Mortality reduction in 46 patients with severe COVID-19 treated with hyperimmune plasma. A proof-of-concept, single-arm, multicenter trial

Cesare Perotti,[1*] Fausto Baldanti,[1,2*] Raffaele Bruno,[1,2] Claudia Del Fante,[1] Elena Seminari,[1] Salvatore Casari,[3] Elena Percivalle,[1] Claudia Glingani,[3] Valeria Musella,[1] Mirko Belliato,[1] Martina Garuti,[3] Federica Meloni,[1,2] Marilena Frigato,[3] Antonio Di Sabatino,[1,2] Catherine Klersy,[1] Giuseppe De Donno[3] and Massimo Franchini[3] on behalf of The collaborative COVID-19 Plasma Task Force

[1]Departments of Immunohematology and Transfusion, Infectious Diseases, Respiratory Diseases, Intensive Care, Virology and Clinical Epidemiology & Biometry, Fondazione IRCCS Policlinico San Matteo, Pavia [2]University of Pavia, Pavia and [3]Departments of Immunohematology and Transfusion, Infectious Diseases, Respiratory Diseases, Carlo Poma Hospital, ASST Mantova, Mantova, Italy.

**CP and FB contributed equally as co-first authors.*

 doi:10.3324/haematol.2020.261784

Received: June 5, 2020.

Accepted: July 15, 2020.

Pre-published: July 23, 2020.

Correspondence: *CATHERINE KLERSY* - klersy@smatteo.pv.it

SUPPLEMENTARY MATERIAL

Table of contents

eTable 1 - Eligibility criteria

Inclusion criteria	
1	Age >=18 years
2	Positive SARS-CoV-2 RT-PCR on nasal swab or deep respiratory sample
3	Diagnosed with moderate-to-severe ARDS (Acute Respiratory Distress Syndrome) for ≤10 days, according to Berlin definition: a. New or worsening of respiratory symptoms within a week of a known clinical insult; b. Radiological imaging (CT, RX, Ultrasound) of bilateral pulmonary opacities not fully explained by effusion, lobar or pulmonary atelectasis, or nodules; c. Respiratory failure not fully explained by heart failure or fluid retention d. PaO2 / FiO2 ≤200 mmHg with PEEP (or CPAP) ≥5 cmH_20
4	Increase in the PCR value of approximately 3.5 times the upper reference limit or above 1.8 mg / dl
5	Need for mechanical ventilation and / or CPAP
6	Patients who signed the informed consent. If there is no possibility of obtaining informed consent for the clinical condition (e.g. patients sedated and treated for acute respiratory failure and consequent mechanical ventilation), the patient's consent will be assumed until manifestly stated otherwise.
Exclusion criteria	
1	Diagnosis of moderate-severe ARDS for > 10 days
2	Patients with proven hypersensitivity or allergic reaction to blood products or immunoglobulins
3	Manifest unwillingness to participate

eTable 2 - Schedule of assessments

Study Period:	**Screening**	**Day 1 (baseline)**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**End of Study**
Informed Consent	(X)								(X)
Inclusion/Exclusion Criteria	X								
Demographics	X								
Medical History	X								
Physical examination		X	X	X	X	X	X	X	
Laboratory		X	X	X	X	X	X	X	
Hemogas analysis		X	X	X	X	X	X		
Viral load (nasal swab, sputum, BAL)		X		X				X	
Chest X rays		X		X				X	
Oxygen Support		X	X	X	X	X	X	X	
Concomitant treatments		X	X	X	X	X	X	X	
COVID-treatment (PLASMA)		**X**		**(X)°**		**(X)°**			
Outcomes									X
Adverse events		X	X	X	X	X	X	X	
immune response		X	X						
Blood sample collection for storage (exploratory biomarkers in future)		X		X				X	

eTable 3 – Control cohort. Patients were enrolled between March 10, 2020 and March 24, 2020 following the same eligibility criteria as for trial cohort (positive nasal swab and Pao2/Fio2<200). All patients were assessed at day 7 . Seven patients died (30%, 80% CI 18-46). CPR decreased by 3.76 mg/dl (95%, CI -12.18 to 4.66) and LDH U/l by 77, (95% CI -181 to 27).

Variable	Patients
Age (years), mean (SD)	63 (13)
Male, n (%)	17 (74)
Comorbidities 2+, n (%)	9 (39)
Oxygen saturation (%), mean (SD)	78 (14)
Pao2/Fio2, mean (SD)	124 (50)
Berlin score severe, n (%)	8 (35)
CRP (mg/dl), median (IQR)	11.5 (6.7-19.0)
Ferritin (ug/l), median (IQR)	1276 (633-1879)
LDH (u/l), median (IQR)	488 (360-589)
Creatinine (mg/dl), median (IQR)	0.81 (0.66-0.97)
Hs-tni (ng/l), median (IQR)	16.5 (7.0-46.0)
Chest radiogram bilateral Multilobe infiltrates, n (%)	23 (100)

Plasma collection from the selected donors and validation procedures

Donors were male or females with no previous pregnancies, aged 18 or above, who had recovered from Covid-19 disease (defined as 2 consecutive negative naso-pharingeal swabs) since not less than 7 days and not more than 30 days. The donors were registered according to the national regulation and thoroughly clinically evaluated by the local physician, with the purpose of highlighting any absolute contraindications to the aphaeresis procedure. All donors will need to test negative for hepatitis A and E RNA, and parvo virus 19 DNA, as well as for hepatitis B, C, HIV and syphilis at the molecular test (according to the current law). All convalescent patients were pre-tested (72 hours in advance) for anti-SARS-CoV-2 neutralizing antibodies title except those living far from the hospital which were tested at the time of donation.

Plasma collection was performed in a dedicated facility, using latest generation cell separator (Trima Accel – Terumo BCT and Amicus –Fresenius Kabi) devices, set according to the donor characteristics, under nurses' supervision. A plasma volume of about 660 ml was collected during each procedure and immediately divided in two bags of equal volume, using a sterile tubing welder. Then, plasma pathogen reduction was performed with the INTERCEPT processing system (Cerus Europe BV) or the Mirasol PRT System (Terumo BCT, Lakewood, CO, USA), as specifically required by the National Centre for Blood and labelled as hyperimmune Covid plasma. Finally, it was stored in a dedicated freezer, at a controlled temperature ranging from -40 to -25°C. Collected plasma had a neutralizing title of 1:160 or more.

As per routine, the plasma was validated and made available for infusion at the completion of all tests. Request of ABO compatible plasma was performed by treating physician using the established local procedures, inclusive of electronic tracking.

CONSORT 2010 checklist of information to include when reporting a pilot or feasibility trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a pilot or feasibility randomized trial in the title	1
	1b	Structured summary of pilot trial design, methods, results, and conclusions (for specific guidance see CONSORT abstract extension for pilot trials)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale for future definitive trial, and reasons for randomized pilot trial	5
	2b	Specific objectives or research questions for pilot trial	5
Methods			
Trial design	3a	Description of pilot trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after pilot trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	suppl
	4b	Settings and locations where the data were collected	5
	4c	How participants were identified and consented	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5-6
Outcomes	6a	Completely defined prespecified assessments or measurements to address each pilot trial objective specified in 2b, including how and when they were assessed	5 suppl
	6b	Any changes to pilot trial assessments or measurements after the pilot trial commenced, with reasons	-
	6c	If applicable, prespecified criteria used to judge whether, or how, to proceed with future definitive trial	7
Sample size	7a	Rationale for numbers in the pilot trial	7
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	-
	8b	Type of randomization(s); details of any restriction (such as blocking and block size)	
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	-
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	-
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	-
	11b	If relevant, description of the similarity of interventions	-

Statistical methods	12	Methods used to address each pilot trial objective whether qualitative or quantitative	7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were approached and/or assessed for eligibility, randomly assigned, received intended treatment, and were assessed for each objective	-
	13b	For each group, losses and exclusions after randomization, together with reasons	na
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the pilot trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	15
Numbers analyzed	16	For each objective, number of participants (denominator) included in each analysis. If relevant, these numbers should be by randomized group	
Outcomes and estimation	17	For each objective, results including expressions of uncertainty (such as 95% confidence interval) for any estimates. If relevant, these results should be by randomized group	9,17
Ancillary analyses	18	Results of any other analyses performed that could be used to inform the future definitive trial	-
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	8,18
	19a	If relevant, other important unintended consequences	-
Discussion			
Limitations	20	Pilot trial limitations, addressing sources of potential bias and remaining uncertainty about feasibility	10
Generalizability	21	Generalizability (applicability) of pilot trial methods and findings to future definitive trial and other studies	10
Interpretation	22	Interpretation consistent with pilot trial objectives and findings, balancing potential benefits and harms, and considering other relevant evidence	10
	22a	Implications for progression from pilot to future definitive trial, including any proposed amendments	10
Other information			
Registration	23	Registration number for pilot trial and name of trial registry	4
Protocol	24	Where the pilot trial protocol can be accessed, if available	suppl
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	none
	26	Ethical approval or approval by research review committee, confirmed with reference number	5

Citation: Eldridge SM, Chan CL, Campbell MJ, Bond CM, Hopewell S, Thabane L, et al. CONSORT 2010 statement: extension to randomized pilot and feasibility trials. BMJ. 2016;355.

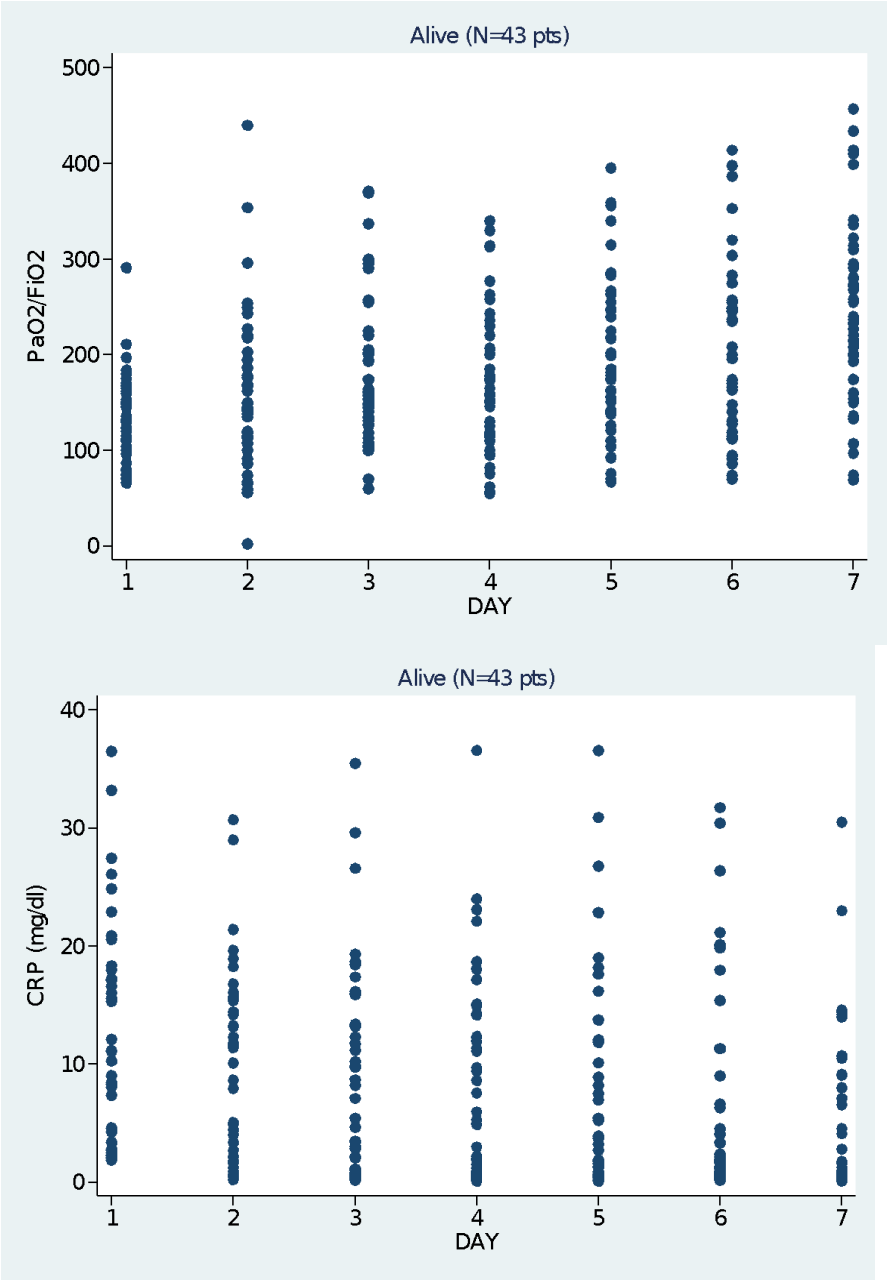
*We strongly recommend reading this statement in conjunction with the CONSORT 2010, extension to randomized pilot and feasibility trials, Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomized trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

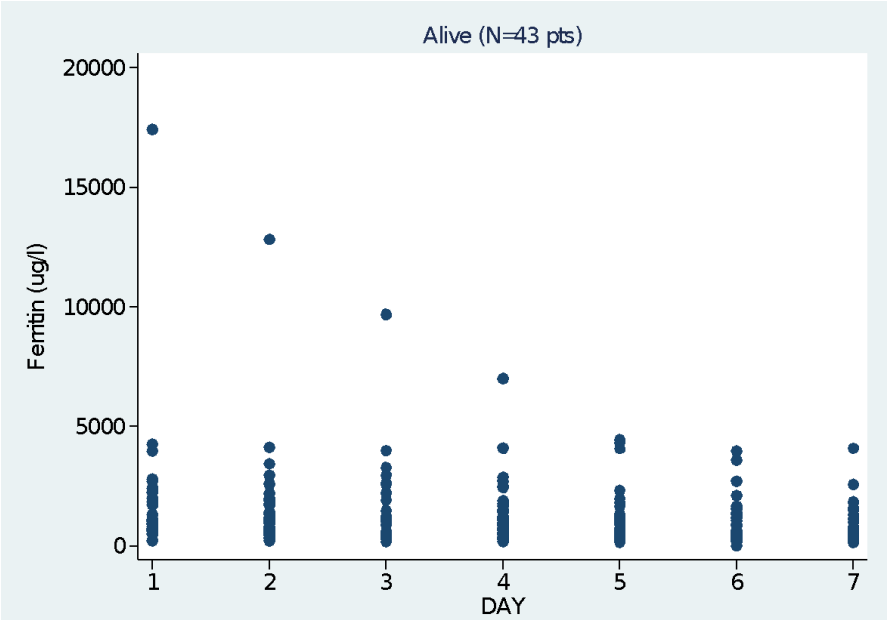
Additional references

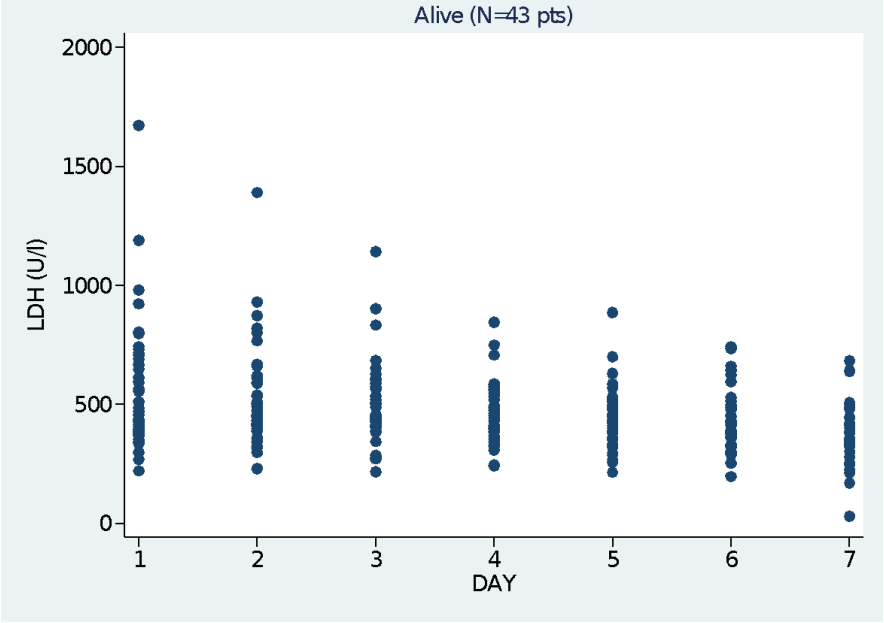
ARDS Definition Task Force; V Marco Ranieri, Gordon D Rubenfeld, B Taylor Thompson, Niall D Ferguson, Ellen Caldwell, Eddy Fan, Luigi Camporota, Arthur S Slutsky. Berlin score:Acute Respiratory. Distress Syndrome: The Berlin Definition
JAMA 2012;307(23):2526-33.

T.R. Fleming.One-sample multiple testing procedure for phase II clinical trials. Biometrics 1982;38:143-151.

Supplemental Figure: Daily individual data for patients who are alive at 7 days




Alive (N=43 pts)
Ferritin (ug/l)
20000
15000
10000
5000
0
1
2
3
4
5
6
7
DAY


Alive (N=43 pts)
LDH (U/l)
2000
1500
1000
500
0
1
2
3
4
5
6
7
DAY