Results of the CAPSID randomized trial for high-dose convalescent plasma in patients with severe COVID-19

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Supplemental Material

Supplementary Tables

Supplementary Figures

Methods

[§] all members of the CAPSID Clinical Trial Group are listed in the Appendix.

Tables

Table 1: Adverse Events

	CCP group (n=53)	Control Group (n=52)	P value
Frequency of adverse events, n (%)			0.62
0	11 (20.8)	9 (17.3)	
1	12 (22.6)	8 (15.4)	
2	7 (13.2)	6 (11.5)	
3	11 (20.8)	7 (13.5)	
4	4 (7.6)	6 (11.5)	
5	1 (1.9)	5 (9.6)	
6	3 (5.7)	5 (9.6)	
≥7	4 (7.6)	6 (11.5)	
Worst AE grade, n (%)			0.18
No AE	11 (20.8)	9 (17.3)	
1	4 (7.6)	0 (0.0)	
2	9 (17.0)	6 (11.5)	
3	7 (13.2)	12 (23.1)	
4	11 (20.8)	8 (15.4)	
5	11 (20.8)	17 (32.7)	
Outcome, n (%)*			
Resolved	32 (60.4)	36 (69.2)	
Ongoing/resolving	19 (35.9)	19 (36.5)	
Resolved with sequelae	4 (7.6)	4 (7.7)	
Death	11 (20.8)	17 (32.7)	
Unknown	6 (11.3)	8 (15.4)	
Causal relationship to IMP, n (%)*, **			
None	34 (64.2)	43 (82.7)	
Unlikely	18 (34.0)	8 (15.4)	
Possible	3 (5.7)	1 (1.9)	
Definitive	0 (0.0)	0 (0.0)	
Patients with SAE, n (%)			
No	31 (58.5)	27 (51.9)	
Yes	22 (41.5)	25 (48.1)	
Number of SAE, n (%)			
1	12 (22.6)	13 (25.0)	
2	6 (11.3)	5 (9.6)	
3	2 (3.8)	5 (9.6)	
4	1 (1.9)	2 (3.9)	
6	1 (1.9)	0 (0.0)	
Reason for Seriousness, n (%)*			
Life threatening	16 (30.2)	15 (28.9)	
Persistent / significant disability	2 (3.8)	1 (1.9)	
Resulted in death	11 (20.8)	17 (32.7)	
Hospitalization / prolongation	7 (13.2)	6 (11.5)	
Important medical event	5 (9.4)	6 (11.5)	

*Multiple answers possible. **Control group patients contain also patients who switched to the plasma arm.

Table 2: Case Fatality Rate on Days 21, 35 and 60.

	CCP-Group (n=53)	Control-Group (n=52)	P Value	Total
Day 21			0.79	
Patient alive	46 (86.8)	44 (84.6)		90 (85.7)
Patient dead	7 (13.2)	8 (15.4)		15 (14.3)
Day 35			0.16	
Patient alive	45 (84.9)	38 (73.1)		83 (79.1)
Patient dead	8 (15.1)	14 (26.9)		22 (21.0)
Day 60			0.19	
Patient alive	42 (79.3)	35 (67.3)		77 (73.3)
Patient dead	11 (20.8)	17 (32.7)		28 (26.7)

Table 3: Time to Event for Secondary Outcome by Transfused Neutralizing Units*

	CCP group (n=53)		Control Group (n=52)
	Low neutralizing units* (n=28)	High neutralizing units* (n=25)	
Time to clinical improvement, days			
N	28	25	52
Median (IQR)	36 (17-NR)	20 (11-NR)	66 (13-NR)
Time to discharge from ICU, days			
N	26	24	49
Median (IQR)	39 (20-NR)	14 (7-39)	42 (12-NR)
Time to discharge from hospital, days			
N	28	25	52
Median (IQR)	39 (21-NR)	21 (13-43)	51 (20-n.r)
Time to first negative SARS-CoV-2 PRC, days			
N	28	25	52
Median (IQR)	14 (5-19)	5 (3-15)	8 (5-21)

*see Methods section for definition of neutralizing units. CCP group was divided by the cumulative amount of neutralizing units per patient (all 3 CCP transfusions) into a low neutralizing unit group (≤ median) and a high neutralizing unit group (> median). NR, not reached.

Table 4: **Time to Event for Secondary Outcome by Inflammation Markers***

	High Inflammation Markers (n=51)**		Low inflammation Markers (n=48)**	
	CCP Group	Control Group	CCP Group	Control Group
Time to Clinical Improvement (days)				
N	25	26	24	24
Median (IQR)	NR (21-NR)	NR (11-NR)	19 (8-NR)	41 (13-NR)
Time to Discharge from ICU (days)				
N	24	26	22	21
Median (IQR)	42 (20-NR)	NR (12-NR)	13 (6-37)	20 (12-69)
Time to Discharge from hospital (days)				
N	25	26	24	24
Median (IQR)	41 (21-NR)	NR (30-NR)	24 (11-NR)	26 (12-NR)
Time to first negative SARS-CoV-2 PCR				
N	25	26	24	24
Median (IQR)	14 (5-18)	15 (5-NR)	5 (3-18)	6 (5-18)

*see Methods section for definition of high / low inflammation markers. The patient group was divided into a low inflammation marker group and a high inflammation marker group. ** Six patients with either missing data on inflammation markers (n=1) or intermediate inflammation markers (n=5) are not included in this table. NR, not reached.

Table 5: Time to Event for Secondary Outcome by Presence or Absence of Neutralizing Antibodies at Baseline*

	anti-SARS-CoV-2 neutralizing antibodies at baseline: positive (n=75)**		anti-SARS-CoV-2 neutralizing antibodies at baseline: negative (n=20)**	
	CCP Group	Control Group	CCP Group	Control Group
Time to Clinical Improvement (days)				
N	37	38	10	10
Median (IQR)	32 (15-NR)	NR (14-NR)	27 (19-NR)	32(9-NR)
Time to Discharge from ICU (days)				
N	35	36	9	9
Median (IQR)	27 (9-NR)	40 (11-NR)	33 (7-NR)	25 (15-NR)
Time to Discharge from hospital (days)				
N	37	38	10	10
Median (IQR)	27 (16-NR)	37 (14-NR)	NR (14-NR)	NR (29-NR)
Time to first negative SARS-CoV-2 PCR				
N	37	38	10	10
Median (IQR)	7 (4-15)	7 (5-22)	16 (4-19)	15 (7-19)

* presence of anti-SARS-CoV-2 neutralizing antibodies with titers of PRNT50 ≥ 1:20 at baseline.

** Ten patients with missing data on neutralizing antibodies (PRNT50) at baseline are not included in this table. NR, not reached.

Table 6: Time to Event for Secondary Outcome by Ventilation Status at Baseline.

	Patients without invasive ventilation / ECMO (n=69)		Patients wit invasive ventilation / ECMO (n=36)	
	CCP Group	Control Group	CCP Group	Control Group
Time to Clinical Improvement (days)				
N	37	32	16	20
Median (IQR)	21 (15-NR)	NR (15-NR)	33 (12-NR)	66 (13-NR)
Time to Discharge from ICU (days)				
N	34	29	16	20
Median (IQR)	16 (7-NR)	16 (8-NR)	NR (32-NR)	NR (29-NR)
Time to Discharge from Hospital (days)				
n	37	32	16	20
Median (IQR)	21 (13-NR)	34 (12-NR)	NR (32-NR)	NR (29-NR)
Time to first negative SARS-CoV-2 PCR				
n	37	32	16	20
Median (IQR)	7 (4-18)	8 (5-26)	7 (5-16)	11 (5-19)

NR, not reached.

Table 7: Baseline Demographics and Clinical Characteristics of Patients in the Control Group with or without Crossover Due to Progressive Disease on Day 14.

	Crossover (n=7)	No crossover (n=45)	P value
Demographic and clinical characteristics			
Median age, years (IQR)	62 (53-63)	62 (55-67)	0.62
Gender, no (%)			1.00
Female	2 (28.6)	15 (33.3)	
Male	5 (71.4)	30 (67.3)	
Body Mass Index, kg/m^2 (IQR)	29.0 (23.2-30.4)	29.4 (26.1-32.00)	0.29
Coexisting Diseases, n (%)			
no other disease	1 (14.3)	3 (6.7)	
BMI >30 kg/m2	5 (71.4)	24 (53.3)	
Hypertension	3 (42.9)	25 (55.6)	
Diabetes	1 (14.3)	14 (31.1)	
COPD, Asthma, other pulmonary disease	1 (14.3)	8 (17.8))	
Thromboembolic disease	1 (14.3)	2 (4.4)	
solid tumor	1 (14.3)	2 (4.4)	
other	4 (57.1)	31 (68.9)	
Point Scale at Study entry, n (%)			0.79
3	0 (0.0)	3 (6.7)	
4	0 (0.0)	8 (17.8)	
5	4 (57.1)	17 (37.8)	
6	0 (0.0)	3 (6.7)	
7	3 (42.9)	14 (31.1)	
Median time from symptom onset of the SARS-CoV-2 infection to randomization, days (IQR)	10 (7-10)	7 (5-11)	0.88
Median time from hospitalization to randomization, days (IQR)	3 (1-8)	2 (1-5)	0.77

SARS-CoV-2 status at baseline			
Result of SARS-CoV-2 PCR nasopharyngeal swab, n (%)			1.0
positive	7 (100.0)	43 (95.6)	
negative	0 (0.0)	1 (2.2)	
missing	0 (0.0)	1 (2.2)	
Humoral immune response at baseline			
SARS-CoV-2 IgA present, n (%)			0.82
Yes	6 (85.7)	32 (71.1)	
No	1 (14.3)	6 (13.3)	
Missing	0 (0.0)	7 (15.6)	
SARS-CoV-2 IgG present, n (%)			0.85
Yes	5 (71.4)	25 (55.6)	
No	2 (28.6)	13 (28.9)	
Missing	0 (0)	7 (15.5)	
Neutralizing antibodies (based on PRNT50 titer ≥20) present			0.63
no	2 (28.6)	8 (17.8)	
yes	5 (71.4)	33 (73.3)	
Missing	0 (0.0)	4 (8.9)	

Laboratory values at baseline		
Inflammation Markers		
Ferritin [µg/L], median (IQR)	1412 (1015-1467)	1096 (639-2496)
CRP [mg/L], median (IQR)	157 (102-227)	126 (66-194)
IL-6 [pg/ml], median (IQR)	142 (44-253)	35 (17-104)
LDH [U/L], median (IQR)	631 (477-789)	481 (362-657)

Table 8: Baseline Demographics and Clinical Characteristics of Patients in the Control Group with Crossover and Patients from the CCP Group Matched by Baseline Characteristics.

	Crossover (n=7)	CCP (n=14)	P value
Demographic and clinical characteristics			
Median age, years (IQR)	62 (53-63)	57 (50-64)	0.66
Gender, no (%)			0.57
Female	2 (28.6)	2 (14.3)	
Male	5 (71.4)	12 (85.7)	
Body Mass Index, kg/m^2 (IQR)	29.0 (23.2-30.4)	30.8 (27.5-93.4)	0.09
Coexisting Diseases, n (%)			
no other disease	1 (14.3)	1 (7.1)	
BMI >30 kg/m2	5 (71.4)	6 (42.9)	
Hypertension	3 (42.9)	9 (64.3)	
Cardiovascular disease	0 (0.0)	3 (21.4)	
Diabetes	1 (14.3)	6 (42.9)	
COPD, Asthma, other pulmonary disease	1 (14.3)	3 (21.2))	
Thromboembolic disease	1 (14.3)	0 (0)	
solid tumor	1 (14.3)	0 (0)	
other	5 (71.4)	7 (50.0)	
Point Scale at Study entry, n (%)			0.92
3	0 (0.0)	1 (7.1)	
4	0 (0.0)	2 (14.3)	
5	4 (57.1)	5 (35.7)	
6	0 (0.0)	1 (7.1)	
7	3 (42.9)	5 (36.7)	
Respiratory support at baseline			0.96
No respiratory support	0 (0)	1 (7.1)	
O_2 by nasal cannula	0 (0)	2 (14.3)	
High flow O_2	2 (28.6)	2 (14.3)	
Non-invasive ventilation	2 (28.6)	3 (21.4)	
Invasive ventilation	3 (42.9)	5 (35.7)	
ECMO	0 (0)	1 (7.1)	
Median time from symptom onset of the SARS-CoV-2 infection to randomization, days (IQR)	10 (7-10)	7 (3-8)	0.17
Median time from hospitalization to randomization, days (IQR)	3 (1-8)	1 (1-2)	0.11
Transfused neutralizing units Median (IQR)	2952 (1688-3348)	5988 (3328-6544)	

Humoral immune response at baseline			
SARS-CoV-2 IgA present, n (%)			1.00
Yes	6 (85.7)	10 (71.4)	
No	1 (14.3)	3 (21.4)	
Missing	0 (0)	1 (7.1)	
SARS-CoV-2 IgG present, n (%)			0.44
Yes	5 (71.4)	5 (35.7)	
No	2 (28.6)	8 (57.1)	
Missing	0 (0.0)	1 (7.1)	
Neutralizing antibodies (based on PRNT50 titer ≥20) present			1.00
no	2 (28.6)	4 (28.6)	
yes	5 (71.4)	10 (71.4)	

Laboratory values at baseline		
Inflammation Markers		
Ferritin [µg/L], median (IQR)	1412 (1015-1467)	1109 (407-1691)
CRP [mg/L], median (IQR)	157 (102-227)	229 (76-284)
IL-6 [pg/ml], median (IQR)	142 (44-253)	94 (43-154)
LDH [U/L], median (IQR)	631 (477-789)	463 (426-571)

Table 9: Baseline Demographics and Clinical Characteristics of Patients in the Control Group with Crossover and Patients from the CCP Group Matched by Ventilation Status on Day 14.

	Crossover (n=6)	CCP (n=6)	P value
Demographic and clinical characteristics			
Median age, years (IQR)	62 (53-63)	64 (59-68)	0.29
Gender, no (%)			1.00
Female	1 (16.7)	0 (0)	
Male	5 (83.3)	6 (100)	
Body Mass Index, kg/m^2 (IQR)	27.7 (23.2-29.1)	27.8 (27.8-30.4)	0.20
Coexisting Diseases, n (%)			
no other disease	1 (16.7)	0 (0.0)	
BMI >30 kg/m2	5 (83.3)	4 (66.7)	
Hypertension	2 (33.3)	5 (83.3)	
Cardiovascular disease	0 (0.0)	2 (33.3)	
Diabetes	0 (0)	2 (33.3)	
COPD, Asthma, other pulmonary disease	1 (16.7)	3 (50.0)	
Thromboembolic disease	1 (16.7)	0 (0)	
solid tumor	1 (16.7)	0 (0)	
other	4 (66.6)	4 (66.6)	
Point Scale at day 14, n (%)			0.18
6	0 (0)	3 (50.0)	
7	6 (100)	3 (50.0)	
Respiratory support at day 14, n (%)			0.24
Invasive ventilation	1 (16.7)	4 (66.7)	
ECMO	5 (83.3)	2 (33.3)	
Median time from symptom onset of the SARS-CoV-2 infection to randomization, days (IQR)	10 (8-10)	5 (2-9)	0.18
Median time from hospitalization to randomization, days (IQR)	4 (2-8)	2 (1-2)	0.26
Transfused neutralizing units Median (IQR)	3092 (2362-3348)	4844 (3384-6336)	

Humoral immune response at baseline			
SARS-CoV-2 IgA present, n (%)			1.00
Yes	6 (100)	6 (100)	
No	0 (0)	0 (0)	
SARS-CoV-2 IgG present, n (%)			1.00
Yes	5 (83.3)	5 (83.3)	
No	1 (16.7)	1 (16.7)	
Neutralizing antibodies (based on PRNT50 titer ≥20) present			1.00
no	1 (16.7)	0 (0)	
yes	5 (83.3)	6 (100)	

Laboratory values at baseline		
Inflammation Markers		
Ferritin [µg/L], median (IQR)	1432 (1237-1467)	1002 (817-1433)
CRP [mg/L], median (IQR)	154 (102-163)	218 (81-300)
IL-6 [pg/ml], median (IQR)	109 (44-155)	82 (74-103)
LDH [U/L], median (IQR)	559 (477-685)	515 (456-537)

Table 10: Proportion of Patients with Low or High Inflammation Markers at Baseline in the CCP Group and the Control Group.

	CCP group			Control group		
	"high"	"low"	missing	"high"	"low"	missing
Ferritin	41.5%	45.3%	13.2%	46.2%	42.3%	11.5%
CRP	52.8%	45.3%	1.9%	46.2%	53.8%	
IL-6	41.5%	35.8%	22.6%	38.5%	44.2%	17.3%

The proportion of patients with baseline values > median ("high") or ≤ median ("low") for ferritin, CRP and IL-6,

Figures

Figure 1A: Distribution of anti-SARS-CoV-2 Titer (PRNT50) in Transfused CCP

The graphs show the relative proportion of patients receiving a CCP with the titer level as indicated on the x-axis for 1., 2. and 3.transfusion of CCP. Titer levels indicate 50% inhibition in the PRNT assay.

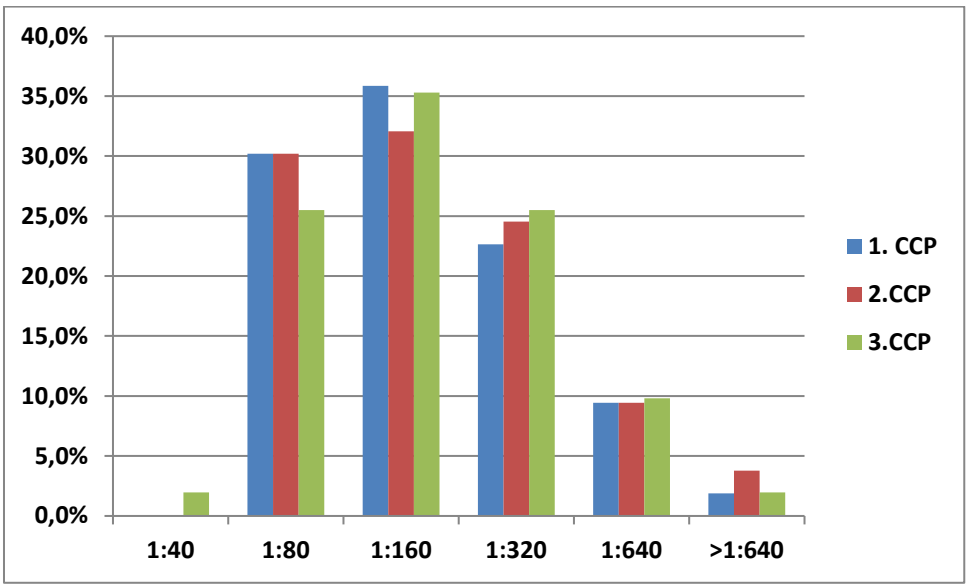


Figure 1B: Distribution of anti-SARS-CoV-2 Titer (PRNT50) in CCP Units Transfused in High Dose and Low Dose CCP Subgroup.

Distribution of the PRNT50 titers of the transfused CCP units in the CCP subgroups which received a high cumulative amount of neutralizing units (upper panel) or a low cumulative amount of neutralizing units (lower panel). The bars represent the relative proportion of transfused CCP in the respective PRNT50 titer group from 1:40 to >1:640. The numbers above the bars denote the relative percentage of CCPs within each subgroup.

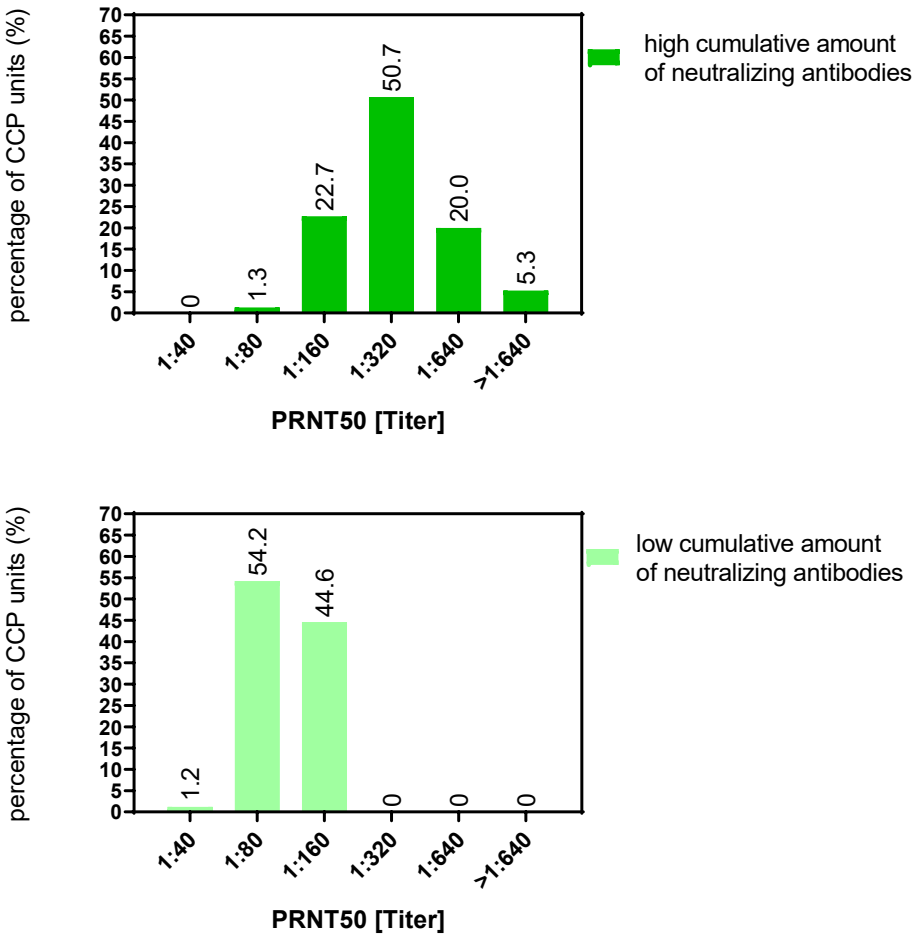


Figure 2A: Probability of First Negative SARS-CoV-2 PCR

Kaplan-Meier estimate of probability of first negative SARS-CoV-2 PCR from nasopharyngeal specimen. Censored patients are indicated by +. P=0.38 (log-rank test).

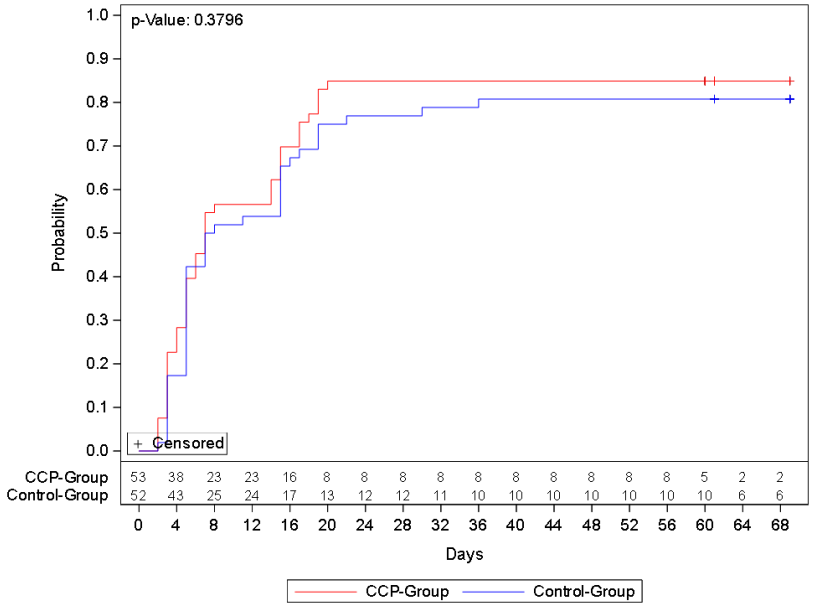


Figure 2B: Probability of First Negative SARS-CoV-2 PCR by Cumulative Amount of Transfused Neutralizing Units

Kaplan-Meier estimate of time to first negative SARS-CoV-2 PCR from nasopharyngeal specimens compared in the CCP subgroup which received a low cumulative amount of neutralizing units (red), the CCP subgroup which received a high cumulative amount of neutralizing units (blue) and the control group (green line). Censored patients are indicated by +. P=0.07 (log-rank test, high amount vs. control group).

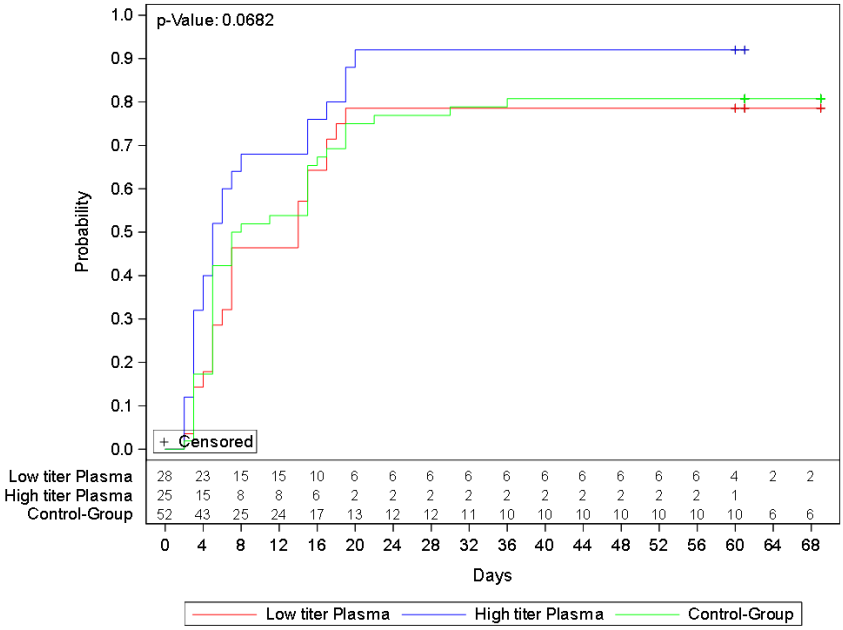


Figure 3A: Probability of Clinical Improvement by Inflammation Markers at Baseline

Kaplan-Meier cumulative estimate of probability of clinical improvement compared in the CCP group with low (purble line) and high inflammation marker (red) and the control group with low (turquoise line) and high inflammation markers (blue line) at baseline. Censored patients are indicated by +. P=0.02 (log-rank test).

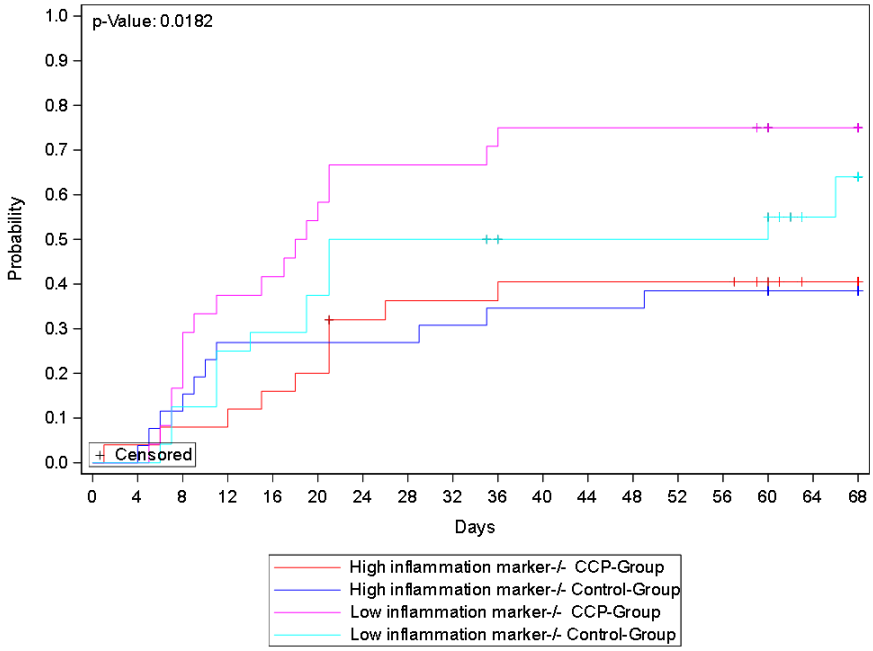


Figure 3B: Probability of Discharge from Hospital by Inflammation Markers at Baseline

Kaplan-Meier cumulative estimate of probability of discharge from hospital compared in the CCP group with low (purble line) and high inflammation marker (red) and the control group with low (turquoise line) and high inflammation markers (blue line) at baseline. Censored patients are indicated by +. P=0.02 (log-rank test).

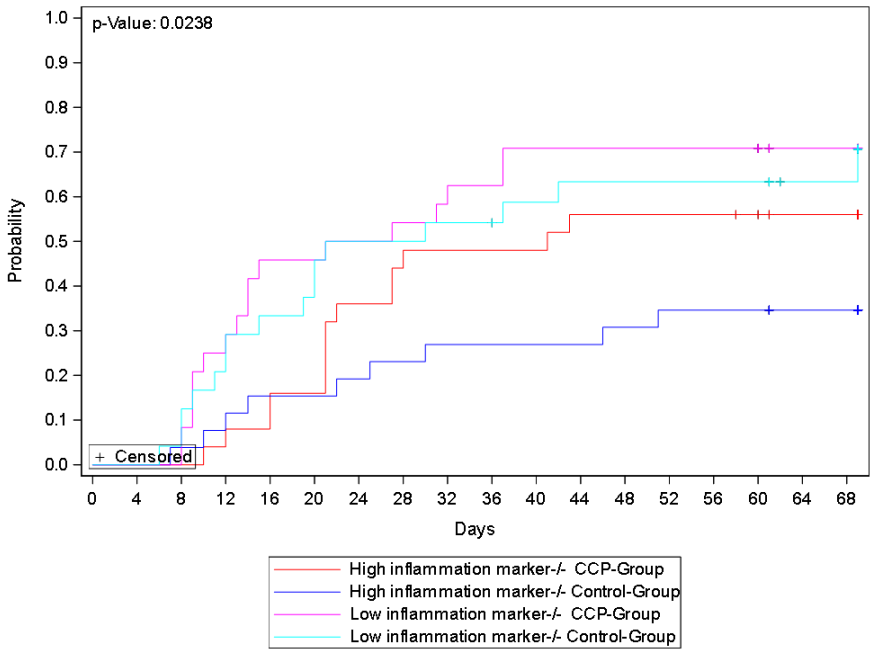
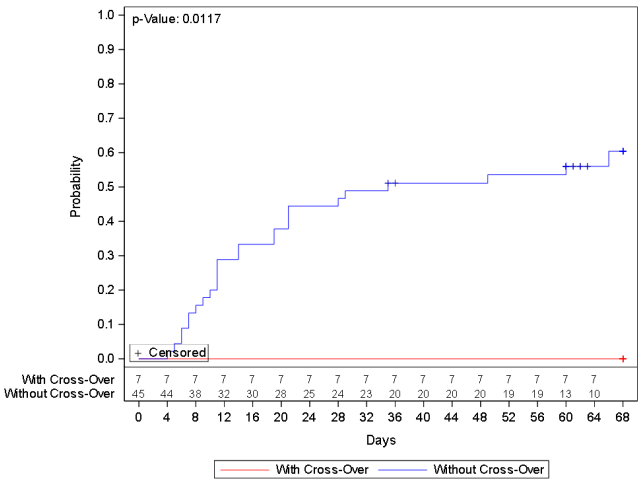


Figure 4: Occurrence of Secondary Outcomes in Control Group Patients with or without Crossover to CCP Treatment

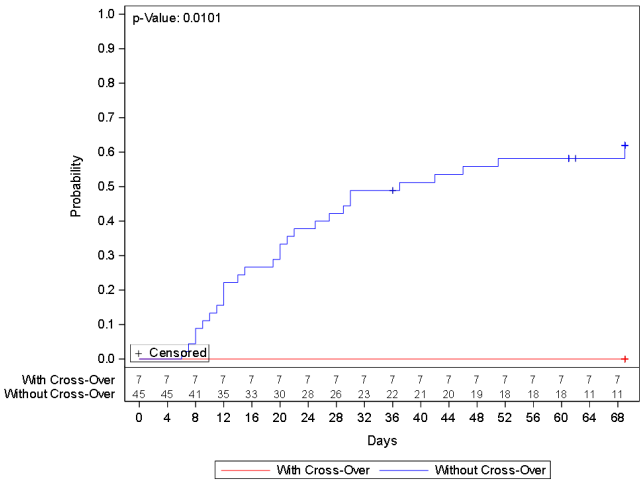
Kaplan-Meier cumulative estimate of probability of

A Probability of clinical improvement



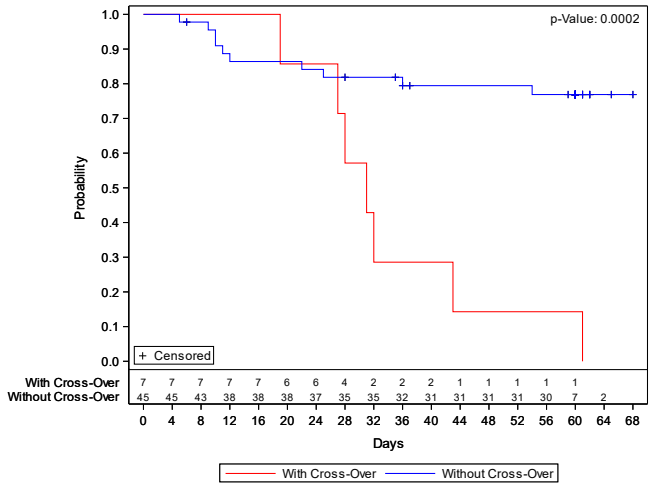
(A) clinical improvement compared in the control group with crossover (red) and without crossover (blue) due to progressive disease on day +14 (CCP treatment on days 15, 17 and 19). Censored patients are indicated by +. P=0.012 (log-rank test).

B Probability of hospital discharge



(B) discharge from hospital compared in the control group with crossover (red) and without crossover (blue) due to progressive disease on day +14 (CCP treatment on days 15, 17 and 19). Censored patients are indicated by +. P=0.01 (log-rank test).

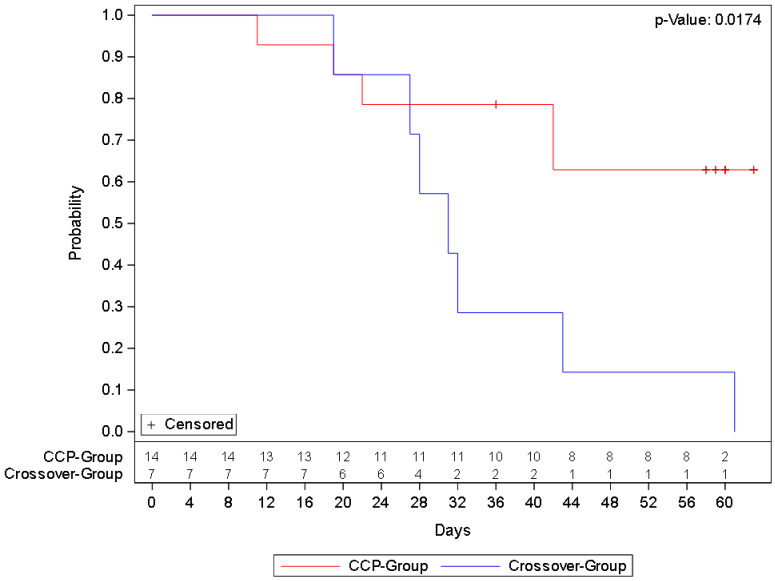
C Probability of overall survival



(C) Overall survival in the control group with crossover (red) and without crossover (blue) due to progressive disease on day +14 (CCP treatment on days 15, 17 and 19). Censored patients are indicated by +. P<0.001 (log-rank test).

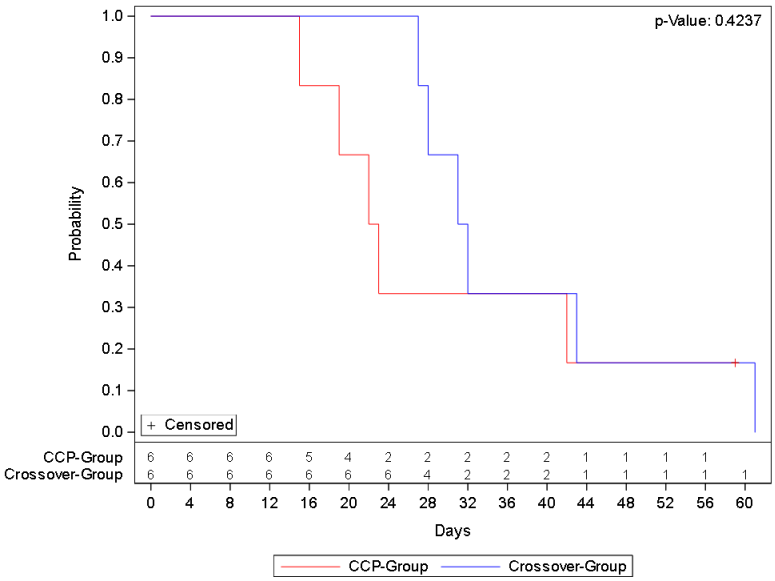
Figure 5: Probability of Overall Survival of Crossover Patients and Matched Patients from the Initial CCP Group.

A: Crossover patients and CCP group patients matched based on baseline characteristics.



Overall survival in patients with crossover (blue line) treated with CCP on day +15, +17 and +19 (n=7) and matched patients treated with CCP on day +1, +3 and +5 (red line)(n=14). The 14 patients of the plasma group were matched according to baseline characteristics. Censored patients are indicated by +. P=0.017 (log-rank test). Variables for propensity score matching are described in Methods in the supplemental material.

B: Crossover patients and CCP group patients matched based on baseline characteristics and ventilation status on day 14



Overall survival in patients with crossover (blue line) treated with CCP on day +15, +17 and +19 (blue line)(n=6) and matched patients treated with CCP on day +1, +3 and +5 (red line)(n=6). The 6 patients of the plasma group were matched according to ventilation status on day 14 and baseline characteristics. Censored patients are indicated by +. p=0.424 (log-rank test). Variables for propensity score matching are described in Methods in the supplemental material.

Methods

Additional Information on Trial Design and Methods

Crossover from Control Group to CCP treatment

Clinical condition in all patients was evaluated on day 14. In case of progressive COVID-19 on day 14 compared to baseline, patients in the control group could be switched to treatment with CCP. A patient switching from the control group to CCP because of progressive COVID-19 on day 14 was considered as failure of the primary outcome. Criteria of progress for the crossover decision were as follows: (a) in patients not requiring invasive ventilation or ECMO at baseline: start of invasive ventilation support or ECMO in the interval from randomization to day 14; (b) in patients requiring invasive ventilation already at baseline: deterioration of ARDS according to the Berlin classification (3): (i) progression to moderate or severe ARDS if ARDS was mild at baseline; (ii) progression to severe ARDS if moderate at baseline and (iii) start of extracorporeal oxygenation in the interval from randomization to day 14. Crossover decisions were confirmed by an independent expert who was experienced in treatment of ARDS and who was not part of the study teams. Seven patients randomized to the control group crossed over to receive CCP after assessment on day 14.

In order to investigate the crossover patients in more detail two subsets of the initial CCP cohort were identified by propensity score matching. The baseline matched subset of ITT matched patients from the initial CCP group to patients from the control group that had a crossover to CCP by the following variables (terms in brackets are possible values)

- age at registration (continuous variable)
- PRNT50 titer at baseline (positive; negative)
- inflammation markers at baseline (high; intermediate; low; missing)
- sex (male; female)
- ventilation support at baseline (ECMO or invasive ventilation; No ECMO or invasive ventilation)
- transfused neutralizing units (>median of all patients; ≤median of all patients).

A second subset included matching of ventilation support on day 14 (D14MS): By propensity score matching patients from the initial CCP group were matched to patients from the control group that had a crossover to CCP by the following variables (terms in brackets are possible values)

- age at registration (continuous variable)
- PRNT50 titer at baseline (positive; negative)
- inflammation markers at baseline (high; intermediate; low; missing)
- sex (male; female)

- ventilation support at day 14 (ECMO or invasive ventilation; no ECMO or invasive ventilation)
- transfused neutralizing units (>median of all patients; ≤median of all patients)

Propensity score matching was performed by using the SAS procedure PROC PSMATCH with default settings using optimal matching to identify matched patients.

Concomitant Treatment

Patients in both groups received other anti-viral treatment and/or supportive treatment according to institutional standard procedures for patients with severe infection with respiratory viruses. Centers were advised to adhere to current recommendations for treatment of severe COVID-19 published by medical societies. Prior and concomitant medication was coded into certain pre-specified categories (corticosteroids, anticoagulation, tocilizumab, NSAIDS, antiviral drugs, others). Medication was evaluated as concomitant medication when the medication was started after a patient's baseline date.

Plaque reduction neutralization test (PRNT) for SARS-CoV-2

Plaque reduction neutralization tests for SARS-CoV-2 were performed as previously described (1,2,4). Briefly, VeroE6 cells ($3.25x10^5$ cell/ml) were seeded in 24-well plates and incubated overnight. Prior to PRNT, patient sera were heat-inactivated at 56°C for 30 minutes. For each dilution step (duplicate), patient sera were diluted in 220 µl OptiPro and mixed 1:1 with 220 µl virus solution containing 100 plaque forming units. The 440 µl serum-virus solution was gently vortexed and incubated at 37°C for 1 hour. Each 24-well was incubated with 200 µl serum-virus solution. After 1 hour at 37°C supernatants were discarded, and cells were supplemented with 1.2% Avicel solution in DMEM. After 3 days at 37°C, supernatants were removed and the 24-well plates were fixed and inactivated using a 6% formaldehyde/PBS solution and stained with crystal violet as described (11). Serum dilutions with a plaque reduction of 50% (PRNT50) and 90% (PRNT90) are referred to as titers. Unless stated otherwise, cut off titers were set at < 1:20.

Enzyme-linked immunosorbent assay (Euroimmun)

The Euroimmun anti-SARS-CoV-2 assay is a classical enzyme-linked immunosorbent assay (ELISA) for the detection of IgG and IgA to the S1 domain of the SARS-COV-2 spike (S) protein. The assay was performed manually according to the manufacturer´s instructions as previously described (1). Results are expressed as optical density (OD) ratios, which were calculated based on the sample and calibrator OD values. For all analytes, a ratio < 0.8 was considered to be non-reactive or negative. An OD-ratio of ≥ 1.1 was considered to be positive.

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