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Clinical Medicine COVID-19

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Between April 4, 2020, and February 5, 2021, 350 patients were randomly assigned to either CP (n = 179) or SOC (n = 171). At 14 days, proportion of patients in categories 5, 6, or 7 was 11.7% in the CP group versus 16.4% in the control group (P = 0.205). The difference was greater at 28 days, with 8.4% of patients in categories 5–7 in the CP group versus 17.0% in […]

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BACKGROUND. Passive immunotherapy with convalescent plasma (CP) is a potential treatment for COVID-19. Evidence from controlled clinical trials is inconclusive.

METHODS. We conducted a randomized, open-label, controlled clinical trial at 27 hospitals in Spain. Patients had to be admitted for COVID-19 pneumonia within 7 days from symptom onset and not on mechanical ventilation or high-flow oxygen devices. Patients were randomized 1:1 to treatment with CP in addition to standard of care (SOC) or to the control arm receiving only SOC. The primary endpoint was the proportion of patients in categories 5 (noninvasive ventilation or high-flow oxygen), 6 (invasive mechanical ventilation or extracorporeal membrane oxygenation [ECMO]), or 7 (death) at 14 days. Primary analysis was performed in the intention-to-treat population.

RESULTS. Between April 4, 2020, and February 5, 2021, 350 patients were randomly assigned to either CP (n = 179) or SOC (n = 171). At 14 days, proportion of patients in categories 5, 6, or 7 was 11.7% in the CP group versus 16.4% in the control group (P = 0.205). The difference was greater at 28 days, with 8.4% of patients in categories 5–7 in the CP group versus 17.0% in the control group (P = 0.021). The difference in overall survival did not reach statistical significance (HR 0.46, 95% CI 0.19–1.14, log-rank P = 0.087).

CONCLUSION. CP showed a significant benefit in preventing progression to noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or death at 28 days. The effect on the predefined primary endpoint at 14 days and the effect on overall survival were not statistically significant.

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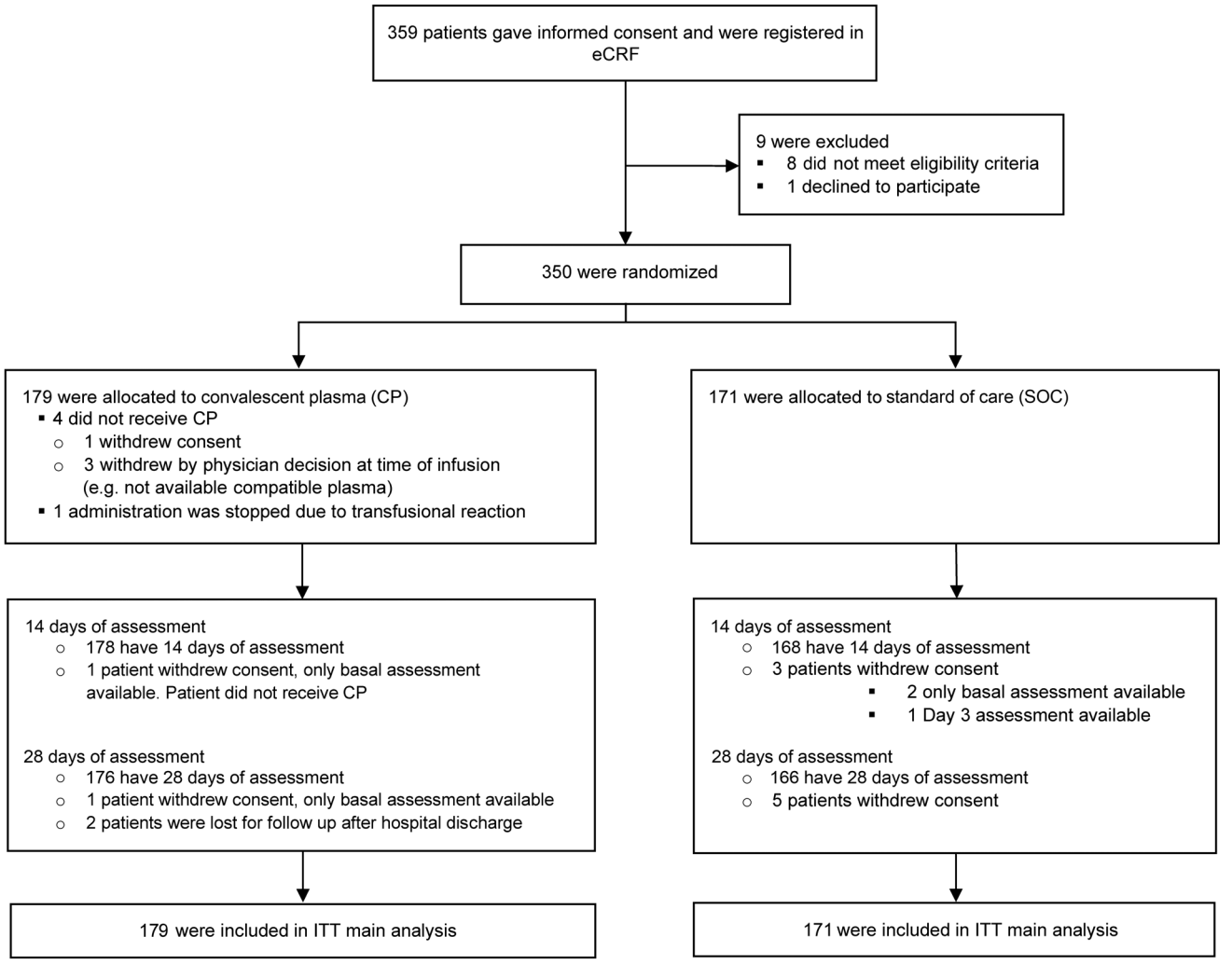

Figure 1. Trial flow diagram.

Introduction

The pressing need for treatments for the COVID-19 pandemic obliged the medical community and authorities to embrace the use of convalescent plasma (CP) as a potentially effective and easily accessible form of passive immunotherapy. Despite the rationale for use, and experience in other viral epidemics (1, 2), several controlled clinical trials and observational studies have provided inconclusive results regarding the beneficial effects of CP in patients hospitalized for COVID-19 (3–12). The evidence suggests that the potential beneficial effects of CP may be limited to the subgroup of noncritical patients who receive high titers of antibodies early in the course of the disease before developing their own humoral immune response (3–8), or to immunocompromised oncohematological patients who may fail to mount an effective immune response (9). Based on this premise, monoclonal and purified antibodies are being developed for outpatients with early and mild disease (13, 14). The ConPlas-19 randomized trial was designed to demonstrate the efficacy and safety of CP used to prevent progression to severe disease or death in noncritical hospitalized patients with early forms of COVID-19.

Results

Patients. Between April 4, 2020, and February 5, 2021, 359 patients were included in the study after giving informed consent, and 350 underwent randomization; 179 were assigned to receive CP in addition to Standard of Care (SOC) and 171 to the control group, which received only SOC. Of the patients randomized to CP, 175 (97.8%) received treatment as assigned (Figure 1) while 4 patients did not receive plasma due to consent withdrawal (1 patient) or physician decision (3 patients). One additional patient stopped CP infusion immediately after start, due to a transfusion-related reaction, and did not complete the treatment according to protocol. Four patients were lost for follow-up before trial day 15 due to consent withdrawal: 1 in the CP group and 3 in the SOC group, all of whom are included in the intention-to-treat analysis.

Patient characteristics are described in Table 1. Median age was 62 years (interquartile range [IQR] 53–75); 65.4% were men and 34.6% were women. Median time interval between symptom onset and randomization was 6 days (IQR 4–7). At baseline, 109 of 350 patients (31.1%) tested positive for anti–SARS-CoV-2 IgG antibodies (Euroimmun Assay, antibodies anti–S1 spike protein).

Table 1. Demographic and clinical characteristics of the patients at baseline

Characteristic	Plasma + SOC (*n* = 179)	SOC (*n* = 171)	All (*N* = 350)
Age, mean ± SD, years	62.7 ± 15.7	63.4 ± 14.9	63.0 ± 15.3
Age, median (IQR), years	63.0 (50.0–75.0)	61.0 (55.0–75.0)	62.0 (53.0–75.0)
Sex, *n* (%)			
Male	118 (65.9)	111 (64.9)	229 (65.4)
Female	61 (34.1)	60 (35.1)	121 (34.6)
Body mass index, mean ± SD	28.6 ± 6.2	28.6 ± 5.8	28.6 ± 6.0
Median time (IQR) from symptom onset to randomization, days	6.0 (4.0–6.0)	6.0 (4.0–7.0)	6.0 (4.0–7.0)
Coexisting conditions, *n* (%)			
Diabetes mellitus	52 (29.1)	38 (22.2)	90 (25.7)
Obesity	31 (17.3)	27 (15.8)	58 (16.6)
Hypertension	90 (50.3)	75 (43.9)	165 (47.1)
Cardiovascular disorder	39 (21.8)	33 (19.3)	72 (20.6)
Chronic lung disease	33 (18.4)	24 (14.0)	57 (16.3)
Chronic kidney disease	9 (5.0)	11 (6.4)	20 (5.7)
Cancer	15 (8.4)	11 (6.4)	26 (7.4)
Immunodeficiency	10 (5.6)	11 (6.4)	21 (6.0)
Neurological or neuromuscular disorder	17 (9.5)	16 (9.4)	33 (9.4)
Chronic liver disease	6 (3.4)	8 (4.7)	14 (4.0)
Bilateral pneumonia at Rx, *n* (%)	148 (82.7)	142 (83.04)	290 (82.9)
Standard of care treatments at baseline, *n* (%)			
Glucocorticoid therapy	127 (70.9)	122 (71.3)	249 (71.1)
Anticoagulants[A]	95 (67.4)	85 (66.4)	180 (66.9)
Remdesivir	41 (22.9)	48 (28.1)	89 (25.4)
Azithromycin	49 (27.4)	45 (26.3)	94 (26.9)
Tocilizumab	10 (5.6)	16 (9.4)	26 (7.4)
Score on ordinal scale, *n* (%)			
Hospitalized, not requiring supplemental oxygen	40 (22.3)	34 (19.9)	74 (21.1)
Hospitalized, requiring supplemental oxygen by mask or nasal prongs	139 (77.7)	137 (80.1)	276 (78.9)
IgG positive for anti SARS-CoV-2 antibodies (Euroimmun) at patient baseline, *n* (%)	48 (26.8)	61 (35.7)	109 (31.1)

[A]Data not available for first 81 patients. Anticoagulation was included as a mandatory variable to be registered in the eCRF with the September 2020 amendment. Data shown correspond to patients of the second (*n* = 187) and third (*n* = 82) waves.

Most patients (78.9%) were receiving supplemental oxygen by mask or nasal prongs and 71.1% were receiving corticosteroids. Baseline data (demographic characteristics, baseline laboratory test results, distribution of ordinal scale scores, and concomitant treatments) were similar in the 2 groups.

Selection of donors and convalescent plasma characteristics. Donors had a prior laboratory-confirmed SARS-CoV-2 infection, were asymptomatic for at least 14 days, and were positive for anti–SARS-CoV-2 IgG (ratio ≥ 1.1 with the Euroimmun assay). Over the course of the study, 233 successful CP aphere-

Table 2. Characteristics of convalescent plasma received by the patients

Characteristic	Patients, *N* = 175[A]
Semiquantitative results for anti–SARS-CoV-2 IgG antibodies anti-S (Euroimmun assay), median (IQR), range	3.4 (1.9–5.9) 0.4[B]–12.1
Patients receiving high-titer plasma (Euroimmun ratio ≥ 3.5)[C]	83/175 (47.4%)
Anti–SARS-CoV-2 IgG antibodies anti-S (ORTHO assay), median (IQR), range	8.2 (4.5–12.0) 0.0[D]–15.4
Patients receiving high-titer plasma (ORTHO clinical S/C ≥ 9.5)[C]	64/175 (36.6%)
NT50 for D614 pseudovirus neutralizing assay[E], median (IQR), range	157 (64–502) 12–3421
Patients receiving high-titer plasma neutralizing Ab[E] (Titer > 336)	59/175 (33.7%)

[A]Four patients assigned to plasma did not receive plasma and are not included. [B]First donors were assessed with several IgG assays that were being validated before Euroimmun was set as the standard assay for the trial. Two donors (4 units) were accepted as positive but resulted negative when reanalyzed with EUROIMMUN. Those units were positive when assessed with ORTHO. [C]Cut-off points proposed by the FDA (ref. 18). [D]One patient received a plasma unit with IgG titer of 0 according to ORTHO assay. This plasma was positive by EUROIMMUN (1.24). All other administered plasma units were positive for anti–SARS-CoV-2 IgG with ORTHO assay. [E]Titers of neutralizing antibodies are expressed as the reciprocal dilution that inhibits 50% of the infection. Thirty-two or less was considered a negative result. High titer arbitrarily set at a titer >336.

Table 3. Main and key secondary outcomes

Outcome	Plasma + SOC (*n* = 179)	SOC (*n* = 171)	RR (95% CI)[A]	*P* value
Primary endpoint				
Noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO or death at 14 days[B], *n*/*N* (%)	21/179 (11.7%)	28/171 (16.4%)	0.94 (0.87–1.03)	0.205
Noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO or death at 28 days, *n*/*N* (%)	15/179 (8.4%)	29/171 (17.0%)	0.91 (0.84–0.99)	0.021
Secondary endpoints				
Death at 14 days, *n*/*N* (%)	6/179 (3.4%)	10/171(5.9%)	0.58 (0.22–1.56)	0.28
Death at 28 days, *n*/*N* (%)	7/179 (3.9%)	14/171(8.2%)	0.49 (0.20–1.17)	0.11

[A]Relative risk (RR) and *P* value obtained from binomial regression model adjusted by center. [B]Primary time point of assessment.

sis procedures were performed after testing 403 donors eligible for screening, and 413 CP units were collected. More detailed information is available in Supplemental Figures 1 and 2; supplemental material available online with this article; https://doi.org/10.1172/JCI152740DS1. The 175 CP units administered in the study were obtained from 116 different donors. Donors were mostly men (87.1%) with a median age of 41.0 years (IQR 30.5–52.0] and the median time elapsed from the end of symptoms to plasmapheresis was 45.0 days (IQR 32.0–54.0).

Several assays were performed to assess antibodies in plasma (Table 2). Anti–SARS-CoV-2 IgG (anti-S) titers in CP units that were administered to trial patients had a median value of 8.2 (IQR 4.5–12.0) as measured by VITROS (Ortho-Clinical Diagnostics, Rochester, New York, USA) with 36.3% of patients receiving high-titer CP units (signal-to-cutoff [S/C] ≥ 9.5). Correlation between the 2 methods used to assess anti–SARS-CoV-2 IgG anti–Spike protein (VITROS Ortho and Euroimmun) are shown in Supplemental Figure 3. Neutraliz-

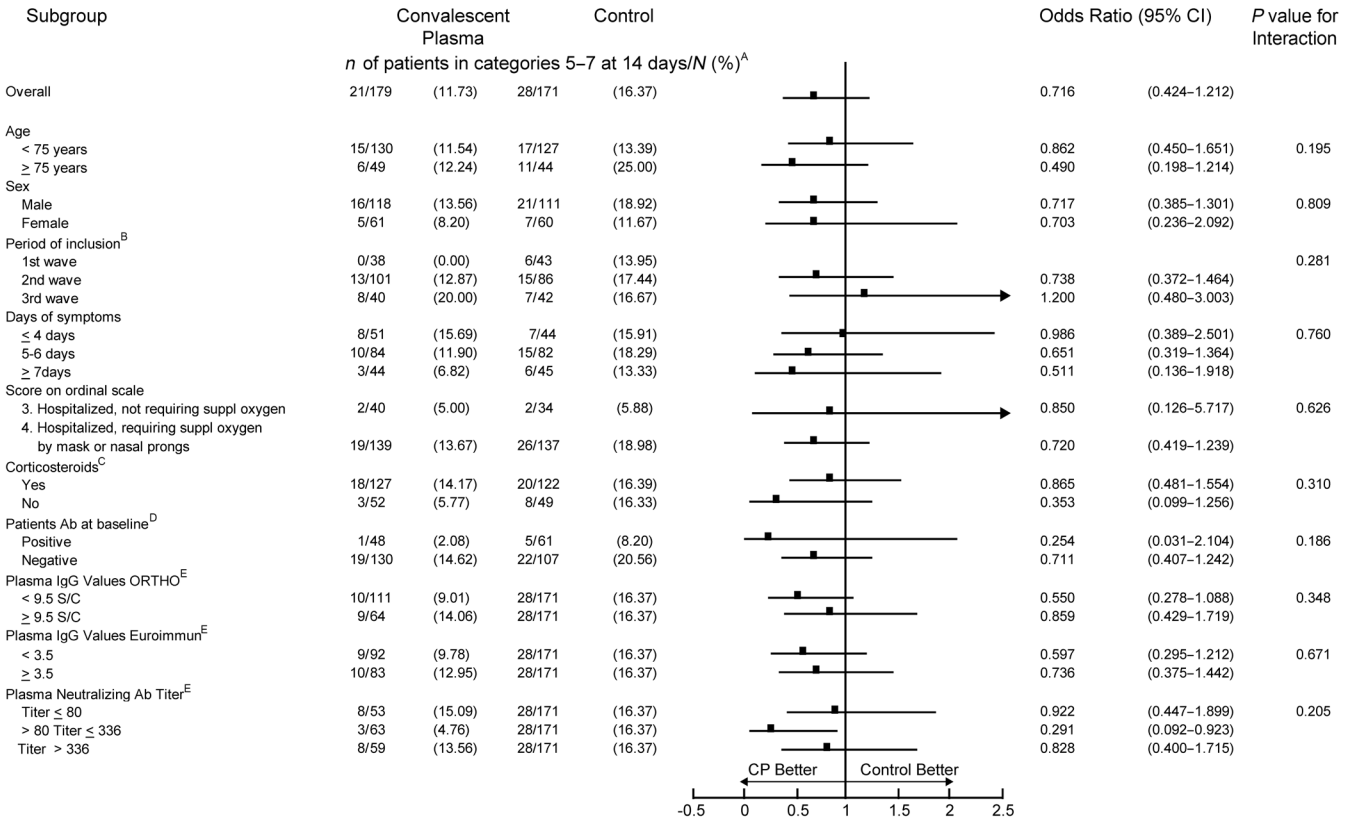

Figure 2. Patients on noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or who died, shown at 14 days according to subgroup analysis. Odds ratio not calculated for subgroups with a zero value. (**A**) *n* indicates patients in categories 5–7 (noninvasive ventilation or high flow oxygen devices; invasive mechanical ventilation or ECMO; death) at that time point, whereas *N* indicates the total number of patients in the group, following the intention-to-treat principle. (**B**) Periods were established according to official information on waves and actual trial recruitment. First wave: April 4, 2020, to July 9, 2020; second wave: September 7, 2020, to December 5, 2020; third wave: December 10, 2020, to February 5, 2021. (**C**) Includes patients receiving corticosteroids on the day of randomization or before. (**D**) Four patients had missing serology values, 146 patients included. (**E**) Four CP patients did not receive plasma (consent withdrawal, not plasma available) and only results from 175 used plasma units are available.

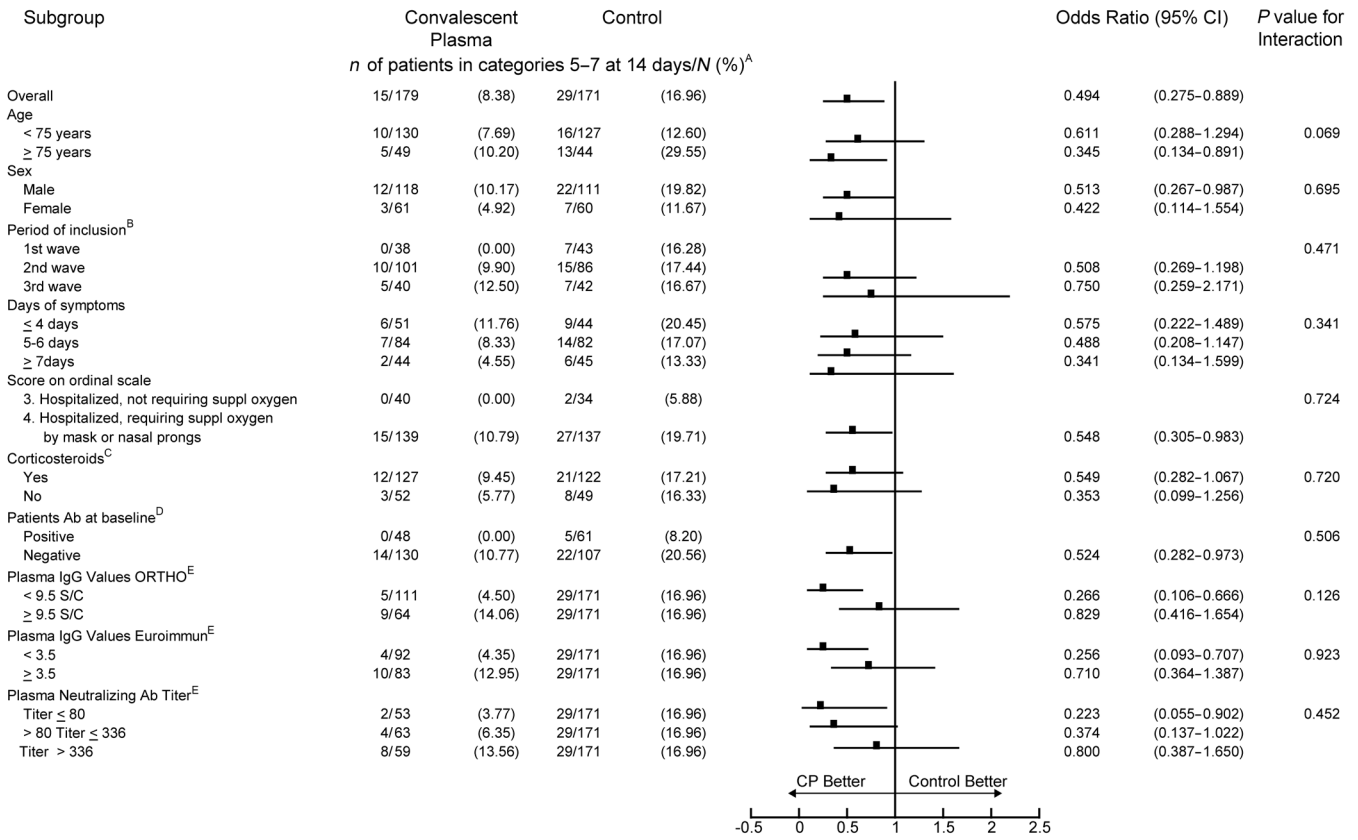

Figure 3. Patients on noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or who died, shown at 28 days according to subgroup analysis. Odds ratio not calculated for subgroups with a zero value. (**A**) *n* indicates patients in categories 5–7 (noninvasive ventilation or high-flow oxygen devices; invasive mechanical ventilation or ECMO; death) at that time point, whereas *N* indicates the total number of patients in the group, following the intention-to-treat principle. (**B**) Periods were established according to official information on waves and actual trial recruitment. First wave: April 4, 2020, to July 9, 2020; second wave: September 7, 2020, to December 5, 2020; third wave: December 10, 2020, to February 5, 2021. (**C**) Includes patients receiving corticosteroids on the day of randomization or before. (**D**) Four patients had missing serology values, 146 patients included. (**E**) Four CP patients did not receive plasma (consent withdrawal, not plasma available) and only results from 175 used plasma units are available.

ing antibody determination by pseudovirus neutralizing ID50 assay showed a median titer of 157 (IQR 64–502).

Primary outcome. The proportion of patients requiring noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or who died (i.e., categories 5–7) at the predefined primary time point of assessment (at 14 days) was 11.7% in the CP group versus 16.4% in the control group ($P = 0.205$). At 28 days, this proportion was 8.4% and 17.0% in the CP and control groups, respectively ($P = 0.021$; Table 3). A preplanned analysis according to basal SARS-CoV-2 antibody status showed that patients who were negative for antibodies at baseline had a worse clinical course. Among patients with anti–SARS-CoV-2 IgG antibodies at the time of inclusion, 2.1% in the CP group and 8.2% in control group were in categories 5–7 at 14 days ($P = 0.23$) and 0% and 8.2% at 28 days, respectively ($P = 0.066$). Among patients without antibodies at inclusion, 14.6% in the CP group and 20.6% in controls were in categories 5–7 at 14 days ($P = 0.23$) and 10.8% and 20.6% at 28 days, respectively ($P = 0.037$). The prespecified subgroup analyses by symptoms duration or anti–SARS-CoV-2 plasma titers did not show consistent findings. Results of the subgroup analyses are shown in Figures 2 and 3 and Supplemental Table 8. The distribution of the clinical status of patients according to the 7-point ordinal scale at 14 and 28 days is shown in Figure 4 as well as in Supplemental Table 2.

Key secondary outcomes. Mortality rates were 3.4 % in the CP arm and 5.9% in the control arm at 14 days ($P = 0.26$) and 3.9% in CP arm and 8.2% in control arm at 28 days ($P = 0.092$). Hazard ratio for death was 0.46 (95% CI 0.16–1.14, log-rank $P = 0.087$; Figure 5). Subgroup analysis, including patient's seroconversion status at inclusion, did not show compelling differences in mortality (Supplemental Figure 7).

There were no significant differences between treatment groups in other secondary variables such as mean change in clinical status, odds of improvement on the ordinal scale, number of days alive and free from mechanical ventilation, time to first clinical deterioration, or time to first improvement (Supplemental Table 7 and Supplemental Figures 4–6). Time to hospital discharge was not different between groups, with a median time to discharge of 9 days in both groups, IQR 8–11 in the CP group, and IQR 8–10 in the control group. The number of rehospitalizations was low in both arms, 3.4% (6/179) in the CP group and 4.1% (7/171) in the control group.

Safety. Thirty-four serious, or grade 3 to 4, adverse events (AEs) were reported in 31 patients: 15 in the CP group and 16 in the control group (Supplemental Table 3). The investigators consid-

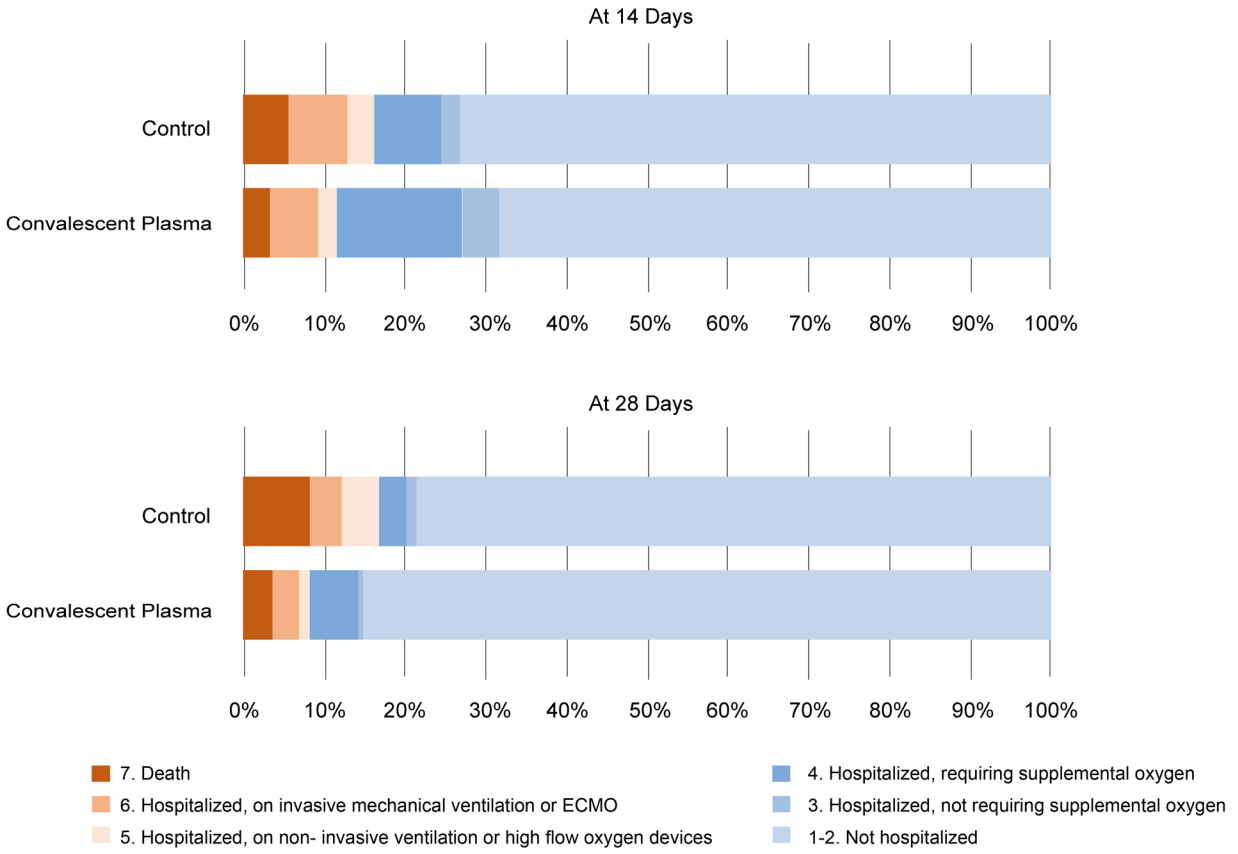

Figure 4. Clinical outcomes of patients treated with convalescent plasma as compared with standard of care. The distribution of the clinical status according to the 7-point ordinal scale is shown at 14 and 28 days. Outcomes at 14 and 28 days after randomization refer to the clinical status at that time, even if the patient was discharged and rehospitalized. In case of discharge before the end of study, patients were scheduled for a visit (either in an outpatient clinic or a phone visit) at 14, 29, and 60 days after randomization. Possible rehospitalizations were actively investigated. Categories 1 and 2 (nonhospitalized) are shown together.

ered all of these AEs to be related to underlying disease or related complications and not to the study treatment. Regarding thrombotic events, 8 patients reported an event, 3 in the CP group and 5 in the control group. The most commonly reported events were pulmonary embolisms ($n = 5$).

CP infusion–related events were reported in 10 patients; 5 were cases of severe worsening of dyspnea, 3 of which were reported by investigators as suspected TRALI (transfusion-related acute lung injury). In these 3 cases, TRALI was ruled out after a full assessment, including negative anti-HLA and antineutrophil antibodies in patients and donors. All patients recovered without sequelae except one patient who died on day 31 due to underlying disease as per investigator causality assessment.

Discussion

Our study suggests that the addition of a single unit of CP to the SOC therapy of patients hospitalized with COVID-19 may reduce their probability of disease progression to noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or death at 28 days (8.4% CP versus 17.0% control ; $P = 0.021$). This difference was not significant for the predefined primary endpoint at 14 days (11.7% CP versus 16.4% control, $P = 0.205$). At the time of study design, the primary endpoint was set at 2 weeks based on the proposal made by the WHO R&D Blueprint initiative (15) and the outcome at 28 days was established as a key secondary endpoint. However, the outcome at 28 days is currently the most widely accepted outcome for COVID-19 trials in hospitalized patients and should be preferentially considered, in line with the recommendations from a broad international workshop on endpoints for COVID-19 trials including all major regulatory agencies (16). The 28-day overall survival did not reach a significant difference between treatment groups (HR 0.46, 95% CI 0.16–1.14, log-rank $P = 0.087$), but our trial was not powered enough to detect differences in mortality results. The rest of secondary variables also showed no differences between groups.

These findings add to results obtained in previous clinical trials with CP in COVID-19. Our hypothesis was that CP would benefit patients early in the course of the disease and who were not critically ill. A trial in elderly patients treated within 72 hours of onset of mild COVID-19 showed that CP decreased the development of severe respiratory disease from 31% to 16% (4). It has been suggested that negative results in some trials could arise from targeting patients with later or more severe forms of the disease, for whom the use of virus neutralizing antibodies comes too late to avoid the inflammatory phase of tissue damage, or in patients who had already developed their own immune response. For instance, the RECOVERY trial included patients with more advanced disease than our ConPlas-19 series,

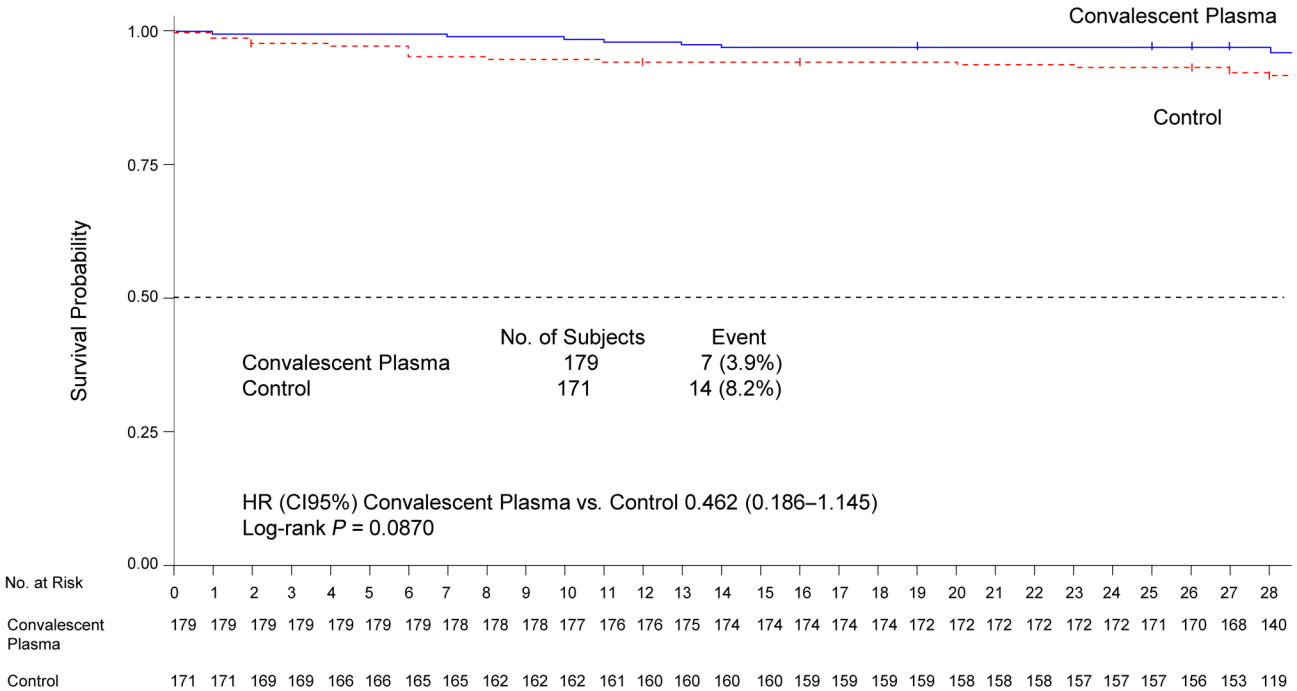

Figure 5. Overall survival by group.

as reflected by their high mortality rate (24% at 28 days) and longer duration of symptoms (median 9 days, IQR 6–12) (10). Likewise, nearly 50% of the patients treated by Simonovich et al. at a median of 8 (range 5–10) days from the onset of symptoms had SARS-CoV-2 antibodies at baseline (5). Other negative trials also included patients with a median duration of symptoms of 10 or more days (3, 8). Time from the onset of symptoms is a parameter frequently used in clinical trials with new medicinal products with antiviral activity, even though it is rather subjective and perhaps not well related to the real-time evolution of the disease, as initial symptoms often go unnoticed. In our trial, which had a median of 6 days after symptom onset, a preplanned subgroup analysis by time from disease onset was not associated with response to CP. Our trial suggests that days of symptoms as reported by patients may be an inaccurate parameter to define the window of opportunity for antiviral products intended for early treatment.

Laboratory assessment of patients' anti–SARS-CoV-2 serological status at the time of treatment might be a more accurate factor for predicting the benefit from passive immunotherapy with CP, as it is currently being explored in trials with commercial monoclonal antibodies. Additionally, a delayed development of SARS-CoV-2 antibodies is a known factor for poor prognosis (17), which could increase the magnitude of the CP benefit. Our results confirmed a higher occurrence of noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or death in patients with pneumonia who were seronegative at hospital admission (17.3% and 15.2% patients at 14 and 28 days, respectively) when compared with patients who had already developed their own immune response at inclusion (5.5% and 4.6% at 14 and 28 days, respectively). In our trial, the benefit from CP was shown in a consistent manner in the seronegative patient subgroup, with a reduced number of clinical worsening at 28 days (10.8% versus 20.6%; $P = 0.037$), and 14.6% versus 20.6% at 14 days ($P = 0.23$). The subgroup of seropositive patients, however, had a lower number of patients and a very low number of events, which results in a very wide CI. This precludes us from concluding on different effects of CP according to serological status.

Our trial showed a more consistent beneficial effect in older patients than in younger ones ($P = 0.069$ for the age effect; Figure 3). In patients 75 years of age or older, disease progression to noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or death at 28 days was 10.2% in the CP group versus 29.6% in the control group ($P = 0.018$) and 12.24% in the CP group and 25% in the control group at 14 days ($P = 0.112$). This is consistent with some previously published results in elderly patients (4).

It has been suggested that CP needs high titers of neutralizing antibodies to be effective (7). Published trials have a substantial heterogeneity in CP characterization, and in some published trials negative results could be explained by the use of CP with very low titers. In one negative trial, no SARS-CoV-2 antibodies were present in one-third of administered plasma and very low titers were present in the remaining two-thirds (median 1:40, IQR 1:30–1:80; ref. 11). At the time of our trial design, it was not possible to have neutralizing antibody titer results in real time to use them for selecting plasma units. Correlation between the available semiquantitative tests for anti–SARS IgG and the titer of functional neutralizing antibodies and accepted cut-off points were still unclear. We decided to use plasma that tested positive for IgG anti–S antibodies, but with a wide spectrum of titers, as this could be closer to future pandemic necessities, with CP collected near the time of its use and unavailability of neutralizing antibody assays. This results in a more pragmatic design aimed at demonstrating real-world efficacy of CP. Our CP selection strategy guaranteed the use of CP with antibodies, but not the use of high titers of neutralizing antibodies. Only one-third of the patients (36.6%) received high-titer CP, as defined by ORTHO anti–SARS-

CoV-2 IgG values ≥ 9.5 (Ortho-Clinical Diagnostics), the cut-off point established by some regulatory agencies (18) or neutralizing antibody titers greater than 336 (33.7%). Up to 24 patients received IgG-positive CP with subsequent neutralizing antibodies results ≤ 32. The subgroup analysis according to plasma titers did not show a greater benefit with the use of high-titer CP, but the limited sample size does not allow us to draw firm conclusions.

Finally, the efficacy of CP could be influenced by the match between the anti–SARS-CoV-2 antibodies in donor CP and the infecting virus variant in the patient, and it has been suggested that to ensure neutralizing activity against circulating virus variants, CP for use in pandemics should be obtained from local sources. The CP used in our study was locally sourced, but mainly obtained from patients infected during the first wave. There is a possibility that this CP may have had a reduced neutralizing potential in subsequent waves of COVID-19 caused by other SARS-CoV-2 variants. The subgroup analysis suggests this is a possibility, as the benefit of CP was substantially greater in the first period of the trial. Nevertheless, at the end of the trial period, the dominant strain in most regions in Spain was the B.1.1.7 variant (19) and some laboratory studies have shown an acceptable retention of neutralizing activity of CP against this variant (20, 21). An alternative explanation for the trend toward a decreasing effect of plasma in successive pandemic periods could be related to the increasing quality of COVID-19 medical care.

This study has limitations. It is not blinded but, nevertheless, blinded randomization was strictly preserved, with a complete blinded electronic allocation performed only after full baseline data of the included patients were registered. After randomization, follow-up was not blinded but potential bias was minimized because all patients underwent well-established standard medical decisions, following the applicable protocols in all centers. In addition, the main results are based on objective variables. No evident between-group differences in treatment after enrollment were observed, either in overall treatments or in specific treatments that could be considered rescue therapies such as corticosteroids, anti–IL-6, anti–IL-1, or JAK inhibitors (Supplemental Table 6). The sample size was not sufficient to address subgroup analyses or differences in some secondary variables such as overall mortality. Recruitment of the numbers of patients required to obtain scientific evidence has been a struggle for all randomized clinical trials in this setting, including ConPlas-19. Meanwhile, many thousands of patients have been treated in open access programs worldwide. From the evidence available now, many of these patients did not benefit from open treatment with CP. If only a small percentage of them had been included in randomized clinical trials, the required evidence would have been compiled much sooner.

In conclusion, with 350 patients hospitalized with COVID-19 randomized relatively early in the course of the disease and excluding severe cases requiring high-flow oxygen devices or mechanical ventilation, our study suggests that patients randomized to receiving CP in addition to SOC had a better outcome at 28 days, particularly patients older than 75 years of age and those with no IgG antibodies detectable at baseline. These results should be interpreted with caution because the primary endpoint at 14 days, overall survival, and other secondary endpoints were not significantly improved. Lack of significance could be influenced by the limited trial sample size, so confirmation from trials adequately powered to assess mortality as well as the effect in particular subgroups of patients is needed. Results from a pooling initiative of individual data from CP randomized trials will be soon available (22) and could help to establish evidence of CP effectiveness in specific groups of patients and to determine the characteristics of CP to be used. Evolving knowledge from the development of monoclonal neutralizing antibodies and purified immunoglobulins will contribute to the clarification of some pending issues regarding the benefits of passive immunotherapy while keeping in mind that monoclonal antibodies are a scarce and expensive commercial product, and not widely accessible, whereas CP is a readily available resource at an acceptable cost worldwide.

Methods

Study design. ConPlas-19 was a multicenter open-label randomized trial conducted in 27 hospitals in Spain. The trial protocol has been published in advance (23). The final version of the protocol with description of amendments is included in the Supplemental Material. Recruitment by clinical site is included in Supplemental Table 1.

All patients were diagnosed with COVID-19 pneumonia and were randomly assigned in a 1:1 ratio to receive a single unit of CP (250–300 mL), or not, as add-on therapy to SOC within first 7 days of symptoms. A description of the SOC followed and its changes over the course of pandemic are included in Supplemental Tables 4 and 5.

Remote data monitoring was performed by dedicated staff, independent of the site investigators, with 100% source data verification performed for all patients recruited for all critical data points that were previously established in the monitoring plan.

An independent Data Safety Monitoring Board (DSMB) was established to oversee the conduct and safety aspects of the trial as well to provide recommendations about continuation of the study based on an unblind analysis of efficacy endpoints at preplanned cut-off points.

Patients. Patients were eligible if hospitalized for laboratory-confirmed SARS-CoV-2 infection and pneumonia diagnosed either by radiographic evidence of pulmonary infiltrates or by clinical evidence plus SpO_2 ≤ 94% on room air, and within 12 days from the onset of symptoms (fever or cough). After the first 81 patients, time window for inclusion was amended to a maximum of 7 days as evolving knowledge indicated that selection of patients with early disease was crucial. Patients were excluded if already on mechanical ventilation (invasive or noninvasive) or high-flow oxygen devices, had Stage 4 severe chronic kidney disease or required dialysis, or if progression to death was imminent and inevitable within 24 hours according to clinical team opinion. The complete description of inclusion and exclusion criteria is available in the Supplement Material.

Laboratory confirmation for inclusion required a RT-PCR or antigen test on samples collected in the ongoing symptomatic COVID-19 period and performed either at a local laboratory or at the trial's central laboratory. Nevertheless, all patients included had a basal blood and oro/naso-pharyngeal swab RT-PCR determination at the trial's central laboratory.

Randomization and masking. After giving informed consent to participate, patients were registered using a web-based electronic Case Report Form (eCRF) performed with ORACLE clinical. After baseline clinical data were recorded and plasma availability confirmed, patients were randomized using a centralized system embedded in the eCRF that ensures allocation concealment. Randomization list was 1:1 ratio, stratified by study site with variable block size multiple of 2 elements and generated using the RERAND system integrated within the eCRF. After randomization, the local clinical team responsible for patient assessment was not blinded to the treatment arm.

Procedures. All patients received SOC, including all supportive and specific treatments used according to local or national recommendations, including off-label medicines. Those patients allocated to add-on CP received a single unit of CP (250–300 mL), which had to be administered immediately after randomization (day 1 of the trial).

CP donors were recruited by the participant hospitals or regional transfusion centers and complied with EU requirements for plasma donors (24), had had laboratory-confirmed SARS-CoV-2 infection, anti–SARS-CoV-2 IgG (ratio ≥1.1 with the Euroimmun ELISA test), and were asymptomatic for at least 14 days. A history of transfusion or pregnancy were exclusion criteria to minimize the risk of TRALI. A 600 mL plasmapheresis was performed to obtain 2 CP units of approximately 300 mL each, that were used in 2 different patients. Each convalescent plasma unit was derived from a single donor. All CP units underwent a pathogen inactivation procedure.

Euroimmun anti–SARS-CoV-2 ELISA IgG assays were performed on donor serum samples at the study's centralized laboratory (Centro Nacional de Microbiología [CNM]), according to the manufacturer's instructions. Microplate wells are coated with recombinant structural protein 1 (S1) of SARS-CoV-2 and the assay detects anti–SARS-CoV-2 IgG against the viral spike protein. The results are evaluated semiquantitatively by calculation of the ratio between the extinction of the sample and calibrator. A ratio < 0.8 is considered negative, ≥ 0.8 and < 1.1 are borderline, and ≥ 1.1 is positive.

CP units used in the trial were further characterized at the study's centralized laboratory using a second method for anti–SARS-CoV-2 IgG levels (VITROS) and a determination of neutralizing antibodies. The VITROS Immunodiagnostic Anti–SARS-CoV-2 ELISA IgG assay (Ortho-Clinical Diagnostics) is a chemiluminescent immunoassay (CLIA) utilizing a recombinant structural protein 1 (S1) of SARS-CoV-2 to measure total antibody presence in serum and plasma. The light signal is read by the system and the results are interpreted qualitatively. An index S/C < 1.0 is considered negative, ≥ 1.0 positive. Finally, as a third assay, we measured neutralizing antibodies in all administered plasma units. Dilutions of participants' plasma samples were preincubated for 1 hour with D614-SARS-CoV2 pseudoviruses (10 ng p24Gag/well) and added to Vero E6 cells in 96-well plates. At 48 hours after infection, viral infectivity was assessed by measuring luciferase activity (Renilla Luciferase Assay, Promega) using a 96-well plate luminometer "Orion II" (Berthold Technologies). The titer of neutralizing antibodies was calculated as 50% inhibitory dose (NT50), expressed as reciprocal of 2-fold serial dilution of heat-inactivated sera (range 1:16–1:8192) resulting in a 50% reduction of pseudovirus infection compared with control without serum. Neutralization ≤ 32 is considered negative. Positive and negative controls were included in the assay and nonspecific neutralization was assessed using a nonrelated pseudovirus expressing the Vesicular Stomatitis Virus envelope.

Neutralizing antibody titer and IgG level results by VITROS assay were only available at the end of the study and were not available for CP unit selection during the study.

Patients were assessed daily during hospitalization. After discharge, follow-up was done in outpatient clinics or by phone at days 14, 28, and 60 after randomization to assess study outcomes, rehospitalizations, and additional safety information. The patient's clinical status was recorded using the 7-category ordinal COVID-19 scale: 1, not hospitalized, no limitations on activities; 2, not hospitalized, limitation on activities; 3, hospitalized, not requiring supplemental oxygen; 4, hospitalized, requiring supplemental oxygen; 5, hospitalized, on noninvasive ventilation or high-flow oxygen devices; 6, hospitalized, on invasive mechanical ventilation or ECMO and 7, death.

Outcomes. The primary outcome was the proportion of patients in categories 5, 6, or 7 (noninvasive ventilation or high-flow oxygen, invasive mechanical ventilation or ECMO, or death) at 14 days (day 15 of the study). Key secondary outcomes included the proportion of patients in categories in categories 5, 6, or 7 at 28 days (day 29 of the study), overall survival, death rate at 14 and 28 days, mean change in the ordinal scale, duration of hospital stay, number of days alive and free from mechanical ventilation, time to discharge, time to first clinical deterioration, time to improvement in one category, and number of rehospitalizations.

Prespecified subgroup analyses of the primary endpoint were planned according to the level of neutralizing antibodies in the administered plasma, duration of symptoms at randomization, positivity of antibodies at patient baseline, and period of patient recruitment according to the different waves of pandemics.

Serious AEs, grade 3 or 4 AEs, and CP infusion–related AEs (within 24 hours after administration) were collected. Investigators were instructed to actively monitor for the appearance of predefined AEs of special interest: TRALI, ADE (antibody-dependent enhancement of infection), and thrombotic events.

Statistics. An initial sample of 278 patients was planned, assuming 20% worsening in patients in the control group and an absolute reduction of 10% in the CP group, with 80% statistical power and 2.5% one-sided alpha level (5% 2-sided). At the time of trial design, there was uncertainty about the proportion of patients worsening to categories 5, 6, or 7 at 14 days. Therefore, one sample size reestimation (at 60% of the trial size) and a series of futility and efficacy interim analyses were planned (at 20%, 40%, 60%, and 80% of the trial size) using statistical boundaries based on rho family spending functions (with rho = 7) calculated by means of the East software v6.5 (Cytel Inc.). The full statistical analysis plan, including stoppage rules for the interim analysis, was completed before the first interim analysis.

The primary analysis follows the intention-to-treat principle and includes all randomized patients. The risk ratios (RRs) with 95% CI and the inferential analysis for the primary endpoint were conducted using a log-binomial regression model including center (grouped by level of recruitment) as a covariate. The ordinal scale changes were analyzed using the Wilcoxon rank-sum test and a shift analysis was performed using a logistic proportional odds model. Survival function was estimated using the Kaplan-Meier method, group comparisons done by log-rank test, and the hazard ratios (and 95% CI) calculated by means of the Cox model. Analysis was performed using SAS v9.4 scientific software (SAS Institute Inc.). The trial was temporarily stopped on July 10, 2020, after the first interim analysis, due to a drastic fall in recruitment (end of first wave in Spain), although prespecified futility or efficacy stop criteria had not been reached. Preliminary results of this first set of patients were publicly reported (25). Nevertheless, the trial recruitment was resumed shortly after, with the surge of the second wave, and the trial was finally completed as planned. On December 3, 2020, the DSMB recommended increasing the sample size by at least a 20% and the new sample size was set at 350 patients. The complete set of patients is reported here.

Study approval. The trial was approved by the Research Ethics Committee of the Hospital Universitario Puerta de Hierro Majadahonda in Madrid, Spain, and conducted in accordance with the principles of the Good Clinical Practice guidelines of the International Conference on Harmonization. A waiver for approval from the Spanish Medicines Agency

was obtained, due to the classification of fresh frozen plasma as a blood product. A specific clinical trial contract was signed with each participating center. Informed consent was obtained from all patients and either written consent or witnessed oral consent followed by written consent, when feasible, were accepted. All donors gave a study-specific written consent.

Author contributions

CAS, JLB, RFD, ARM, EMR, and BRA conceived the study and led protocol development. RMM, FT, CPH and JRPP contributed to study design. JCP, AFC, ADS, JLT, MML, IP, ALMG, MJNO, MLPL, PV, and JRPP contributed to data acquisition, clinical follow-up of patients, analysis, and/or interpretation. IRM, MLP, JVE, MISS, MCJF, JPO, ARG, LB, MEMS, MRB, JAMC, and JLB contributed to convalescent plasma collection, qualification, and release, and to hemovigilance procedures. AVI coordinated study activities. CPH was in charge of safety procedures and EMD performed and interpreted specific laboratory safety assessments. ICF performed and interpreted virus laboratory assays. MPO performed and interpreted serology assays. JGP and JA performed and interpreted neutralization assays. FT, CAS, JLB, RFD, and BRA designed and supervised statistical analyses. CAS and RFD wrote the first version of the manuscript. All authors contributed to critical revision of the manuscript and approved its final version.

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