and non Severe COVID-19 cases. Colure code indicate proportion of respective age range in years as indicated in the figure.

Figure S1B: Distribution of sex in severe and non Severe COVID-19 cases. Colure code indicate proportion of respective sex as indicated in the figure.

Figure S1C: Distribution of Death in severe COVID-19 cases without and with co-morbidity. Colure code indicate proportion of death or survived as indicated in the figure.