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Antibody Correlates of Protection for COVID-19 Convalescent Plasma Associated with Reduced Outpatient Hospitalizations

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ABSTRACT

SARS-CoV-2 antibody levels associated with reduced hospitalization risk remain undefined. Our outpatient COVID-19 convalescent plasma (CCP), placebo-controlled trial observed SARS-CoV-2 antibody levels decreasing 22-fold from matched donor units into post-transfusion seronegative recipients. Unvaccinated recipients were jointly stratified by a) early or late transfusion (≤ 5 or >5 days from symptom onset) and b) high or low post-transfusion SARS-CoV-2 antibody levels ($<$ or $\geq$ geometric mean). Early treatment with high post-transfusion antibody levels reduced hospitalization risk-0/102 (0%) compared to all other CCP recipients-17/370 (4.6%; Fisher exact p=0.03) and to all control plasma recipients-35/461 (7.6%; Fisher exact p=0.001). A similar donor upper/lower antibody level and early late transfusion stratified analyses indicated significant hospital risk reduction. Pre-transfusion nasal viral loads were similar in CCP and control recipients regardless of hospitalization outcome. Therapeutic CCP should comprise the upper 30% of donor antibody levels to provide effective outpatient use for immunocompromised and immunocompetent outpatients.

INTRODUCTION

The threshold of antibody correlating with reduced severe disease progression varies and needs to be independently determined for diverse infectious diseases, from protozoans, like *Plasmodium falciparum*[1,2] to viruses, like SARS-CoV-2[3,4]. Correlates of protection for antibody levels demarcating vaccine efficacy for COVID-19 hospitalization risk reduction have been represented in relation to COVID-19 convalescent plasma (CCP) equivalents[5-7]. Likewise, a model[8] utilizing vaccine, monoclonal antibody, and CCP data from clinical trials was created based on the reference CCP equivalents for 50% hospitalization risk reduction[9-11]. Monoclonal antibody outpatient randomized controlled trials (RCTs) demonstrate 50%-80% efficacy in hospital risk reduction[12-15]. However, the same monoclonal antibody doses failed to show disease progression reduction in COVID-19 inpatients[14,16-18]. Like the monoclonal antibodies, intravenous remdesivir demonstrated greater efficacy in outpatients versus inpatients[19,20]. In summary, both early timing and dose of antiviral antibodies and drugs matter for outpatient reduction of hospitalization risk.

Convalescent persons recovering from pre-Alpha period COVID-19 have varying antibody levels spanning a 3-log10 range from undetectable antibody to positive after a 3 to 5 thousand dilution[21]. Individuals with both boosted vaccines and recent COVID-19 (i.e., hybrid immunity) have considerably higher antibody levels (i.e., detectable even over 50,000 inverse dilutional geometric mean titers) than either individuals with antibodies from infection or vaccination alone [22,23]. Our CCP outpatient treatment within 9 days of symptom onset, showed greater than 50% hospitalization relative risk reduction, which increased to more than 80% risk reduction (point estimate) when given within 5 days of symptom onset[24]. Hospitalized participants had the same donor unit range of SARS-CoV-2 antibody levels as those not hospitalized.

A prespecified analysis from the parent outpatient CCP RCT aimed to compare antibody levels in donor-recipient pairs to define dilutional decrease and to correlate both donor and recipient antibody levels to disease progression culminating in hospitalization. In this substudy, we analyzed the risk of hospitalization among unvaccinated COVID-19 outpatients, comparing control plasma recipients to four different CCP groups jointly stratified by early or late treatment

171   (i.e., $\leq$ 5 or >5 days from symptom onset), and by high or low post-transfusion recipient or donor
172   antibody levels (i.e., < or $\geq$ geometric mean, GM).

RESULTS

Trial population

175   At 23 sites throughout the USA, the previously reported outpatient CCP clinical trial (CSSC-004,
176   NCT04373460) transfused 1181 COVID-19 outpatients over 16 months (June 3, 2020 to October
177   1, 2021), to show that receipt of CCP, compared to control plasma, reduced the risk of
178   hospitalization for COVID-19 by 54%[24]. Written and signed informed consent was obtained
179   from all participants. Hospitalizations occurred in 53 of 964 unvaccinated and 1 of 58 partially
180   vaccinated participants. None of the 159 fully vaccinated individuals were hospitalized[24]. We
181   focused our correlation of antibody level analysis and hospitalization on those unvaccinated with
182   evaluable antibody data, for which 479 received control plasma and 472 CCP (Table 1). The
183   unvaccinated participant's mean age was 44, with more females than males, approximately 40%
184   obese with BMI 30 or over, and approximately 40% with one or more pre-existing comorbidities
185   for severe COVID-19 risk. Unvaccinated CCP recipients, both screen seropositive or
186   seronegative were further jointly stratified by early ($\leq$5 days of symptom onset) or late (>5 days
187   of symptoms onset) time to transfusion as well as post-transfusion antibody levels (measured
188   within 30 minutes after transfusion completion) in the upper or lower half of screened
189   seronegative CCP recipients (Table 1).

Donor CCP and recipient screen and post-transfusion antibody levels in unvaccinated participants

193   Approximately 40% of all potential CCP study donors were excluded for low antibody levels,
194   thus transfusion units represented the upper 60% of all convalescent plasma donors. Because of
195   the greater correlation with virus neutralization antibody (nAb), we measured the plasma anti-
196   Spike-Receptor Binding Domain (S-RBD) IgG antibodies levels by dilutional titer and the more
197   precise area under the curve (AUC) on both the donors at collection and in over 5,000 recipient
198   samples at pre-transfusion screen, post-transfusion and follow-up visits[21]. The anti-S-RBD IgG
199   titer threshold for seronegative was 180 titer or below. The donor anti-S-RBD IgG GM titer of
200   6,678 and anti-S-RBD IgG AUC of 3,172 separated donor antibody levels' upper and lower half
201   (Fig. 1A). The donor control plasma was either collected in 2019 (211 units) or blood bank tested
202   SARS-CoV-2 seronegative when collected in 2020-2021.

204   To experimentally determine the dilution factor associated with the single ~200 mL CCP
205   administration in 338 unvaccinated seronegative recipients, measurements of matched donor and
206   recipient anti-S-RBD IgG AUC were performed. The matched transfusion donor antibody levels
207   proportionately decreased 22-fold (approximately 5%) from the GM donor anti-S-RBD IgG
208   3286 AUC to recipient post-transfusion antibody levels at GM 147 AUC measured within 30
209   minutes of transfusion completion (Fig. 1B). The decrease was parallel such that matched donor
210   antibody levels below 1000 AUC were also consistently below 150 AUC post-transfusion in
211   recipients. Similarly, 15 seronegative hospitalized CCP recipients had post-transfusion antibody
212   levels 19 times lower than their matched donors (Fig. 1C).

214   The unvaccinated study participants included a screen seropositive participant subset (199/951
215   (21%) (107 in the CCP and 92 in the control plasma groups), with 83% of the 199 seropositive

216   participants already having pre-transfusion antibody levels above the GM 150 AUC for
217   seronegative post-transfusion CCP recipients (Fig.1D). The pre-transfusion seropositive
218   transfusion antibody levels were near 500 to 1000 GM anti-S-RBD IgG AUC which is 3 to 6
219   times lower than the donor anti-S-RBD IgG antibody levels of 3172 AUC. The low post-
220   transfusion recipient antibodies (early or late) were predictably lower in frequency and GM AUC
221   levels. Segregating the unvaccinated seropositive recipients by days from symptom onset to
222   transfusion revealed a trend of a higher frequency of participants being seropositive after day 3,
223   with higher GM antibody levels approximating 1000 anti-S-RBD IgG AUC, which was
224   associated with the early development of virus-specific immunity also observed by Wolfel[25]
225   (Fig. 1E).
226
227   ## Recipient antibody level post-transfusion benchmarks of protection that prevents
228   ## hospitalization
229   To correlate post-transfusion antibody levels among unvaccinated participants (both screen
230   seronegative and seropositive) to hospital outcome, we stratified the post-transfusion anti-S-RBD
231   IgG AUC by early or late treatment and high or low antibody levels for the CCP group (Fig. 2,
232   Extended Table 1). The recipient antibody geometric mean of 150 AUC delineated high versus
233   low antibody levels. Among the CCP participants treated early (symptom onset within 5 days)
234   with high post-transfusion recipient antibody levels, there were no hospitalizations, 0/102
235   participants. In those unvaccinated CCP recipients treated early with low post-transfusion
236   antibody levels (anti-S-RBD IgG AUC below 150 AUC), we observed 5/104 (5%) participant
237   hospitalizations (Fig. 2). There were 12 total hospitalizations in the two groups receiving
238   transfusions late—6/164 (3.7%) recipients with high antibody levels and 6/102 (6%) recipients
239   with antibodies below anti-S-RBD IgG 150 AUC. We compared the proportion of
240   hospitalizations of the early treatment with high post-transfusion antibody level CCP group with
241   that of the other three CCP groups (17/270, 4.6%; Fishers exact p=0.03), the control plasma
242   group (36/479, 7.5%; Fishers exact p=0.001), and the early control plasma group (24/207,
243   11.6%; Fishers exact p=0.00005; Extended Table 1). Similarly, delineating high and low post-
244   transfusion recipient antibody levels above the geometric mean of 540 anti-S-RBD IgG titer,
245   although less precise than AUC, revealed that participants receiving early treatment with high
246   titer CCP also had no hospitalizations (0/45, 0%; Extended Fig. 1 and Table 1).
247
248   ## Donor antibody level pre-transfusion benchmarks of protection that prevent
249   ## hospitalization
250   To correlate matched CCP donor units in the unvaccinated recipients to hospital outcome, we
251   next investigated if the corresponding donor antibody levels would also translate into protection
252   from hospitalization. The unvaccinated CCP recipients were again delineated by symptom onset
253   to transfusion, but instead of post-transfusion antibody level representation, the matched
254   corresponding donor antibody levels were stratified by donor anti-S-RBD IgG antibodies at GM
255   3286 AUC for all 592 recipients (Fig. 3A) and virus neutralization antibody (nAb) levels ≥60
256   AUC (Fig. 3B). In all antibody level metrics, we compared the GM upper to lower as high and
257   low antibody levels, respectively. The numbers remained statistically significant by the Fishers
258   exact test compared to both control plasma groups (Fig. 3 and Extended Table 1). Similar to the
259   analyses with AUC values, donor anti-S-RBD IgG and nAb GM titer values, although less
260   precise, revealed their respective recipients with early treatment and high donor antibody titers
261   having the lowest proportion of hospitalizations (Extended Fig. 2 and Table 2). We also stratified

262   donor antibody levels by the commercial assay EUROIMMUN ratio of optical density expressed
263   above or below 6 AU or 600 BAU/mL (Extended Fig 3 and Extended Table 1), a level that also
264   demarcates the upper portion of study donors[24].

265

266   ## Screen seropositive hospitalizations in the unvaccinated
267   The screen seropositive recipients with subsequent hospitalizations were investigated, despite
268   low incidence. 7/54 unvaccinated participants, subsequently hospitalized, were seropositive at
269   the initial screen, two in the CCP group and five in the control group (Extended Table 2). Three
270   of the five hospitalized participants from the control group had screen anti-S-RBD IgG antibody
271   titers at 540 and AUC less than 150, fitting the early/low seronegative data described above. In
272   the unvaccinated CCP group, 2/107 (1.9%) who were screen seropositive were hospitalized
273   versus 15/378 (4%) seronegative at screen (Chi square p=0.29; CCP seropositive versus
274   seronegative). Among the control group of whom were hospitalized, there were 5/92 (5.4%)
275   screen seropositive versus 29/381 (7.6%) screen seronegative (chi square p=0.47 control-
276   seropositive to seronegative). Of those screened seropositive, there was no statistical difference
277   in hospitalization between those who received CCP and control plasma (chi square p=0.17; CCP-
278   seropositive to control-seropositive). The single screen seronegative and partially vaccinated
279   hospitalized participant transfused with control plasma received their first vaccine dose the same
280   day as symptom onset. Among the 159 fully vaccinated and not hospitalized, 158 (99%) were
281   screen seropositive, while 30/58 (69%) partially vaccinated participants were seropositive.

282

283   ## Pre-transfusion nasal SARS-CoV-2 viral RNA loads
284   Nasal viral load might independently determine risk of hospitalization. Quantitative RT-PCR
285   was performed on the screen nasal swabs collected before plasma transfusion to correlate the
286   SARS-CoV-2 nasal viral RNA load to hospitalization. All unvaccinated individuals subsequently
287   receiving either control plasma or CCP had indistinguishable screen viral loads (i.e., near 10,000
288   copies) (Fig. 4A). Analyzing nasal viral loads of early/high, early/low, late/high, and late/low
289   recipient groups revealed, as expected, that early transfusions closer to symptom onset were
290   associated with higher viral loads than late transfusions. The CCP early/high and early/low
291   recipient pre-transfusion nasal viral loads were indistinguishable. The viral load was similar
292   among unvaccinated at screen, regardless of subsequent hospitalization outcome (Fig. 4B).
293   While the inclusion criteria required a documented positive molecular SARS-CoV-2 test (87%
294   by RNA detection and 13% by antigen detection), the interval between the study inclusion
295   SARS-CoV-2 test (performed outside the study) and the research study nasal swab collection
296   prior to transfusion may have been up to a week.

297

298   Stratifying nasal viral load by days from symptom onset to transfusion time among unvaccinated
299   participants infected prior to the Delta variant, which has different viral load kinetics[26,27],
300   indicated a decline in pre-transfusion viral burden among seronegative individuals after 5 days
301   from symptom onset (Fig. 4C). Seropositive individuals had lower viral loads compared to
302   seronegative individuals at all timepoints, with a majority of seropositive viral loads below the
303   limit of detection (330 copies) by day 4 post-symptom onset (Fig. 4D). The screen viral RNA
304   copy data suggest that seropositivity at the time of transfusion correlated with lower viral loads,
305   but differences in viral RNA load did not impact hospitalization outcome.

306

Differences in antibody levels weeks after transfusion

Passive transfer of SARS-CoV-2 specific antibodies was postulated to possibly down-modulate subsequent SARS-CoV-2 antibody responses. This clinical study was designed to measure antibody responses up to 90 days post-transfusion to compare antibody kinetics between CCP and control plasma recipients. There were no differences in antibody levels between CCP and control plasma recipients at or beyond 14 days post-transfusion (Fig. 5). Longitudinal differences in antibody levels observed were due to hospitalization rather than treatment. The multivariate linear mixed-effects regression, adjusted for variant, age, sex, and BMI, showed no differences in antibody levels between CCP and control plasma recipients beyond 14 days post-transfusion (Fig. 5). There were neither sex, age, BMI, nor comorbidity differences in antibody levels between CCP and control groups.

Within the control group, there were eight immunocompetent recipients with undetectable SARS-CoV-2 antibody levels during all follow-up visits. Three of the 8 had persistent COVID-19 symptoms after 90 days of study follow-up. All CCP recipients had detectable antibodies at or after the day 14 visit.

Variant period differences in screen seropositivity, nasal viral load and day 90 antibody levels

We investigated how SARS-CoV-2 variants influenced seropositivity, nasal viral load and 3-month antibody levels in the setting of ongoing vaccination after December 2020. We separated participants into three periods: pre-Alpha (June 3, 2020 to January 31, 2021), Alpha (February 1, 2021 to July 15 2021), and Delta (July 16, 2021 to October 1, 2021). Participants recruited in the Delta period were younger, with fewer medical conditions, and more than 60% fully vaccinated (Extended Table 3). Screen seropositivity rates among unvaccinated individuals were low (~ 20%), during pre-Alpha and Alpha periods rising to 46% during the Delta period. The baseline screen anti-S-RBD AUC GM antibody levels in the unvaccinated seropositive participants were 529, 482, and 7042 anti-S-RBD AUC during the pre-Alpha, Alpha and Delta periods, respectively compared to the 3286 AUC in the mainly pre-Alpha study donors (Extended Fig. 4A).

Pre-transfusion nasal viral loads were similar between CCP and control during the pre-Alpha, Alpha, and Delta periods (Extended Fig. 4B). When unvaccinated participants were stratified based on seropositivity at the time of transfusion, viral loads were consistently lower in seropositive individuals compared to seronegative individuals regardless of the time period in which CCP was administered (Extended Fig. 4C). During the Delta period, unvaccinated seropositive individuals also had lower viral loads compared to seronegative individuals, but the participant numbers stratified by symptom onset day were low (Extended Fig. 5A, B). Fully vaccinated and seropositive participants at screen also showed a drop in viral load when transfusion occurred more than 5 days after symptom onset (Extended Fig. 5C).

Comparing CCP and control day 90 antibody levels in those unvaccinated at screen (excluding the 165 vaccinated during the follow-up visits), indicated no difference between treatment groups or groups stratified by early or high CCP antibody treatment (Extended Figure 6A). Those unvaccinated participants, subsequently hospitalized (41/54, 76%) during the pre-Alpha period, had a GM of 13007 AUC with very few having antibody levels below the mean for unvaccinated participants in the pre-Alpha period at day 90 compared to the non-hospitalized

353　unvaccinated pre-Alpha participants GM 2691 AUC antibody level, 5 times less than those
354　hospitalized (Extended Fig. 6B). Unvaccinated recipients during the Alpha period had similar
355　day 90 antibody levels (geometric mean AUC=6683) to those infected during the Delta period
356　(geometric mean AUC=5929), but near double the pre-Alpha period participants. Fully
357　vaccinated recipients at screen who had breakthrough infection during Delta period showed a
358　GM AUC of 53813, which was 20 times greater than the GM AUC of day 90 pre-Alpha period
359　recipients.

Discussion

361　This jointly stratified study analysis by both time to transfusion and antibody levels found that
362　post-transfusion anti-S-RBD IgG AUC levels at or over 150 AUC and transfusion within 5 days
363　of symptom onset, resulted in no hospitalization in unvaccinated recipients. In seronegative
364　recipients, the post-transfusion upper 50% antibody levels were proportionately matched to
365　upper 50% donor antibody levels measurements by three different test methodologies-anti-S-
366　RBD IgG ELISA, direct live virus neutralization, and EUROIMMUN anti-SARS-CoV-2 ELISA.
367　Given that we selected the upper 60% of donors for study qualification and by all antibody
368　measurements for stratified analysis, the upper half of qualified donors which equals the upper
369　30%, had similar impact on hospitalization risk reduction. The four-quadrant graphic depiction
370　of hospitalizations was similar in the recipient antibody levels compared to the 3 separate donor
371　antibody level measurements. This implicates the upper 30% of all potential pre-Alpha CCP
372　donors as effective for reducing hospitalizations prior to the Delta variant.

374　An individual's circulating plasma volume is, on average, 3 liters, which predicts a 15-fold
375　proportional antibody dilution for a 200 mL CCP transfusion. A prior infection prevention study
376　transfused CCP into healthy nonSARS-CoV-2 infected individuals and similarly measured 20-
377　fold SARS-CoV-2 antibody dilution[28]. This suggests that the antibody compartment volume is
378　similar between non-infected individuals and those with COVID-19 during the first week of
379　illness. We did not observe a measurably higher volume of distribution, suggesting that
380　extravascular transfer of high levels of antibodies to lower the post-transfusion antibody levels
381　occurred. The donor and recipient antibody levels are directly proportional in Fig. 1B-C where
382　the lines are parallel with the 22-fold dilution. From the recipient post-transfusion levels, one can
383　predict matched donor levels are proportionately greater by a factor of 20. The screen
384　seronegative participants had a clean negative antibody background. Those who were screen
385　seropositive in Fig. 1D already had greater antibody levels than more than half of the post-
386　transfusion seronegative participants. We have not explored whether CCP has better neutralizing
387　abilities than the early host immune response when antibodies are just starting to measurably
388　increase after 4 days from symptom onset for screen seropositive participants (low antibodies in
389　the 500 to 1000 range) (Fig. 1E).

391　Antivirals (antibodies or small molecule antivirals) administered early in the COVID-19 course
392　protect from progression to severe disease requiring hospitalization. At least three factors interact
393　to predict risk of outpatient hospitalization: 1) viral load and variant properties; 2) human host
394　risk factors for severe disease (age, obesity, or medical comorbidities), and 3) quantity and
395　quality (degree of match to variants) of SARS-CoV-2 specific antibodies at disease onset, due to
396　active vaccination or immune status (i.e., hybrid immunity, breakthrough, etc.).

398 We found no differences in nasal viral loads between control and CCP recipients at screening to
399 account for differences in hospitalization rates. Importantly, those participants who were
400 subsequently hospitalized had similar viral loads at the time of transfusion when compared to
401 those not hospitalized. Unvaccinated recipients who were seropositive at screening had lower
402 viral loads than seronegative participants. We also observed lower viral loads in those screened
403 late. Specifically, those who were screened and transfused late ($\geq$5 days after symptom onset)
404 had lower viral loads compared to those who were transfused early, suggesting that the
405 administration of antiviral drugs or CCP early (i.e., during the period of higher viral loads
406 measured by nasal sampling) maximizes the therapeutic effect. Viral load dynamics in the lung
407 or other tissues may differ, and treatment timing should be explored further.

409 The jointly stratified subgroup analysis was matched for patients' age, demographics, obesity
410 and one or more medical conditions for severe COVID-19 progression to hospitalization. Our
411 study population was also younger, less obese and with fewer risk factors for disease progression
412 than other published RCTs of CCP outpatient use[29]. Our study population also had a higher
413 prevalence of seronegative participants upon transfusion than the Emergency Department based
414 study (C3PO) where the seropositive rate was near 50%, which may be indicative of a different
415 patient population[30].

417 In this study, there are COVID-19 participants with a 100-fold range of antibody levels. CCP
418 units from the top 30% of all convalescent donors are necessary to confer protection against
419 hospitalization with severe COVID-19, such that when antibody levels are diluted approximately
420 20-fold, the recipient antibody levels remain above the CCP GM. These data suggest that
421 convalescent plasma has been underdosed in prior infectious disease outbreaks and that future
422 pandemic patients should receive higher therapeutic donor antibody levels. The current study
423 introduces the concept that therapeutic donor CCP levels must be in the upper half or higher to
424 fully account for the dilution effect in the recipient. Plasma from approximately 330 unique CCP
425 donations were transfused into 592 CCP participants. These donor units were previously
426 characterized for full-length anti-Spike IgG with GM titers of 13,053 and more precise AUC GM
427 of 7938 which equals 243 BAU/mL using the international standards[31]. The median nAb titer
428 was 80 with GM titer of 58 and nAb AUC of 51, equaling GM 27 IU/mL. The commercial
429 EUROIMMUN arbitrary units (AU) mean was 6 for the unique units. The donor anti-S-RBD
430 IgG GM titer was 6,678 and anti-S-RBD IgG GM AUC of 3,172. These thresholds separate the
431 upper and lower portion of donor antibody levels and was also approximately 50% of total full
432 length Spike titer antibody levels, but 100 times the nAb GM titer levels.

434 Unvaccinated participants who received CCP after 5 days (late) or received units with low
435 antibody levels still had near significant reduction in hospital risk of about 4% rather than 7.6%
436 in all controls. The parent study included fully vaccinated and partially vaccinated participants in
437 the analyses, which lowered risk of hospitalization to 6.3%. Here, we observed an 11.6% risk of
438 hospitalization among unvaccinated controls transfused early within 5 days of symptom onset.
439 The effect of early CCP transfusion and high post-transfusion antibody is even greater among
440 this subgroup of unvaccinated recipients.

442 The antibody level that affords protection is not absolute, as not even monoclonal antibody or
443 small molecule antiviral therapy affords 100% reduction in risk of hospitalization. There were

444 two screen seropositive participants in the CCP group and two in the control group with antibody
445 levels also above the anti-S-RBD IgG antibody threshold of inverse dilution titer over 540 and
446 AUC over 150, presumptively with newly formed antibodies. The finding that early
447 administration of antibodies to SARS-CoV-2 is beneficial in reducing progression of disease is
448 consistent with the observation that those who mount early antibody responses have lower rates
449 of death after hospitalization[32,33] and that inducement of humoral immunity by vaccination
450 reduces severity of disease and death[34].
451
452 When CCP was first deployed in 2020, there were concerns that specific antibody administration
453 to individuals in the early stages of COVID-19 could interfere with the development of
454 endogenous immune responses[35]. However, our findings show that transfusion of CCP, as
455 compared to control plasma, was not associated with differences in the development of a
456 humoral immune response in recipients, reassuring for the immunological safety of CCP in
457 humans. The C3PO convalescent plasma study also did not see an antibody level difference
458 between CCP and saline infusions[30,36].
459
460 While our study had predominately SARS-CoV-2 naïve recipients enrolled prior to the Omicron
461 variant who were largely unvaccinated, the findings are applicable to immunocompromised
462 patients today who lack SARS-CoV-2 antibodies. Another limitation is the low number of
463 seronegative participants transfused within 5 days of symptom onset with post-transfusion above
464 the geometric mean donor antibody levels in our study population (approximately 100
465 participants). The parent study was not powered to look at these stratified quadrants.
466
467 In summary, our results support and reconfirm the adage that for antibody therapy to be
468 effective, sufficient amounts of pathogen specific antibody should be dosed early[37]. No
469 hospitalizations were observed in those treated at ≤5 days of symptom onset with high titer CCP
470 indicating that this is the optimal combination for effective CCP use. Early treatment alone is
471 insufficient as hospitalizations were still observed in the group treated ≤5 days with lower titer
472 units, necessitating both early treatment and adequate antibody dosing for optimal efficacy. Our
473 results provide evidence for the best use of CCP. We advocate that CCP units used for therapy
474 comprise the upper 30% of donor antibody levels. These levels should set the threshold for future
475 therapeutic CCP. When humanity faces its next pandemic, there is a high likelihood that
476 convalescent plasma will be used again until better specific therapies become available. Our data
477 provide a roadmap for optimal early, high dose convalescent plasma deployment in such future
478 emergencies.
479

Online Methods
481 Study Ethics
482 Johns Hopkins served as the single-IRB (sIRB). For the Center for American Indian Health sites,
483 the protocol was also independently reviewed and approved by the Navajo Nation Health Human
484 Research Review Board and the National Indian Health Service IRB. The protocol was also
485 approved by the Department of Defense (DoD) Human Research Protection Office (HRPO).
486 An independent medical monitor who was unaware of the trial group assignments reviewed all
487 serious adverse events, and an independent panel of three physicians who were unaware of the
488 trial-group assignments adjudicated Covid 19 related hospitalizations and severity. An

489 independent data and safety monitoring board provided interim safety and efficacy reviews. The
490 trial was conducted in accordance with the principles of the Declaration of Helsinki, the Good
491 Clinical Practice guidelines of the International Council for Harmonisation, and all applicable
492 regulatory requirements.
493
494 Study Population
495 In this multicenter, double-blind, randomized, controlled trial, we evaluated the efficacy and
496 safety of COVID-19 convalescent plasma, as compared with control plasma, in symptomatic
497 adults (≥18 years of age) who had tested positive for severe acute respiratory syndrome
498 coronavirus 2, regardless of their risk factors for disease progression or vaccination status.
499 Participants were enrolled within 8 days after symptom onset and received a transfusion within 1
500 day after randomization. The primary study outcome (reported previously) was COVID-19–
501 related hospitalization within 28 days after transfusion. There were no obvious imbalances
502 between the trial groups in the parent trial with respect to baseline characteristics, including
503 coexisting conditions, COVID-19 vaccination status, vital signs, and clinical laboratory results.
504
505 Study Center(s):
506 Anne Arundel Medical Center; Ascada Research; Baylor College of Medicine; Johns Hopkins
507 Center for American Indian Health; Johns Hopkins Bloomberg School of Public Health; Johns
508 Hopkins University; Lifespan/Brown University Rhode Island Hospital; Mayo Clinic, Phoenix;
509 MedStar Washington Hospital Center; NorthShore University Health System; The Bliss Group;
510 The Next Practice Group; University of California, Los Angeles Health; University of Alabama
511 at Birmingham; University of California, Irvine Health; University of California, San Diego;
512 University of Cincinnati Medical Center; University of Massachusetts Worcester; University of
513 Miami; University of New Mexico; University of Rochester; University of Texas Health Science
514 Center at Houston; University of Utah Health; Vassar Brothers Medical Center; Wayne State
515 University; Western Connecticut Health Network, Danbury Hospital; Western Connecticut
516 Health Network, Norwalk Hospital.
517
518 Study Plasma
519 The study qualified donor plasma with SARS-CoV-2 positive antibodies after a 1:320 dilution
520 under FDA IND 19725 protocol. After July 2021, the transfused plasma donor units met the
521 existing FDA Emergency Use Authorization (EUA) criteria for high titer at EUROIMMUN
522 arbitrary unit (AU) over 3.5. Many identical apheresis donor plasma units were transfused into 2,
523 3, or 4 separate recipients. Plasma from 333 unique CCP donations was transfused into the 592
524 CCP participants. Seventy-five percent of the donor collections were before September 2020
525 with more than 90% by January 2021 and the last 25 collections by March 2021. These donor
526 units were previously characterized for full-length anti-Spike IgG geometric mean (GM) titers of
527 13,053, which corresponded with a more precise area under the curve (AUC) geometric mean of
528 7938, equaling 243 BAU/mL using the international standards[24]. The median neutralizing
529 antibody (nAb) titer was 80, with a geometric mean titer of 58, and nAb AUC of 51, equaling
530 GM 27 IU/mL[24]. The commercial EUROIMMUN arbitrary units (AU) mean was 6 for the
531 unique donor units[24].
532
533 Study visits and time periods

534   In these studies, antibody levels were measured at screen before transfusion, within 30 minutes
535   of transfusion, and various timepoints up to 90 days post-transfusion. Participants were
536   transfused during pre-Alpha (June 3, 2020 to January 31, 2021), Alpha (February 1, 2021 to July
537   14, 2021), and Delta (July 15 to October 1, 2021) variant periods. There were just three
538   participants transfused from July 2 to July 9, 2021 which decreased the number of false
539   designations. The first Alpha (B1.1.7) confirmed by sequencing was from a participant
540   transfused February 18, 2021.
541
542   **EUROIMMUN ELISA Assay**
543   The EUROIMMUN anti–SARS– CoV-2 ELISA for IgG (cat. EI2606-9601G) was validated in a
544   Clinical Laboratory Improvement Amendments–certified (CLIA-certified) laboratory for donor
545   qualification as positive after a 1:320 dilution as per IND 19725 protocol. The assay was
546   performed according to the manufacturer's specifications. In a separate research laboratory, the
547   donor optical density (OD) was measured at 1:101 dilution. The ratio calculated by dividing the
548   sample OD by the OD of the calibrator from that run constitutes the AU.To measure anti–SARS–
549   CoV-2 IgG binding, each plate had the following components: 100 μL plasma (1:101 dilution,
550   per the manufacturer's protocol) and 100 μL undiluted positive, negative, and calibrator controls.
551   Plates were washed 3 times, followed by the manufacturer's protocol for addition of conjugate
552   and substrate. Ratios of 0.8 or higher were considered positive.
553
554   **Indirect ELISA**
555   The ELISA protocol was adapted from a protocol published by the Florian Krammer
556   laboratory[38].The 96-well plates (Immulon 4HBX, Thermo Fisher Scientific-Cat#-3855) were
557   coated with S-RBD of the parent strain at a volume of 50 μL of 2 μg/mL diluted antigen in
558   filtered, sterile 1 × PBS (Thermo Fisher Scientific) at 4°C overnight. The coating buffer was
559   removed, and the plates were washed 3 times with 300 μL 1 × PBS plus 0.1% Tween-20 (PBST)
560   wash buffer (Thermo Fisher Scientific) and then blocked with 200 μL PBST with 3% nonfat
561   milk (milk powder, American Bio) by volume for 1 hour at room temperature. All plasma
562   samples were heat-inactivated at 56°C on a heating block for 1 hour before use and diluted 1:2 in
563   PBS. Negative control samples were prepared at 1:10 dilutions in PBST in 1% nonfat milk and
564   plated at a final dilution of 1:100. A mAb against the SARS-CoV-2 Spike protein was used as a
565   positive control (1:5000 dilution; Sino Biological, 40150- D001). Plasma samples were prepared
566   in 3-fold serial dilutions starting at 1:20 in PBST in 1% nonfat milk. Blocking solution was
567   removed, and 100 μL diluted plasma was added in duplicate to the plates and incubated at room
568   temperature for 2 hours. Plates were washed 3 times with PBST wash buffer, and 50 μL of
569   secondary antibody was added to the plates and incubated at room temperature for 1 hour.
570   Antihuman secondary antibody, Fc-specific total IgG HRP (1:5000 dilution; Thermo Fisher
571   Scientific, Invitrogen, A18823), was prepared in PBST plus 1% nonfat milk. Plates were washed,
572   and all residual liquid was removed before the addition of 100 μL SIGMAFAST OPD (o-
573   phenylenediamine dihydrochloride) solution (MilliporeSigma) to each well, followed by
574   incubation in darkness at room temperature for 10 minutes. To stop the reaction, 50 μL 3M HCl
575   (Thermo Fisher Scientific) was added to each well. The OD of each plate was read at 490 nm
576   (OD490) on a SpectraMax i3 ELISA Plate Reader (BioTek Instruments). The positive cutoff
577   value for each plate was calculated by summing the average of the negative values and 3 times
578   the SD of the negatives. Limits of detection (LOD) were set to half the lowest AUC value at or

14

579 below 20 titer. The anti-S-RBD IgG titer threshold for seronegative was 180 titer or below. The
580 seropositive anti-S-RBD IgG ELISA titers represent 3-fold dilutions from 540 to 393,660.
581
582 **SARS-CoV-2 Viral Load**
583 Nasopharyngeal specimens obtained at screen were stored in 5 mL of virus transport media at -
584 70˚C on site, then shipped to the central storage facility at Johns Hopkins University. RNA was
585 extracted from 200 μL transport media with either the Qiagen viral RNA extraction kit (Qiagen,
586 Hilden, Germany), or the chemagic Viral RNA/DNA 300 H96 kit with chemagic 360 nucleic
587 acid extraction system (Perkin Elmer), according to manufacturer recommended protocols. Real-
588 time reverse transcriptase quantitative PCR (RT-qPCR) assays targeting the SARS-CoV2
589 nucleocapsid (N) gene and the human RNaseP gene were performed based on the methods
590 described by the US CDC[39]. Primer and FAM-labelled probe sets for CDC nCoV_N1 and
591 RNaseP assays were purchased from IDT (Integrated DNA Technologies) as part of the SARS-
592 CoV2 Research Use Only RUO qPCR primer and probe kit (part number 10006713, 2019_nCoV
593 RUO kit). Single-plex assays with equivalent volumes of RNA (or Positive Control, Plasmid-
594 RNA Standards or Nuclease Free H2O for No Template Controls (NTCs)) were performed using
595 the TaqPath 1-Step RT-qPCR MasterMix (Applied Biosystems, ThermoFisher Scientific) in a
596 QuantStudio 5 Real-Time PCR system (ThermoFisher Scientific). The SARS-CoV-2 nCoV-N
597 control plasmid comprised the complete nucleocapsid gene of SARS-CoV-2 isolate Wuhan-Hu-
598 1, complete genome (GenBank: NC_045512.2), and the HsRPP30 Positive control contained a
599 portion of the RNAseP (RPP30) gene. Both plasmid controls were purchased from IDT.
600 Standards for quantitative analysis were prepared from serial dilutions of the nCoV-N and
601 HsRPP30 plasmid controls for which target copy number was known. The range covered was
602 200,000 copies to 320 copies. Standard curve analysis of nCoV_N1 Ct values was performed by
603 the QuantStudio Design and Analysis software to determine RNA copies of viral genome. Only
604 samples with quantities within the standard curve range were given a COVID-19 call/score
605 "positive". A Ct value for the RNaseP gene was used to verify that human RNA was present in
606 each specimen. For samples that did not amplify viral genome or any host cell RNA, a repeat
607 RT-qPCR was performed and subsequently assigned as "undetermined".
608
609 **SARS-CoV-2 Virus Neutralization Assay**
610 Plasma neutralizing antibodies were determined against WA-1 (SARS-CoV-2/USA-WA1/2020
611 EPI_ISL_404895), which was obtained from BEI Resources, as described previously[22,40]. Two-
612 fold dilutions of plasma (starting at a 1:20 dilution) were made and infectious virus was added to
613 the plasma dilutions at a final concentration of 1×10^5 TCID$_{50}$/mL (100 TCID$_{50}$ per 100 μL).
614 The samples were incubated with the virus for 1 hour at room temperature, and then 100 μL of
615 each dilution was added to 1 well of a 96-well plate of VeroE6-TMPRSS2 cells in hexaplicate.
616 The cells were incubated for 6 hours at 37°C, 5% CO_2. The inocula were removed, fresh
617 infection media (IM) was added, and the cells were incubated at 37°C, 5% CO_2 for 2 days. The
618 cells were fixed by the addition of 100 μL of 4% formaldehyde per well, incubated for at least 4
619 hours at room temperature, and then stained with Napthol Blue Black (MilliporeSigma). The
620 neutralizing antibodies titer was calculated as the highest serum dilution that eliminated the
621 cytopathic effect in 50% of the wells (NT50), and the AUC was calculated using Graphpad
622 Prism.
623
624 **Statistical analysis**

625 Spearman correlation was used to calculated strength of association between titer and AUC units
626 for antibody measurement. Fold drops in anti-S-RBD IgG AUC between donor and
627 corresponding recipients were calculated by dividing the geometric mean of donors by that of
628 recipients. Statistical differences between donor and post-transfusion recipient antibody levels
629 were determined by non-parametric Mann-Whitney tests. Using viral load or antibody data,
630 multiple comparisons across vaccination status, variant, serostatus, or treatment groups were
631 performed using non-parametric Kruskall-Wallis tests with Dunn's post-hoc corrections. Fishers
632 exact tests were performed to compare the association of timing of treatment (early or late) and
633 antibody levels (high or low) with hospitalization using unvaccinated, CCP recipient post-
634 transfusion (D0) antibody data. Longitudinal recipient antibody data were first $\log_{10}$-transformed
635 and analyzed using a linear mixed-effects regression model, adjusted for variant, age, sex, and
636 BMI. An interaction term was included to examine how antibody levels changed over time by
637 treatment (control or CCP) and hospitalization status for unvaccinated participants. Marginal
638 effects were graphed with 95% confidence intervals. Statistical analyses were performed using
639 GraphPad Prism 8 (GraphPad Software) and Stata 17 (StataCorp).

640 Data Availability
641 Source data for figures included in supplementary source data file. Data is available from individual
642 authors upon request with reply expected in 14 days. Deidentified data from clinical trial will be
643 deposited in the Vivli server for public access before end of 2023.

644 Code Availability
645 Unique software or computational code was not created for this study.

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756

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HS.P., C.B., A.Yin, A.C., A.P., S.L.K., D.J.S drafted the manuscript. All authors provided final
approval of the version to be published.

Competing Interests

798 Tables

799 Table 1 Baseline characteristics for unvaccinated control and CCP recipients and the joint
800 stratification based upon symptom onset to transfusion as early or late transfusion (≤5 or >5 days
801 from symptom onset) and high or low antibody levels above or below recipient anti-S-RBD IgG
802 at 150 AUC.

Unvaccinated	Control	CCP	CCP early high	CCP early low	CCP late high	CCP late low	CCP late (high&low) and early low	Control early
Number	479	472*	102	104	164	102	370	207
Median age (IQR)	44 (33-55)	42 (32-54)	42 (30-510)	46 (33-56)	41 (31-55)	44 (33-54)	44 (32-55)	43 (32-56)
Mean Age	44	43	41	45	43	44	44	45
Age Category, n (%)								
18-49 yr	285 (60)	310 (66)	74 (73)	63 (61)	107 (64)	66 (65)	236 (64)	127 (61)
50-85 yr	194 (40)	162 (34)	28 (27)	41 (39)	57 (34)	36 (35)	134 (36)	80 (39)
Sex, n (%)								
Female	283 (59)	255 (54)	58 (57)	52 (50)	90 (55)	55 (54)	197 (53)	115 (56)
Male	196 (41)	217 (46)	44 (43)	52 (50)	74 (45)	47 (46)	173 (47)	92 (44)
Race, n (%)								()
Asian	17 (4)	18 (4)	0 (0)	5 (5)	10 (6)	3 (3)	18 (5)	13 (6)
Black	66 (14)	74 (16)	12 (12)	14 (13)	34 (20)	14 (14)	62 (17)	30 (14)
American Indian	11 (2)	9 (2)	1 (1)	3 (3)	3 (2)	2 (2)	8 (2)	8 (4)
Pacific Islander	2 (0)	2 (0)	0 (0)	0 (0)	1 (1)	1 (1)	2 (1)	1 (0)
Other race/not reported	7 (1)	5 (1)	2 (2)	1 (1)	1 (1)	1 (1)	3 (1)	4 (2)
White	386 (81)	367 (78)	86 (84)	83 (80)	117 (70)	81 (79)	281 (76)	159 (77)
Ethnicity, n (%)								
Hispanic or Latino	72 (15)	69 (15)	20 (20)	12 (12)	23 (14)	14 (14)	49 (13)	37 (18)
BMI Category, n (%)								
BMI <30	261 (58)	274 (61)	64 (65)	56 (57)	97 (63)	57 (59)	210 (60)	112 (57)
BMI ≥30	190 (42)	172 (39)	34 (35)	43 (43)	56 (37)	39 (41)	138 (40)	85 (43)
Any Comorbidity, n (%)	196 (41)	193 (41)	45 (44)	45 (43)	56 (34)	47 (46)	148 (40)	88 (43)
Hypertension, n (%)	117 (24)	119 (25)	27 (26)	27 (26)	33 (20)	32 (31)	92 (25)	52 (25)
Diabetes, n (%)	47 (10)	37 (8)	5 (5)	8 (8)	14 (8)	10 (10)	32 (9)	19 (9)
Asthma, n (%)	59 (12)	51 (11)	13 (13)	11 (11)	17 (10)	10 (10)	38 (10)	25 (12)
HIV, n (%)	12 (3)	11 (2)	2 (2)	4 (4)	4 (2)	1 (1)	9 (2)	4 (2)
Pregnancy, n (%)	0 (0)	2 (0)	0 (0)	1 (1)	0 (0)	1 (1)	2 (1)	0 (0)
Serostatus at Screen, n (%)								
Seronegative*	368 (77)	361 (76)	74 (73)	97 (93)	97 (59)	93 (91)	287 (78)	167 (81)
Seropositive*	92 (19)	107 (23)	28 (27)	6 (6)	66 (40)	7 (7)	79 (21)	30 (14)
No screen bloods	19 (4)	4 (1)	0 (0)	1 (1)	1 (1)	2 (2)	4 (1)	10 (5)

803 *13 CCP participants were excluded due to missing post-transfusion anti-S-RBD IgG levels.

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Extended Table 1 Segregation of time (early or late) and antibody level (high or low) to hospitalizations in both unvaccinated groups. Early is symptom onset to transfusion within 5 days and late is after 5 days. The antibody levels are measured by recipient anti-S-RBD IgG inverse dilutional titer and AUC as well as donor anti-S-RBD IgG titer and AUC along with virus neutralizations from donor with titers above the median and AUC at or above the geometric mean. Data are n (%). P values by Fisher's exact tests were calculated by comparing proportion of early and high hospitalizations/non-hospitalizations to the remaining CCP participants, to all unvaccinated controls and to the unvaccinated controls transfused within 5 days of symptom onset.

	Early/ high CCP	Early/ low CCP	Late/ high CCP	Late/ low CCP	Early/low and Late/high and low	Unvaccinated control plasma	Unvaccinated early control plasma
Recipient anti-S-RBD IgG AUC ≥150					p=0.03	p=0.001	p=0.00005
Hospitalized, n (%)	0 (0)	5 (4.8)	6 (3.7)	6 (5.9)	17 (4.6)	36 (7.5)	24 (11.6)
Total	102	104	164	102	370	479	207
Donor anti-S-RBD IgG AUC ≥3286					p=0.21	p=0.02	p=0.0007
Hospitalized, n (%)	1 (1)	4 (3.6)	6 (4.8)	5 (3.6)	15 (4.0)	36 (7.5)	24 (11.6)
Total	96	110	125	140	375	479	207
Donor nAb AUC ≥58					p=0.016	p=0.0006	p=0.00002
Hospitalized, n (%)	0 (0)	5 (5.6)	6 (3.8)	5 (4.8)	16 (4.4)	36 (7.5)	24 (11.6)
Total	113	90	159	104	353	479	207
Donor EUROIMMUN AU ≥6					p=0.052	p=0.003	p=0.0002
Hospitalized, n (%)	0 (0)	5 (4.2)	5 (4.8)	6 (3.8)	16 (4.2)	36 (7.5)	24 (11.6)
Total	86	119	105	158	382	479	207
Recipient anti-S-RBD IgG titer>540					p=0.39	p=0.06	p=0.01
Hospitalized, n (%)	0 0)	5 (3.1)	3 (3.1)	9 (3.7)	17 (5.2)	36 (7.5)	24 (11.6)
Total	45	161	97	169	427	479	207
Donor anti-S-RBD IgG titer >4860					p=0.33	p=0.02	p=0.0011
Hospitalized, n (%)	1 (1.1)	4 (3.5)	6 (5.3)	5 (3.3)	15 (3.9)	36 (7.5)	24 (11.6)
Total	91	115	114	151	380	479	207
Donor nAb titer ≥80					p=0.03	p=0.001	p=0.00005
Hospitalized, n (%)	0 (0)	5 (4.9)	6 (4.1)	5 (4.3)	16 (4.4)	36 (7.5)	24 (11.6)
Total	101	102	148	115	365	479	207
Donor EUROIMMUN BAU ≥600					p=0.052	p=0.003	p=0.0002
Hospitalized, n (%)	0 (0)	5 (4.2)	5 (4.8)	6 (3.8)	16 (4.2)	36 (7.5)	24 (11.6)
Total	86	119	105	158	382	479	207

Extended Table 2
Unvaccinated screen seropositive participants who were hospitalized

Participant No.	Treatment group	Screen nasal viral load	Days from symptom onset to transfusion	Variant	Screen RBD Titer	Screen RBD AUC
1	Control	320	5	pre-Alpha	540	134
2	Control	320	4	pre-Alpha	4860	1605
3	Control	23271	4	pre-Alpha	540	87
4	Control	12168	3	Alpha	540	123
5	Control	498	9	Alpha	14580	4302
6	CCP	320	7	pre-Alpha	1620	406
7	CCP	4219	7	Alpha	1620	239

Extended Table 3.
Full study participant characteristics during variant periods

	Pre-Alpha n (%)	Alpha n (%)	Delta n (%)	Total n (%)
Number	693	260	228	1181
Treatment				
CCP	349 (50)	128 (49)	115 (50)	592 (50)
Control	344 (50)	132 (51)	113 (50)	589 (50)
Median Age (IQR)	47 (35-58)	40 (31-49)	36 (29-47)	43 (32-54)
Mean Age	47	40	38	44
Age Category				
18-49 yr	388 (56)	197 (76)	185 (81)	770 (65)
50-85 yr	305 (44)	63 (24)	43 (19)	411 (35)
Sex				
Female	384 (55)	151 (58)	140 (61	675 (57)
Male	309 (45)	109 (42)	88 (39)	506 (43)
Race				
Asian	25 (4)	11 (4)	8 (4)	44 (4)
Black	78 (11)	45 (17)	40 (18)	163 (14)
American Indian	16 (2)	0 (0	1 (0)	17 (1)
Pacific Islander	3 (0)	1 (0)	0 (0)	4 (0)
Other/Not Reported	8 (1)	2 (1)	6 (3)	16 (1)
White	560 (81)	201 (77)	173 (76)	934 (79)
Hispanic or Latino	79 (11)	40 (15)	51 (22)	170 (14)
BMI Category				
BMI<30	395 (57)	139 (53)	145 (64)	679 (57)
BMI =>30	250 (36)	116 (45)	79 (35)	445 (38)
Any Comorbidity	310 (45)	98 (38)	51 (22)	459 (39)
Hypertension	199 (29)	52 (20)	25 (11)	276 (23)
Diabetes	72 (10)	18 (7)	9 (4)	99 (8)
Asthma	84 (12)	27 (10)	21 (9)	132 (11)
HIV	17 (2)	7 (3	1 (0	25 (2)
Pregnant	1 (0)	0 (0)	2 (1)	3 (0)
Vaccination Status at Screen				
Unvaccinated	632 (91)	200 (77)	79 (35)	911 (77)
Seronegative*	501 (82)	154 (77)	42 (54)	697 (78)
Seropositive*	110 (18)	46 (23)	36 (46)	192 (22)

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No screening samples	21	0	1	22
Partly Vaccinated	20 (3)	28 (11)	9 (4)	57 (5)
Seronegative*	6 (30)	9 (32)	2 (22)	17 (30)
Seropositive*	14 (70)	19 (68)	7 (78)	40 (70)
Fully Vaccinated	0 (0)	21 (8)	138 (61)	159 (13)
Seronegative*	0 (0)	0 (0)	1 (1)	1 (1)
Seropositive*	0 (0)	21 (100)	129 (99)	150 (99)
No screening samples	0	0	8	8
Vaccinated During Study	100 (14)	60 (23)	5 (2)	165 (14)
Hospitalized	41(6)	11 (4)	2 (1)	54 (5)
Seronegative*	35 (90)	8 (1-PV) (73)	1 (100)	44 (86)
Seropositive*	4 (10)	3 (27)	0	7 (14)
No screening samples	2	0	1	3

Figures

Fig. 1 **Screen seropositive participants and post-transfusion seronegative participants.** A) Spearman correlation of unique donor anti-S-RBD titer and AUC (n=318) B) Unvaccinated and not hospitalized recipient screen seronegative anti-S-RBD AUC (n=338) with corresponding donor unit antibody levels transfused into matched recipients and C) Seronegative recipients (n=15) subsequently hospitalized after transfusion. D) Antibody levels in the 199 (21%) of unvaccinated, but seropositive recipients at screening before transfusion in the jointly stratified groups. There were no significant differences in screening antibody levels between control and CCP recipients. E) The same 199 unvaccinated seropositive participant antibody levels stratified by days from symptom onset to transfusion. All point estimates are shown with error bars indicating the geometric mean with geometric SD. Numbers above the x-axis represent geometric mean (GM), the number in the group (n). ***p<0.001 by non-parametric Kruskal-Wallis multiple comparisons test with Dunn's post-hoc corrections or Mann-Whitney test. The dashed line in B-E represents the upper post-transfusion 150 AUC recipient's threshold.
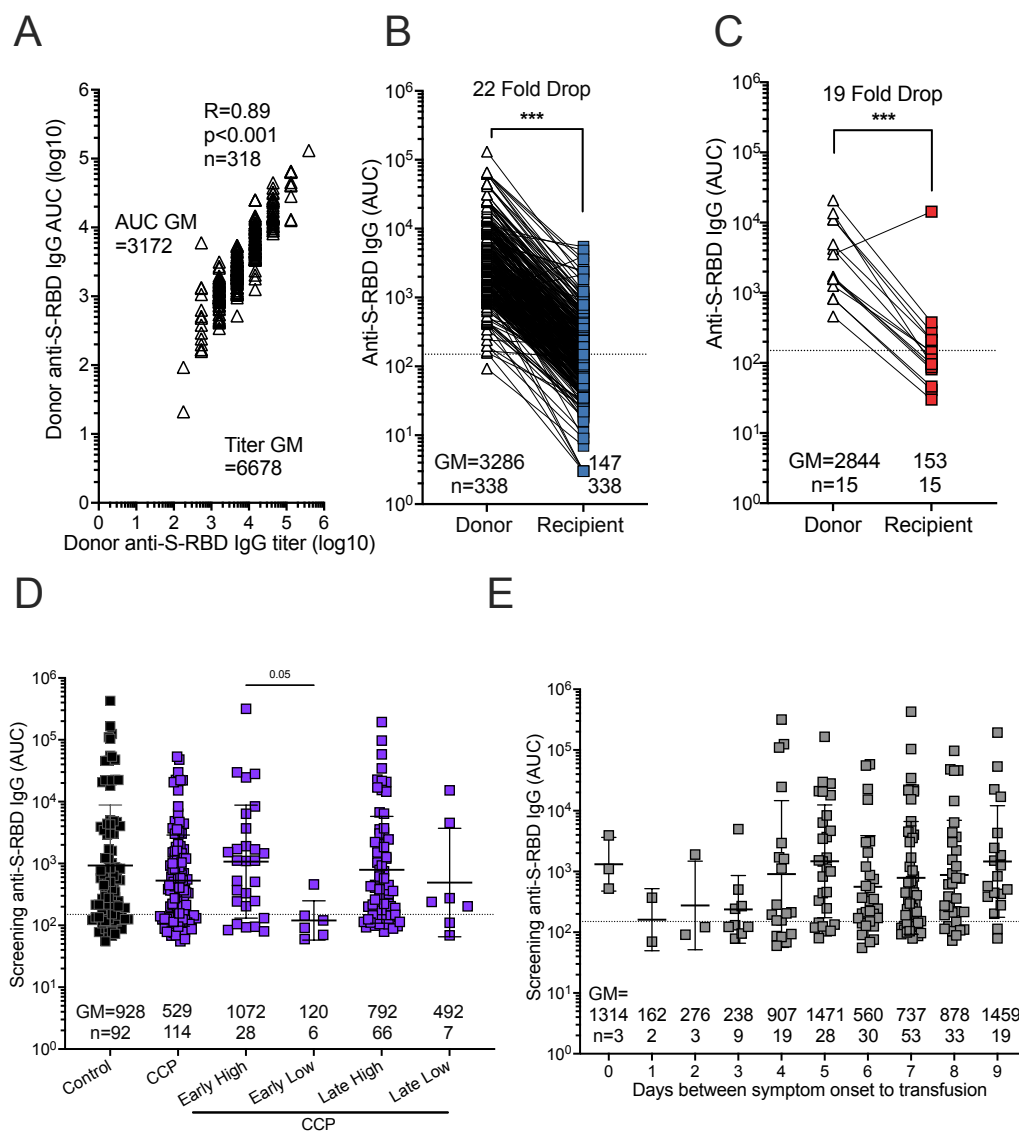
Fig. 2 **CCP participant post-transfusion recipient antibody levels stratified by duration from symptom onset to transfusion.** Post-transfusion recipient antibody levels segregated by duration from symptom onset to transfusion. Recipient post-transfusion anti-S-RBD IgG AUC levels of subsequently hospitalized (red dots) and non-hospitalized within 28 days from transfusion (blue dots) were plotted by days from symptom onset to transfusion, segregating by early (≤5 days of symptom onset) or late (>5 days after symptom onset) transfusion and high (≥150 AUC) or low (<150 AUC) antibody levels. Early transfusion with high antibody levels measured 30 minutes post-transfusion (D0) among unvaccinated, CCP recipients had the lowest proportion of hospitalizations (0%) whereas late transfusion with low antibody levels had the greatest proportion of hospitalizations (5.9%). Numbers in each quadrant represent the proportion of hospitalization and sample size for each category.
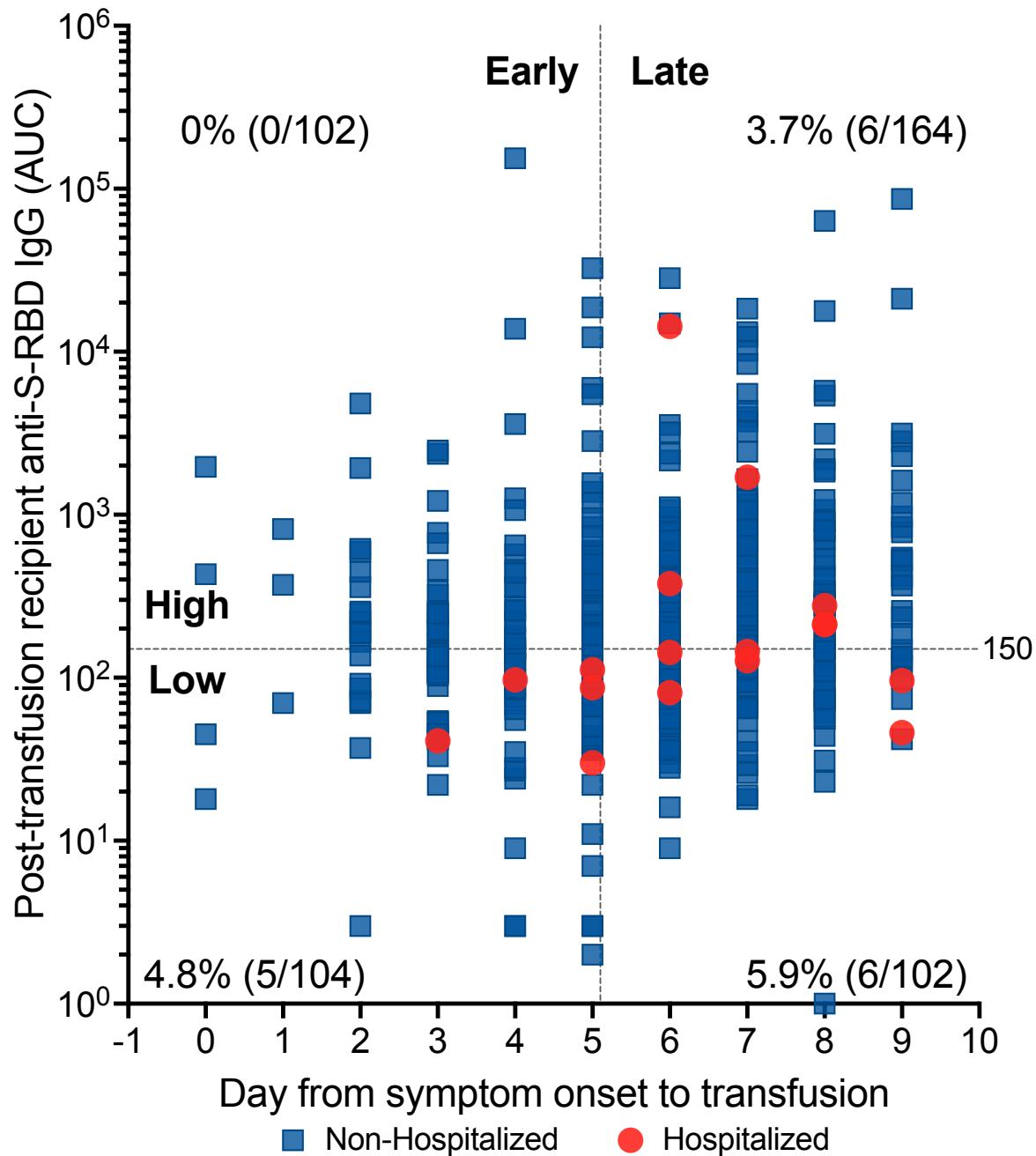

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Fig. 3 **Mapped recipients with donor unit antibody levels stratified by duration from symptom onset to transfusion.** Donor unit antibody levels that correspond with unvaccinated, CCP recipient antibody data segregated by duration from symptom onset to transfusion. A) Early recipients with donor anti-S-RBD IgG AUC levels over 3286 were found to correlate with the lowest proportion of hospitalizations (1.1%). B) Early recipients with donor neutralization antibody (nAb) AUC levels greater than the geometric mean of 58 also had the lowest proportion of hospitalizations (0%). The percentage of recipients hospitalized is indicated in each quadrant of the graph. Numbers in each quadrant represent the proportion of hospitalization and sample size for each category.
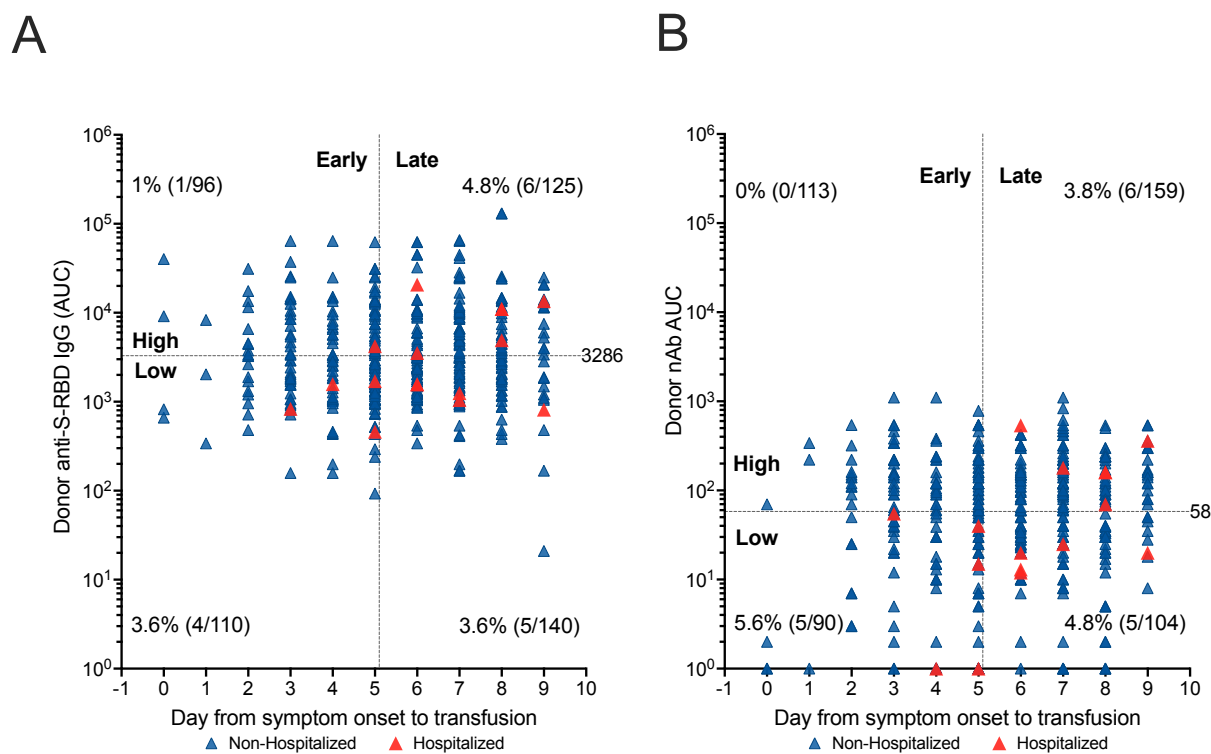
Fig. 4 **Screen pre-transfusion nasal swab viral load determinations** segregated by A) the unvaccinated jointly stratified early/late or high/low groups. B) CCP or control plasma administration in those hospitalized or not hospitalized. Unvaccinated pre-Delta period participants were segregated into C) seronegative and D) seropositive populations by symptom duration in days to transfusion. Numbers above the x-axis represent geometric mean (GM), the number in the group (n), and percentage of PCR-positive samples (%) for each category. *** $p<0.001$, ** $p=0.002$ and * $p=0.033$ by non-parametric Kruskal-Wallis multiple comparisons test with Dunn's post-hoc corrections. All point estimates are shown with error bars indicating the geometric mean with geometric SD. The dashed lines indicate samples below the limit of detection of 330 viral copies.
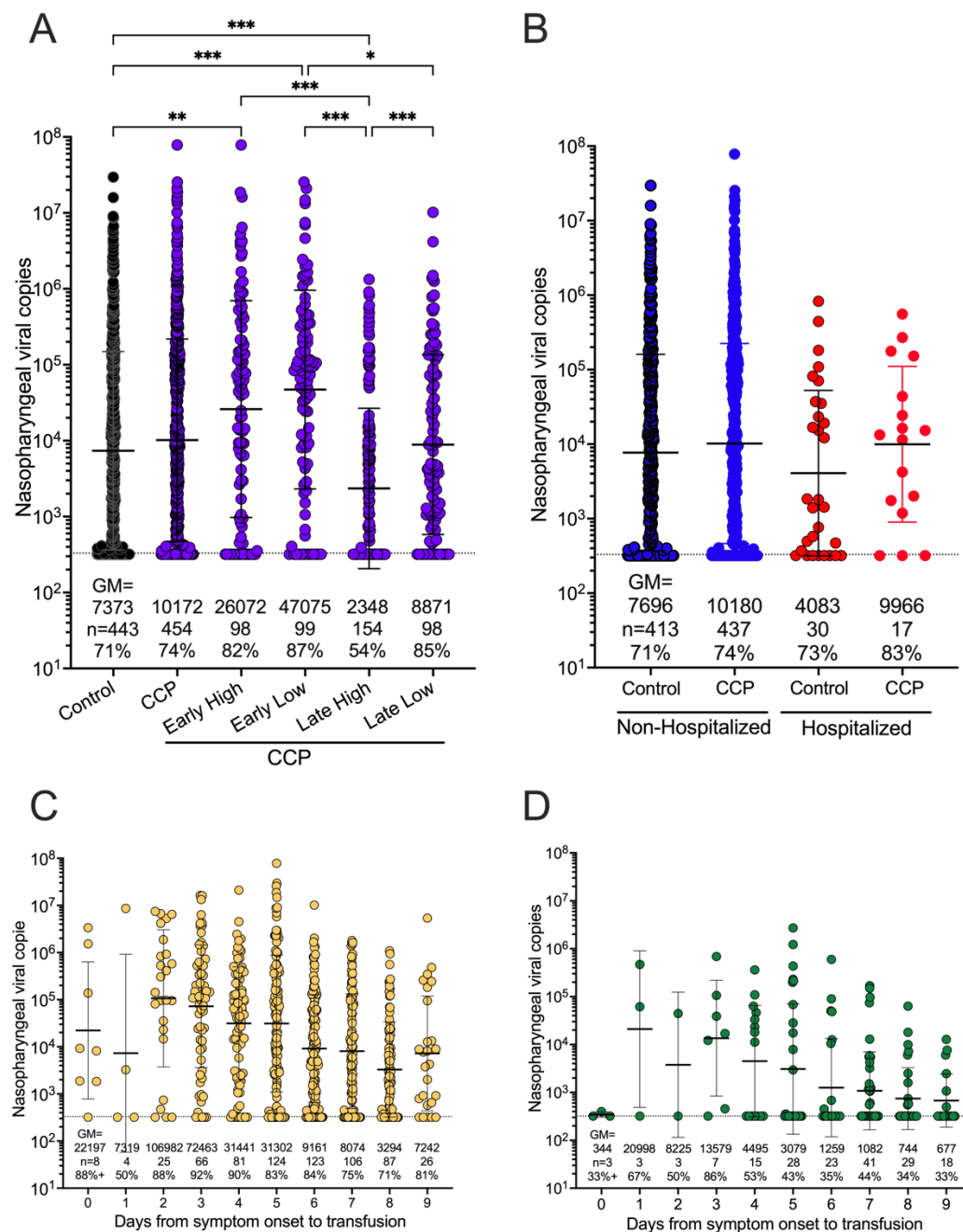
Fig. 5 **Antibody levels over three months past transfusion in those hospitalized or not further segregated by CCP or control plasma.** Log_{10}-transformed antibody levels up to 90 days post-transfusion were segregated by treatment and hospitalization status of recipients using a linear mixed effects regression model, adjusted for variant, age, sex, and BMI. CCP recipients have greater AUC levels on D0, but by D14, the hospitalized recipients have greater AUC levels than non-hospitalized. The average days from transfusion to hospitalization was 3.05 days, with all post-transfusion hospitalizations occurring between D0 and D14. The dashed line represents the log-transformed cutoff (1.924) for seropositivity. This diagnostic threshold equals the anti-S-RBD IgG log_{10}-transformed value at a 180 titer.
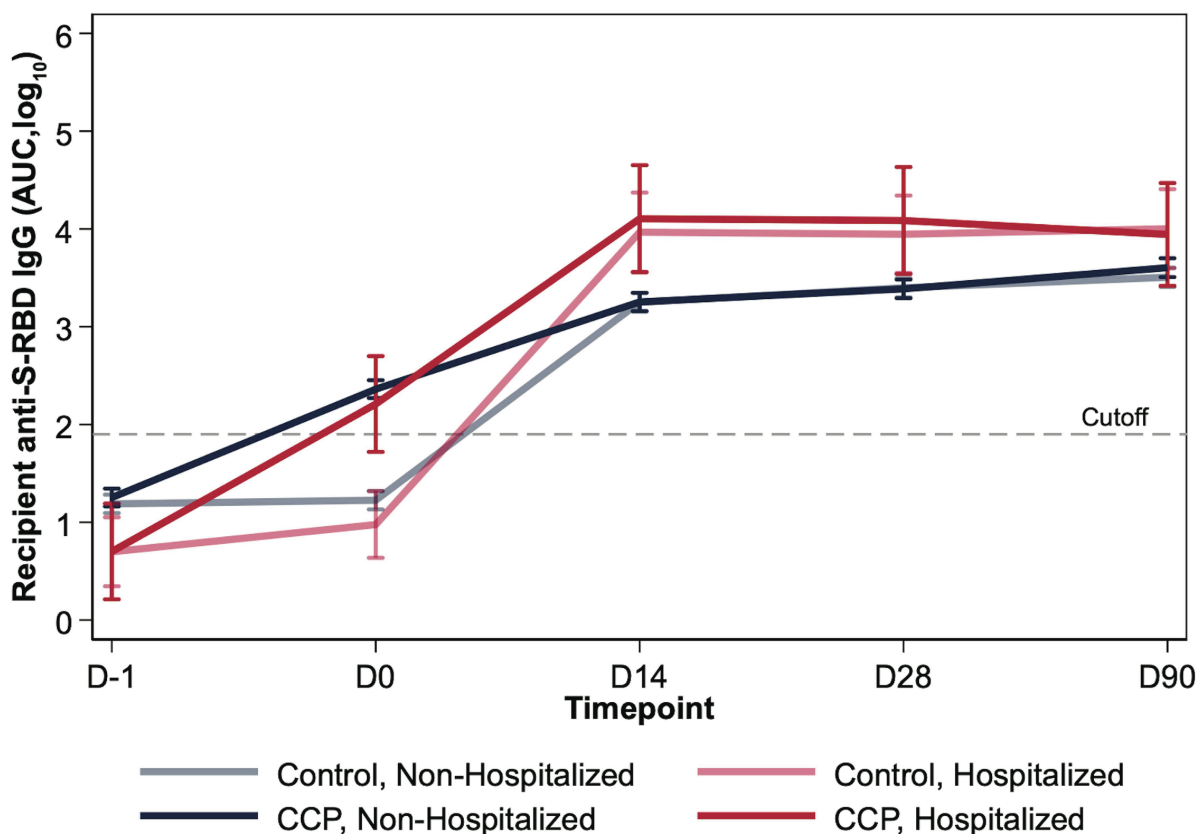

| Group	Comparison	Timepoint	Contrast	P Value
CCP	Hospitalized vs. Non-Hospitalized	D0	-0.144	0.573
Control	Hospitalized vs. Non-Hospitalized	D0	-0.243	0.180
Hospitalized	CCP vs. Control	D0	1.23	<0.001
Non-Hospitalized	CCP vs. Control	D0	1.13	<0.001
CCP	Hospitalized vs. Non-Hospitalized	D14	0.819	0.003
Control	Hospitalized vs. Non-Hospitalized	D14	0.686	0.001
Hospitalized	CCP vs. Control	D14	0.129	0.702
Non-Hospitalized	CCP vs. Control	D14	-0.003	0.963
CCP	Hospitalized vs. Non-Hospitalized	D28	0.649	0.020
Control	Hospitalized vs. Non-Hospitalized	D28	0.576	0.005
Hospitalized	CCP vs. Control	D28	0.061	0.856
Non-Hospitalized	CCP vs. Control	D28	-0.012	0.857

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Extended Figures

Extended Fig. 1 **Post-transfusion recipient antibody levels stratified by duration from symptom onset to transfusion.** Anti-S-RBD IgG titer levels 30 minutes post-transfusion for unvaccinated CCP recipients are plotted by recipients' days from symptom onset to transfusion, colored by hospitalized (red dots) or non-hospitalized (blue dots). Early ($\leq$5 days of symptom onset) or late (>5 days after symptom onset) transfusion is indicated by a dashed line on the x-axis. High (>540 AUC) or low ($\leq$540 AUC) levels of antibody are indicated by the dashed line on the y-axis. CCP recipients with early transfusion with high measured anti-S-RBD IgG titer levels shortly after transfusion had the lowest proportion of hospitalization (0%) whereas those with late transfusion and low measured antibody levels had the greatest (5.3%), consistent with the data in AUC units.


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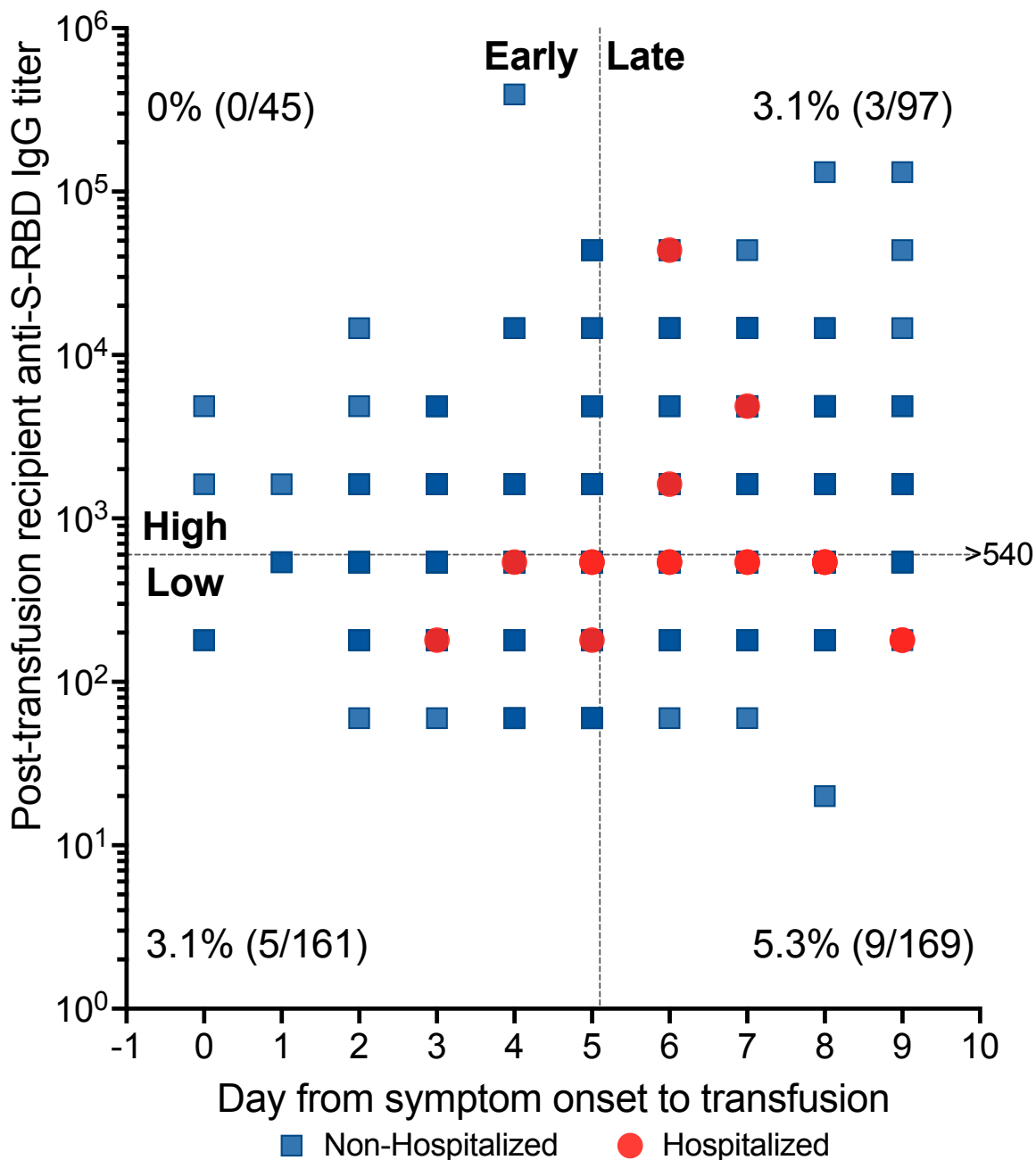
Extended Fig. 2 **Recipients stratified by duration from symptom onset to transfusion matched to donor unit titer antibody levels.** Donor unit antibody levels that correspond with unvaccinated, CCP recipient antibody data segregated by duration from symptom onset to transfusion. A) Early recipients with donor anti-S-RBD IgG titer levels over 4860 were found to correlate with the lowest proportion of hospitalizations (1.1%). B) Early recipients with donor neutralization antibody (nAb) titer levels greater than the median of 80 also had the lowest proportion of hospitalizations (0%). The percentage of recipients hospitalized is indicated in each quadrant of the graph. Numbers in each quadrant represent the proportion of hospitalization and sample size for each category.
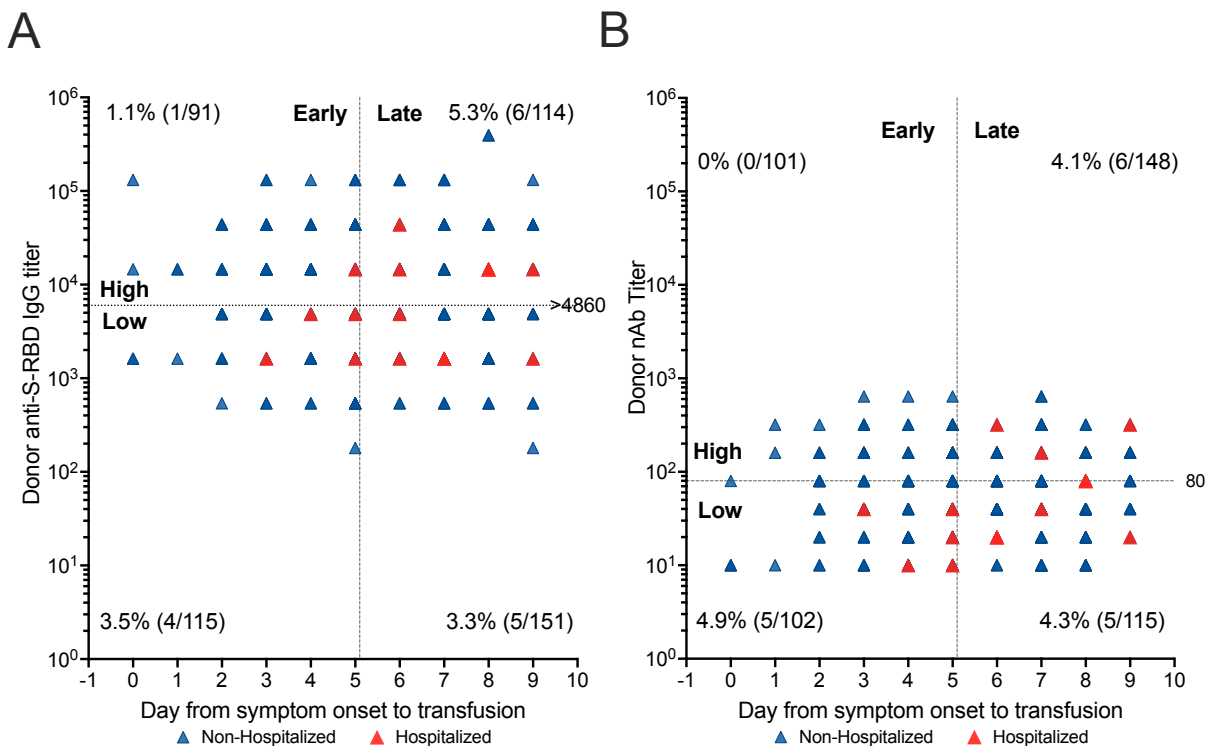
Extended Fig. 3 **Recipients segregated by duration from symptom onset to transfusion matched to donor unit antibody levels measured by EUROIMMUN.** Donor unit antibody levels that correspond with unvaccinated, CCP recipient antibody data segregated by duration from symptom onset to transfusion. A) Early recipients with donor EUROIMMUN over mean of 6 AU were found to correlate with the lowest proportion of hospitalizations (0%). B) Early recipients with EUROIMMUN BAU/mL greater than the geometric mean of 600 also had the lowest proportion of hospitalizations (0%). The percentage of recipients hospitalized is indicated in each quadrant of the graph. Numbers in each quadrant represent the proportion of hospitalization and sample size for each category.
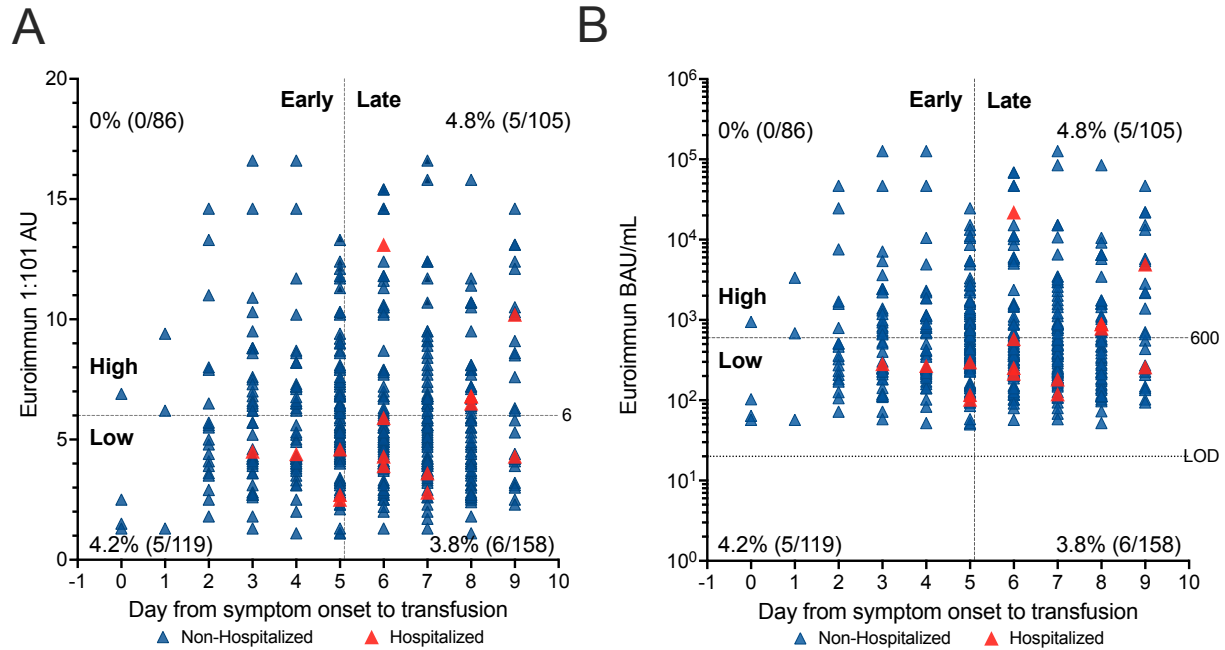
Extended Fig 4 **Antibody levels and viral loads by variant period** A) Unvaccinated and fully vaccinated, but seropositive recipient antibody levels (anti-S-RBD AUC) at screening pre-transfusion as well as fully vaccinated seropositive participants. B) Nasal swab viral load determinations for both vaccinated and unvaccinated segregated by B) CCP or control plasma administration C) serostatus of vaccinated and unvaccinated participants. ***p<0.001 and *p=0.033 by non-parametric Kruskal-Wallis multiple comparisons test with Dunn's post-hoc corrections. All point estimates are shown with error bars indicating the geometric mean with geometric SD. Numbers above the x-axis in B and C represent the geometric means (GM), the number in the group (n) and percentage of samples PCR positive (%). The dashed line in A represents the upper portion post-transfusion 150 AUC recipient's threshold. The dashed lines indicate samples below the limit of detection of 330 viral copies.
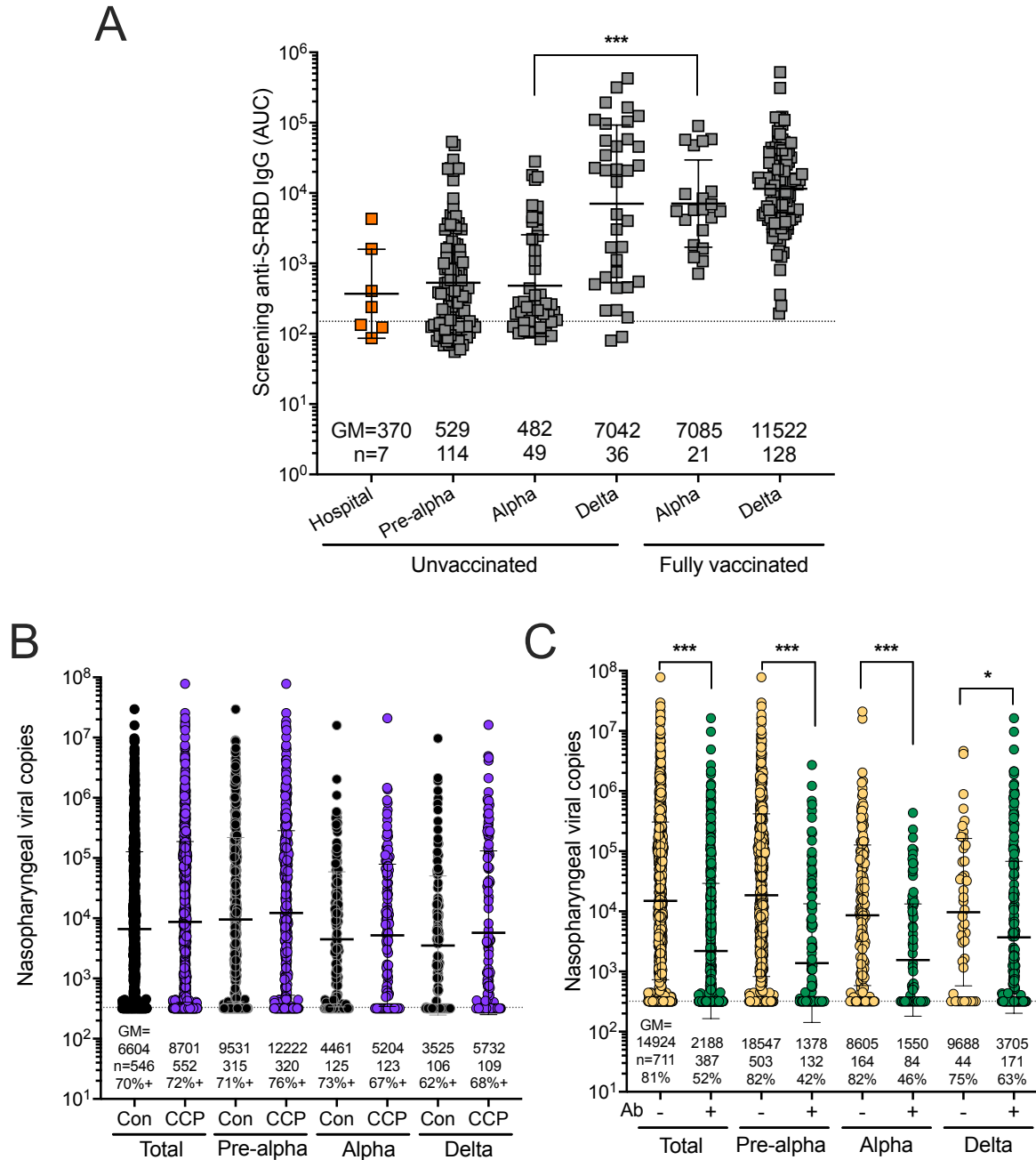
Extended Fig. 5 **Screen viral loads during the Delta period.** During the Delta period there were only 77 (34%) participants unvaccinated to segregate into A) seronegative (n=42) and B) seropositive (n=35) groups by duration from symptom onset to transfusion. C) During the Delta period fully vaccinated participants (n=128) were antibody positive with an additional single recipient fully vaccinated, but seronegative with nasal viral load on day 0 of 320 (not graphed). All point estimates are shown with error bars indicating the geometric mean with geometric SD. Numbers above the x-axis represent geometric means (GM), the number in the group (n), and the percentage of samples PCR positive (%). The dashed lines indicate samples below the limit of detection of 330 viral copies.
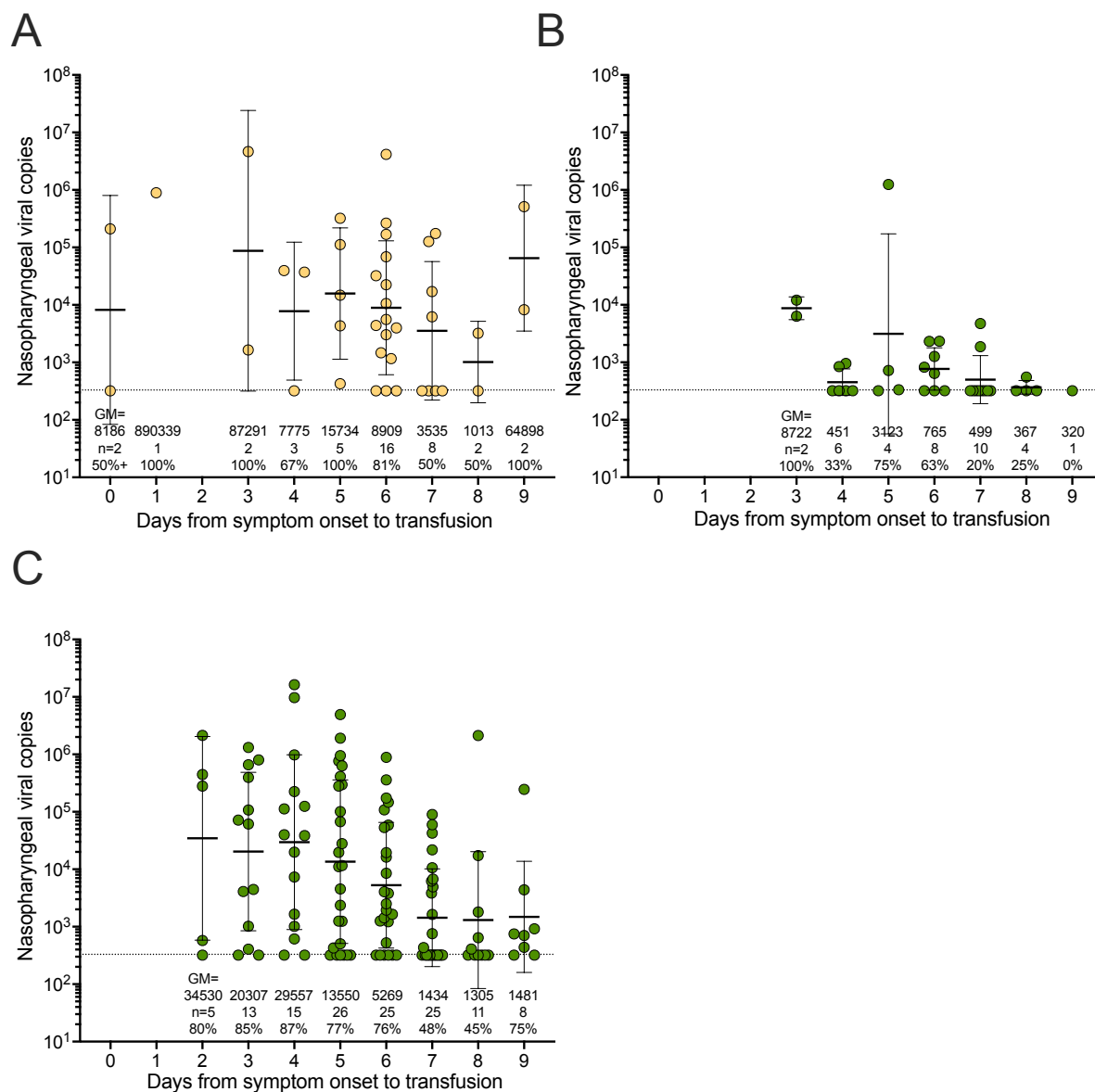
Extended Fig. 6 **Antibody levels three months post-transfusion.** recipients anti-S-RBD AUC antibody
levels at Day 90 post-transfusion (excluding the 165 vaccinated during the follow-up visits) separated by
A) Jointly stratified unvaccinated early and late treatment with high or low post-transfusion antibody
levels B) both CCP and control recipients by SARS-CoV-2 variant period and vaccination status. Clear
squares indicate donor, red squares indicate hospitalized recipients, and gray squares indicate both CCP
and control non-hospitalized recipients. ***p<0.001 and *p=0.033 by non-parametric Kruskal-Wallis
multiple comparisons test with Dunn's post-hoc corrections. All point estimates are shown with error bars
indicating the geometric mean with geometric SD. Numbers above the x-axis represent each category's
geometric mean (GM) and number in the group (n). The dashed line in A, B represents the upper portion
post-transfusion 150 AUC recipient's threshold, GM donor 3286 AUC and GM donor 6678 titer.