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Effect of Convalescent Plasma on Mortality among Hospitalized Patients with COVID-19: Initial Three-Month Experience

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43 **Key Points**

44 **Question.** Does transfusion of human convalescent plasma reduce mortality among
45 hospitalized COVID-19 patients?

46 **Findings.** Transfusion of convalescent plasma with higher antibody levels to
47 hospitalized COVID-19 patients significantly reduced mortality compared to transfusions
48 with low antibody levels. Transfusions within three days of COVID-19 diagnosis yielded
49 greater reductions in mortality.

50 **Meaning.** Embedded in an Expanded Access Program providing access to COVID-19
51 convalescent plasma and designed to assess its safety, several signals consistent with
52 efficacy of convalescent plasma in the treatment of hospitalized COVID-19 patients
53 emerged.

54 **Abstract**

55 **Importance:** Passive antibody transfer is a longstanding treatment strategy for
56 infectious diseases that involve the respiratory system. In this context, human
57 convalescent plasma has been used to treat coronavirus disease 2019 (COVID-19), but
58 the efficacy remains uncertain.

59 **Objective:** To explore potential signals of efficacy of COVID-19 convalescent plasma.

60 **Design:** Open-label, Expanded Access Program (EAP) for the treatment of COVID-19
61 patients with human convalescent plasma.

62 **Setting:** Multicenter, including 2,807 acute care facilities in the US and territories.

63 **Participants:** Adult participants enrolled and transfused under the purview of the US
64 Convalescent Plasma EAP program between April 4 and July 4, 2020 who were
65 hospitalized with (or at risk of) severe or life threatening acute COVID-19 respiratory
66 syndrome.

67 **Intervention:** Transfusion of at least one unit of human COVID-19 convalescent plasma
68 using standard transfusion guidelines at any time during hospitalization. Convalescent
69 plasma was donated by recently-recovered COVID-19 survivors, and the antibody
70 levels in the units collected were unknown at the time of transfusion.

71 **Main Outcomes and Measures:** Seven and thirty-day mortality.

72 **Results:** The 35,322 transfused patients had heterogeneous demographic and clinical
73 characteristics. This cohort included a high proportion of critically-ill patients, with 52.3%
74 in the intensive care unit (ICU) and 27.5% receiving mechanical ventilation at the time of
75 plasma transfusion. The seven-day mortality rate was 8.7% [95% CI 8.3%-9.2%] in
76 patients transfused within 3 days of COVID-19 diagnosis but 11.9% [11.4%-12.2%] in
77 patients transfused 4 or more days after diagnosis ($p<0.001$). Similar findings were
78 observed in 30-day mortality (21.6% vs. 26.7%, $p<0.0001$). Importantly, a gradient of
79 mortality was seen in relation to IgG antibody levels in the transfused plasma. For
80 patients who received high IgG plasma (>18.45 S/Co), seven-day mortality was 8.9%
81 (6.8%, 11.7%); for recipients of medium IgG plasma (4.62 to 18.45 S/Co) mortality was
82 11.6% (10.3%, 13.1%); and for recipients of low IgG plasma (<4.62 S/Co) mortality was

83 13.7% (11.1%, 16.8%) ($p=0.048$). This unadjusted dose-response relationship with IgG
84 was also observed in thirty-day mortality ($p=0.021$). The pooled relative risk of mortality
85 among patients transfused with high antibody level plasma units was 0.65 [0.47-0.92]
86 for 7 days and 0.77 [0.63-0.94] for 30 days compared to low antibody level plasma
87 units.

88 **Conclusions and Relevance:** The relationships between reduced mortality and both
89 earlier time to transfusion and higher antibody levels provide signatures of efficacy for
90 convalescent plasma in the treatment of hospitalized COVID-19 patients. This
91 information may be informative for the treatment of COVID-19 and design of
92 randomized clinical trials involving convalescent plasma.

93 **Trial Registration:** ClinicalTrials.gov Identifier: NCT04338360

94 **Introduction**

95 Passive antibody transfer, including convalescent plasma or serum, has
96 previously been used to treat infectious diseases that involve the respiratory system¹⁻³.
97 This therapeutic approach was established early in the last century and included
98 widespread use of convalescent plasma for treatment of the 1918 influenza⁴. In this
99 context, the coronavirus disease 2019 (COVID-19) pandemic has revived interest in the
100 use of convalescent plasma for the treatment of hospitalized patients with COVID-19.
101 Although there is substantial interest in the use of COVID-19 convalescent plasma, the
102 efficacy signals are preliminary^{5,6}.

103 In response to the global COVID-19 pandemic and need for access to treatments
104 possibly providing benefit while randomized clinical trials were in various stages of
105 development and enrollment, the Mayo Clinic initiated the US Expanded Access
106 Program (EAP) for convalescent plasma, which resulted in widespread use of
107 convalescent plasma to treat COVID-19 in the U.S. The EAP received collaborative and
108 financial support from the Biomedical Advanced Research and Development Authority
109 (BARDA). Although the charter of the EAP was to provide access to and assess the
110 safety of COVID-19 convalescent plasma, we performed exploratory analyses on the
111 efficacy of this agent. We hypothesized, based on historical data that earlier
112 administration of convalescent plasma with high antibody levels would be associated
113 with reduced mortality. To address this hypothesis, we evaluated seven and 30-day
114 mortality in 35,322 hospitalized adults transfused with COVID-19 convalescent plasma
115 by asking two questions. First, was earlier treatment of patients with convalescent
116 plasma after diagnosis of COVID-19 associated with reduced mortality compared to
117 later treatment in the course of disease? Second, were higher antibody levels in the
118 transfused convalescent plasma associated with reduced mortality?

119 **Methods**

120 ***Design and Oversight***

121 As described previously^{7,8}, the EAP was a US Government-sponsored, national,
122 pragmatic intervention conducted as a multicenter, open-label protocol in hospitalized
123 adults with COVID-19. All hospitals or acute care facilities in the US and any physician
124 licensed in the US were eligible to participate provided they agreed to adhere to the
125 treatment protocol, FDA, and state regulations.

126 Mayo Clinic served as the academic research organization conducting the study. The
127 Mayo Clinic Institutional Review Board (IRB) was the central IRB, approved the protocol
128 all modifications, and performed regulatory oversight for all sites and investigators. The
129 principal investigator served as the regulatory sponsor. A Data and Safety Monitoring
130 Board oversaw the safety analyses and advised the regulatory sponsor and the Mayo
131 Clinic IRB on risk. Written informed consent was obtained from the participant or a
132 legally-authorized representative prior to enrollment, except for those patients who
133 necessitated use of an emergency consent process defined in collaboration with the US
134 FDA.

135 ***Participants***

136 Eligible patients were aged 18 years or older, hospitalized with a laboratory confirmed
137 diagnosis of infection with severe acute respiratory syndrome coronavirus 2 (SARS-
138 CoV-2), and had (or were judged by a healthcare provider to be at high risk of
139 progression to) severe or life-threatening COVID-19. Inclusion criteria and the clinical
140 symptoms defining severe or life-threatening COVID-19 are outlined in **Supplement 1**.

141 ***Plasma Collection***

142 Convalescent plasma was obtained from a registered or licensed blood collector, and
143 COVID-19 antibody levels were unknown at the time of plasma collection. Convalescent
144 plasma was donated by COVID-19 survivors with confirmed diagnosis via clinical
145 laboratory test whom were symptom free for 14 days, or more according to standard
146 blood center procedures⁹. An aliquot of plasma or serum was shipped from a subset of
147 blood collection centers for later antibody testing. At the time of collection, each plasma

148 unit was assigned a standardized identifying number (ISBT 128 code) specific to the
149 *donor*, which was used to link antibody levels with study outcomes corresponding to the
150 plasma *recipient(s)*.

151 ***Plasma Transfusion***

152 Compatible COVID-19 convalescent plasma was administered intravenously according
153 to individual institutional protocols. The transfusion dose of COVID-19 convalescent
154 plasma was at least one unit (approximately 200 mL), with the option to administer
155 additional doses if clinically justified.

156 ***Data Entry***

157 Web-based, standardized data reporting surveys were completed to assess the clinical
158 status of patients using the Research Electronic Data Capture system (REDCap,
159 v.9.1.15 Vanderbilt University, Nashville, TN)^{10,11}, with FDA authorization, as previously
160 described^{7,8}. Given the rapidity at which the EAP was implemented and considering the
161 stress on clinical staff at participating sites during this on-going pandemic, the web-
162 based case reporting forms were designed to optimize convenience. Additionally,
163 although the patient inclusion criteria were specific to hospitalized patients, these
164 criteria were exceptionally broad (**Supplement 1**). While these elements of the EAP
165 may be atypical, they are perhaps understandable in a crisis of the magnitude of the
166 COVID 19 pandemic.

167 ***Antibody testing***

168 Binding antibody levels from sera were tested using the Ortho-Clinical Diagnostics
169 VITROS Anti-SARS-CoV-2 IgG chemiluminescent immunoassay (CLIA) in accordance
170 with manufacturer instructions¹². The Ortho-Clinical IgG CLIA is a qualitative assay
171 based on a recombinant form of the SARS-CoV-2 spike subunit 1 protein. Results of
172 this assay are based on the sample signal-to-cut-off (S/Co) ratio, with values <1.0 and
173 ≥1.00 corresponding to negative and positive results, The S/Co values reflect relative
174 levels of anti-SARS-CoV-2 antibodies.

175 **Statistics**

176 The sample size for the EAP was not determined *a priori* and patient accrual has not
177 concluded at the time of this writing. The sample sizes for these analyses varied by the
178 availability of linked antibody data, and in some cases, missing data. For the analyses
179 not associated with antibody data, all transfusions on or before July 4, 2020 were
180 included (i.e., three months after the first confirmed transfusion in the EAP). The
181 database was locked for this study report on August 5, 2020 to allow all included
182 patients to have up to 30 days of follow up after transfusion. For the subset of patients
183 with remnant samples suitable for antibody analysis, all patients matched by the
184 standardized identifying number (ISBT 128 code) were included, with some caveats
185 detailed below.

186 Based on insights from the pre-antibiotic era that antibody therapy was most effective
187 when given early^{2,13}, our cohort was stratified into categories based on the days from
188 COVID-19 diagnosis to plasma transfusion, including: 0, 1-3, 4-10, and 11 or more days
189 and for some graphical presentations and analyses, dichotomized into 0-3 vs. 4 or more
190 days. The timing of death was recorded within the precision of a calendar day, so
191 adjustments were needed to develop survival estimates. For deaths that occurred on
192 the same day of plasma transfusion, a death indicator representing 0.5 days was
193 assigned. Otherwise, the number of days between plasma transfusion and death was
194 calculated for each patient. Transfused patients were assumed to be alive unless death
195 was recorded via web-based reporting survey.

196 Given that patients may have had more than one unit of plasma from different donors
197 and the days from diagnosis to transfusion were heterogeneous, decision rules were
198 required for analyses of the antibody data. To control for the potential confounding
199 effects of plasma volume and non-uniform antibody levels between multiple plasma
200 units in the analysis, plasma recipients with a single unit, defined as 150 – 250 mL of
201 plasma, were included in the analysis. Finally, plasma from a single donor may have
202 been fractioned into multiple plasma units and transfused to multiple recipients. The
203 analysis did not adjust for the potential clustering that may have occurred in doing so.
204 For the semi-quantitative Ortho-Clinical IgG assay, low, medium and high relative

205 binding antibody levels were established by setting thresholds for low and high based
206 on the ~20th and ~80th percentiles of the distribution for the S/Co ratios, respectively.
207 Accordingly, the thresholds were set at 4.62 S/Co and 18.45 S/Co.

208 Unadjusted (crude) mortality and adjusted mortality estimates were constructed. For the
209 unadjusted mortality, or case fatality rate, tabulations of the number of mortality events
210 recorded divided by the total number at risk were computed. Score confidence intervals
211 were estimated. For analysis within subgroups, crude mortality was also estimated by
212 grouping the events on key strata variables (e.g., time to transfusion; time epoch of the
213 study)

214 With the study being non-randomized and containing multiple sources of possible
215 confounding, adjusted estimates of point mortality were also estimated. Two
216 approaches to adjusting for confounding were implemented. First, the general process
217 of generating crude estimates by strata was used to estimate the relative risk by stratum
218 and then a pooled (common) estimate over all strata was estimated using the Mantel-
219 Haenszel estimator. The second approach for adjusted point estimates was developed
220 as an extension of the methods used for estimating adjusted survival, using a baseline
221 Cox regression model fitted to the data. Without loss of generality, we assumed a
222 single variable of direct interest (e.g., days to transfusion) and a set of covariates to be
223 controlled for within the estimate. Using the 'conditional' method for estimating adjusted
224 survival curves¹⁴, an adjusted estimate of the mortality at Day 7, for example, was
225 obtained. To estimate the confidence interval for the adjusted survival curve, the
226 bootstrap method was used. For each of the bootstrap replicates, the original full data
227 set was used to determine the reference distribution for standardization of the mortality
228 estimate. This approach was extended to provide an estimate of the relative risk over
229 one or more variables of interest. The posterior distribution of potential relative risks was
230 constructed by a Cartesian merge of the posterior adjusted survival estimates for each
231 group. The 2.5th and 97.5th percentiles of this distribution were used as the bootstrap
232 confidence interval for the relative risk. No p-values were provided for this method. The
233 adjustment variables used in these analyses were as follows: time epoch (as shown in
234 **Table 1**), gender, race, age at enrollment (as categories), and indicator variables for

235 having already developed one or more severe COVID-19 conditions (as shown in **Table**
236 **1**), being on a ventilator, use of hydroxychloroquine, use of remdesivir, and use of
237 steroids prior to transfusion.

238 Descriptive statistics are presented as frequencies and percentages. Analytic data are
239 presented as point estimates and 95% confidence intervals. P-values less than 0.05
240 were considered statistically significant and no correction for multiple testing has been
241 applied to reported p-values. All statistical analyses were completed using R version
242 3.6.2.

243 **Results**

244 ***Patient Characteristics***

245 Between April 4 and July 4, 2020, 47,047 patients were enrolled in the EAP, of whom
246 36,226 were transfused with COVID-19 convalescent plasma. Of the 1,959 registered
247 sites with at least one patient enrolled, 1,809 sites had transfused at least one patient
248 (92.3%) and 928 sites had transfused at least ten patients (47.4%), **Figure 1**. Data were
249 included for 35,322 transfused patients with 7-day and 30-day follow-up. Key patient
250 characteristics are presented in **Table 1**, stratified into three groups delineating the time
251 period of the study and COVID-19 pandemic. The data set represented a non-
252 probability sample of hospitalized COVID-19 patients with diverse representation of
253 gender, age, weight status, race, and ethnicity. As shown in **Table 1**, the patients
254 transfused early in the study period (before May 01) were more critically-ill (higher rates
255 of mechanical ventilation, ICU admissions and septic shock), had higher concomitant
256 treatment with hydroxychloroquine and azithromycin, and lower concomitant treatment
257 with remdesivir compared with groups transfused later in the study period.

258 ***Unadjusted Analyses***

259 Since the initiation of the EAP, there has been a reduction in both the seven-day crude
260 mortality rate and a pronounced shift of the time to transfusion towards more rapid
261 transfusion of convalescent plasma. The crude seven-day mortality rate was reduced in
262 patients transfused within 3 days (8.7%, 8.3%-9.2%) of COVID-19 diagnosis compared
263 to patients transfused 4 or more days after COVID-19 diagnosis (11.9%, 11.4%-12.3%;
264 $P<0.001$), **Table 2**. Similar trends were seen for unadjusted 30-day mortality. Table 2
265 presents several additional analyses by risk modifiers (e.g., age and ventilation status at
266 time of transfusion). As a means for controlling for study epoch, the time to transfusion
267 association is presented further stratified by study period. More favorable estimates for
268 mortality were found for all early transfusions (3 or fewer days) across both 7- and 30-
269 day mortality for all three study months ($P<0.001$; **Table 2**).

270 ***Adjusted Analysis including Antibodies***

271 In a subset of 3,082 transfused patients who received only a single unit of plasma (150
272 – 250 mL), the unadjusted antibody association with mortality is presented in **Table 2**.

273 **Supplemental Table 2** presents the key demographic data by antibody groups (low,
274 medium and high) for these patients. While there were some statistically significant
275 differences among the antibody level groupings, this table shows that patients were well
276 balanced across the antibody level groupings as a whole. The associations of mortality
277 with antibody levels was found at both 7- and 30-days ($p < 0.05$ for both) and when
278 antibody levels were stratified by time to transfusion, a pronounced separation in
279 mortality was found between the extremes of the classification (early transfusion, high
280 antibody levels vs. late transfusion, low antibody levels) albeit the associations for 7-day
281 mortality was at the threshold for statistical significance ($p = 0.05$). **Supplemental Table**
282 **2** presents additional estimates of crude mortality on the subset of patients with
283 matched antibody data.

284 **Figure 2A** presents the adjusted analyses with antibody groupings alone whereas
285 **Figure 2B** presents these same data allowing for the timing of the transfusion to be
286 integrated directly into the analysis. These data demonstrate a clear “dose” dependent
287 relationship of reduced 7-day mortality with the higher antibody levels. **Figure 2C** and
288 **2D** replicate these findings using 30-day mortality data. While some confidence intervals
289 include the null value of relative risk of 1.0, the magnitude of relative risks, particularly
290 after adjustment, is an important finding of the study.

291 **Figure 3** presents an alternate analytical approach to estimate the effect of the antibody
292 levels. The stratified Mantel-Haenszel approach estimates the relative risk for both 7-
293 and 30-day mortality for patient profiles in the analysis. This stratification approach
294 provides direct analytical control for the potential confounders as each row in the figure
295 represents homogeneity with respect to the factors listed. Overall, there is a consistent
296 signal of a protective effect of the high antibody levels across the strata. The pooled, or
297 common, relative risk for 7-day and 30-day mortality were 0.65 (0.47 to 0.92) and 0.77
298 (0.63 to 0.94). For this analysis only patients transfused with units containing antibody
299 levels over 18.45 S/Co or less than 4.62 S/Co were included.

300 **Discussion**

301 In our cohort of over 35,000 hospitalized patients with COVID-19, several signals
302 consistent with effectiveness for convalescent plasma were observed in a broad sample
303 of acute care facilities across the US. Earlier use of convalescent plasma was
304 associated with lower observed rates of 7-day and 30-day mortality. The use of
305 convalescent plasma with higher antibody levels was associated with reduced 7-day
306 and 30-day mortality. These findings were supported by two different analytical methods
307 used to control for confounding. The finding of a dose response between antibody levels
308 and reduction in mortality provides strong evidence that specific antibody is the active
309 agent in convalescent plasma for treatment of COVID-19. All data considered as a
310 whole, these findings are consistent with the notion that the quality and manner in which
311 convalescent plasma is administered to patients hospitalized with COVID-19 may
312 reduce mortality.

313 Given the historical efficacy of passive antibody therapy for infectious diseases, the
314 primary objective of the EAP was to facilitate access to convalescent plasma for
315 hospitalized COVID-19 patients across the US. The other major goal was to assess
316 safety. With these goals met^{7,8}, we analyzed the data from 1,809 sites and noted there
317 was variability in time to transfusion after diagnosis. Initially, we had no information
318 about the antibody levels in the convalescent plasma being administered. These
319 factors provided elements of inherent randomization in the data collected and formed
320 the basis of an exploratory analysis for signals associated to efficacy. They are also
321 consistent with key principles of antibody therapy recognized during the heyday of this
322 treatment modality in the 1920s and 30s^{15,16}, supporting their use as a framework to
323 explore the efficacy of convalescent plasma in COVID-19.

324 ***Time to Treatment***

325 Both 7-day and 30-day mortality adjusted for disease severity and demographic factors
326 were reduced in patients transfused within 3 days of COVID-19 diagnosis compared to
327 patients transfused 4 or more days after COVID-19 diagnosis. Additionally, the declining
328 week-to-week trends in crude mortality (as previously observed⁷) were temporally
329 associated with more rapid treatment. Prior to the antibiotic era, treatment of respiratory

330 infections with antibody therapy was most effective if initiated within three days of
331 hospitalization. Thus, we used a similar timeframe, relative to date of diagnosis rather
332 than hospitalization, for stratifying the current data. Along similar lines, 7 and 30 day
333 survivors received on average higher volumes of plasma in unadjusted analyses. This is
334 of interest because we had no knowledge of the volume of plasma which might
335 constitute an efficacious dose prior to beginning this study.

336 ***Antibody Assessment***

337 Seven and 30 day mortality rates were reduced in patients who received plasma with
338 higher antibody levels. This finding is more limited than the time data as only a subset of
339 the plasma units had remnant samples preserved that were suitable for assaying
340 antibody levels. The survival benefit became more pronounced when the analysis was
341 restricted to less severely ill patients treated early. Because of the multifactorial nature
342 of antibody-mediated effects and the potential for other disease modifying factors to be
343 present in convalescent plasma, further assay development to more fully characterize
344 the mechanisms in which plasma confers anti-viral properties is warranted. We also
345 note that there was no evidence of worsening outcomes or increased mortality in
346 patients treated with very high antibody levels indicating that antibody dependent
347 enhancement of disease was unlikely. Finally, it is important to recognize that the
348 antibody levels we obtained were on repurposed remnant biospecimens collected for
349 blood banking quality assurance. Thus there was potential variability in a number of
350 factors related to biospecimen handling and storage that might influence the
351 measurement of antibody levels in the specimens available to us. Of note adjusted 30-
352 day mortality was 30% in patients treated with plasma with low antibody levels (IgG) 4
353 or more days after COVID-19 diagnosis. By contrast 30-day mortality was 20% in
354 patients treated within 3 days of diagnosis with plasma with high antibody levels. The
355 pooled estimate from the stratified analysis estimated a 23% relative reduction in
356 mortality at 30 days across a wide range of sub-strata within the study. This reduction in
357 mortality is similar to that observed in a number of small randomized trials and
358 retrospective matched control studies¹⁷.

359 **Limitations**

360 The design of the EAP has been criticized because it was not a randomized placebo,
361 controlled trial (RCT)¹⁸. We started the EAP in late March 2020. It was designed to
362 provide access to convalescent plasma largely at hospitals and acute care facilities that
363 were not already part of a RCT or did not have the infrastructure to support complex
364 RCTs. We also envisioned modest total enrollment and our original IRB approval was
365 for 5,000 patients. In this context, our primary goal was to report on the safety of
366 convalescent plasma and to perform an exploratory analysis for potential signals of
367 efficacy. As described earlier, the EAP was a pragmatic study design, organized to
368 allow routine clinical care to dictate the timing and administration of plasma with the
369 collection of real world data. We did not prespecify which medications patients should
370 be on to participate. The enrollment and data collection forms were streamlined to make
371 participation easy for sites engulfed in the work of a pandemic. The use of a central,
372 academic IRB allowed for consistent data evaluation and oversight. We streamlined PI
373 credentialing and IRB reliance processes. All forms were web-based at a time when
374 some believed that SARS-CoV-2 might be transmitted via paper contaminated with the
375 virus. We did not randomly assign treatment strategies or use of adjunctive medications.
376 Nonetheless, there were some elements of randomization or pseudo-randomization in
377 our study. Physicians could choose the timing of convalescent plasma, the number of
378 units administered, any repeat therapies and whether ICU or mechanically ventilated
379 patients were included. Furthermore, the degree of immune activity within the units of
380 convalescent plasma (i.e. specific IgG levels) was not known. It was assumed that
381 patients would receive plasma with low, medium and high antibody levels in a pseudo-
382 randomized manner and that would enable assessment of efficacy.

383 We acknowledge that RCTs produce evidence of the highest quality in most but not all
384 clinical situations. RCTs can occur when a number of specific criteria are present which
385 allow their conduct. First, RCTs necessitate a stable supply of investigational product
386 (i.e. convalescent plasma) or placebo/comparator which can be pre-positioned at all
387 participating sites. The supply of convalescent plasma in April was not sufficient for
388 such collection and pre-positioning. Second, RCTs require sufficient numbers of sites
389 which have an appropriate patient base to approach for the study. The COVID-19

390 pandemic has migrated across different US regions every few weeks, making it
391 challenging to predict where sites should be selected and prepared for a RCT. Third,
392 sites must be validated and activated. This work requires training of the investigators
393 and study team members as well as typically on-site visits. The crises of the COVID-19
394 pandemic were not compatible with these site training and activation activities; travel
395 within the US has been restricted and staff sent to activate sites would likely have been
396 quarantined for two weeks before being able to go to another region to activate sites.
397 Fourth, the very nature of a RCT requires subject willingness to be randomized to active
398 treatment or placebo or a comparator agent. There was no consensus in April nor is
399 there a global consensus now regarding what would be an appropriate placebo-control
400 to use. Fifth, many COVID-19 patients would likely have been distrustful of being
401 randomized to a placebo based upon historical precedent. Sixth, the number of sites
402 who could have participated in a RCT is limited; who was the appropriate ethical entity
403 to pick those sites and to exclude other sites? Our design allowed any willing hospital,
404 PI and patient to be included in the pragmatic, real-world data study. Finally, there were
405 ongoing small RCTs when we started this program. Physicians, hospitals and patients
406 have the choices of this program versus a RCT. It is clear that over 90,000 patients and
407 over 10,000 physicians elected to participate in the pragmatic, real-world evidence
408 study design. We did not indicate our study would prove efficacy or even offer potential
409 help. It was clear that it was a research investigation and informed consent was
410 obtained in all subjects prior to the transfusion of plasma. Perhaps the current design
411 can inform trialists and RCT advocates of the importance of study designs which are
412 easy and simple to join/enroll and which make the workload of participation as easy and
413 clinically relevant as possible.

414 ***Conclusion***

415 The relationships between mortality and both time to plasma transfusion, and antibody
416 levels provide a signature that is consistent with efficacy for the use of convalescent
417 plasma in the treatment of hospitalized COVID-19 patients.

418 ***Disclaimer***

419 The views and opinions expressed in this manuscript are those of the authors and do
420 not reflect the official policy or position of the US Department of Health and Human
421 services and its agencies including the Biomedical Research and Development
422 Authority and the Food and Drug Administration, as well as any agency of the U.S.
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461 **References**

- 462 1. Luke TC, Casadevall A, Watowich SJ, Hoffman SL, Beigel JH, Burgess TH. Hark back:
463 passive immunotherapy for influenza and other serious infections. *Crit Care Med.*
464 2010;38(4 Suppl):e66-73.
- 465 2. Casadevall A, Scharff MD. Serum therapy revisited: animal models of infection and
466 development of passive antibody therapy. *Antimicrob Agents Chemother.*
467 1994;38(8):1695-1702.
- 468 3. Casadevall A, Scharff MD. Return to the past: the case for antibody-based therapies in
469 infectious diseases. *Clin Infect Dis.* 1995;21(1):150-161.
- 470 4. Luke TC, Kilbane EM, Jackson JL, Hoffman SL. Meta-analysis: convalescent blood
471 products for Spanish influenza pneumonia: a future H5N1 treatment? *Ann Intern Med.*
472 2006;145(8):599-609.
- 473 5. Li L, Zhang W, Hu Y, et al. Effect of Convalescent Plasma Therapy on Time to Clinical
474 Improvement in Patients With Severe and Life-threatening COVID-19: A Randomized
475 Clinical Trial. *JAMA.* 2020.
- 476 6. Salazar E, Perez KK, Ashraf M, et al. Treatment of Coronavirus Disease 2019 (COVID-
477 19) Patients with Convalescent Plasma. *Am J Pathol.* 2020.
- 478 7. Joyner MJ, Bruno KA, Klassen SA, et al. Safety Update: COVID-19 Convalescent
479 Plasma in 20,000 Hospitalized Patients. *Mayo Clinic Proceedings.* 2020;in press.
- 480 8. Joyner MJ, Wright RS, Fairweather D, et al. Early safety indicators of COVID-19
481 convalescent plasma in 5000 patients. *J Clin Invest.* 2020.
- 482 9. Bloch EM, Shoham S, Casadevall A, et al. Deployment of convalescent plasma for the
483 prevention and treatment of COVID-19. *J Clin Invest.* 2020.
- 484 10. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international
485 community of software platform partners. *J Biomed Inform.* 2019;95:103208.
- 486 11. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic
487 data capture (REDCap)--a metadata-driven methodology and workflow process for
488 providing translational research informatics support. *J Biomed Inform.* 2009;42(2):377-
489 381.
- 490 12. Theel ES, Harring J, Hilgart H, Granger D. Performance Characteristics of Four High-
491 Throughput Immunoassays for Detection of IgG Antibodies against SARS-CoV-2. *J Clin*
492 *Microbiol.* 2020;58(8).
- 493 13. Flexner S. The Results of the Serum Treatment in Thirteen Hundred Cases of Epidemic
494 Meningitis. *J Exp Med.* 1913;17(5):553-576.
- 495 14. Therneau TM, Crowson CS, Atkinson EJ. Adjusted survival curves. In: January; 2015.
- 496 15. Casadevall A, Scharff MD. "Serum Therapy" revisited: Animal models of infection and
497 the development of passive antibody therapy. *Antimicrob Agents Chemotherap.*
498 1994;38:1695-1702.
- 499 16. Casadevall A, Scharff MD. Return to the past: the case for antibody-based therapies in
500 infectious diseases. *Clin Infect Dis.* 1995;21:150-161.
- 501 17. Joyner MJ, Klassen SA, Senefeld JW, et al. Evidence favouring the efficacy of
502 convalescent plasma for COVID-19 therapy *in review.* 2020.
- 503 18. Califf RM, Hernandez AF, Landray M. Weighing the Benefits and Risks of Proliferating
504 Observational Treatment Assessments: Observational Cacophony, Randomized
505 Harmony. *JAMA.* 2020.

506

Table 1. Patient Characteristics Stratified by Time Period of COVID-19 Convalescent Plasma Transfusion.

	Apr 04 - May 01 (N=6,990)	May 01 - Jun 04 (N=14,846)	Jun 04 - Jul 04 (N=13,486)	Total Patients (N=35,322)	P value
Age at Enrollment (years)					<0.001
18 to 39	539 (7.7%)	1,337 (9.0%)	1,596 (11.8%)	3,472 (9.8%)	
40 to 59	2,424 (34.7%)	4,938 (33.3%)	4,806 (35.6%)	12,168 (34.4%)	
60 to 69	2,007 (28.7%)	3,791 (25.5%)	3,170 (23.5%)	8,968 (25.4%)	
70 to 79	1,358 (19.4%)	2,879 (19.4%)	2,467 (18.3%)	6,704 (19.0%)	
80 or older	662 (9.5%)	1,901 (12.8%)	1,447 (10.7%)	4,010 (11.4%)	
Gender					<0.001
Female	2,546 (36.5%)	5,961 (40.2%)	5,489 (40.8%)	13,996 (39.7%)	
Male	4,416 (63.4%)	8,838 (59.7%)	7,961 (59.1%)	21,215 (60.2%)	
Undisclosed	6 (0.1%)	11 (0.1%)	11 (0.1%)	28 (0.1%)	
Weight Status					<0.001
Underweight	69 (1.2%)	286 (1.9%)	156 (1.2%)	511 (1.5%)	
Normal Weight	1,010 (17.4%)	2,601 (17.6%)	1,744 (12.9%)	5,355 (15.7%)	
Overweight	1,723 (29.7%)	4,096 (27.8%)	3,647 (27.1%)	9,466 (27.8%)	
Obese	2,997 (51.7%)	7,761 (52.6%)	7,926 (58.8%)	18,684 (54.9%)	
Race					<0.001
White	3,330 (47.6%)	7,299 (49.2%)	7,178 (53.2%)	17,807 (50.4%)	
Asian	456 (6.5%)	628 (4.2%)	390 (2.9%)	1,474 (4.2%)	
Black or African American	1,301 (18.6%)	2,971 (20.0%)	2,379 (17.6%)	6,651 (18.8%)	
Other or Unknown	1,903 (27.2%)	3,948 (26.6%)	3,539 (26.2%)	9,390 (26.6%)	
Ethnicity					<0.001
Hispanic/Latino	2,391 (34.2%)	5,297 (35.7%)	5,875 (43.6%)	13,563 (38.4%)	
Not Hispanic/Latino	4,599 (65.8%)	9,549 (64.3%)	7,611 (56.4%)	21,759 (61.6%)	
Clinical Status					
Current severe or life-threatening COVID-19	5,584 (79.9%)	9,761 (65.7%)	8,157 (60.5%)	23,502 (66.5%)	<0.001
Intensive Care Unit (ICU) care prior to infusion	4,601 (65.8%)	7,908 (53.3%)	5,952 (44.1%)	18,461 (52.3%)	<0.001
Mechanical Ventilation prior to infusion	3,217 (49.9%)	4,143 (27.9%)	2,213 (16.4%)	9,573 (27.5%)	<0.001
Severe Risk Factors^a					
Respiratory failure	4,063 (72.8%)	6,352 (65.1%)	4,760 (58.4%)	15,175 (64.6%)	<0.001
Dyspnea	3,543 (63.4%)	6,976 (71.5%)	6,476 (79.4%)	16,995 (72.3%)	<0.001
Blood oxygen saturation ≤ 93%	3,507 (62.8%)	7,063 (72.4%)	6,394 (78.4%)	16,964 (72.2%)	<0.001
Lung infiltrates > 50% within 24 to 48 hours	2,415 (43.2%)	4,151 (42.5%)	3,015 (37.0%)	9,581 (40.8%)	<0.001
Respiratory frequency ≥ 30/min	2,205 (39.5%)	4,174 (42.8%)	3,366 (41.3%)	9,745 (41.5%)	<0.001
PaO ₂ :FiO ₂ ratio < 300	1,905 (34.1%)	3,075 (31.5%)	1,952 (23.9%)	6,932 (29.5%)	<0.001
Multiple organ dysfunction or failure	1,062 (19.0%)	1,200 (12.3%)	560 (6.9%)	2,822 (12.0%)	<0.001
Septic shock	844 (15.1%)	960 (9.8%)	475 (5.8%)	2,279 (9.7%)	<0.001
Number of Severe Risk Factors					<0.001
None	1,407 (20.1%)	5,085 (34.3%)	5,331 (39.5%)	11,823 (33.5%)	
Limited (1 to 4)	3,895 (55.7%)	6,992 (47.1%)	6,190 (45.9%)	17,077 (48.3%)	
Many (5+)	1,688 (24.1%)	2,769 (18.7%)	1,965 (14.6%)	6,422 (18.2%)	

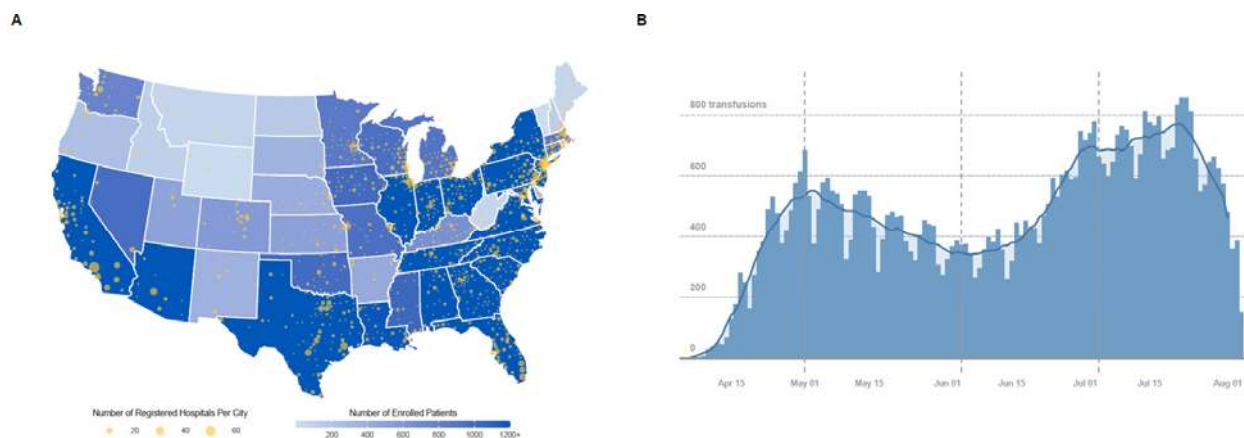
Table 1. Patient Characteristics Stratified by Time Period of COVID-19 Convalescent Plasma Transfusion.

	Apr 04 - May 01 (N=6,990)	May 01 - Jun 04 (N=14,846)	Jun 04 - Jul 04 (N=13,486)	Total Patients (N=35,322)	P value
Medications during hospital stay					
Angiotensin Receptor Blocker	397 (5.7%)	839 (5.7%)	779 (5.8%)	2,015 (5.7%)	0.90
Ace Inhibitor	467 (6.7%)	1,130 (7.6%)	1,023 (7.6%)	2,620 (7.4%)	0.032
Azithromycin	3,811 (54.5%)	5,717 (38.5%)	5,456 (40.5%)	14,984 (42.4%)	<0.001
Remdesivir	329 (4.7%)	4,066 (27.4%)	6,240 (46.3%)	10,635 (30.1%)	<0.001
Steroids	3,736 (53.4%)	6,137 (41.3%)	7,735 (57.4%)	17,608 (49.8%)	<0.001
Chloroquine	33 (0.5%)	22 (0.1%)	6 (0.0%)	61 (0.2%)	<0.001
Hydroxychloroquine	4,356 (62.3%)	2,437 (16.4%)	245 (1.8%)	7,038 (19.9%)	<0.001
Time to Transfusion					<0.001
0 days	141 (2.0%)	598 (4.0%)	625 (4.6%)	1,364 (3.9%)	
1 to 3 days	1,590 (22.7%)	5,748 (38.7%)	6,705 (49.7%)	14,043 (39.8%)	
4 to 10 days	2,843 (40.7%)	6,244 (42.1%)	5,271 (39.1%)	14,358 (40.6%)	
11+ days	2,416 (34.6%)	2,256 (15.2%)	885 (6.6%)	5,557 (15.7%)	

^aThese data include a subset of the sample (n = 23,502), only those patients that currently have severe or life-threatening COVID-19. Data was not available for Gender (n=83), Weight Status (n=1,306) and Mechanical Ventilation prior to infusion (n=544).

Table 2. Crude Mortality (7 and 30 day) of patients transfused with COVID-10 Convalescent Plasma.

	Seven-day Mortality				Thirty-day Mortality			
	Sample, No	Events, No	Estimate, 95% CI	P-value	Sample, No	Events, No	Estimate, 95% CI	P-value
Overall Mortality	35,322	3,706	10.5% (10.2%, 10.8%)		35,322	8,652	24.5% (24.0%, 24.9%)	
Age				<0.0001				<0.0001
18 - 39 y	3,472	109	3.1% (2.6%, 3.8%)		3,472	261	7.5% (6.7%, 8.4%)	
40 - 59 y	12,168	662	5.4% (5.1%, 5.9%)		12,168	1,837	15.1% (14.5%, 15.7%)	
60 - 69 y	8,968	897	10.0% (9.4%, 10.6%)		8,968	2,431	27.1% (26.2%, 28.0%)	
70 - 79 y	6,704	1,023	15.3% (14.4%, 16.1%)		6,704	2,367	35.3% (34.2%, 36.5%)	
80 y or older	4,010	1,015	25.3% (24.0%, 26.7%)		4,010	1,756	43.8% (42.3%, 45.3%)	
On Ventilator Prior to Infusion				<0.0001				<0.0001
No	25,205	1,932	7.7% (7.3%, 8.0%)		25,205	4,523	17.9% (17.5%, 18.4%)	
Yes	9,573	1,685	17.6% (16.9%, 18.4%)		9,573	3,924	41.0% (40.0%, 42.0%)	
Missing	544	89	16.4% (13.5%, 19.7%)		544	205	37.7% (33.7%, 41.8%)	
Days to Transfusion				<0.0001				<0.0001
<= 3 days	15,407	1,340	8.7% (8.3%, 9.2%)		15,407	3,329	21.6% (21.0%, 22.3%)	
4+ days	19,915	2,366	11.9% (11.4%, 12.3%)		19,915	5,323	26.7% (26.1%, 27.3%)	
Study Period and Days to Transfusion				<0.0001				<0.0001
Apr 04 - May 01 (<= 3 days)	1,731	232	13.4% (11.9%, 15.1%)		1,731	526	30.4% (28.3%, 32.6%)	
Apr 04 - May 01 (4+ days)	5,259	853	16.2% (15.2%, 17.2%)		5,259	1,821	34.6% (33.4%, 35.9%)	
May 01 - Jun 04 (<= 3 days)	6,346	659	10.4% (9.7%, 11.2%)		6,346	1,452	22.9% (21.9%, 23.9%)	
May 01 - Jun 04 (4+ days)	8,500	1,060	12.5% (11.8%, 13.2%)		8,500	2,260	26.6% (25.7%, 27.5%)	
Jun 04 - Jul 04 (<= 3 days)	7,330	449	6.1% (5.6%, 6.7%)		7,330	1,351	18.4% (17.6%, 19.3%)	
Jun 04 - Jul 04 (4+ days)	6,156	453	7.4% (6.7%, 8.0%)		6,156	1,242	20.2% (19.2%, 21.2%)	
Ortho IgG				0.0483				0.0208
Low	561	77	13.7% (11.1%, 16.8%)		561	166	29.6% (26.0%, 33.5%)	
Medium	2,006	233	11.6% (10.3%, 13.1%)		2,006	549	27.4% (25.5%, 29.4%)	
High	515	46	8.9% (6.8%, 11.7%)		515	115	22.3% (18.9%, 26.1%)	
IgG - Time to Transfusion				0.0500				<0.0001
<= 3 days (Low)	190	25	13.2% (9.1%, 18.7%)		190	48	25.3% (19.6%, 31.9%)	
<= 3 days (Medium)	727	73	10.0% (8.1%, 12.4%)		727	166	22.8% (19.9%, 26.0%)	
<= 3 days (High)	180	11	6.1% (3.4%, 10.6%)		180	30	16.7% (11.9%, 22.8%)	
4+ days (Low)	371	52	14.0% (10.9%, 17.9%)		371	118	31.8% (27.3%, 36.7%)	
4+ days (Medium)	1,279	160	12.5% (10.8%, 14.4%)		1,279	383	29.9% (27.5%, 32.5%)	
4+ days (High)	335	35	10.4% (7.6%, 14.2%)		335	85	25.4% (21.0%, 30.3%)	



507

508 **Figure 1. Participation in the US COVID-19 Convalescent Plasma Expanded Access**

509 **Program (EAP). A.** Choropleth map displaying the number of cumulatively enrolled patients in

510 the EAP within each state of the contiguous US, with lower enrollment values displayed in a

511 lighter hue and higher enrollment values displayed in a darker hue of blue. Registered acute

512 care facilities are represented as filled yellow circles, with larger circles indicating greater

513 number of registered facilities within the metropolitan area of a city. The choropleth map does

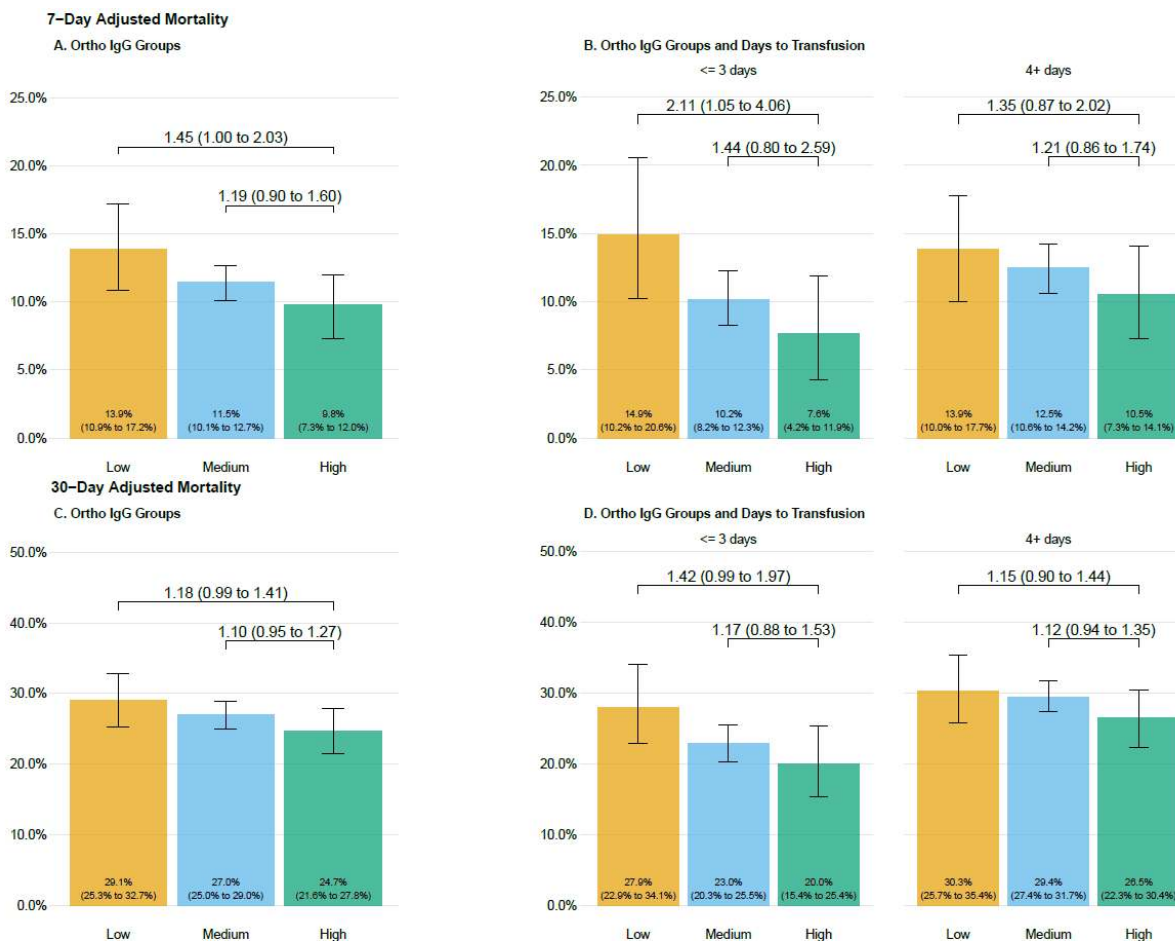
514 not display data from non-contiguous US locations, including registered facilities in Puerto Rico,

515 Hawaii, Alaska, Guam, and Northern Mariana Islands. **B.** The chronological graph represents

516 the number of patients that have received a COVID-19 convalescent plasma transfusion,

517 including daily counts (blue bars) and 7-day average (blue line). The dashed vertical reference

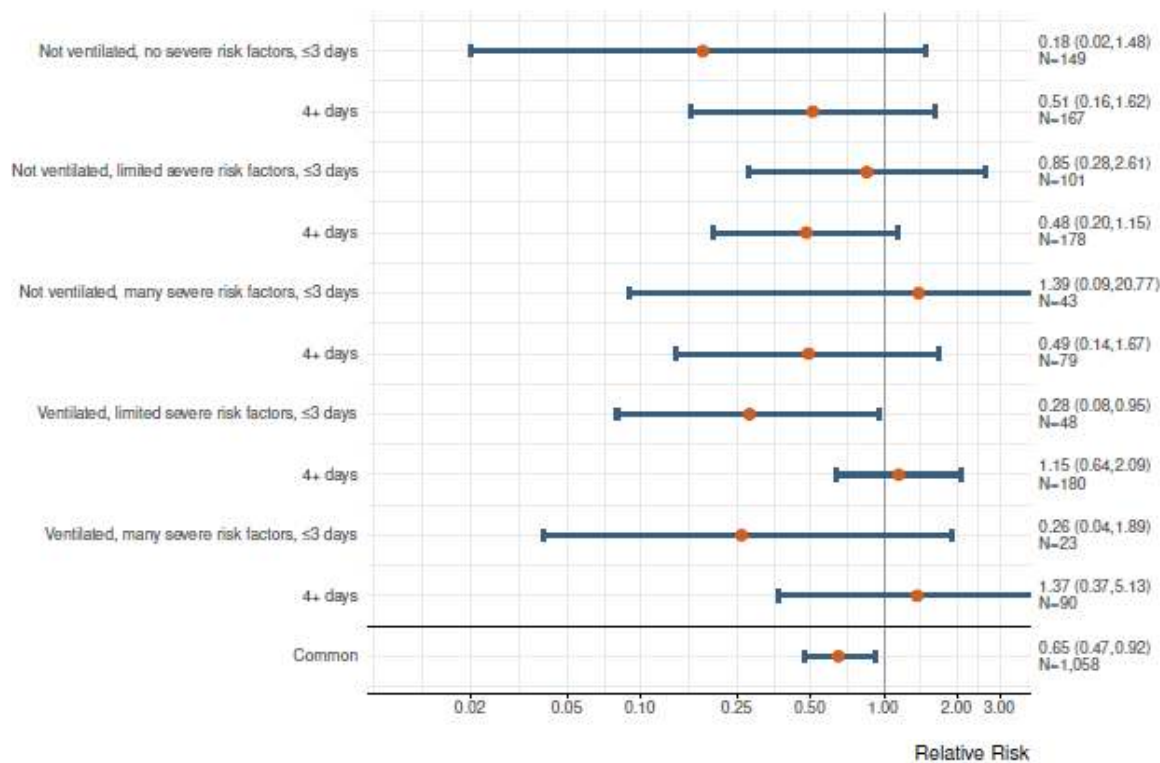
518 lines delineate the three study epochs.



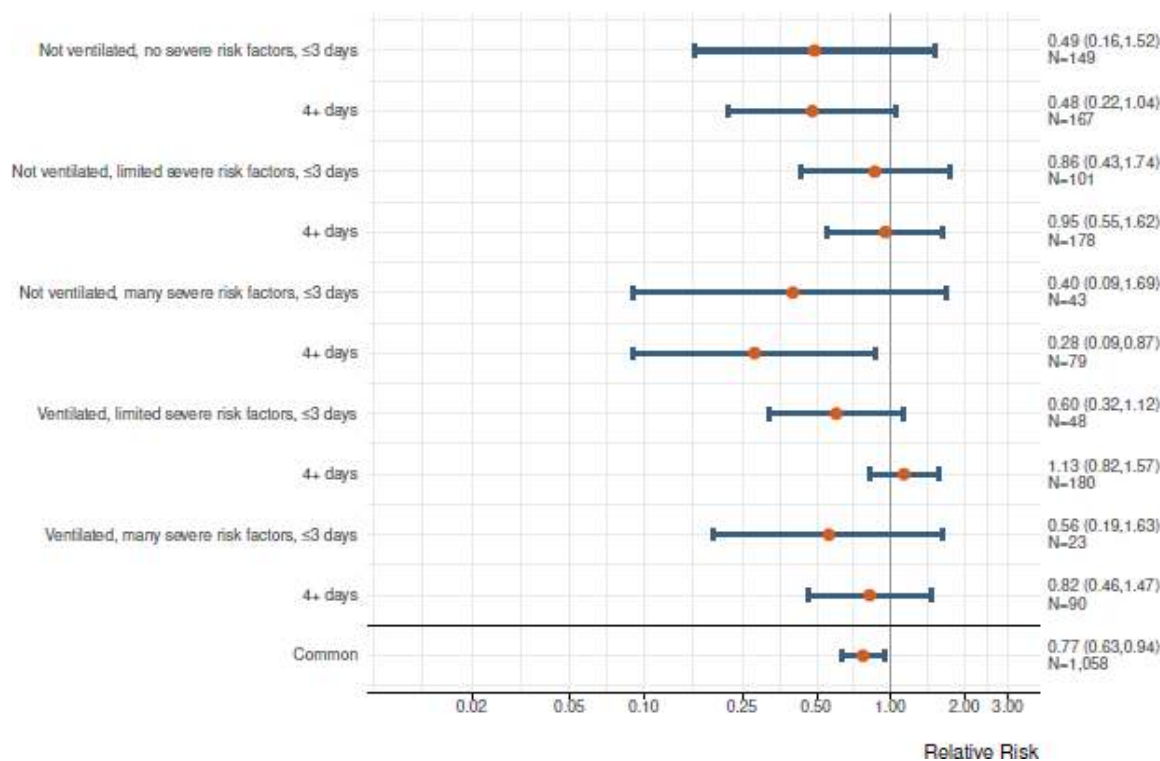
519

520 **Figure 2. Seven day (A, B) and 30-day (C, D) adjusted mortality stratified by antibody**
 521 **groupings in patients transfused with COVID-19 convalescent plasma.** Adjusted mortality
 522 rate is presented on the vertical axis, and the height of each bar graph represents adjusted
 523 mortality with 95% confidence interval denoted. Data are stratified by groupings of antibody
 524 levels with semiquantitative groupings of low (<4.62 S/Co, orange bars), medium (4.62 to 18.45
 525 S/Co, blue bars) and high (> 18.45 S/Co, green bars). Values presented as text within the boxes
 526 are the estimated adjusted mortality rates. Values connecting various categories shown with the
 527 overbraces are bootstrapped estimates of relative risk and 95% bootstrap confidence intervals.
 528 Refer to the methods for the variables in the adjustment and the calculation of the relative risks.

A. 7-Day Mortality



B. 30-Day Mortality



530 **Figure 3. Forest plots of relative risks for 7-day (A) and 30-day (B) mortality for high**
531 **versus low antibody concentration.** Each row in the figure represents 10 mutually exclusive
532 categorizations of patients transfused with convalescent plasma with measured antibody levels.
533 Estimates are the relative risk for mortality for patients who received convalescent plasma with
534 IgG S/co > 18.45 vs. patients that received < 4.62 S/Co. Patients that received units with IgG
535 S/Co values between 4.62 and 18.45 are not included in this analysis as the planned
536 comparison was to highlight the potential efficacy of high IgG containing units vs. units with low
537 levels of detectable antibodies. The bottom row in each figure represents the common (pooled)
538 estimate based on the Mantel-Haenszel estimator. The number of severe risk factors was
539 categorized as none (n=0), limited (n=1 – 4) or many (5 or more), as defined in **Table 1**.

540

541 **Supplement 1**

542 **Trial Protocol**

543 **1 | Study Objectives**

544 Convalescent plasma is a potential disease altering therapy for hospitalized patients
545 with COVID-19 infections. There is strong historical precedence for its use in respiratory
546 infections suggesting it may be effective in the treatment of COVID-19. Additionally, the
547 administration of convalescent plasma is considered well-tolerated and safe, both
548 historically and within the context of the current COVID-19 pandemic.

549 **1.2 | Primary Objectives**

550 The primary outcome of this Expanded Access Program was to provide access to
551 COVID-19 convalescent plasma, assessed as the availability of convalescent plasma.

552 **1.3 | Secondary Objectives**

553 The secondary outcome of this Expanded Access Program was to determine the safety
554 of transfusion of COVID-19 convalescent plasma assessed as the case-rate and
555 relatedness of serious adverse events.

556 **1.4 | Tertiary Objectives**

557 The tertiary outcome of this Expanded Access Program was to explore the efficacy of
558 transfusion of COVID-19 convalescent plasma.

559 **2 | Study Intervention**

560 This Expanded Access Program was a national, pragmatic intervention conducted as a
561 multicenter, open-label protocol in hospitalized adults with COVID-19. All patients
562 received the study intervention (COVID-19 convalescent plasma transfusion). Primary
563 study endpoints included:

- 564 1. Hospital discharge
 - 565 2. Death
 - 566 3. 30 days of observation after COVID-19 convalescent plasma transfusion
- 567

568 **2.1 | Study Intervention Description**

569 Compatible COVID-19 convalescent plasma was administered intravenously according
570 to accepted transfusion guidelines used for fresh frozen plasma.

571 **2.2 | Dosing and Administration**

572 For practical purposes in the current outbreak, one – two units of compatible COVID-19
573 convalescent plasma were administered. Convalescent plasma was obtained from a
574 registered or licensed blood collector and was collected preferably by apheresis or, if
575 necessary, by conventional methods. Individual institutional guidelines for the
576 administration of plasma were followed, including the use of any premedications, such
577 as acetaminophen or diphenhydramine.

578 **2.3 | Preparation and Packaging**

579 Compatible convalescent plasma units were obtained from a registered or licensed
580 blood collector following registration of a patient under the auspices of the Expanded
581 Access Program. COVID-19 convalescent plasma was supplied as an investigational
582 blood product for the treatment of COVID-19. The plasma container label of the COVID-
583 19 convalescent plasma unit included the following statement, “Caution: New Drug –
584 Limited by Federal (or United States) law to investigational use.” (21 CFR 312.6(a)).

585 **3 | Research Population**

586 Eligible patients for this Expanded Access Program were identified by their treating
587 providers. The patient inclusion criteria were specific to hospitalized patients, these
588 criteria were exceptionally broad.

589 **3.1 | Inclusion Criteria**

Supplemental Table 1. Inclusion Criteria

1. Age at least 18 years
 2. Laboratory confirmed diagnosis of infection with SARS-CoV-2
 3. Admitted to an acute care facility for the treatment of COVID-19 complications
 4. Severe or life threatening COVID-19, or judged by the treating provider to be at high risk of progression to severe or life-threatening disease
 5. Informed consent provided by the patient or healthcare proxy
-

Severe or Life-threatening COVID-19 is defined by one or more of the following:

- dyspnea
 - respiratory frequency $\geq 30 \cdot \text{min}^{-1}$
 - blood oxygen saturation $\leq 93\%$
 - partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300
 - lung infiltrates $> 50\%$ within 24 to 48 hours
 - respiratory failure
 - septic shock
 - multiple organ failure
-

590

591 **3.2 | Exclusion Criteria**

592 None.

593
594

Supplement 2

Supplemental Table 2. Patient Characteristics Stratified by IgG.

	Low (N=561)	Medium (N=2,006)	High (N=515)	Total Patients (N=3,082)	P value
Age at Enrollment (years)					0.078
18 to 39	59 (10.5%)	155 (7.7%)	53 (10.3%)	267 (8.7%)	
40 to 59	185 (33.0%)	689 (34.3%)	183 (35.5%)	1,057 (34.3%)	
60 to 69	152 (27.1%)	503 (25.1%)	143 (27.8%)	798 (25.9%)	
70 to 79	102 (18.2%)	418 (20.8%)	86 (16.7%)	606 (19.7%)	
80 or older	63 (11.2%)	241 (12.0%)	50 (9.7%)	354 (11.5%)	
Gender					0.14
Female	201 (36.0%)	774 (38.7%)	221 (42.9%)	1,196 (38.9%)	
Male	357 (63.9%)	1,227 (61.3%)	293 (56.9%)	1,877 (61.0%)	
Undisclosed	1 (0.2%)	1 (0.0%)	1 (0.2%)	3 (0.1%)	
Weight Status					0.064
Underweight	7 (1.4%)	32 (1.7%)	3 (0.6%)	42 (1.5%)	
Normal Weight	87 (17.0%)	334 (17.7%)	84 (17.1%)	505 (17.5%)	
Overweight	154 (30.1%)	545 (28.8%)	115 (23.5%)	814 (28.1%)	
Obese	263 (51.5%)	980 (51.8%)	288 (58.8%)	1,531 (52.9%)	
Race					0.54
White	266 (47.4%)	967 (48.2%)	234 (45.4%)	1,467 (47.6%)	
Asian	23 (4.1%)	77 (3.8%)	15 (2.9%)	115 (3.7%)	
Black or African American	125 (22.3%)	443 (22.1%)	135 (26.2%)	703 (22.8%)	
Other or Unknown	147 (26.2%)	519 (25.9%)	131 (25.4%)	797 (25.9%)	
Ethnicity					0.24
Hispanic/Latino	223 (39.8%)	747 (37.2%)	179 (34.8%)	1,149 (37.3%)	
Not Hispanic/Latino	338 (60.2%)	1,259 (62.8%)	336 (65.2%)	1,933 (62.7%)	
Severe Risk Factors					
Current severe or life-threatening COVID-19	382 (68.1%)	1,286 (64.1%)	341 (66.2%)	2,009 (65.2%)	0.19
Intensive Care Unit (ICU) care prior to infusion	344 (61.3%)	1,226 (61.1%)	298 (57.9%)	1,868 (60.6%)	0.38
Mechanical Ventilation prior to infusion	183 (33.4%)	666 (33.9%)	158 (31.0%)	1,007 (33.3%)	0.45
Clinical symptoms^a					
Respiratory failure	265 (69.4%)	919 (71.5%)	231 (67.7%)	1,415 (70.4%)	0.36
Dyspnea	265 (69.4%)	910 (70.8%)	241 (70.7%)	1,416 (70.5%)	0.87
Blood oxygen saturation \leq 93%	269 (70.4%)	909 (70.7%)	233 (68.3%)	1,411 (70.2%)	0.70
Lung infiltrates > 50% within 24 to 48 hours	194 (50.8%)	588 (45.7%)	147 (43.1%)	929 (46.2%)	0.097
Respiratory frequency \geq 30/min	177 (46.3%)	580 (45.1%)	157 (46.0%)	914 (45.5%)	0.89
PaO ₂ :FiO ₂ ratio < 300	137 (35.9%)	451 (35.1%)	93 (27.3%)	681 (33.9%)	0.017

Supplemental Table 2. Patient Characteristics Stratified by IgG.

	Low (N=561)	Medium (N=2,006)	High (N=515)	Total Patients (N=3,082)	P value
Multiple organ dysfunction or failure	65 (17.0%)	227 (17.7%)	48 (14.1%)	340 (16.9%)	0.29
Septic shock	56 (14.7%)	188 (14.6%)	44 (12.9%)	288 (14.3%)	0.71
Number of Severe Risk Factors					0.042
None	179 (31.9%)	720 (35.9%)	174 (33.8%)	1,073 (34.8%)	
Limited (1 to 4)	239 (42.6%)	868 (43.3%)	243 (47.2%)	1,350 (43.8%)	
Many (5+)	143 (25.5%)	418 (20.8%)	98 (19.0%)	659 (21.4%)	
Medications during hospital stay					
ARB	27 (4.8%)	107 (5.3%)	24 (4.7%)	158 (5.1%)	0.77
Ace Inhibitor	40 (7.1%)	175 (8.7%)	35 (6.8%)	250 (8.1%)	0.23
Azithromycin	277 (49.4%)	923 (46.0%)	226 (43.9%)	1,426 (46.3%)	0.18
Remdesivir	164 (29.2%)	538 (26.8%)	130 (25.2%)	832 (27.0%)	0.32
Steroids	251 (44.7%)	899 (44.8%)	209 (40.6%)	1,359 (44.1%)	0.21
Chloroquine	4 (0.7%)	4 (0.2%)	1 (0.2%)	9 (0.3%)	0.12
Hydroxychloroquine	174 (31.0%)	595 (29.7%)	99 (19.2%)	868 (28.2%)	<0.001
Time to Transfusion					0.34
0 days	16 (2.9%)	58 (2.9%)	16 (3.1%)	90 (2.9%)	
1 to 3 days	174 (31.0%)	669 (33.3%)	164 (31.8%)	1,007 (32.7%)	
4 to 10 days	251 (44.7%)	846 (42.2%)	244 (47.4%)	1,341 (43.5%)	
11+ days	120 (21.4%)	433 (21.6%)	91 (17.7%)	644 (20.9%)	
Time Epoch					<0.001
Apr 04 to May 01	146 (26.0%)	543 (27.1%)	86 (16.7%)	775 (25.1%)	
May 01 to Jun 04	348 (62.0%)	1,242 (61.9%)	359 (69.7%)	1,949 (63.2%)	
Jun 04 to Jul 04	67 (11.9%)	221 (11.0%)	70 (13.6%)	358 (11.6%)	

^aThese data include a subset of the sample (n = 2,009), only those patients that currently have severe or life-threatening COVID-19

Data was not available for Gender (n=6), Weight Status (n=190) and Mechanical Ventilation prior to infusion (n=61).

Supplemental Table 3. Crude Mortality (7 and 30 day) of patients with IgG transfused with COVID-10 Convalescent Plasma.

	Seven-day Mortality				Thirty-day Mortality			
	Sample, No	Events, No	Estimate, 95% CI	P-value	Sample, No	Events, No	Estimate, 95% CI	P-value
Overall Mortality	3,082	356	11.6% (10.5%, 12.7%)		3,082	830	26.9% (25.4%, 28.5%)	
Age				<0.0001				<0.0001
18 - 39 y	267	10	3.7% (2.0%, 6.8%)		267	27	10.1% (7.0%, 14.3%)	
40 - 59 y	1,057	83	7.9% (6.4%, 9.6%)		1,057	187	17.7% (15.5%, 20.1%)	
60 - 69 y	798	89	11.2% (9.2%, 13.5%)		798	243	30.5% (27.4%, 33.7%)	
70 - 79 y	606	97	16.0% (13.3%, 19.1%)		606	217	35.8% (32.1%, 39.7%)	
80 y or older	354	77	21.8% (17.8%, 26.3%)		354	156	44.1% (39.0%, 49.3%)	
On Ventilator Prior to Infusion				<0.0001				<0.0001
No	2,014	170	8.4% (7.3%, 9.7%)		2,014	382	19.0% (17.3%, 20.7%)	
Yes	1,007	177	17.6% (15.4%, 20.0%)		1,007	421	41.8% (38.8%, 44.9%)	
Missing	61	9	14.8% (8.0%, 25.7%)		61	27	44.3% (32.5%, 56.7%)	
Days to Transfusion				0.0371				<0.0001
<= 3 days	1,097	109	9.9% (8.3%, 11.8%)		1,097	244	22.2% (19.9%, 24.8%)	
4+ days	1,985	247	12.4% (11.1%, 14.0%)		1,985	586	29.5% (27.6%, 31.6%)	
Study Period and Days to Transfusion				0.0470				<0.0001
Apr 04 - May 01 (<= 3 days)	138	14	10.1% (6.1%, 16.3%)		138	36	26.1% (19.5%, 34.0%)	
Apr 04 - May 01 (4+ days)	637	95	14.9% (12.4%, 17.9%)		637	219	34.4% (30.8%, 38.2%)	
May 01 - Jun 04 (<= 3 days)	773	77	10.0% (8.0%, 12.3%)		773	172	22.3% (19.5%, 25.3%)	
May 01 - Jun 04 (4+ days)	1,176	137	11.6% (9.9%, 13.6%)		1,176	327	27.8% (25.3%, 30.4%)	
Jun 04 - Jul 04 (<= 3 days)	186	18	9.7% (6.2%, 14.8%)		186	36	19.4% (14.3%, 25.6%)	
Jun 04 - Jul 04 (4+ days)	172	15	8.7% (5.4%, 13.9%)		172	40	23.3% (17.6%, 30.1%)	
Ortho IgG				0.0483				0.0208
Low	561	77	13.7% (11.1%, 16.8%)		561	166	29.6% (26.0%, 33.5%)	
Medium	2,006	233	11.6% (10.3%, 13.1%)		2,006	549	27.4% (25.5%, 29.4%)	
High	515	46	8.9% (6.8%, 11.7%)		515	115	22.3% (18.9%, 26.1%)	
IgG - Time to Transfusion				0.0500				<0.0001
<= 3 days (Low)	190	25	13.2% (9.1%, 18.7%)		190	48	25.3% (19.6%, 31.9%)	
<= 3 days (Medium)	727	73	10.0% (8.1%, 12.4%)		727	166	22.8% (19.9%, 26.0%)	
<= 3 days (High)	180	11	6.1% (3.4%, 10.6%)		180	30	16.7% (11.9%, 22.8%)	
4+ days (Low)	371	52	14.0% (10.9%, 17.9%)		371	118	31.8% (27.3%, 36.7%)	
4+ days (Medium)	1,279	160	12.5% (10.8%, 14.4%)		1,279	383	29.9% (27.5%, 32.5%)	
4+ days (High)	335	35	10.4% (7.6%, 14.2%)		335	85	25.4% (21.0%, 30.3%)	