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Efficacy of convalescent plasma for treatment of COVID-19 in Uganda

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ABSTRACT

Rationale Convalescent plasma (CCP) has been studied as a potential therapy for COVID-19, but data on its efficacy in Africa are limited.

Objective In this trial we set out to determine the efficacy of CCP for treatment of COVID-19 in Uganda.

Measurements Patients with a positive SARS-CoV-2 reverse transcriptase (RT)-PCR test irrespective of disease severity were hospitalised and randomised to receive either COVID-19 CCP plus standard of care (SOC) or SOC alone. The primary outcome was time to viral clearance, defined as having two consecutive RT-PCR-negative tests by day 28. Secondary outcomes included time to symptom resolution, clinical status on the modified WHO Ordinal Clinical Scale (≥1-point increase), progression to severe/critical condition (defined as oxygen saturation <93% or needing oxygen), mortality and safety.

Main results A total of 136 patients were randomised, 69 to CCP+SOC and 67 to SOC only. The median age was 50 years (IQR: 38.5–62.0), 71.3% were male and the median duration of symptom was 7 days (IQR=4–8). Time to viral clearance was not different between the CCP+SOC and SOC arms (median of 6 days (IQR=4–11) vs 4 (IQR=4–6), p=0.196). There were no statistically significant differences in secondary outcomes in CCP+SOC versus SOC: time to symptom resolution (median=7 (IQR=5–7) vs 7 (IQR=5–10) days, p=0.450), disease progression (9 (22.0%) vs 7 (24.0%) patients, p=0.830) and mortality (10 (14.5%) vs 8 (11.9%) deaths, p=0.476).

Conclusion In this African trial, CCP therapy did not result in beneficial virological or clinical improvements. Further trials are needed to determine subgroups of patients who may benefit from CCP in Africa.

Trial registration number NCT04542941.

Key messages

► Convalescent plasma has been studied as a potential therapy for COVID-19, but data on its efficacy in Africa are limited.

► In this African trial, COVID-19 convalescent plasma therapy did not result in beneficial virological or clinical improvements.

► COVID-19 convalescent plasma has been evaluated as a potential COVID-19 treatment in several randomised and non-randomised trials with mixed results on both clinical and virological outcomes.

and since then 88 674 cases and 2203 deaths have been reported.[1]

Infection prevention measures are key to COVID-19 control resulting in flattening of infection curves in several countries.[2] Recent scientific advances such as vaccine development and roll-out have created hope to ending the pandemic. However, cost, logistical challenges with vaccine cold chain and emergence of viral variants justify continued efforts to find effective COVID-19 treatments.[3 4] With the exception of anti-inflammatory agents such as dexamethasone, interleukin blocking agents such as tocilizumab and anti-SARS-CoV-2 monoclonal antibodies, several other repurposed medications for treatment of COVID-19, such as hydroxychloroquine and remdesivir, have not been found to be conclusively beneficial.[5–11]

Convalescent plasma (CCP), a form of passive immunisation, has been used for treatment of infections for over 100 years.[12] COVID-19 CCP has also been evaluated as a potential COVID-19 treatment in several randomised and non-randomised trials with mixed results (both clinical and virological

INTRODUCTION

COVID-19 has posed a significant global health emergency especially in under-resourced health settings. Uganda reported its first case of COVID-19 on 21 March 2020,

outcomes). Some trials reported benefit, while others did not.[13–22] Li et al[19] found that patients treated with CCP achieved significantly higher rates of viral clearance at 24, 48 and 72 hours than those not treated with CCP.[19]

CCP exerts its antiviral effects through a number of mechanisms.[23] The antibodies in plasma block glycoproteins on the surface of the viruses, thereby inhibiting fusion and entry into the cell, preventing the release of progeny virions from the infected cells and inhibiting extracellular proteolytic cleavage of viral protein and infected cells clearance.[24 25] Other antibody-mediated pathways contribute to viral clearance, such as antibody-dependent cell-mediated cytotoxicity, complement activation and cytotoxicity and phagocytic clearance of complement-coated targets.[26] This rapid viral clearance eliminates the stimulus for the cytokine release cascade which is believed to perpetuate tissue injury post viral replication.

We conducted a randomised, open-label clinical trial to determine if administration of CCP to patients who were reverse transcriptase (RT)-PCR-positive at the time of hospitalisation would lead to earlier clearance of SARS-CoV-2 and better clinical outcomes.

METHODS

Trial design

The CCP trial was an open-label, randomised clinical trial conducted at Mulago National Referral Hospital (MNRH) COVID-19 Treatment Unit. The trial included patients with documented SARS-CoV-2-positive RT-PCR performed at the trial laboratory of Makerere University Department of Immunology and Molecular Biology. We excluded patients with a prior diagnosis of IgA deficiency and those unable to participate in follow-up procedures. Permuted block randomisation with varying sizes of blocks was used to randomly assign eligible participants to receive either CCP plus standard of care (CCP +SOC) or standard of care only (SOC). All participants provided written informed consent prior to enrolment.

Patient and public involvement

Participants' involvement in the baseline survey assisted us in identifying individuals who recovered from SARS-CoV-2. These formed a pool of participants from whom we obtained CCP. For results dissemination, we informed plasma donors and recipients about the outcome of the clinical trials through follow-up visits to the hospital.

Intervention

The intervention was CCP. We obtained details of COVID-19 survivors from the national database and contacted them by telephone to donate plasma for use in the trial. Initial screening was done telephonically using the Uganda blood transfusion checklist. Potentially eligible donors underwent further screening at the donor centre set up at MNRH.[27] The details of the process of acquiring and processing the CCP have been previously published.[27 28] The lower limit of anti-SARS-CoV-2 IgG antibody titres for plasma units was 27.5 AU/mL, which was equivalent to 2.2 µg/mL. Neutralising antibody titre testing was not performed due to logistical reasons. For randomisation, we used permuted blocks with varying sizes of blocks to randomly assign eligible participants in a 1:1 ratio to receive eituher CCP +SOC or SOC. The SOC was according to the Uganda COVID-19 case management guidelines.[5] Donor CCP was cross-matched with the patient's red blood cells to ensure compatibility. CCP was administered over look 2–3 hours at a rate of 1.4–2 mL/min and a second aliquot transfused at the same rate 3 hours after completion of the first one.

Outcomes

The primary outcome was time to viral clearance, defined by two consecutive negative SARS-CoV-2 RT-PCR test results. Prespecified secondary outcomesb included time to symptom resolution, clinical status on the modified WHO Ordinal Clinical Scale for clinical improvement (≥1-point increase) and progression to severe/critical condition (defined as oxygen saturation (SPO_2 <93% or needing oxygen). Clinical status was assessed daily during hospitalisation and on day 28 postenrolment. Adverse events were defined as any medical occurrence post intervention, graded as mild, moderate, severe and life-threatening, and the relationship with the intervention was classified as unrelated, possibly related, probably or definitely related to the intervention. Specifically for plasma transfusion we monitored for transfusion-related risks such as allergic transfusion reactions, anaphylaxis, febrile reactions, transfusion-related acute lung injury, transfusion-associated circulatory overload and ABO incompatibility haemolysis.

Sample size estimation

The primary outcome of this randomised clinical trial was viral clearance. The trial was planned to detect a minimum hazard reduction in the primary outcome of 40% in the CCP (intervention) arm, equivalent to an HR of 0.6. With a power of 80%, a two-tailed type 1 error (alpha) of 0.05, a patient accrual period of 3 months, a total study time of 6 months, a ratio of accrual to total time of 0.5, with no anticipated cross-over (no dropout of, or drop-in, the intervention arm), a ratio of n1 to n2 of 1:1, and equal enrolment rate in both arms, the required unadjusted sample size per group/arm was estimated to be 66 patients, giving a total of 132. Given that the trial was testing a potential therapy for a disease with no proven therapy and that all participants will be inpatients, a minimal loss-to-follow-up of 3% is anticipated, and after adjusting for it the total number of patients to be enrolled and randomised totalled to 136 (68 per arm).

Randomisation

Randomisation was performed using permuted block randomisation with varying block sizes ranging from 4 to 8. Generation of randomisation numbers was done by the study biostatistician, who did not have contact with participants. The biostatistician printed individual randomisation numbers with their corresponding treatment assignment and placed each number in an opaque envelope. The following steps were then followed:

► Prebatched random number envelopes were availed to the study coordinator who kept custody of them and passed them to clinicians who administered the randomisation. Each enrolled participant was asked to pick the envelope on top of the batch.
► Participants were not allowed to pick an envelope from any other position of the batch; this was done to avoid breaking the randomisation code and assignment probabilities.
► Prior to picking an envelope, the randomising clinician explained to the participant that the randomisation is up to chance, like the flip of a coin, which gives each participant an equal chance of receiving either intervention. The clinician also informed the participant that numbers corresponding to study arms were generated by the computer and arranged randomly and batched, and that each participant will randomise himself/herself by picking the top most envelope from the batch availed to them.
► After picking an envelope each participant was asked to open it to find the arm she/he had been randomised. Then the clinician explained to the participant the study arm that the participant would have randomised themselves to.
► After randomisation, the clinician indicated the study arm onto the participant's enrolment form and stuck the study arm assignment sheet (that was obtained from the envelope) on to the enrolment form. Used envelopes were collected and logged in each day.

Laboratory and radiology evaluation

Viral RNA extraction was done using the Qiagen Viral RNA Mini Kit. RT-PCR for SARS-CoV-2 was performed using the TaqPath COVID-19 RT-PCR Kit (Thermo Fisher Scientific) on the Applied Biosystems 7500 RT-PCR instrument following the manufacturer's instructions. SARS-CoV-2 RT-PCR was done at baseline and on days 3, 5, 7, 14 and 28 post randomisation or until two consecutive negative RT-PCR results were obtained, whichever occurred first.

Antibody titres were determined using the Acro Biosystems Anti-SARS-CoV-2 antibody IgG titre serological ELISA assay kit (spike protein Receptor Binding Domain (RBD))[29] following the manufacturer's instructions.

The resultant IgG titres (ng/mL) were converted to arbitrary units per millilitre by dividing by the background concentration (ng/mL), multiplied by 3, of the negative control samples obtained on the same ELISA plate. This was done for convenience of interpretation according to the standard operating procedures of our laboratory.

Clinical evaluation, laboratory tests and chest X-ray (CXR) were performed at baseline and whenever clinically indicated. CXRs were scored using the Brixia score as published.[30]

Statistical analysis

The characteristics of participants were described using mean (SD) and median (IQR) for continuous variables and proportions for categorical variables. Comparisons across the study arms were done using t-tests and Wilcoxon rank-sum test for continuous variables, and χ^2 or Fisher's exact tests for categorical ones. Time-to-event data were analysed using Kaplan-Meier (KM) curves and Cox proportional hazards regression models. Associations were estimated by risk ratio (RR) and HR with corresponding 95% CI. Intention-to-treat analysis was performed using STATA V.14.

RESULTS

Patient characteristics

Screening for entry into this trial began on 21 September 2020 and the last patient was screened on 2 December 2020. During this period 403 patients were screened and 160 of them had a positive RT-PCR test. Of these 160, 8 were excluded due to lack of ABO-compatible plasma, 15 did not consent for randomisation and 1 was unable to return for follow-up, leaving 136 patients for randomisation. The first patient was randomised on 23 September 2020. The trajectory of the 136 randomised patients is shown in figure 1. Baseline characteristics of study participants are shown in table 1.

The median age of the patients was 50 years (IQR: 38.5–62.0) and the majority (71.3%) of the respondents were male. A total of 123 (90.4%) patients reported at least one COVID-19-related symptom and the median duration of symptoms was 7 (IQR 4–8) days. The most common symptoms were cough (62.5%), difficulty breathing (51.5%), chest pain (41.9%) and fever (33.1%). One patient had documented fever (ie, temperature ≥37.5°C), 38 (27.9%) had an SPO_2 of <93%, 45 (33.1%) had tachycardia, 40 (29.4%) had hypertension (blood pressure ≥130/90 mm Hg) and 15 (33.3%) had body mass index greater than 30. More than half (58.1%) of the participants reported at least one comorbidity (hypertension 36.0%, diabetes 23.5% and HIV 11.0%). At enrolment, 66 patients (48.5%) were on supplemental oxygen, 80 (58.8%) were on systemic corticosteroids mainly dexamethasone, and 80 (58.8%) were on anticoagulants mainly low molecular weight heparin. One participant was on non-invasive ventilation.

The median (IQR) antibody titre of transfused plasma was 139.5 (84.3–195.4) AU. Logistical constraints precluded the performance of some key COVID-19 care laboratory tests in all patients. The results of some tests are
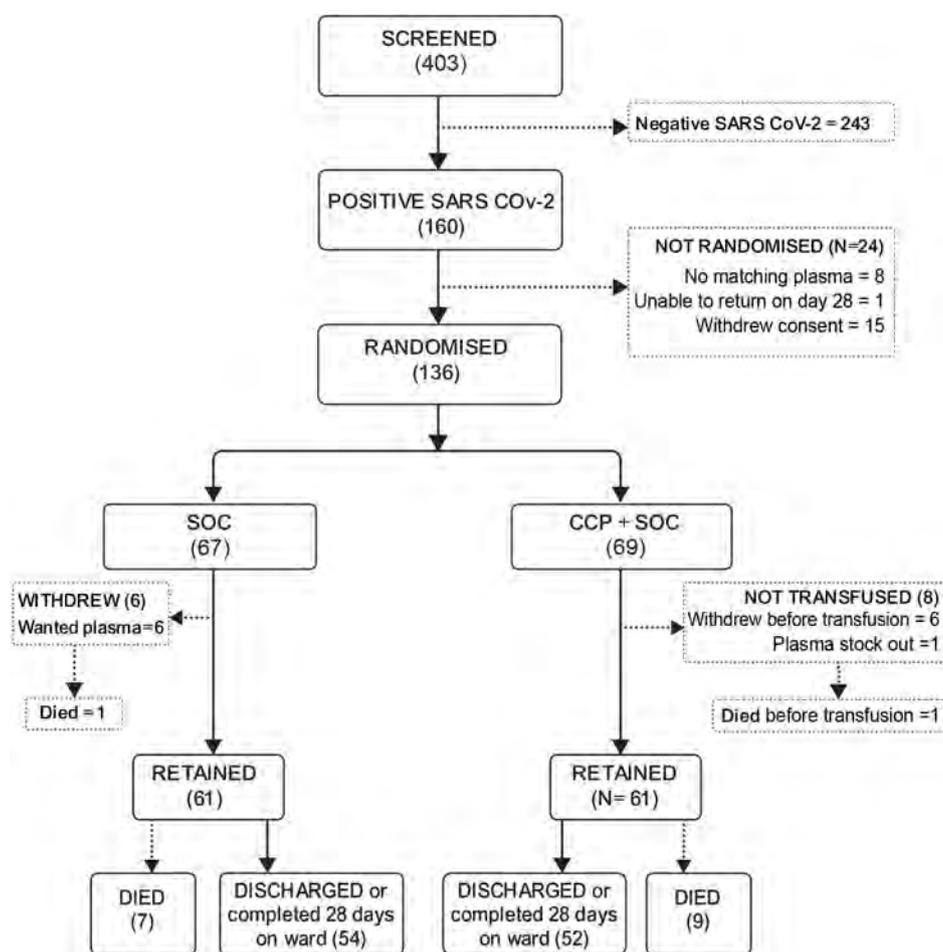
Figure 1 Enrolment and randomisation into the trial. CCP, convalescent plasma; SOC, standard of care.

shown in table 1. Complete blood count was performed in 36 patients, out of which 9 (25%) had leucocytosis, 5 (13.9%) had leucopenia and 21 (58.3%) had lymphopaenia, which was more common among those in the CCP arm (65% vs 50%, p=0.0126). Liver, renal functional and C reactive protein tests were obtained for 113 of the patients. Rates of liver enzyme elevation and hyperbilirubinaemia were 37% for aspartate transferase, 7% for alanine transferase and 68.1% for hyperbilirubinaemia, all not statistically different between the arms. Creatinine ≥1.2 g/dL was observed in 17.7% of patients, while C reactive protein ≥10 mg/L was observed in 71.7%, both not statistically different between the arms. The mean (SD) CXR COVID-19 quantitative score among the 34 patients with CXRs was 8.9 (6.4), and no statistical differences were observed between the arms.

Outcomes
Efficacy
Time to viral clearance was not different between the CCP+SOC and SOC arms: median (IQR) of 6 (4–11) vs 4 (4–6) days (p=0.196) (table 2 and figure 2). We found no significant difference in the proportions of patients with one or two consecutive negative RT-PCR results between the CCP arm and the SOC arm (online

supplemental table 1). Similarly, viral clearance did not differ by day of sample collection (online supplemental table 2). We adjusted the primary outcomes for several baseline characteristics and found no statistically significant association with all factors analysed, including comorbidities such as HIV (online supplemental table 3). Antibody titres of transfused plasma were available for 56 patients. We compared trial outcomes by percentiles of antibody titres. The results from this analysis are presented in online supplemental table 4. Time to two negative consecutive RT-PCR results was shortest for those patients in the lowest percentile, but this difference was not statistically significant (p=0.643). The same result was observed for time to symptom resolution (p=0.061) and disease progression (p=0.052). There was no death among patients who received plasma with the lowest antibody titre.

A total of 123 patients (90.4%) had at least one symptom. We computed the median time to resolution of these symptoms and compared this between the arms. The median (IQR) time to symptom resolution was 7 (5–7) days for CCP arm vs 7 (5–10) days for SOC (p=0.450). Online supplemental figure 1 shows the KM curves of time to resolution of major symptoms by intervention group. At enrolment 70 patients had SPO_2 greater than

Table 1 Patients' baseline characteristics

Characteristics	Overall (N=136)	Arm	
		CCP+SOC (n=69)	SOC (n=67)
Age, median (IQR)	50 (38.5–62)	48 (35–64)	53 (44–61)
Sex, male, n (%)	97 (71.3)	48 (69.6)	49 (73.1)
At least one symptom	123 (90.4)	61 (88.4)	62 (92.5)
Fever	45 (33.1)	22 (31.9)	23 (34.4)
Sore throat	10 (7.4)	7 (10.1)	3 (4.5)
Cough	85 (62.5)	38 (55.1)	47 (70.2)
Difficulty with breathing	70 (51.5)	37 (53.6)	33 (49.3)
Chest pain	57 (41.9)	29 (42.0)	28 (41.8)
Loss of taste	12 (8.8)	8 (11.6)	4 (6.0)
Rhinorrhoea	2 (1.5)	1 (1.5)	1 (1.5)
Others	18 (13.2)	8 (11.6)	10 (14.9)
Symptom length, M (IQR)	7 (4–8)	7 (3–7)	7 (5–10)
Temperature ≥37.5°C, n (%)	1 (0.7)	0 (0.0)	1 (1.5)
SPO$_2$ <93%, n (%)	38 (27.9)	19 (27.5)	19 (28.4)
Pulse rate ≥100, n (%)	45 (33.1)	20 (29.0)	25 (37.3)
BP ≥130/90, n (%)	40 (29.4)	20 (29.0)	20 (29.9)
BMI >30, n/N (%)	15/45 (33.3)	8/24 (33.3)	7/21 (33.3)
At least one comorbidity	79 (58.1)	39 (56.5)	40 (59.7)
Hypertension	49 (36.0)	24 (34.8)	25 (37.3)
Diabetes mellitus	32 (23.5)	17 (24.6)	15 (22.4)
Asthma	5 (3.7)	5 (7.3)	0 (0.0)
Tuberculosis	4 (2.9)	1 (1.5)	3 (4.5)
HIV	15 (11.0)	4 (5.8)	11 (16.4)
Others	16 (11.8)	9 (13.0)	7 (10.5)
Medications, n (%)			
Corticosteroids	80 (58.8)	36 (52.2)	44 (65.7)
Anticoagulant	80 (58.8)	35 (50.7)	45 (67.2)
Oxygen therapy	66 (48.5)	28 (40.6)	38 (56.7)
Oxygen 1–5 L/min	35 (53.0)	13 (46.4)	22 (57.9)
Oxygen >5 L/min	31 (47.0)	15 (53.6)	16 (42.1)
Non-invasive ventilation	1 (0.7)	1 (1.5)	0 (0.0)
WCC ≥10 x 10^9/L, n=36	9 (25)	4 (20.)	5 (31.3)
WCC ≤4000/mm^3	5 (13.9)	3 (15)	2 (12.5)
Neutrophil ≤1500/mm^3	2 (5.6)	2 (10)	0 (0)
Lymphocyte ≤1500/mm^3	21 (58.3)	13 (65)	8 (50)
Platelets <150 000/mm^3	6 (16.7)	5 (25)	1 (6.3)
Haemoglobin ≤1 g/L	0	0	0
AST >40 U/L, n/n=113 (%)	37 (32.7)	14 (25.0)	23 (40.4)
ALT >40 U/L, n/N (%)	7 (6.2)	5 (8.9)	2 (3.5)
Creatinine ≥1.2 g/dL, n/N (%)	20 (17.7)	11 (19.5)	9 (15.8)
Bilirubin total >1.2 mg/dL (%)	77 (68.1)	40 (71.4)	37 (64.9)
CRP ≥10 mg/L, n/N (%)	81 (71.7)	39 (69.5)	42 (73.7)
CXR score, median (SD), n=34	8.9 (6.4)	7.4 (7.3)	10.2 (5.3)

ALT, alanine transferase; AST, aspartate transferase; BMI, body mass index; BP, blood pressure; CCP, convalescent plasma; CRP, C reactive protein; CXR, chest X-ray; SOC, standard of care; SPO$_2$, oxygen saturation; WCC, white cell count.

Table 2 Trial outcomes of patients randomised to convalescent plasma (CCP) compared with standard of care (SOC)

Outcome	Overall	CCP	SOC	RR*	P value
Primary outcome					
Time to two consecutively negative reverse transcriptase-PCR, median (IQR), days	n=136 5.5 (4–8)	n=69 6 (4–11)	n=67 4 (4–6)		0.196
Secondary outcomes					
Time to symptom resolution among symptomatic patients, median (IQR)	n=120 2 (1–4)	n=59 2 (1–4)	n=61 2 (1–4)		0.772
Progression to severe/critical disease (oxygen saturation <93% or needing oxygen)	n=70 16 (22.9)	n=41 9 (22.0)	n=29 7 (24.1)	0.91 (0.38 to 2.16)	0.830
Mortality	n=136 18 (13.2)	n=69 10 (14.5)	n=67 8 (11.9)		0.661

*Unadjusted risk ratio.

93% and were not on oxygen therapy. The SPO$_2$ deteriorated to less than 93%, requiring oxygen therapy among 16 (22.9%) patients. Disease progression occurred in nine patients (22.0%) in the CCP+SOC arm compared with seven (24.0%) in the SOC arm (p=0.830) (table 2). Online supplemental figure 2 shows the KM curves of time to progression to severe/critical disease by intervention group (p=0.869). Adjusting for baseline characteristics revealed that disease progression increased with age irrespective of intervention. Compared with patients >65 years, the relative risk of deterioration was 0.22 for those in the 26–35 years' age group and 0.39 for those in the 56–65 years' age group (online supplemental table 5).

A total of 24 (17.7%) patients deteriorated at least once based on the modified WHO Ordinal Clinical Scale and this did not differ by arm: 12 (17.4%) in the CCP arm vs 12 (17.9%) in the SOC arm (p=0.937) (online supplemental table 6 and figure 3A). Mortality rates in the
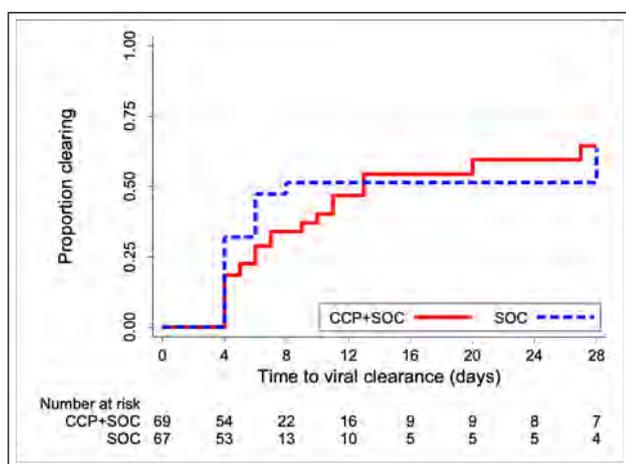

Figure 2 Kaplan-Meier curve showing time to viral clearance by intervention group (p=0.378). CCP, convalescent plasma; SOC, standard of care.

trial are presented in table 2 and figure 3B. Overall 18 patients died in the trial. Ten (14.5%) patients died in the CCP arm compared with eight patients (11.9%) in the SOC arm, but this difference did not reach statistical significance (p=0.661) (table 2). We adjusted mortality for key baseline characteristics including gender, age, commodities and severity according to baseline oxygen needs, among others (online supplemental table 7). Mortality decreased with decreasing age and increased with increasing oxygen need.

Safety
A total of 29 patients experienced adverse events listed in online supplemental table 8A (15 in the CCP arm and 14 in the control arm). The relatedness of the adverse events to the study product is presented in online supplemental table 8B. Three adverse events were judged definitely related and three judged to be possibly related to plasma transfusion, while the rest were thought to be unrelated to plasma transfusion.

DISCUSSION
The results of this open-label, randomised clinical trial of CCP indicate that when compared with SOC alone, CCP plus SOC showed no effect on viral or symptom clearance, disease progression and mortality. This differs from what has been reported in some previous studies.[19 22 31] Rajendran et al,[31] in a systematic review of mainly observational cohorts, case reports and series, report that CCP was associated with a significant decrease in viral load. Li et al[19] studied 103 patients with COVID-19 in China and found that rates of viral clearance at 24, 48 and 72 hours in patients treated with CCP were all significantly higher than those not treated with CCP.[19] Agarwal et al[22] reported that the proportion of patients with negative RT-PCR results at day 7
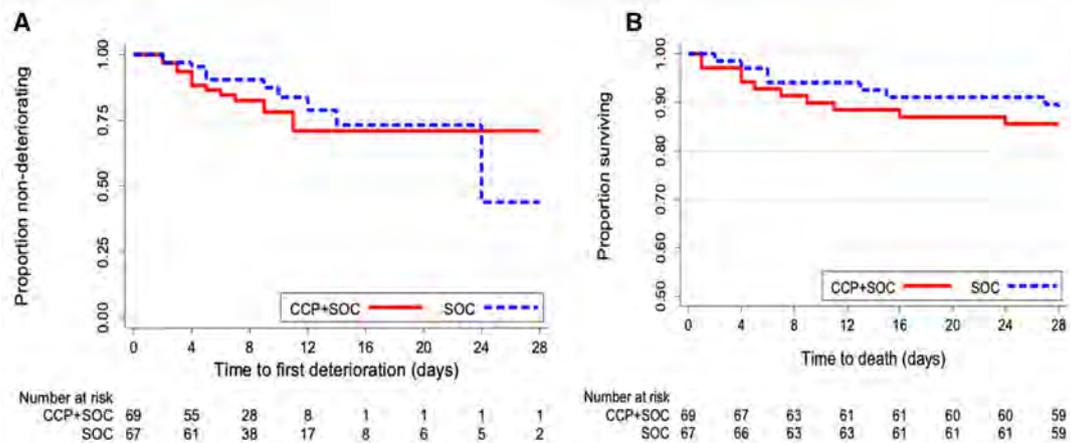
Figure 3 Kaplan-Meier curves showing time to first deterioration (A) and death (B) by intervention group. There were no significant differences: first deterioration (p=0.372) and death (p=0.633). CCP, convalescent plasma; SOC, standard of care.

was higher in the CCP arm than in the control (68% vs 55%, RR 1.2, 95% CI 1.04 to 1.5).

Plasma also had no effect on clinical outcomes, in line with findings from several recent randomised trials.[17 22 32] In Argentina, Simonovich et al[17] randomised 228 and 105 patients to receive plasma or placebo, respectively. Results from this trial show no benefit of plasma on mortality (10.96% in the plasma group vs 11.43% in the placebo group). Moreover they observed that CCP appeared to be associated with a worse outcome among patients less than 65 years.[17] The median age in our trial patients was 50 years and we observed higher mortality in the CCP arm, although the differences did not show statistical significance. Contrary to the findings from randomised trials including the current trial, several non-randomised trials found plasma beneficial.[18 20 31 33 34] For example a systematic review by Rajendran et al[31] found plasma beneficial in terms of clinical outcomes and viral clearance. A retrospective analysis of 35 322 patients who received CCP at 2807 acute care facilities in the USA also found that plasma significantly reduced mortality.[20] The conflicting results based on study design highlight the need for caution while adopting intervention in the absence of well-conducted trials.

The reasons why CCP in our trial did not lead to faster viral clearance are not clear, but probably reflect the lack of efficacy of CCP in COVID-19 treatment, as has been reported in a number of recent trials.[17 22] However, two factors have been studied as potential effect modifiers: timing of transfusion and the level of antibody titres in the transfused plasma. Joyner et al[20] found a dose–response relationship between levels of antibody titres in transfused plasma and outcomes, showing that among patients who received high IgG plasma (>18.45 s/Co), 7-day mortality was 8.9% (6.8%, 11.7%); for recipients of medium IgG plasma (4.62–18.45 s/Co) mortality was 11.6% (10.3%, 13.1%); and for recipients of low IgG plasma (<4.62 s/Co) mortality

was 13.7% (11.1%, 16.8%) (p=0.048). Another study by Libster et al[18] enrolled patients older than 75 years with non-severe disease and symptoms for less than 3 days (early disease) and randomised them to receive high-titre plasma. Results from this trial found that severe respiratory disease developed in 13 of 80 patients (16%) who received CCP and 25 of 80 patients (31%) who received placebo (relative risk, 0.52; 95% CI 0.29 to 0.94; p=0.03), with a relative risk reduction of 48%. Most plasma units used in our trial had antibody titres more than the recommended 1:160.

In terms of timing, the study by Joyner et al[20] described above found that the 7-day mortality rate was 8.7% (95% CI 8.3% to 9.2%) in patients transfused within 3 days of COVID-19 diagnosis compared with 11.9% (11.4% to 12.2%) in patients transfused 4 or more days after diagnosis (p<0.001), and similar findings were observed in 30-day mortality (21.6% vs 26.7%, p<0.0001). These results are also supported by those from the study by Libster et al,[18] which found benefit when higher-titre plasma was transfused within 3 days of symptom onset. Plasma effects are mainly mediated by the neutralising antibodies it contains.[24 25] It is therefore likely that timing and age may have something to do with whether the plasma recipients have developed their own antibodies or not because research shows that most patients with COVID-19 have neutralising antibodies with levels mediated by disease severity (more severe disease being associated with high antibodies).[35] Cheng et al[33] report better outcomes among plasma recipients who were PCR positive and seronegative at the time of transfusion (66.7% vs 20%, p=0.001). In another study in the Netherlands, 44 of the 56 (79%) patients had neutralising antibodies comparable with those of the donors (ie, 1:160 vs 1:160, p=0.40) at a median of 10 days of symptoms.[36] The study further found no difference in mortality (p=0.95), hospital stay (p=0.68) or day-15 disease severity (p=0.58) between plasma-treated patients and patients on SOC.[36] In our trial we

explored the effect of timing in a subset of patients who were randomised within 3 days of symptom onset, but did not observe any difference in outcomes.

Our trial had limitations, including lack of antibody assay for trial patients at randomisation, inability to perform clinical laboratory tests and chest radiology for all trial patients, as well as inability to perform conventional neutralising antibody assays. We also note that the arms were unbalanced on some baseline characteristics, such as the use of anticoagulants and oxygen, although these imbalances were not statistically significant. The sample size was kept to the minimum due to logistical reasons, which has made our trial underpowered. Our trial was an open-label trial without placebo control, which could have introduced bias in the measurement of some outcomes. There are several strengths. This study is one of the few trials on CCP in Africa, included a young population, focused on viral clearance as a primary outcome and included the full spectrum of COVID-19 infection including mild asymptomatic cases.

In conclusion CCP therapy did not result in beneficial virological or clinical improvements in this trial. Further trials are needed to determine subgroups of patients who may benefit from COVID-19 CCP in Africa.

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REFERENCES
1 Uganda Ministry of health Covid 19 pandemic. Available: https://www.health.go.ug/covid/about-corona-virus/ [Accessed 20 Mar 2021].
2 Liu Y, Morgenstern C, Kelly J, *et al*. The impact of non-pharmaceutical interventions on SARS-CoV-2 transmission across 130 countries and territories. *BMC Med* 2021;19:1–12.
3 Callaway E, Ledford H. How to redesign COVID vaccines so they protect against variants. *Nature* 2021;590:15–16.
4 Mahase E. Covid-19: what new variants are emerging and how are they being investigated? *BMJ* 2021;372:n158.
5 Government of Uganda. National Guidelines for Management of Covid-19 - Ministry of Health. Available: https://www.health.go.ug/cause/national-guidelines-for-management-of-covid-19/ [Accessed 10 Mar 2021].
6 Xu X, Han M, Li T, *et al*. Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci U S A* 2020;117:10970–5.
7 Medicine R-CIJNEJo. Interleukin-6 receptor antagonists in critically ill patients with Covid-19. *N Engl J Med* 2021.
8 Horby PW, Pessoa-Amorim G, Peto L. Tocilizumab in patients admitted to hospital with COVID-19 (recovery): preliminary results of a randomised, controlled, open-label, platform trial. *Medrxiv* 2021.

9 NIo H. *The COVID-19 treatment guidelines panel's statement on the emergency use authorization of convalescent plasma for the treatment of COVID-19*, 2020.

10 Medicine RCGJNEJo. *Dexamethasone in hospitalized patients with Covid-19—preliminary report*, 2020.

11 Wang Y, Zhang D, Du G, *et al*. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020;395:1569–78.

12 RAMBAR AC. Convalescent serum and pooled plasma in communicable diseases. *U S Nav Med Bull* 1946;46:93–6.

13 Garraud O, Heshmati F, Pozzetto B, *et al*. Plasma therapy against infectious pathogens, as of yesterday, today and tomorrow. *Transfus Clin Biol* 2016;23:39–44.

14 Winkler AM, Koepsell SA, SAJCoih K. The use of convalescent plasma to treat emerging infectious diseases. *Curr Opin Hematol* 2015;22:521–6.

15 Budhiraja S, Dewan A, Aggarwal R, *et al*. Effectiveness of convalescent plasma in Indian patients with COVID-19. *Blood Cells Mol Dis* 2021;88:102548.

16 Olivares-Gazca JC, Priesca-Marín JM, Ojeda-Laguna M, *et al*. Infusion of convalescent plasma is associated with clinical improvement in critically ill patients with COVID-19: a pilot study. *RIC* 2020;72:159–64.

17 Simonovich VA, Burgos Pratx LD, Scibona P, *et al*. A randomized trial of convalescent plasma in Covid-19 severe pneumonia. *N Engl J Med* 2021;384:619–29.

18 Libster R, Pérez Marc G, Wappner D. Early high-titer plasma therapy to prevent severe Covid-19 in older adults 2021.

19 Li L, Zhang W, Hu Y, *et al*. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19. *JAMA* 2020;324:460.

20 Joyner MJ, Senefeld JW, Klassen SA, *et al*. Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: initial three-month experience. *medRxiv* 2020. doi:10.1101/2020.08.12.20169359. [Epub ahead of print: 12 Aug 2020].

21 Gharbharan A, Jordans CCE, GeurtsvanKessel C, *et al*. Effects of potent neutralizing antibodies from convalescent plasma in patients hospitalized for severe SARS-CoV-2 infection. *Nat Commun* 2021;12:1–12.

22 Agarwal A, Mukherjee A, Kumar G, *et al*. Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID trial). *BMJ* 2020;151:m3939.

23 Dai W, Gu H, Hao S. Potential benefits, mechanisms, and uncertainties of convalescent plasma therapy for COVID-19. *Blood Sci* 2020;2:71–5.

24 Flyak AI, Ilinykh PA, Murin CD, *et al*. Mechanism of human antibody-mediated neutralization of Marburg virus. *Cell* 2015;160:893–903.

25 Lu C-L, Murakowski DK, Bournazos S, *et al*. Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. *Science* 2016;352:1001–4.

26 Liu Q, Fan C, Li Q, *et al*. Antibody-dependent-cellular-cytotoxicity-inducing antibodies significantly affect the post-exposure treatment of Ebola virus infection. *Sci Rep* 2017;7:1–11.

27 Muttamba W, Lusiba J, Namakula LO, *et al*. Feasibility of collecting and processing of COVID-19 convalescent plasma for treatment of COVID-19 in Uganda. *PLoS One* 2021;16:e0252306.

28 Muttamba W, Lusiba J, Namakula LO. Feasibility of collecting and processing of COVID-19 convalescent plasma for treatment of COVID-19 in Uganda 2020.

29 SARS-CoV-2 (COVID-19) antibody titer assay kit (Spike protein RBD) - ACROBiosystems. Available: https://www.acrobiosystems.com/P3184-SARS-CoV-2-%28COVID-19%29-antibody-titer-assay-kit-%28Spike-protein-RBD%29.html [Accessed 05 Sep 2020].

30 Borghesi A, Maroldi R, RJLrm M. COVID-19 outbreak in Italy: experimental chest X-ray scoring system for quantifying and monitoring disease progression. *Radiol Med* 2020;125:509–13.

31 Rajendran K, Krishnasamy N, Rangarajan J, *et al*. Convalescent plasma transfusion for the treatment of COVID-19: systematic review. *J Med Virol* 2020;92:1475–83.

32 Li L, Zhang W, Hu Y, *et al*. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. *JAMA* 2020;324:460–70.

33 Cheng Y, Wong R, Soo YOY, *et al*. Use of convalescent plasma therapy in SARS patients in Hong Kong. *Eur J Clin Microbiol Infect Dis* 2005;24:44–6.

34 Abolghasemi H, Eshghi P, Cheraghali AM, *et al*. Clinical efficacy of convalescent plasma for treatment of COVID-19 infections: results of a multicenter clinical study. *Transfus Apher Sci* 2020;59:102875.

35 Ko J-H, Joo E-J, Park S-J, *et al*. Neutralizing antibody production in asymptomatic and mild COVID-19 patients, in comparison with pneumonic COVID-19 patients. *J Clin Med* 2020;9:2268.

36 Gharbharan A, Jordans CC, GeurtsvanKessel C. Convalescent plasma for COVID-19. A randomized clinical trial. *Medrxiv* 2020.