Sustained response after remdesivir and convalescent plasma therapy in a B-cell depleted patient with protracted COVID-19

Jakob Malsy[1*], Luzia Veletzky[1*], Janna Heide[1,8], Annette Hennigs[1], Ines Gil-Ibanez[1], Alexander Stein[2,3], Marc Lütgehetmann[4,8], Ulrich Rosien[5], Dorothea Jasper[5], Sven Peine[6], Jens Hiller[6], Friedrich Haag[7], Stefan Schmiedel[1], Samuel Huber[1], Sabine Jordan[1], Marylyn M. Addo[1,8], Julian Schulze zur Wiesch[1,8°]

1 I. Department of Medicine, Gastroenterology and Hepatology, with the Sections Infectious Diseases and Tropical Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany.

2 Hämatologisch-Onkologische Praxis Eppendorf, HOPE

3 University Cancer Center Hamburg (UCCH), Hamburg, Germany

4 Institute of Medical Microbiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

5 Department of Medicine, Israelitisches Krankenhaus, Hamburg, Germany

6 Institute of Transfusion Medicine, University Medical Center Hamburg-Eppendorf, Germany

7 Department of Clinical Immunology, University Medical Center Hamburg-Eppendorf, Germany

8 German Center for Infection Research (DZIF), Hamburg-Lubeck-Borstel-Riems, Germany

*these authors contributed equally, °corresponding author

Corresponding author: PD Dr. Schulze zur Wiesch | Martinistraße 52, 20246 Hamburg, Germany phone: + 49 40 7410 20977 | fax: + 49 40 7410 55128 | j.schulze-zur-wiesch@uke.de

Abstract

We provide detailed clinical, virological and immunological data of a B-cell depleted patient treated with obinutuzumab for follicular lymphoma with protracted COVID-19 and viremia. A sustained response was achieved after two courses of remdesivir and subsequent convalescent plasma therapy. Immunocompromised patients might require combined and prolonged antiviral treatment regimens.

Key words: COVID-19, obinutuzumab, follicular lymphoma, convalescent plasma, remdesivir

Introduction

The majority of patients with coronavirus disease 2019 (COVID-19) show mild to moderate symptoms, while a minority develops a more severe disease course that can include complications like ARDS, septic shock, cardiac injury and thrombosis [1,2]. Immunocompromised patients are prone to prolonged disease courses, but at the same time might be protected from severe disease mediated by an overstimulation of the immune system [3,4]. First case reports of COVID-19 patients with B-cell depletion after anti-CD20 therapy seem to confirm this notion [5,6]. The nucleotide analogue remdesivir is the only antiviral agent approved for the treatment of COVID-19 to date and the efficacy of convalescent plasma therapy is currently being assessed [7]. Here, we report about a prolonged COVID-19 course with SARS-CoV-2 viremia in a patient who received the $CD20^+$ cell depleting antibody obinutuzumab after chemotherapy for follicular lymphoma prior to infection with SARS-CoV-2. This patient did not produce SARS-CoV-2-specific antibodies and did not clear viremia on her own despite the detection of SARS-CoV-2-specific T-cells. After subsequent combination therapy with remdesivir and convalescent plasma, this patient now displays a sustained virological control of COVID-19.

Case description

On March 23, 2020 a 53-year-old woman returned from a ski trip and presented to our emergency department with recurrent fever, myalgias, asthenia, loss of appetite, a dry cough and mild dyspnea. A nasopharyngeal swab had tested positive for SARS-CoV-2 RNA (**Fig.1**). In 2019 the patient had been treated with obinutuzumab-CHOP for a follicular lymphoma (grade I, Ki67 40%, stage III). Starting November 2019, she received monotherapy with obinutuzumab (last dose in January 2020, 1000mg i.v.). On admission, the results of her physical exam were as follows: body temperature 37.8° Celsius, blood pressure 120/60 mm Hg, heart rate 89 beats per minute, respiratory rate of 14 breaths per minute and oxygen saturation of 98% while breathing ambient air. A chest radiograph showed faint patchy consolidations in the lower lungs. Laboratory findings included anemia (Hb 11.8 g/dl), leukocytopenia (2.4 x 10^9/l), and lymphocytopenia (0.48 x 10^9/l). CRP, LDH and IL-6 were elevated with 26 mg/l, 284 U/l and 26.6 ng/l respectively, B-cells were undetectable and T-cell counts were low (374 /µl). Both $CD4^+$ and $CD8^+$ T-cell numbers were decreased (293 /µl and 61 /µl respectively) with a $CD4^+/CD8^+$ ratio of 4.78 and a normal concentration of regulatory T-cells (8.4 %) (**Fig.1A, Tab.1**).

The patient was discharged following the resolution of her fever and improvement of asthenia and myalgias after one week. However, she was readmitted after two days with renewed fever, nausea, progressive dyspnea and unchanged elevated laboratory parameters (**Tab.1**). Regularly collected nasopharyngeal swabs were negative since day 21 after the onset of symptoms. Hereafter, the sputum remained positive. A CT-scan on day 35 revealed a progression of the infection from the upper to the lower airways with detection of bilobular pulmonary infiltrations (**Fig.1A and Fig.1E**). Comprehensive microbiological tests remained negative. For the next 40 days the patient remained hospitalized due to intermittent dyspnea, fever, asthenia, elevated markers of inflammation and received symptomatic therapy. 49 days after the initial onset of symptoms, her fever eventually subsided and the patient was again discharged (**Fig.1A**). 5 days later, a scheduled outpatient follow-up appointment revealed a Hb of 7.2 mg/dl, progressive dyspnea and liquid diarrhea. After readmission to a local hospital, the patient received blood transfusions and antibiotic therapy but

remained febrile with hypotensive episodes (90/60 mm Hg) and was consecutively transferred to our hospital. SARS-CoV-2 PCR revealed viral RNA in the patients' blood and stool (**Fig.1A**). IL-6 and CRP as well as proBNP were elevated (81.1 pg/ml, 198 mg/dl and 2250 ng/l respectively; **Tab.1**). T-cell counts further declined with a preferential loss of CD8+ cells, resulting in an elevated $CD4^+/CD8^+$ T-cell ratio of 6.62. Transthoracic echocardiography revealed normal cardiac function. A pulmonary embolism was ruled out by CT-imaging which revealed progressive bi-pulmonary consolidations and ground glass opacities (**Fig.1E**). 63 days after onset of symptoms, intravenous remdesivir treatment was initiated with a loading dose of 200 mg followed by nine days of 100 mg per day as part of the open-label GS-US-540-5773 trial. The fever quickly resolved and her cough and diarrhea improved. After ten days of remdesivir therapy no SARS-CoV-2-RNA was detectable in her blood and stool and inflammatory parameters normalized (**Fig.1A, Tab.1**). A CT-scan showed regression of pulmonary infiltrates and the patient was discharged (**Fig. 1E: Pictures G-I**).

Eight days after cessation of treatment with remdesivir, fever, cough, diarrhea and asthenia reoccurred and the patient was readmitted to our hospital with detectable SARS-CoV-2-RNA in blood and sputum, as well as elevated inflammatory markers and again increased lymphocytopenia (**Fig.1A, Tab.1**). Under the impression of a recrudescence of COVID-19, including detectable SARS-CoV-2 viremia, and having ruled out other infectious complications, the patient received 100 mg remdesivir per day for five days within the framework of a compassionate use program. Again, symptoms rapidly improved. To prevent a second relapse in the absence of an intrinsic antibody-response it was then decided in agreement with the patient, to administer convalescent plasma therapy as an individual healing attempt. Two courses of six units of convalescent plasma were transfused, each consisting of 2 units per day administered every other day (**Fig.1B**). Three weeks after the last plasma administration, the patient was asymptomatic and SARS-CoV-2 PCR from swabs and blood remained negative. Since then the patient has achieved a sustained control of viremia and has remained asymptomatic. Anti-SARS-CoV-2 IgG serum-concentrations were detectable directly after the transfusions but decreased over the following weeks (**Fig.1B)**.

Material

Written informed consent was obtained from the patient. PCR to detect SARS-CoV-2 RNA and detection of specific antibodies were performed as described previously [8,9].

138 peptides overlapping by ten amino acids corresponding to the complete SARS-CoV-2 membrane-, envelope-, and nucleocapsid amino acid sequence were synthesized and pooled into 13 pools [9]. High definition human leukocyte antigen class I and II typing was performed by PCR-sequence-specific oligonucleotide probing. 30-50 x 10^6 fresh peripheral blood mononuclear cells were stimulated with one of the 13 peptide pools for 10 days. After 10 days, cells were re-stimulated with single SARS-CoV-2-peptides and subsequently assayed for interferon-γ production by Enzyme-linked immunosorbent spot and intracellular cytokine staining as previously described [9,10].

Discussion

Anti-CD20 therapy has been reported to impair cytokine production, B-/T-cell interactions and to reduce cell populations including T- and B-memory cells [11]. Although production of immunoglobulins is not immediately impaired by obinutuzumab - since plasma-cells do not express CD20 - CD20-mediated depletion of earlier B-cell differentiation stages attenuates the humoral immune response to neoantigens [11]. In accordance with these observations our patient showed immunoglobulin levels at the lower limits of normal (**Tab.1**) and no SARS-CoV-2-specific antibodies were detectable prior to the treatment with convalescent plasma. While the virus was cleared from the upper airways within twenty days, the patient was unable to control SARS-CoV-2 viremia despite a broad SARS-CoV-2 T-cell response (**Fig.1D**). CT-scans at days 35 and 62 revealed progressive lung infiltrations (**Fig.1E**), and repeated SARS-CoV-2-specific PCR from sputum, blood and stool tested positive (**Fig.1A**) – indicating first a shift from the upper to the lower airways and eventually dissemination of the virus to different body compartments. Unfortunately, routine blood and stool SARS-CoV-2 PCR testing became only available during the course of her hospitalization, and the kinetics of viral dissemination could not be determined. A reinfection with SARS-CoV-2 could be an alternative explanation of the reoccurrence of symptoms after the first course of remdesivir. However, the patient had stayed in strict home isolation after discharge and had repeated negative nasopharyngeal swabs after readmission (**Fig.1A**). Therefore, the possibility of a reinfection was considered very unlikely.

Remdesivir shortens recovery time in immunocompetent patients, likely by inhibiting proliferation of viral RNA [7]. Efficacy data on convalescent plasma for treatment of COVID-19 are scarce but suggest an improved outcome in immunocompetent patients [12]. However, in this case the rationale for plasma administration was to substitute the specific antibodies that the patient was unable to produce herself.

While remdesivir did not appear to sufficiently suppress the viral replication on its own, the combination of remdesivir, followed by convalescent plasma therapy, coincided with a sustained virological response. After plasma transfusion, anti-SARS-CoV-2 IgG remained detectable in decreasing concentrations and were most probably transfused allogenic antibodies.

Whether plasma transfusion alone would have been sufficient to clear the virus can only be speculated. The overall efficacy of plasma therapy in viremic SARS-CoV-2 patients alone or in combination with other antivirals has not yet been reported. In this current case, the rationale of applying plasma therapy was to prevent another recrudescence of COVID-19 after remdesivir therapy in the absence of intrinsic SARS-CoV-2-antibodies.

It is not clear in how far patients lacking a B-cell response are protected from reinfection by their immune system. Also, it is unknown, whether and at which point a sustained viral response can be assumed in SARS-CoV-2 infections after therapy-induced clearance of the virus.

Successful treatment of SARS-CoV-2 infection in immunocompromised patients might require combined and prolonged antiviral treatment regimens that need to be established in future prospective trials that focus on this vulnerable patient population.

NOTES

Acknowledgments

The authors thank the UKE ID COVID-19 study team. They extend their gratitude to the patient for allowing us to report this interesting case. We thank Diana Brainard for critical discussions.

Funding

This work was supported by Deutsche Forschungsgemeinschaft (DFG) and German Center for Infection (DZIF). DFG SFB841 to JSzW, ML and SH, DFG SFB1328 to JSzW, FH and SH.

Disclosure of Conflicts of Interest

JSzW previously received speaker´s fees from Gilead. The other authors declare no competing interests. MMA acted as investigator of the Gilead-sponsored, open-label GS-US-540-5773 trial. A.H. reports that patient was part of the Trial GS-US-540-5773 (as mentioned in the report) and received the first course of Remdesivir through the trial from Gilead Sciences. S.P. reports personal fees from Cerus Corporation, outside the submitted work. All other authors have no potential conflicts.

References

1. Guan W, Ni Z, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med **2020**; 382:1708–1720.

2. Wang D, Hu B, Hu C, et al. Clinical Characteristics of 138 Hospitalized Patients with 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA **2020**; 323:1061–1069.

3. Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. Virol Sin **2020**; 35:266–271.

4. Decker A, Welzel M, Laubner K, et al. Prolonged SARS-CoV-2 shedding and mild course of COVID-19 in a patient after recent heart transplantation. Am J Transplant **2020**; 20:1–7.

5. Suwanwongse K, Shabarek N. Benign course of COVID-19 in a multiple sclerosis patient treated with Ocrelizumab. Mult Scler Relat Disord **2020**; 42:102201.

6. Woo MS, Steins D, Häußler V, et al. Control of SARS-CoV-2 infection in rituximab-treated neuroimmunological patients. J Neurol **2020**; 267:1–3.

7. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 — Preliminary Report. N Engl J Med **2020**; :NEJMoa2007764.

8. Pflüger LS, Bannasch JH, Brehm TT, et al. Clinical evaluation of five different automated SARS-CoV-2 serology assays in a cohort of hospitalized COVID-19 patients. J Clin Virol **2020**; :104549.

9. Pfefferle S, Reucher S, Nörz D, Lütgehetmann M. Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. Eurosurveillance **2020**; 25:1–5.

10. Heide J, Wildner NH, Ackermann C, et al. Detection of EXP1-Specific CD4+ T Cell Responses Directed Against a Broad Range of Epitopes Including Two Promiscuous MHC Class II Binders During Acute Plasmodium falciparum Malaria. Front Immunol **2019**; 10:3037.

11. Aguado JM, Manuel O. Editorial for ESCMID Study Group for Infections in Compromised Hosts (ESGICH) Consensus Document on the safety of targeted and biological therapies: an infectious diseases perspective. Clin Microbiol Infect **2018**; 24 Suppl 2:S1.

12. Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci U S A **2020**; 117:9490–9496.

		days after onset of symptoms																				
laboratory parameter	**normal range**	**12**	**23**	**29**	**31**	**36**	**45**	**58**	**63**	**68**	**70**	**74**	**76**	**79**	**84**	**92**	**97**	**103**	**110**	**114**	**124**	**138**
WBC count, x 10^9/l	3.8-11.0	2.4		3	2.7	2.6	2.2	4.6	4.6	3.9	4.6	6.2	5.4	3.7	3.8	5.9		3.9	4.2	3.4	4.2	2.8
Neutrophil count, x 10^9/l	1.5-7.7	1.52		2.42	2.28	2.06	1.64	4.21	4.3	3.05	3.52	4.52	3.82	2.21	3.16	4.87		2.42	2.78	2.12	1.41	53.9
Lymphocyte count Mrd/ll	1.1-3.4	0.48	0.37	0.38	0.21	0.31	0.3	0.24	0.12	0.38	0.51	0.63	0.51	0.36	0.31	0.41		0.69	0.54	0.47	1.76	0.72
Lymphocyte count, not-activated /µl	6-307	87		87	78			66		129	163	142	262	76	57		133	152			194	23
Lymphcyte count, activated /µl	<290	52		25	61			32		63	80	99	152	44	33		62	92			157	40
T-lymphocytes /µl	900-2900	374	295	271	191		156	203		422	453	405	379	334	150			609	563		1850	697
$CD4^+$ lymphocytes /µl	500-1350	293	230	222	156		118	171		318	343	288	268	230	105		217	380	382		613	422
$CD8^+$ lymphocytes /µl	290-930	61	52	40	27		29	26		87	92	95	89	84	39		119	206	161		1194	254
CD4/CD8 ratio	0.6-3.6	4.78	4.43	5.56	5.79		4.07	6.62		3.67	3.72	3.02	3	2.74	2.72		1.82	1.85	2.36		0.51	1.66
Regulatory T-cells, %	5,7-10.1	8.4			3		5.8	4.7		8.9	5.1	5.8			7.2		5.3	7.7			7.9	6.96
B-lymphocytes /µl	80-500	0		0	0			0		0	0	0	0	0	0		1	0			0	0
NK-cells /µl	35-350	67	82	33	33		74	11		47	50	84	91	96	55		46	95	140		147	97
IgG, g/l	6.5-16.0	6.36	5.87			5.37			5.34		5.39	5.71	5.61		4.67			6.1			6.64	6.35
IgA, g/l	0.4-3.5	1.81	1.81			1.73		1.66			1.73		1.71		1.36		1.56				1.71	1.66
IgM, g/l	0.5-3	0.5	0.42			0.37		0.34		0.3	0.31	0.32	0.32		0.27			0.36			0.4	0.41
IL-6 level pg/ml	<0.7	26.6	23.1	19.4	17.4	27.7	44.2	55	81.1	5.9	5.8	5.9	11.5	17.1	34.8			2.9	2.4	52.6	1.8	1.9
CRP, mg/dl	0-5	26	29	49	59	74	85	142	198	27	14	8	9	14	69	24		<4		<4	<4	<4
C3 mg/dl	90-170	175	203							186	160	167	189	198								156
C4 mg/dl	12-36	62.5	71.3							60	50.7	54.1	66.4	67.8								43.7
Calprotectin, in stool, µg/g	<50										15.7	27.4	9.19									
Hemoglobin, g/l	12.3-15.3	11.8		10.4	9.9	9.4	8.3	9.3	8.7	10.4	10.1	10.3	10.7	10.3	9.1	9.3		9.8	9.8	10.5	10.9	11.9
Platelet count, x	150-400	307		332	315	289	318	355	330	478	401	369	331	292	320	534		467	434	460	354	364

10^9/l																						
ALT, U/l	<35	16	22	38	20	44	26	45	43	60	50	33	25	29	29	48	54	48	40	34	30	
AST, U/l	<35	27	29	39	31	38	28	73	38	77	50	37	31	32	52	62	69	44	32	36	21	
Total bilirubin, mmol/l	0.3-1.2	0.4			0.2		0.2	0.2		0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.4	0.2	0.3	
Creatinine, mg/dl	0.55-1.02	0.69	0.77	0.9	0.87	0.66	0.64	0.63	0.52	0.54	0.53	0.55	0.52	0.59	0.6	0.56	0.56	0.52	0.51	0.62	0.64	
Sodium, mmoll/l	135-145	141	138	132	134	137	135	140		142	138	136	140	139	132	135	142	140	139	143	142	
Potassium, mmol/l	3.5-5.0	3.6	4.1	3.7	4	4.3	4.5	3.8		3.5	3.8	4.2	4	4	4.1	3.8	3.8	4	3.6	4	4.1	
Hypersensitive Tn T, pg/ml	<14	9			11	11	8	12	16													
Hypersensitive Tn I, pg/ml										5	6	6	7		7	5	3	3	3	3	5	
proBNP, ng/l	< 125				291	195	154	1319		169	115	68	108	154	435	78	435	283	111	184	96	
INR		1			1			1	1.1				1		1		0.9			0.9	0.9	
aPTT, s	23-30	22			26			19	32			24	24		26		20			21	21	
Fibrinogen, g/l	1.6-3.4	4.17			5.78			8.75	7.89			5.13	6.09	6.58	6.55		4.82			3.41	3.45	
D-dimer, mg/dl	0.21-0.52	0.32	1.16	1.15	0.82	1.06	0.7	1.25	1.03	0.44	0.51	0.82	0.55	0.56	0.52	0.46	0.5	0.41	0.3	0.66	0.38	
Procalcitonin, µg/l	0-0.5				0.31	0.06	<0.02	0.04	0.22	0.06	0.07	0.05	0.05	0.04	0.08	0.04	0.03	<0.02	<0.02	<0.02	<0.02	
Lactate dehydrogenase, U/l	120-246	284	255	289	283	310	280	591	400	483	420	406	288	281	455	363	400	324	242	295	250	
Alb, g/l	34-50	35.4			28.8			26.8							32.8		35.9			39.5	41	

Table 1. Laboratory data

Figure legends:

Figure 1: Timeline of clinical, laboratory and immunological findings of prolonged COVID-19.

A: Overview of clinical and laboratory findings. Microbiological findings, body temperature, CRP-levels, IL-6 levels, lymphocyte counts, and therapies given. '+' indicate positive, '-' indicate negative PCR results for SARS-CoV-2 RNA in different specimen. **B: Overview of the convalescent plasma therapy.** Concentrations of anti-SARS-CoV-2 IgG in the patient's serum (curve) and in the plasma administered (vertical bars) are shown. The convalescent plasma had been collected in Germany by a standardized apheresis protocol from voluntary blood donors with a history of COVID-19 and a positive SARS-CoV2-antibody screening test. C: No peripheral B-cells and low concentrations of T-cells, with dominant $CD8^+$ populations were observed on admission. **D: SARS-CoV-2-specific T-cell response.** Out of 138 tested SARS-CoV-2 peptides, 79 showed a broad T-cell response against CD4+ and 12 against CD8+ T-cells. Representative CD4+ and CD8+ T-cell responses against single peptides of the SARS-CoV-2 antigens envelope, membrane and nucleocapsid protein are shown. The left FACS-plots show the negative control (no peptide response) and the right FACS-plots the positive peptide response. Amino acid sequences of viral peptides are shown above the corresponding plots. **E**: **Progressive pulmonary consolidations resolved after remdesivir therapy.** Chest CT imaging on day 35 after onset of symptoms with bilateral, multiple ground-glass opacities (A-C). Contrast medium chest CT imaging on day 62 after onset of symptoms with progressive consolidations (D-F). Chest CT imaging on days 74 (G-I) and 112 (J-M) after onset of symptoms showed resolving infiltrations.

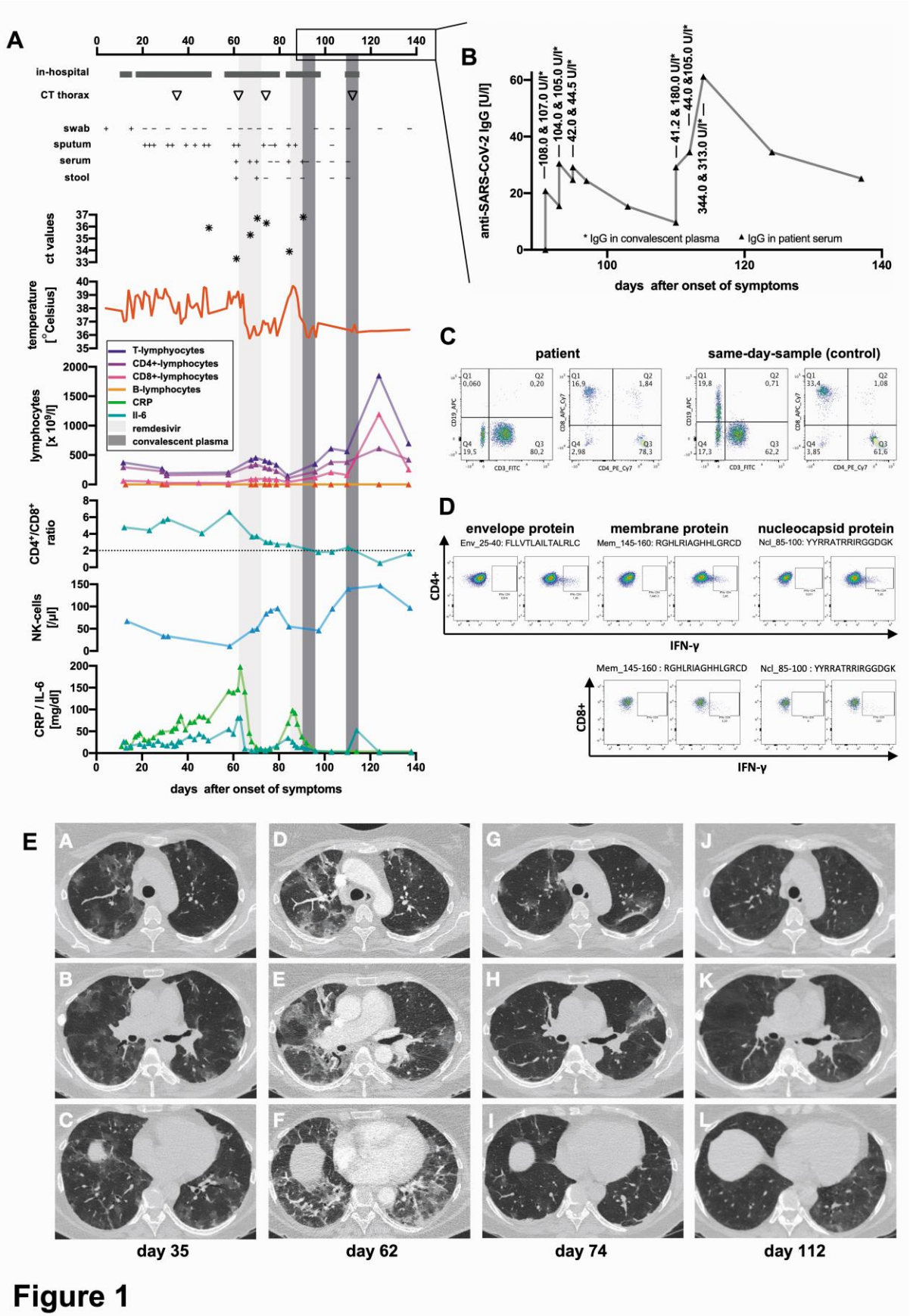
A
in-hospital
CT thorax
swab
sputum
serum
stool
ct values
temperature [°Celsius]
T-lymphyocytes
CD4+-lymphocytes
CD8+-lymphocytes
B-lymphocytes
CRP
Il-6
remdesivir
convalescent plasma
lymphocytes [x 10⁹/l]
CD4⁺/CD8⁺ ratio
NK-cells [/µl]
CRP / IL-6 [mg/dl]
days after onset of symptoms
B
anti-SARS-CoV-2 IgG [U/l]
108.0 & 107.0 U/l*
104.0 & 105.0 U/l*
42.0 & 44.5 U/l*
41.2 & 180.0 U/l*
44.0 &105.0 U/l*
344.0 & 313.0 U/l*
* IgG in convalescent plasma
IgG in patient serum
days after onset of symptoms
C
patient
same-day-sample (control)
CD19_APC
CD3_FITC
CD8_APC_Cy7
CD4_PE_Cy7
D
envelope protein
Env_25-40: FLLVTLAILTALRLC
membrane protein
Mem_145-160: RGHLRIAGHHLGRCD
nucleocapsid protein
Ncl_85-100: YYRRATRRIRGGDGK
CD4+
IFN-γ
Mem_145-160 : RGHLRIAGHHLGRCD
Ncl_85-100 : YYRRATRRIRGGDGK
CD8+
IFN-γ
E
day 35
day 62
day 74
day 112

Figure 1