Passive immunotherapy for the treatment and prevention of COVID-19

Andrea Alemany Ortiz

Passive immunotherapy for the treatment and prevention of COVID-19

Doctoral thesis dissertation presented by **Andrea Alemany Ortiz** to apply for the degree of doctor at the University of Barcelona

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Als meus pares, a la Maria i al Darren

per tot l'amor

Nothing in life is to be feared, it is only to be understood.

Now is the time to understand more, so that we may fear less.

Marie Curie

AUTORIZATION FOR THE PRESENTATION OF THE THESIS

Dr Oriol Mitjà Villar, Researcher at the Fight Infections Foundation, Hospital Universitari Germans Trias i Pujol and Universitat de Barcelona.

DECLARES THAT:

The thesis memory submited by Ms. Andrea Alemany Ortiz with title "Passive immunotherapy for the treatment and prevention of COVID-19", has been developed under my supervision. I authorize its deposit for being defended and judged by a tribunal.

Signed on the day 04th September 2023.

Director Oriol Mitjà Villar

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Dr Oriol Mitjà Villar, Researcher at the Fight Infections Foundation, Hospital Universitari Germans Trias i Pujol and Universitat de Barcelona; and the doctoral candidate Andrea Alemany Ortiz

DECLARE THAT

The doctoral thesis entitled "Passive immunotherapy for the treatment and prevention of COVID-19", is original, containing its own results and information, without plagiarism from other thesis, publications or research from other authors. They also confirm that ethical codes and good practices have been followed for its preparation They declare that they consent that the thesis may be submitted to procedures to verify its originality.

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Candidate Andrea Alemany Ortiz

DECLARATION OF AUTHORSHIP OF THE THESIS

The doctoral candidate Ms Andrea Alemany Ortiz

DECLARES THAT

She is the author of the doctoral thesis entitled “Passive immunotherapy for the treatment and prevention of COVID-19”.

Signed on the day 04th September 2023.

The candidate Andrea Alemany Ortiz

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Abbreviations and acronyms

ACE2	Angiotensin-Converting Enzyme 2
ADCC	Antibody-Dependent Cellular Cytotoxicity
ADCP	Antibody-Dependent Cellular Phagocytosis
ADE	Antibody Dependent Enhancement
AU	Arbitrary Units
ARDS	Acute Respiratory Distress Syndrome
BAU	Binding Antibody Units
CCP	Covid-19 Convalescent Plasma
CI	Contraindicated
CLIA	Chemiluminescence Immunoassay
COVID-19	Coronavirus Disease 2019
CP	Convalescent Plasma
EAP	Expanded Access Program
ELISA	Enzyme Linked Immunosorbent Assay
EMA	European Medicines Agency
EpAbs	Equine Polyclonal Antibodies
EU	European Union
EUA	Emergency Use Authorization
EVD	Ebola Virus Disease
Fc	Fragment Crystallizable
FDA	Food and Drug Administration
HAI	Hemagglutination Inhibiting Antibody
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
hIG	Hyperimmune Immunoglobulin
hIVIG	Hyperimmune Intravenous Immunoglobulin
HIV	Human Immunodeficiency Virus
HR	Hazzard Ratio
ICU	Intensive Care Unit

ID50	Inhibitory Dilution 50%
IG / Ig	Immunoglobulin
IM	Intramuscular
IU	International Units
IV	Intravenous
IVIG	Intravenous Immunoglobulin
MA	Meta-Analysis
mAb	Monoclonal Antibody
MB	Methylene Blue
MDR	Multi-Drug Resistant
MERS	Middle East Respiratory Syndrome
MERS-CoV	Middle East Respiratory Syndrome Coronavirus
mg	milligram
ml	Milliliter
NA	Not Available
nAb	Neutralizing Antibody
Non-CP	Non-Convalescent Plasma
OD	Odds Ratio
PEP	Post-Exposure Prophylaxis
PHEIC	Public Health Emergency of International Concern
PrEP	Pre-Exposure Prophylaxis
PRNT	Plaque Reduction Neutralization Test
RBD	Receptor Binding Domain
RCT	Randomized Clinical Trial
RF	Risk Factor
RR	Risk Ratio
RSV	Respiratory Syncytial Virus
RU	Relative Units
S	Spike
SARS	Severe Acute Respiratory Syndrome
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus

SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SC	Subcutaneous
SOC	Standard Of Care
TACO	Transfusion-Associated Circulatory Overload
TRALI	Transfusion-Related Acute Lung Injury
UK	United Kingdom
USA	United States America
VOC	Variant Of Concern
VZV	Varicella-Zoster Virus
WHO	World Health Organization

List of articles in the thesis

This thesis follows the compendium of publications format.

The thesis consists of two objectives and four articles.

Specific objectives:

Objective 1: To assess the efficacy of a single intravenous infusion of high-titre COVID-19 convalescent plasma (as defined by FDA) in preventing hospitalization by day 28 among adult outpatients with confirmed symptomatic SARS-CoV-2 infection, regardless of comorbidities, within 9 days from the onset of symptoms, as compared to placebo.

Objective 2: To assess the efficacy of a subcutaneous infusion of 1g and 2g of 20% hyperimmune immunoglobulin C19-IG20% in preventing the progression to symptomatic COVID-19 among early asymptomatic adults with confirmed SARS-CoV-2 infection, regardless of comorbidities, within 5 days from diagnosis, as compared to placebo.

Thesis articles relevant to objective 1:

- **Article 1**

Alemany A*, Millat-Martinez P*, Corbacho-Monné M, Malchair P, Ouchi D, Ruiz-Comellas A, Ramírez-Morros A, Rodríguez Codina J, Amado Simon R, Videla S, Costes G, Capdevila-Jáuregui M, Torrano-Soler P, San José A, Bonet Papell G, Puig J, Otero A, Ruibal Suarez JC, Zarauza Pellejero A, Llopis Roca F, Rodriguez Cortez O, Garcia Garcia V, Vidal-Alaball J, Millan A, Contreras E, Grifols JR, Ancochea À, Galvan-Femenia I, Piccolo Ferreira F, Bonet M, Cantoni J, Prat N, Ara J, Forcada Arcarons A, Farré M, Pradenas E, Blanco J, Àngel Rodriguez-Arias M, Fernández Rivas G, Marks M, Bassat Q, Blanco I, Baro B, Clotet B, Mitjà O; CONV-ERT Group. High-titre methylene blue-treated convalescent plasma as an early treatment for outpatients with COVID-19: a randomised, placebo-controlled trial. ***Lancet Respir Med.*** *2022 Mar;10(3):278-288*.
Impact Factor 2022: 102.642; Quartile: Q1

- **Article 2:**

Millat-Martinez P*, Gharbharan A*, **Alemany A***, Rokx C, Geurtsvankessel C, Papageourgiou G, van Geloven N, Jordans C, Groeneveld G, Swaneveld F, van der Schoot E, Corbacho-Monné M, Ouchi D, Piccolo Ferreira F, Malchair P, Videla S, García García V, Ruiz-Comellas A, Ramírez-Morros A, Rodriguez Codina J, Amado Simon R, Grifols JR, Blanco J, Blanco I, Ara J, Bassat Q, Clotet B, Baro B, Troxel A, Zwaginga JJ, Mitjà O, Rijnders BJA; CoV-Early study group; COnV-ert study group. Prospective individual patient data meta-analysis of two randomized trials on convalescent plasma for COVID-19 outpatients. ***Nat Commun.*** *2022 May 11;13(1):2583.*
Impact Factor 2022: 17.694; Quartile: Q1

- **Article 3:**

Levine AC, Fukuta Y, Huaman MA, Ou J, Meisenberg BR, Patel B, Paxton JH, Hanley DF, Rijnders BJA, Gharbharan A, Rokx C, Zwaginga JJ, **Alemany A**, Mitjà O, Ouchi D, Millat-Martinez P, Durkalski-Mauldin V, Korley FK, Dumont LJ, Callaway CW, Libster R, Marc GP, Wappner D, Esteban I, Polack F, Sullivan DJ. Coronavirus Disease 2019 Convalescent Plasma Outpatient Therapy to Prevent Outpatient Hospitalization: A Meta-Analysis of Individual Participant Data From 5 Randomized Trials. ***Clin Infect Dis.*** *2023 Jun 16;76(12):2077-2086.*

Impact Factor 2022: 20.999; Quartile: Q1

Thesis articles relevant to objective 2:

- **Article 4:**

Alemany A, Millat-Martinez P, Corbacho-Monné M, Suñer C, Galvan-Casas C, Carrera C, Ouchi D, Prat N, Ara J, Nadal N, Riel R, Funollet B, Ojeda-Ciurana C, Balague LE, Salvador-González B, Arcarons AF, Vidal-Alaball J, Del Cura-González MI, Barrientos RR, Ramos-Blanes R, Bou AA, Mondou E, Torres M, Campins N, Sanz A, Tang Y, Rodriguez-Arias MÀ, Bassat Q, Clotet B; GC2010 STUDY GROUP; Mitjà O. Subcutaneous anti-COVID-19 hyperimmune immunoglobulin for prevention of disease in asymptomatic individuals with SARS-CoV-2 infection: a double-blind, placebo-controlled, randomised clinical trial. ***EClinicalMedicine.*** *2023 Mar 10;57:101898.*

Impact Factor 2022: 17.033; Quartile: Q1

Thesis summary

Thesis summary in English

Title: Passive immunotherapy for the treatment and prevention of COVID-19

Introduction: Passive immunotherapy has been proposed as a potential treatment and prevention for several emerging viral diseases, such as Influenza, SARS, MERS, and Ebola. Since the start of the COVID-19 pandemic, passive immunotherapies, including the use of COVID-19 convalescent plasma (CCP), hyperimmune immunoglobulins (hIG), and monoclonal antibodies (mAbs), have been employed in attempts to reduce disease progression and mortality.

Hypothesis: Passive immunotherapy, including CCP and hIG, is efficacious for treating and preventing COVID-19 when administered early in the course of SARS-CoV-2 infection. First, a single intravenous infusion of high-titre CCP is efficacious in preventing hospitalization by day 28 in COVID-19 outpatients within 9 days from symptom onset. Second, a subcutaneous infusion of 1g and 2g of 20% hyperimmune immunoglobulin C19-IG20% is efficacious in preventing the development of symptomatic COVID-19 in early asymptomatic adults with confirmed SARS-CoV-2 infection within 5 days.

Objectives: Two main objectives are evaluated in this thesis. The first objective is to assess the efficacy of a single intravenous infusion of high-titre COVID-19 convalescent plasma (as defined by FDA) in preventing hospitalization by day 28 among adult outpatients with confirmed symptomatic SARS-CoV-2 infection, regardless of comorbidities, within 9 days from the onset of symptoms, as compared to placebo. The second objective is to assess the efficacy of a subcutaneous infusion of 1g and 2g of 20% hyperimmune immunoglobulin C19-IG20% in preventing the progression to symptomatic COVID-19 among early asymptomatic adults with confirmed SARS-CoV-2 infection, regardless of comorbidities, within 5 days of diagnosis, as compared to placebo.

Methods: This thesis comprises four studies that assess the two objectives of the thesis. The first objective was addressed in three studies, including one randomized controlled trial (RCT) and two meta-analyses, while the second objective was evaluated in the fourth study, which was also an RCT. The first study, the COnV-ert trial, was a multicentre, double-blind, randomized, placebo-controlled clinical trial conducted in four health-care centres in Catalonia, Spain to evaluate the early treatment with 250-300 ml of ABO-compatible high-titre methylene blue-treated CCP for COVID-19 outpatients. The second study involved a meta-analysis of individual participant data from two RCTs, the COnV-ert and COV-Early trials, conducted in Spain and the Netherlands, respectively, focusing on CCP for COVID-19 outpatients. The third study was a meta-analysis of all international RCTs reporting on the efficacy of CCP for COVID-19 outpatients, that used individual participant data from five RCTs, including the COnV-ert trial. Lastly, the fourth study, the GC2010 trial, was a multicentre, double-blind, randomized, placebo-controlled clinical trial conducted in Spain to assess the efficacy of subcutaneous 20% hyperimmune immunoglobulin C19-IG20% in preventing progression of asymptomatic individuals with SARS-CoV-2 infection to symptomatic COVID-19.

Main results: The first study (COnV-ert trial) enrolled 376 COVID-19 outpatients and observed no differences in hospitalization rates at day 28 between CCP and placebo groups (12% vs 11%, RR 1.05; p=0.76) or in the mean change in viral load from baseline to day 7 (crude difference -0.10 $\log_{10}$, p=0.42). The second study included a total of 797 COVID-19 outpatients from two RCTs and showed no differences between the two groups in improved disease severity, as measured through a 5-point disease severity scale (OR 0.936, 95% CI 0.667 to 1.311) and a composite of hospitalization or death by day 28 (OR 0.919, 95% CI 0.592 to 1.416). The third study included 2963 COVID-19 outpatients from five RCTs and found a 30.1% relative risk reduction for all-cause hospitalization with the early treatment with high-titre CCP (8.5% vs 12.2%, 3.7% absolute risk reduction, 95% CI, 1.3% to 6.0%). Moreover, the study found the greatest reduction in hospitalization (51.4% relative risk reduction) in participants who received earlier transfusion (≤5 days after symptom onset) of CCP with higher antibody titres (above the median titre for each individual RCT). The fourth study (GC2010 trial) enrolled 461 participants and reported no differences in the proportion of individuals who remained

asymptomatic through day 14 after infusion (59.9% vs 64.7% vs 63.7%; p=0.53 and p=0.85).

Conclusions: Both CCP and the hIG C19-IG20% have shown to be well-tolerated; however, their clinical effectiveness has been inconsistent in COVID-19 patients. CCP has drawn mixed efficacy results, which could be explained in the context of highly variable populations, timing of administration and CCP SARS-CoV-2 antibody levels, testing methods, dose, and pathogen inactivation methods (i.e., methylene blue). Nonetheless, considering the overall evidence generated in this thesis, CCP may be a valuable option for the early treatment of COVID-19 outpatients at hight risk of disease progression. On the other hand, subcutaneous hIG C19-IG20% at doses of 1 and 2 g cannot be recommended for asymptomatic individuals with SARS-CoV-2 infection to prevent the development of symptomatic COVID-19. Key insights gained from the use of CCP and hIG for COVID-19 emphasize the importance of early administration and high neutralization capacity in the product, which can be achieved from vaccinated donors with recent infection. Future research should prioritize the evaluation of CCP and hIG for COVID-19 in immunocompromised vaccinated individuals, particularly in light of the emergence of new variants.

Resumen en castellano

Título: Inmunoterapia pasiva para el tratamiento y prevención de la COVID-19.

Introducción: La inmunoterapia pasiva se ha utilizado como potencial tratamiento y prevención de varias epidemias causadas por enfermedades virales emergentes, incluyendo Influenza, SARS, MERS y Ébola. Desde el inicio de la pandemia de COVID-19, las inmunoterapias pasivas, incluido el uso de plasma convaleciente de COVID-19 (CCP), inmunoglobulinas hiperinmunes (hIG) y anticuerpos monoclonales (mAb), se han empleado en intentos de reducir la progresión de la enfermedad y la mortalidad.

Hipótesis: La inmunoterapia pasiva, incluyendo el CCP y las hIG, es eficaz para tratar y prevenir la COVID-19 cuando se administra de forma precoz en el curso de la infección por SARS-CoV-2. En primer lugar, una infusión endovenosa única de CCP con títulos altos de anticuerpos es eficaz para prevenir la hospitalización a día 28 en pacientes ambulatorios con COVID-19 durante los primeros 9 días desde el inicio de los síntomas. En segundo lugar, una infusión subcutánea de 1g y 2g de inmunoglobulina hiperinmune al 20% C19-IG20% es eficaz para prevenir el desarrollo de COVID-19 sintomático en individuos asintomáticos con infección precoz confirmada por SARS-CoV-2 durante los primeros 5 días.

Objetivos: Dos objetivos principales son evaluados en esta tesis. El primer objetivo es evaluar la eficacia de una infusión única endovenosa de plasma convaleciente de COVID-19 con títulos altos de anticuerpos (según la definición de la FDA) para prevenir la hospitalización a día 28 en pacientes adultos ambulatorios con infección sintomática confirmada por SARS-CoV-2, independientemente de las comorbilidades, durante los 9 primeros días desde el inicio de los síntomas, en comparación con placebo. El segundo objetivo es evaluar la eficacia de una infusión subcutánea de 1g y 2g de la inmunoglobulina hiperinmune al 20% C19-IG20% para prevenir el desarrollo de COVID-19 sintomático en adultos asintomáticos con infección precoz confirmada por SARS-CoV-2 durante los 5 primeros días, independientemente de las comorbilidades, en comparación con placebo.

Métodos: Esta tesis consta de cuatro estudios que evalúan los dos objetivos de la tesis. El primer objetivo se abordó en tres estudios, incluyendo un ensayo clínico controlado aleatorizado (ECA) y dos metaanálisis, mientras que el segundo objetivo se evaluó en el cuarto estudio, que también fue un ECA. El primer estudio, el estudio COnV-ert, fue un ensayo clínico multicéntrico, doble ciego, aleatorizado y controlado con placebo realizado en cuatro centros sanitarios de Cataluña, España, para evaluar el tratamiento precoz con 250-300 ml de CCP con compatibilidad ABO tratado con azul de metileno con títulos altos de anticuerpos para pacientes ambulatorios de COVID-19. El segundo estudio implicó un metaanálisis de datos de participantes individuales de dos ECA, los estudios COnV-ert y COV-Early, sobre CCP para pacientes ambulatorios con COVID-19, realizados en España y los Países Bajos, respectivamente. El tercer estudio fue un metaanálisis de todos los ECA internacionales informando sobre la eficacia del CCP para pacientes ambulatorios con COVID-19, que usó datos de participantes individuales de cinco ECA, incluido el estudio COnV-ert. Por último, el cuarto estudio, el estudio GC2010, fue un ensayo clínico multicéntrico, doble ciego, aleatorizado y controlado con placebo realizado en España para evaluar la eficacia de la inmunoglobulina hiperinmune al 20% subcutánea C19-IG20% para prevenir la progresión de individuos asintomáticos infectados por SARS-CoV-2 a COVID-19 sintomático.

Resultados principales: El primer estudio (estudio COnV-ert) incluyó a 376 pacientes ambulatorios con COVID-19 y no observó diferencias en las tasas de hospitalización a día 28 entre el grupo CCP y placebo (12 % frente a 11 %, RR 1,05; *p*=0,76) ni en la reducción media en la carga viral (diferencia bruta -0,10 log10, *p*=0,42). El segundo estudio incluyó un total de 797 pacientes ambulatorios con COVID-19 de dos ECA y no mostró diferencias entre ambos grupos en la mejora de la gravedad de la enfermedad, medida a través de una escala de gravedad de la enfermedad de 5 puntos (OR 0,936, IC del 95 % 0,667 a 1,311) y una escala compuesta de hospitalización o muerte a día 28 (OR 0,919, IC 95% 0,592 a 1,416). El tercer estudio incluyó un total de 2963 pacientes ambulatorios con COVID-19 de cinco ECA y encontró una reducción del riesgo relativo del 30,1% para la hospitalización por cualquier causa con el tratamiento con CCP con títulos altos de anticuerpos (8,5% frente al 12,2%, reducción del riesgo absoluto del 3,7%, IC del 95%, 1,3% a 6,0%). Además, el estudio observó una mayor reducción de la

hospitalización (reducción del riesgo relativo del 51,4%) en aquellos participantes que recibieron una transfusión más temprana (≤5 días después del inicio de los síntomas) de CCP con títulos más altos de anticuerpos (por encima del título medio para cada ECA individual). El cuarto estudio (GC2010) reclutó a 461 participantes y no informó diferencias en la proporción de participantes que permanecieron asintomáticos hasta el día 14 después de la infusión (59,9 % frente a 64,7% frente a 63,7 %; $p = 0,53$ y $p = 0,85$).

Conclusiones: Tanto el CCP como la hIG C19-IG20% han demostrado ser bien tolerados, pero la efectividad clínica ha sido inconsistente en pacientes con COVID-19. El CCP ha obtenido resultados mixtos de eficacia, que podrían explicarse en el contexto de variaciones relevantes en las poblaciones, el tiempo de la administración y los niveles de anticuerpos contra el SARS-CoV-2 del CCP, los test para la medición de anticuerpos, la dosis y los métodos de inactivación de patógenos usados (azul de metileno). Sin embargo, de acuerdo con la evidencia global generada en esta tesis, el CCP puede considerarse como una opción valiosa para el tratamiento precoz de pacientes ambulatorios con COVID-19 con alto riesgo de progresión de la enfermedad. Por otro lado, la hIG C19-IG20% subcutánea a la dosis de 1 y 2 g no se puede recomendar en personas asintomáticas con infección por SARS-CoV-2 para prevenir el desarrollo de COVID-19 sintomático. Los conocimientos clave obtenidos del uso del CCP y las hIG para COVID-19 enfatizan la importancia de una administración temprana y una alta capacidad neutralizante del producto, que se puede lograr a partir de donantes vacunados y con infección reciente. Las investigaciones futuras deberían priorizar la evaluación del CCP y las hIG para COVID-19 en individuos vacunados inmunocomprometidos, particularmente a la luz de la aparición de nuevas variantes.

Introduction

1. Passive immunotherapy for infectious diseases

Key messages Panel 1:

- Passive immunotherapies encompass the use of convalescent plasma (CP), hyperimmune immunoglobulins (hIG), and monoclonal antibodies (mAbs).
- Passive immunotherapies have been used since the 1890s for the treatment and prevention of infectious diseases.
- Their use in modern medicine is limited, due to the development of effective antimicrobial therapies, vaccines, and antiviral drugs.
- Main current indications include toxin neutralization and prophylaxis of viral infections.
- More recently, they had been proposed as potential therapies for several epidemics of emerging viral diseases, including influenza, SARS, MERS, and Ebola, with limited efficacy results.

1.1. Overview

Passive immunotherapy involves administering antibodies against a specific agent directly to a susceptible individual to provide immediate immunity for the prevention or treatment of an infectious disease caused by that specific agent. In contrast, active immunotherapy aims to stimulate the host's immune response to produce a lasting, durable response.[1]

Passive immunotherapies, including the use of convalescent plasma, (hyperimmune) immunoglobulins, and monoclonal antibodies, have been used for prevention and treatment of several infectious diseases since the early twentieth century. Antibody therapies can influence the course of bacterial, fungal, and viral infections and are usually pathogen-specific treatments.

1.2. History

Serum therapy was introduced in the 1890s after Emil von Behring first demonstrated its effectiveness in treating diphtheria.[2,3] In 1890, Behring and Kitasato were the first to discover that sera from rabbits infected with *Clostridium tetani* conferred protection to naïve mice against live *Tetanus bacilli* and against tetanus toxin.[4] In 1893, Paul Ehrlich joined Behring to develop high-quality anti-diphtheria serum from horses, which in 1894 was used in a clinical trial that found the treatment efficacious in reducing mortality in children with diphtheria, especially if administered early after diagnosis.[5,6] Passive antibody administration then became the standard of care for prophylaxis and treatment of many pathogen-mediated and toxin-mediated diseases, until the introduction of antimicrobial therapy in the 1940s. At the time, passive immunotherapy was used to treat bacterial infections (e.g., *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae,* Group A *Streptococcus, Bordetella pertussis, Bacillus anthracis, Clostridium botulinum, Clostridium tetani, Brucella abortus, Shigella dysenteriae, Francisella tularensis, Corynebacterium diphtheriae*) and viral infections (e.g., measles, poliomyelitis, mumps, varicella zoster).[3] Taking into account the limitations of the data from historical studies, which were not conducted according to current evidence-based medicine standards, serum therapy showed higher efficacy when used as prophylaxis or as early treatment after infection.[7] Antibody therapy was considered to follow three main principles: (1) specificity, or specific antibody content for the pathogen treated, (2) quantity, or sufficient antibody content, and (3) temporality, consisting in early administration, both before and after infection.[8] Regarding the source of therapeutic antibody preparations, animal-derived serum was most frequently used, whereas human-derived serum was used for diseases affecting only humans or when animal immunization was not practical (e.g., viral diseases).[2] Animal serum was preferred because larger amounts of the product could be collected and standardized in laboratory tests.

With the advent of modern drug development in the first half of the XXth century, the generalized use of polyclonal serum therapy for treating bacterial diseases was abandoned and replaced with antimicrobial therapy. An important disadvantage of polyclonal serum, which was mainly animal-derived, was the high rate of adverse reactions. Most common

adverse reactions included fever, chills, and allergic reactions and, in some cases, a self-limited syndrome (known as "serum sickness") characterized by rash, proteinuria, and arthralgias. Another risk was the transmission of blood-borne pathogens, such as hepatitis virus, due to the lack of availability of screening procedures at the time. Aside from toxicity, other drawbacks of serum therapy included highly efficacy variability, technical limitations of collection and administration, difficulties for making an early specific diagnosis as a requirement to use type-specific sera, and high cost.[8]

1.3. Types of passive immunotherapies

Passive immunotherapies encompass (1) convalescent plasma, (2) immunoglobulins, and (3) monoclonal antibodies.

1.3.1. Convalescent plasma

Convalescent plasma (CP) refers to blood plasma collected from patients who have recovered from an infection or individuals who have been vaccinated against an infection and have developed humoral immunity against that specific pathogen. CP is typically obtained in blood donor centres from volunteers by two primary methods: automated apheresis or fractionation. The identification, selection, and recruitment of potential donors might be challenging, as convalescent subjects must also meet donor selection criteria, in compliance with regional policies and routine procedures.[2] CP contains polyclonal antibodies, that bind to multiple epitopes and are usually made by several different antibody-secreting plasma cell lineages. Main advantages of CP include its low cost and straightforward production, which provide a readily available supply of antibody content that is extremely useful in emergency situations. Moreover, the polyclonal nature of its antibodies makes CP less susceptible to escape variants. Disadvantages of CP include lack of standardization in dose, affinity, and specificity of antibodies, which lead to high inter-unit variability. Moreover, the overall dose of specific antibodies is generally lower, thus high volumes administered intravenously are required.

1.3.2. Immunoglobulin

Immunoglobulin (IG) preparations refer to a therapeutic product derived from pooling plasma collected from either multiple human donors or animals with the specific desired antibody. There are two main types of immunoglobulin preparations: normal or nonspecific, obtained from unselected donors, and hyperimmune or specific, obtained

from selected donors. Hyperimmune immunoglobulins (hIG) are manufactured using plasma with high-titre specific antibodies, predominantly IgG.[5] Immunoglobulins have several benefits over plasma treatment: they contain a standardized and controlled titre of polyclonal antibodies, they require smaller volumes and there is no ABO blood matching needed. IG products are available for intramuscular (IMIG) injection and subcutaneous (SCIG) and intravenous infusion (IVIG). The main drawback of IG is that technical requirements and costs are higher than those of CP.

1.3.3. Monoclonal antibodies

A monoclonal antibody (mAb) product refers to antibodies produced by a single clone of B cells and therefore identical in structure and specificity for binding to the same epitope. Monoclonal antibodies may be derived from the isolation of memory B cells from convalescent patients or from animals after direct inoculation with the pathogen.[5] mAb products could be murine, chimeric, humanized, and human.[9] In contrast to polyclonal antibody products (i.e., CP and hIG), mAbs offer certain advantages. They are homogeneous and reproducible reagents that can be generated into large amounts, and have significantly higher specific activity and affinity, thus requiring lower volumes. Additionally, mAbs are available for intramuscular, subcutaneous, and intravenous administration. However, mAbs also have some disadvantages over polyclonal antibody products. First, the efficacy of mAbs can be diminished by antigenic changes in the targeted pathogen. Nevertheless, this limitation can be mitigated by employing cocktails of mAbs directed at multiple antigenic targets thus minimizing susceptibility to escape variants. Second, the high cost and complexity of mAb production is a challenge to the widespread global use of this strategy.

The characteristics and differences between CP, IG and mAb are summarized in **Table 1**.

Table 1. Characteristics of different types of passive immunotherapies

	Convalescent plasma (CP)	Immunoglobulin (IG)	Monoclonal antibody (mAb)
Antibody content	Polyclonal	Polyclonal	Monoclonal
Specificity	Multiple epitopes	Multiple epitopes	Single epitope (multiple*)
Isotype	Multiple isotypes (IgG, IgM, IgA)	Multiple IgG subclasses	Single isotype (multiple*)
Affinity	Variable Low	Variable Low	Defined High
Volume	High	Low	Low
Administration route	IV	IV, IM, SC	IV, IM, SC
Escape variant susceptibility	Low	Low	High (low*)
Source	Immune host (human)	Immune host (human, animal)	Cells (*in vitro*)
Serum half-life	Variable	Variable	Defined
Cost	Low	High	High
Technical requirement	Low	High	High
Time to deployment	Days – weeks - months**	Months	Months - years

Legend: *For mAbs combination/cocktail; **Depending on regional quarantine requirements

Adapted from Casadevall et al., 2021[8]

1.4. Modern use of passive immunotherapies for infectious diseases

In modern medicine, the use of CP and hIG as treatment for infectious diseases became limited, mainly as a result of the development of highly effective vaccines that drastically reduced the incidence of many of these diseases, and the development of antimicrobial and antiviral drugs. However, antibody therapy retained a niche for toxin neutralization and eventually gained importance for a variety of conditions, mainly viral infections and as replacement therapy in patients with immunoglobulin deficiencies.[2,3]

In addition to improvements in passive immunotherapy, in 1975, Köhler and Milstein introduced the hybridoma technique,[10] which made it possible to obtain pure mAbs in large amounts. The first mAb for clinical use (muromonab-CD3) was approved in 1986, and therapeutic mAbs have become the predominant class of new drugs developed in recent years, mainly for treating cancers and immunological diseases.[9] In the field of infectious diseases, interest in this approach has been limited until recent years,[11] when

the interest has grown exponentially as a potential therapy for emerging viral infections causing epidemics.

Currently, there are several applications and approved indications for passive immunotherapy that span from toxin neutralization to prophylaxis against viral infections, as well as addressing immunodeficiency syndromes. Currently approved passive immunotherapies for toxin neutralization are summarized in **Table 2**. Many of these therapies are included in the World Health Organization's List of Essential Medicines and there is supply shortage for some of them in the context of an increase in vaccination rates and decline in disease incidence.

Table 2. Passive immunotherapies for toxin neutralization

Disease	Etiologic agent	Antibody therapy	Administration	Indication
Botulism	Neurotoxin *Clostridium botulinum*	**HBAT** Heptavalent Botulinum antitoxin*	IV	**PEP** **Treatment**
Diphtheria	Exotoxin *Corynebacterium diphtheriae*	**DAT** Diphtheria antitoxin*	IV	**Treatment**
Tetanus	Toxin *Clostridium tetani*	**TIG** Anti-tetanus immunoglobulin** **IVIG***	IV IM	**PEP** **Treatment**
Snake envenomation	Toxin Snake venom	Antivenom immunoglobulins*	IV IM	**PEP** **Treatment**

Legend: IV: intravenous; IM: intramuscular; PEP: Post-exposure prophylaxis

*Equine-derived; **Human; ***If TIG not available

References for Table 1: [12–18]

The use of hIG preparations has been established for the prophylaxis of several viral infections, including measles, rubella, hepatitis A, hepatitis B, rabies, and varicella, frequently in combination with specific vaccines (**Table 3**). More recently, some mAbs have also been approved for prophylaxis and/or treatment of HIV and rabies.

Table 3 (I). Currently approved passive immunotherapies for viral infections

Disease **Etiologic agent**	**Antibody therapy**	**Administration**	**Indication**
Measles Measles virus	**HNIM** Human Normal Immunoglobulin	IV IM	**PEP** -Preferable within 6 days after exposure -If vaccination CI or high-risk individuals*
Rubella Rubella virus	**HNIM** Human Normal Immunoglobulin	IM	**PEP** -Within 72 hours after exposure -Non-immune pregnant women where termination is unacceptable
Hepatitis A HAV	**HNIM** Human Normal Immunoglobulin	IM	**PEP** -Within 2-4 weeks after exposure -High-risk individuals**
Hepatitis B HBV	**HBIG** Human hepatitis B specific immunoglobulin	IM	**PEP** -Preferable within 24-48 hours, <1 week after exposure -Non-immune/nonresponder individuals
Rabies Rabies virus	**HRIG** Human Rabies Immune Globulin	Infiltrated around the bite site IM	**PEP** -Non-immunized exposed individuals
	Rabishield Single human IgG1 mAb **Twinrab** Murine dual IgG1 and IgG2B mAb	IM	**PEP**[¥]

Table 3 (II). Currently approved passive immunotherapies for viral infections

Disease **Etiologic agent**	**Antibody therapy**	**Administration**	**Indication**
Varicella VZV	**VZIG** Human varicella-zoster immunoglobulin **IVIG**	IM IV	**PEP** -Significant exposure to high-risk individuals*
HIV	**Ibalizumab** (Trogarzo®) Humanized IgG4 mAb	IV	**Treatment**$^{\Sigma}$ -MDR HIV infection with treatment failure with current regimen

Legend: IV: intravenous; IM: intramuscular; PEP: Post-exposure prophylaxis; CI: contraindicated; MDR: multidrug resistant

*High risk individuals: infants <6 months of age, immunocompromised, susceptible pregnant women; **High-risk individuals: ≥40-60 years old, chronic liver disease, immunocompromised; $^{¥}$Currently licensed only in India; $^{\Sigma}$US FDA approval in 2018 (for use in adults in combination with other ARV drugs); not approved by EMA

References for Table 3: [19–28]

Antibody therapy, mainly in the form of human normal immunoglobulin for intravenous administration (IVIG), is used for a variety of non-infectious illnesses as replacement therapy (i.e., primary immunodeficiency syndromes [PID] with impaired antibody production, secondary immunodeficiencies [SID] in patients who suffer from severe or recurrent infections, ineffective antimicrobial treatment and specific antibody failure or serum IgG level of <4 g/L) and for immunomodulation (i.e., primary immune thrombocytopenia [ITP] in patients at high risk of bleeding, Guillain-Barré Syndrome [GBS], Kawasaki's disease, Chronic inflammatory demyelinating polyradiculoneuropathy [CIDP], multifocal motor neuropathy [MMN]).[29]

1.5. Passive immunotherapies for epidemics of emerging viral diseases

Both the emergence of new pathogens and the increasing number of immunocompromised individuals in the last decades have renewed interest in passive immunotherapy. Since 1918 H1N1 pandemic, antibody-based therapeutics have been proposed as potential treatments for several outbreaks, epidemics, and pandemics of emerging viral diseases, for which there is no therapy, with mixed efficacy results[8] (**Table 4**).

1.5.1. Influenza

First, during the Spanish flu H1N1 pandemic in 1918, convalescent human blood products (whole blood, plasma, or serum) were transfused with the aim to reduce morbidity and mortality in hospitalized patients.[7] A meta-analysis of eight studies (none of which were blinded, randomized, or placebo-controlled trials) involving 1,703 patients suggested that transfusion with influenza-convalescent human blood products was effective in reducing mortality in hospitalized patients with H1N1 influenza complicated by pneumonia.[30] Moreover, efficacy was highest when treatment was administered early (<4 days of pneumonia complications).

Immune plasma was administered to a patient in China with severe H5N1 influenza-pneumonia and multiorgan failure in 2006 with a favourable outcome.[31]

Following the re-emergence of H1N1 influenza in 2009, the production and use of CP was included in the pandemic preparedness measures of the World Health Organization (WHO),[32] especially as an urgent response in settings where vaccination and/or effective antiviral therapy were lacking. A cohort study evaluated the efficacy of CP for influenza A (H1N1) and found an association with a reduction in mortality, viral load, and serum cytokine response.[33] However, mortality in the control arm was remarkably higher than previously reported, precluding accurate estimates of the impact of CP on changes in mortality trends. A more recent randomized controlled trial conducted in 2019 at 31 US medical centres showed no benefit of high-titre anti-influenza plasma (2 units of CP with HAI antibody titres ≥1:80) in reducing mortality when administered to hospitalized children and adults with severe influenza A.[34] An explanation for the lack of benefit of CP in this trial could be that participants included had advanced disease at the time of antibody administration.[8]

Aside from CP, at least eight mAb products have been tested in clinical trials for the treatment of influenza, with mixed and inconclusive results regarding their efficacy.[35]

1.5.2. Argentine haemorrhagic fever

For Argentine haemorrhagic fever, a zoonotic infectious disease caused by Junín virus, passive immunotherapy was associated with a reduction in mortality. Thus, current standard of care consists of the early transfusion of CP in standardized high doses of neutralizing antibodies.[36]

1.5.3. Respiratory syncytial virus

Respiratory syncytial virus immune globulin intravenous (RSV-IGIV, RespiriGam) was approved by the FDA for use in the prevention of severe RSV infections in high-risk infants and children aged up to 24 months following two studies that showed a 40 to 65% reduction in hospitalization rates. However, RSV-IVIG was replaced by the monoclonal antibody palivizumab in 2004, which was approved in over 45 countries for the immune-prophylaxis of RSV of high-risk children.[37,38] Recently, a new long-acting mAb, Nirsevimab (Beyfortus®), has been approved in the EU, the UK and by the EMA for the prevention of RSV lower respiratory tract disease in neonates and infants during their first RSV season.[39]

1.5.4. SARS and MERS

CP and hIVIG were used to treat severe acute respiratory syndrome (SARS) during the 2003 SARS outbreak,[40–42] with promising preliminary results. However, conclusive evidence from randomized clinical trials regarding the treatment efficacy is lacking.

CP was infused for treating Middle East respiratory syndrome (MERS) during the outbreak in Korea in 2015, with no beneficial effects and several challenging points reported.[43]

1.5.5. Ebola

In the wake of several outbreaks of Ebola virus disease (EVD) in many African countries, the WHO prioritized the evaluation of passive immunotherapies. A nonrandomized study conducted in Guinea in 2016 revealed that the transfusion of up to 500 ml of CP (with unknown levels of neutralizing antibodies) within two days of diagnosis in 84 patients with confirmed EVD was not associated with a significant improvement in survival.[44]

Low antibody content could have contributed to the negative results. In 2018, a randomized clinical trial conducted in the Democratic Republic of Congo assessing the efficacy of four investigational therapies reported a reduction of mortality with the mAb products Mab114 (Ansuvimab, Ebanga®) and REGN-EB3 (Inmazeb®), compared to the mAb cocktail ZMapp and the antiviral drug remdesivir.[45] Following the results of this trial, both monoclonal human IgG1 products were approved in late 2020.

Table 4 (I). Passive immunotherapies for epidemics of emerging viral diseases

Disease Etiologic agent Epidemics	Antibody therapy	Indication	Efficacy	Type of study Sample size References
Influenza H1N1 Pandemic 1918	**CP** 125-250 ml 1 or 2 units	Hospitalized patients with influenza complicated pneumonia	Mortality reduction (21%)	Meta-Analysis n=1,703 Luke TC et al., 2006[30]
Influenza Avian H5N1 Outbreak 2006	**CP** 200 ml nAb titre 1:80	Hospitalized patients	Inconclusive evidence	Case report n=1 Zhou et al., 2007[31]
Influenza H1N1 Pandemic 2009	**CP**	Hospitalized patients	Mortality reduction (63%)	Cohort study n=93 Hung et al., 2011[33]
Influenza H1N1 Outbreak 2019	**CP** 2 units HAI antibody titres ≥1:80	Hospitalized adults and children with severe influenza A	No statistically significant mortality reduction	RCT n=200 Beigel et al., 2019[34]
Argentine Haemorrhagic Fever Junin virus Outbreaks	**CP** nAb titre 3500/kg	Within 8 days of symptom onset	Mortality reduction (93%)	RCT n=217 Maiztegui et al., 1979[46]; Enria et al., 1994[47]; Enria et al., 1984[48]

Table 4 (II). Passive immunotherapies for epidemics of emerging viral diseases

Disease Etiologic agent Epidemics	Antibody therapy	Indication	Efficacy	Type of study Sample size References
Respiratory syncytial virus RSV Outbreaks	**RSV-IVIG** nAb titres 1:2048 to 1:8102	Adult and paediatric bone marrow transplantation with RSV pneumonia	Inconclusive evidence	Observational n=19; n=11 Whimbey et al., 1995[49]; DeVincenzo et al., 2000[50]
	Palivizumab (Synagis®) mAb humanized IgG1	PrEP High-risk neonates	Hospitalization reduction	Meta-Analysis n=2,831; n=15,000 Andabaka et al., 2013[51]; Checchia et al., 2011[52]
	Nirsevimab (Beyfortus®) mAb humanized IgG1k long acting	PrEP Neonates and infants during their first RSV season	Hospitalization reduction	RCTs n=1,453; n=1,490 Griffin et al., 2020[53]; Hammitt et al., 2022[54]
SARS SARS-CoV Outbreak 2003	**CP** **IVIG**	Hospitalized patients	Inconclusive evidence	Systematic review n=NA Stockman et al., 2006[55]
MERS MERS-CoV Outbreak 2015	**CP**	Hospitalized patients	Inconclusive evidence	Observational n=3 Ko et al., 2017[43]

Table 4 (III). Passive immunotherapies for epidemics of emerging viral diseases

Disease Etiologic agent Epidemics	Antibody therapy	Indication	Efficacy	Type of study Sample size References
Ebola EBOV Outbreak 2014-2016 Outbreak 2018-2019	**CP** nAbs unknown titres	Hospitalized patients within 2 days from diagnosis	No mortality reduction	Trial (non-randomized) n=84 van Griensven et al., 2016[44]
	REGN-EB3 (Inmazeb®) Human IgG1; 3 mAb cocktail: atoltivimab, maftivimab, odesivimab-ebgn **Ansuvimab** (Ebanga®; mAb114) Human IgG1; single mAb	Hospitalized patients	Mortality reduction (compared to ZMapp and remdesivir)	RCT n=681 Mulangu et al., 2019[45]

Legend: CP: Convalescent plasma; ml: millilitres; nAb: neutralizing antibody; HAI: Hemagglutination Inhibiting Antibody; RCT: randomized clinical trial; mAb: monoclonal antibody; IgG: immunoglobulin G; PrEP: pre-exposure prophylaxis; IVIG: intravenous immunoglobulin; SARS: severe acute respiratory syndrome; SARS-CoV: severe acute respiratory syndrome coronavirus; MERS: Middle East respiratory syndrome; MERS-CoV2: Middle East respiratory syndrome

2. Coronavirus disease 2019 (COVID-19)

2.1. Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, a novel coronavirus that was first identified in December 2019 in Wuhan, a city in the Hubei Province of China, as the cause of a cluster of pneumonia cases. The virus rapidly spread, resulting in an epidemic throughout China, followed by a global pandemic. The WHO declared a Public Health Emergency of International Concern (PHEIC) in January 2020, and characterized the outbreak as a pandemic in March 2020.[56] On May 2023, more than three years into the pandemic, the WHO declared an end to the COVID-19 PHEIC.[57]

2.2. Virology

SARS-CoV-2 belongs to the family of coronaviruses, which are important human and animal pathogens, known to cause illnesses ranging from common cold to severe diseases, such as MERS and SARS. SARS-CoV-2 is a single-stranded, positive-sense RNA (+ssRNA) virus, which belongs to lineage B of the genus *Beta-coronavirus* in the *Coronaviridae* family.[58]

SARS-CoV-2 presents four major structural proteins: spike glycoprotein (S), nucleocapsid protein (N), membrane protein (M) and envelope protein (E). The S protein can be further separated into S1 and S2 subdomains, with S1 binding the host receptor and S2 mediating membrane fusion.[58] SARS-CoV-2 can enter the host cell by two different ways: (1) receptor-mediated plasma membrane fusion and (2) receptor-mediated endocytosis. Receptor proteins on the host cell surface are crucial for virus attachment to host cells for both fusion and endocytosis.[58,59] The host receptor for SARS-CoV-2 cell entry is the angiotensin-converting enzyme 2 (ACE2), which is widely expressed in the cells of the lung, intestine, liver, heart, vascular endothelium, testis, and kidney. SARS-CoV-2 binds to ACE2 through the receptor-binding domain (RBD) of its spike protein (S).[59] After RBD-ACE2 interaction, the S protein undergoes proteolytic cleavage, that is catalysed by several host proteases, including furin, TMPRSS2, and cathepsin B/L. Depending on the entry route taken by SARS-CoV-2, the S2' site is cleaved by different proteases: transmembrane protease (TMPRSS2) at the cell surface and cathepsins

following endocytosis. Proteolytic processing activates the S protein and allows viral-host membrane fusion, followed by the release of viral RNA into the host cytoplasm, where viral RNA replicates and assembles new viral particles.

2.3. Epidemiology

Since the first reports of cases from Wuhan, over 750 million confirmed cases and over 6.9 million deaths have been reported globally.[60] Initially, there was a very rapid transmission of SARS-CoV-2 and high mortality, as a result of a very effective respiratory transmission, viral virulence and susceptibility of the population, as well as a shortage of preventive medical devices, limited availability of diagnostic tests, and lack of effective treatments and vaccines. Most affected countries went into lockdown to prevent largescale transmission within the region and to other regions. The COVID-19 pandemic has caused multiple epidemic waves of confirmed cases and deaths worldwide, which have varied widely among different countries and regions in terms of number and severity of cases, as well as overall fatality rate. The impact and distribution of these waves have depended on several factors, such as public health measures and interventions, including lockdowns, the use of novel therapeutic tools, and vaccination policies, as well as the prevalence of the primary viral variant. The most important public health measure to mitigate the pandemic has been vaccination, which began by the end of 2020, and has resulted in a remarkable reduction in number of cases, hospital and ICU admissions and deaths.

2.4. Transmission

The primary mode of transmission of SARS-CoV-2 is respiratory transmission via respiratory particles or droplets (direct person-to-person) and, less commonly, via aerosols (longer distances). While direct contact and fomite transmission are presumed, they are likely to represent unusual modes of transmission. The role of other modes of transmission, such as transplacental (vertical), fecal-oral, sexual, and blood-borne transmission are thought to be rare or uncertain.[61] Risk of transmission varies by the type and duration of exposure, the use of preventive measures, individual index case factors, such as the viral load in respiratory secretions,[62] viral factors, such as different variants, and individual contact factors, such as vaccination status. Transmission dynamics are heterogeneous, and reported evidence has suggested that superspreading events (events

with persons in close proximity in indoor settings with poor ventilation for extended periods) have had a major role in sustaining the epidemic.

2.5. Pathogenesis

COVID-19 pathogenesis starts with an early viral phase, characterized by SARS-CoV-2 virus replication and variable symptoms, which triggers an endogenous antibody response around days 10-12 of infection. The viral phase can progress to a life-threatening inflammatory phase, that may clear the virus but impairs pulmonary gas exchange and, in some cases, causes respiratory failure and death.

2.6. Immune responses following infection

SARS-CoV-2 infection induces protective SARS-CoV-2 specific antibodies and cell-mediated responses. Regarding humoral immunity, most patients develop detectable serum antibodies to the RBD of the viral S protein within 1-2 weeks after symptom onset and neutralizing titres peak over day 23. A higher magnitude of antibodies has been associated with increased disease severity. After clinical recovery, neutralizing antibodies decline over time, although neutralizing activity has been detected up until 12 months after infection. Memory B cells specific to the spike- and receptor-binding domain that increase over time, along with spike protein-specific plasma cells have been identified in the few months following infection. This indicates the possibility of a durable long-term memory humoral response. Regarding cell-mediated immunity, evidence indicates the development of SARS-CoV-2 specific CD4 and CD8 T cell responses in patients recovered from COVID-19 and in vaccinated individuals, suggesting the potential for durable T cell immune response.[63,64]

2.7. Clinical features

The clinical spectrum of SARS-CoV-2 infection ranges from asymptomatic infection to critical and fatal disease. The proportion of asymptomatic infections is uncertain and has varied in the context of different variants and vaccination uptake. Most symptomatic infections are mild, and most common symptoms include cough, myalgias, headache, fever, fatigue, sore throat, diarrhoea, mild upper respiratory symptoms (e.g., nasal congestion, sneezing), and loss of smell and taste. Severe disease presents with dyspnoea, pneumonia with bilateral infiltrates and hypoxia, followed by respiratory failure, shock or multiorgan dysfunction in some cases. Acute respiratory distress syndrome (ARDS) is

the major complication in patients with severe COVID-19, and other complications of severe illness include thromboembolic events, acute cardiac injury, kidney injury, and inflammatory complications. Persistent symptoms following acute COVID-19 have also been described and characterized in the long COVID-19 syndrome, which includes fatigue, dyspnoea, chest pain, cough, musculoskeletal pain, and psychological and cognitive symptoms, among others.

The incubation period from the time of exposure until the onset of COVID-19 symptoms is three to five days on average and up to 14 days and depends on the variant. For instance, the median incubation period for the Omicron variant appears to be slightly shorter, around three days.

2.8. Risk factor for severe COVID-19 outcomes

Age is one of the strongest risk factors for severe COVID-19 outcomes, with risk rising markedly with increasing age, and risk elevating substantially at ages >65 years. Being unvaccinated or not up to date with COVID-19 vaccinations also increases the risk. Other risk factors include asthma and chronic lung disease, heart conditions, obesity, cancer, chronic kidney disease, chronic liver disease, diabetes mellitus, and cerebrovascular disease.[65] A higher risk of severe COVID-19 outcomes is associated with a variety of immunocompromising conditions, including HIV, primary immunodeficiencies, solid organ or blood stem cell transplantation, use of corticosteroids or use of other immunosuppressive medications. Some immunodeficiencies seem to increase the risk above and beyond traditional risk factors, including individuals who make autoantibodies to type I interferons and use of T cell-depleting or T-cell suppressing agents (e.g., antithymocyte globulin, calcineurin inhibitors, mycophenolate mofetil, belatacept) or B cell-depleting agents (e.g., rituximab, ocrelizumab, obinutuzumab). Prolonged shedding of SARS-CoV-2 has also been reported in patients who are immunocompromised.

Some laboratory variables have also been associated with more severe COVID-19 outcomes, including a low lymphocyte count, and elevations in D-dimer, C-reactive protein (CRP), lactate dehydrogenase (LDH), troponin and ferritin, among others. However, the prognostic value of these laboratory features has not been definitively established.

2.9. Diagnostic

Diagnostic tests to detect SARS-CoV-2 infection comprise two main types: viral tests and antibody or serology tests. Viral tests are used to detect current infection and include nucleic acid amplification tests (NAATs), such as PCR test, and antigen tests, whereas serology tests (antibody detection) are used for the diagnosis of prior infection or vaccination. NAATs and antigen tests are performed using respiratory tract specimens, mainly nasopharyngeal swabs and nasal swabs, but nasal or nasopharyngeal washes, oropharyngeal swabs and saliva are also included as recommended specimen types for diagnosis in current guidelines.[66] Sensitivity and specificity vary between different tests and different settings,[67] and clinical performance depends on the type and quality of the specimen and the duration of illness at the time of testing. Testing has been used for diagnostic of symptomatic individuals and close contacts and screening of selected asymptomatic individuals. Diagnostic tests, testing strategies, criteria and priority for testing and guidelines have been adapted throughout the pandemic, according to newly generated evidence and the epidemiological situation.[68]

2.10. Variants of concern

SARS-CoV-2 has been spreading globally and has consistently mutated over the course of the pandemic, resulting in variants that are different from the original SARS-CoV-2 virus. Some variants (variants of concern) have been important because of an increased transmissibility, greater risk of severe disease, significant immune evasion, or reduction in neutralization by antibodies generated during previous infections or vaccination, and reduced effectiveness of treatments or vaccines. Different variants of concern identified include Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2) variants. Since 2022, Omicron (B.1.1.529) variants, including evolving sublineages (e.g., BA.1, BA.2, B1.4, BA.5, BQ.1, BQ.11, BF.7, BA.2.75, XBB, XBB.1, XBB.1.5) have been the predominant circulating variants globally.[69]

2.11. Prevention

Prevention strategies that have been recommended during the COVID-19 pandemic in the setting of community transmission of SARS-CoV-2 include early identification, early diagnosis and early isolation of cases, mask use, hand washing and respiratory hygiene,

adequate ventilation of indoor spaces, social distancing, screening in selected high-risk settings, and vaccination.

2.12. Vaccination

COVID-19 vaccines have been essential for reducing the spread and the severity and mortality caused by COVID-19. Development of effective vaccines has been extremely accelerated, as a major international response to address the COVID-19 pandemic. The high efficacy of several COVID-19 vaccines in preventing symptomatic SARS-CoV-2 infections was found in large-scale phase III trials and each vaccine that has received emergency use listing by the WHO has gone through the standard preclinical and clinical stages of development, with stringent safety criteria.

Currently, several COVID-19 vaccines are available globally. COVID-19 vaccines have been developed using several different platforms, such as (1) inactivated virus (e.g., CoronaVac Sinovac, BBIBP-CoV/HB02 Sinopharm, Covaxin Biotech) or (2) live attenuated viruses (COVI-VAC), (3) recombinant proteins (e.g., Novavax, HIPRA), (4) vector virus (e.g., Janssen/Johnson & Johnson, AstraZeneca, Sputnik V), and (5) mRNA (e.g., Pfizer-BioNTech, Moderna) and (6) DNA vaccines (e.g., Zydus Cadila)[70]. Some of these platforms are traditional approaches (e.g., inactivated and attenuated viruses), while some others are new approaches that had never previously been used in a licensed vaccine, such as mRNA and DNA vaccines. The major antigenic target for COVID-19 vaccines is the surface spike protein.

Immunogenicity and effectiveness of COVID-19 vaccines appear lower in immunocompromised individuals compared with the general population. In this context, specific vaccination guidance for this population have been proposed.[71]

Initially, vaccination was prioritized for high-risk individuals, such as older people, health care workers, and individuals with chronic diseases, according to the availability of vaccines. Later, vaccination was proposed to the general population, achieving very high rates of vaccination worldwide. More recently, original (monovalent) vaccines have been updated (bivalent vaccines) to protect against both the original virus and the Omicron variant BA.4 and BA.5. Since the beginning of vaccination campaign, as of 30 May 2023, a total of 13 billion vaccine doses have been administered globally.[60]

2.13. COVID-19 treatments

At the beginning of the COVID-19 pandemic, the urgency for medical treatments spread the compassionate-use of unproven medicines based on the rationale for potential efficacy. Many agents with antiviral effect *in vitro* against SARS-CoV-2, such as lopinavir-ritonavir, hydroxychloroquine, azithromycin and ivermectin, were empirically used until evidence from randomized clinical trials demonstrated their lack of efficacy.[72,73]

Several RCT have been conducted worldwide, aiming to evaluate the efficacy of antiviral and immunomodulatory drugs in both hospitalized and nonhospitalized COVID-19 patients. However, only a few treatments have shown to improve clinical outcomes and are currently approved for use as COVID-19 treatments. Remdesivir is the only antiviral drug that is approved by the FDA for the treatment of COVID-19, currently recommended for the management of hospitalized and nonhospitalized patients after showing a reduction in time to recovery and hospitalization, respectively, in RCT.[74,75] Ritonavir-boosted nirmatrelvir (Paxlovid) and molnupiravir have received EUAs from the FDA for the treatment of mild to moderate COVID-19 in high-risk individuals. Both antiviral drugs have shown 89% and 31% reduction of hospitalization or death in COVID-19 outpatients, respectively.[76,77] Moreover, these antivirals have shown to retained *in vitro* activity against omicron variant and its subvariants.[78,79] Some immunomodulatory agents are currently recommended for the treatment of hospitalized patients. Multiple RCT have shown that systemic corticosteroids improve clinical outcomes and reduce mortality in hospitalized patients with COVID-19 who require supplemental oxygen.[80–82] The anti-IL-6 receptor mAbs tocilizumab and sarilumab have also been assessed as treatment for hospitalized COVID-19 patients who had systemic inflammation (rapidly increasing oxygen needs and systemic inflammation despite dexamethasone use) showing improvement of survival.[83–85] Janus kinase (JAK) inhibitors, such as baricitinib and tofacitinib, have shown a reduction of mortality when use for treatment of hospitalized COVID-19 patients who require conventional oxygen, high-flow nasal canula, NIV, or mechanical ventilation.[86–88] Some repurposed drugs that showed antiviral or immunomodulatory activity for COVID-19 *in vitro* have also been evaluated in RCTs,

with mixed or inconclusive efficacy results, such as metformin, colchicine, fluvoxamine, and inhaled budesonide.[89–95]

3. Passive immunotherapy for COVID-19

Key messages Panel 3:

- Passive immunotherapy has been used to try to prevent disease progression and mortality since the start of the COVID-19 pandemic, when effective therapies were not yet available.
- The COVID-19 pandemic has represented the first large-scale opportunity to comprehensively evaluate the efficacy of passive immunotherapies through an evidence-based medicine approach.
- Before initiating the studies integrated into this thesis, there existed a scarcity of evidence on passive immunotherapy for COVID-19.
- To better contextualize the results of this thesis, the introduction will comprehensively discuss the existing evidence up to the time of composing this thesis report.
- Several trials have investigated the use of COVID-19 convalescent plasma (CCP) for both in-patients and out-patients with COVID-19, yielding mixed results and efficacy notably driven by early administration of products with high antibody titres.
- Very few studies have evaluated the efficacy of hIG for COVID-19, yielding inconclusive results.
- Several trials have demonstrated the efficacy of mAbs for the treatment of COVID-19 outpatients and for prophylaxis, as well as for selected groups of hospitalized patients prior to the advent of Omicron variant.
- Evidence generated from the studies contained in this thesis add to the existing body of knowledge on CCP and hIG for COVID-19, and might be relevant for future viral epidemics.

3.1. COVID-19 convalescent plasma

3.1.1. Overview

CP has the ability to be used as a first-line response, as soon as there are convalescing survivors. Thus, COVID-19 convalescent plasma (CCP) was firstly deployed in March 2020 in countries that experienced the early waves of COVID-19, such as China and Italy.[96–98] Since then, it has been widely available for COVID-19 patients, mainly through extended and compassionate use.

3.1.2. Mechanism of action

The postulated primary mechanism for the clinical benefit of CCP is through a direct antiviral effect by SARS-CoV-2 neutralization, which occurs when antibodies bind to the spike protein, thereby preventing its binding to the host cell receptors. In addition to the direct antiviral effects, non-neutralizing Fc-mediated functions, such as antibody-dependent cellular cytotoxicity, phagocytosis and complement activation, are also thought to play a role.[99] Moreover, CCP also contains other proteins such as anti-inflammatory cytokines, clotting factors, natural antibodies, defensins and pentraxins that could have an additional immunomodulatory effect.[100] Recent work suggests that antibody-mediated antigen catalysis of the spike protein is another mechanism by which CCP can neutralize SARS-CoV-2, and that this phenomenon could be durable despite antigenic drift of viral variants.[101]

3.1.3. Preclinical studies in animal models

Preclinical studies in hamster model have shown that CCP with high neutralizing antibody titres administered one day after infection significantly reduced SARS-CoV-2 replication in the lungs and the incidence and severity of pneumonia.[102]

Mixed results have been obtained in therapeutic studies with CCP in nonhuman primates. A macaque model study found that passive transfer of IgG from convalescent rhesus macaques could provide protection against SARS-CoV-2 infection, as well as therapeutic efficacy following viral challenge, in a dose-dependent manner. However, the study showed therapeutic benefit only when using high serum neutralizing antibody (nAb) titres, potentially at levels that exceed typical serum nAbs achieved in human recipients of convalescent human plasma.[103] Another study treating rhesus macaques with pooled

human CCP with moderate antibody titre infused one day after virus inoculation failed to reduce virus replication.[104] In contrast, administration of high-titre CCP from convalescent African green monkeys to African green monkeys 10 hours after the challenge of virus inoculation showed benefits in reducing the severity of virus-associated lung pathology and reductions in coagulopathy and inflammatory processes.[105] Another study tested a pooled very-high neutralizing antibody titre (RVPN NT50 value of 3,003) human CCP administered to rhesus macaques one day after infection and compared it to animals treated with pooled control plasma. Results showed low levels of antiviral antibodies achieved in treated animals, leading to minimal effects on reduction of viral replication in the upper and lower respiratory tract secretions, but a significant effect on the reduction of lung inflammation, as measured by lung histology. Importantly, CCP therapy was inferior compared to a monoclonal antibody-based therapy previously tested in the same animal model under the same experimental conditions.[106]

3.1.4. Human clinical observational studies

Several observational studies have assessed the safety and efficacy of CCP for the treatment of COVID-19 in patients across all stages of the disease.

Regarding safety, a first analysis of 5,000 hospitalized adults with severe or life-threatening COVID-19 patients treated with CCP as part of the Expanded Access Program (EAP) showed a low incidence of serious adverse events in the first 4 hours after transfusion.[107] A second analysis including the first 20,000 patients treated as part of the EAP confirmed the safety of CCP, showing a low rate of serious adverse events (SAE) (i.e., <1% transfusion reactions, <1% thromboembolic events, 3% cardiac events), most of which were considered unrelated to the plasma transfusion and related to severe COVID-19.[108] The observed rate of SAE was similar between the infusion of CCP and the conventional plasma that is commonly transfused to severely ill patients without COVID-19.

Regarding clinical efficacy, a report from the EAP described a modest survival benefit at 30 days following infusion associated with early use of CCP with high anti-SARS-CoV-2 IgG titres in hospitalized patients not receiving mechanical ventilation.[109] The retrospective study included 3,082 patients and antibody titres were measured by the Ortho VITROS IgG assay, with cutoff >18.45 of antibody levels categorizing as high

(80th percentile of the distribution for the signal-to-cutoff ratio). 7-day mortality in a subset of non-intubated patients younger than 80 years old and treated within 72 hours after diagnosis was 6.3% in those receiving high-titre CCP vs. 11.3% in those receiving low-titre CCP.[109] A very large retrospective study from 176 community hospitals affiliated with Hospital Corporation of America (HCA) Healthcare that included 44,770 patients hospitalized with COVID-19 (3,774 treated with CCP and 10,687 matching patients receiving standard treatment) confirmed substantial mortality reduction in hospitalized patients receiving CCP within 3 days of admission (aHR 0.71, $p<0.001$).[110] Several other observational and non-randomized studies have been published, some of which have shown signals of efficacy with early, high-titre CCP, or in specific subgroups.[111–116] However, results have been variable, and minimal or no benefit has been seen in late disease once respiratory failure has progressed to the stage of requiring mechanical ventilation/intubation. No observational studies have investigated the benefits of CCP in outpatients.

3.1.5. Randomized clinical trials

Available evidence on the efficacy of CCP for COVID-19 before the design and implementation of the first study included in this thesis (COnV-ert trial) consisted of only few observational studies and one randomized controlled trial (RCT) conducted in China.[98] The promising efficacy results observed in those studies, along with preclinical data and the previous FDA authorization for emergency use of CCP for patients with severe or life-threatening COVID-19, provided the rationale for conducting our study.

Altogether, cumulative evidence from RCT of CCP for COVID-19 available by the time of writing this thesis report shows highly variable efficacy results, resulting in uncertainty. Efficacy has been driven by early administration after disease onset and use of high-titre antibody content. Evidence from RCT will be summarized according to hospitalization status and presence of immunosuppression at the time of infusion.

3.1.5.1. Hospitalized patients (inpatients) that are immunocompetent

As previously mentioned, evidence before the studies included in this thesis consisted of a single RCT evaluating the efficacy of CCP in 103 hospitalized patients with severe and life-threatening COVID-19. Although the study found no differences in clinical

improvement at day 28 in the overall study population, a benefit in clinical improvement in the subset of severe COVID-19 patients and a reduction of viral load were observed.[98]

Currently, more than twenty randomized clinical trials have been reported assessing the efficacy of CCP in hospitalized patients worldwide. These trials have presented relevant differences in variables associated with efficacy, including: (1) the time of administration of CCP (ranging from 4 to 14 days from symptom onset, and sometimes measured from date of hospital or ICU admission instead), (2) the disease severity of participants at baseline (measured by the WHO clinical progression scale score and ranging from 4 - hospitalized with no oxygen requirements- to 9 - hospitalized with mechanical ventilation requirements), (3) the comparator used (standard of care, non-convalescent plasma or saline as placebo), (4) the volume of CCP infused (reported as total volume, number of units and time between infusion of different units), (5) the titres of total IgG antibodies and neutralizing antibodies contained in the CCP (low, high and non-available titres), (6) the assays used to measure antibody titres (several different neutralization assays and binding antibody immunoassays), and (7) the primary outcomes measured (e.g., mortality, clinical improvement, disease progression, oxygen requirements, WHO clinical progression scale, viral load). Overall, most trials have found no survival benefit for CCP in hospitalized patients with COVID-19 (**Table 5**). However, some signals of efficacy have been observed in some of the studies in subgroups of participants with less severe COVID-19 or receiving an earlier administration of CCP from symptoms onset, and when using CCP with higher titres of neutralizing antibodies, as well as in subsets of immunosuppressed individuals.

The results of RECOVERY, the largest clinical trial of CCP for any infectious indication, showed no evidence that high-titre CCP improved survival or other prespecified clinical outcomes in patients hospitalized with COVID-19. Moreover, results were consistent across subgroups of age, sex, duration of symptoms to randomization, baseline serostatus, levels of respiratory support received at baseline, and use of corticosteroids.[117] Results from the other two largest randomized controlled trials that assessed CCP in hospitalized patients (CONCOR-1[118] and REMAP-CAP[119]) are consistent with evidence reported from RECOVERY, showing no reduction of disease severity and mortality with high-

titre CCP in hospitalized patients with COVID-19. Limitations of these studies are that all three were open-label trials that were stopped early due to futility.

All RCT of CCP in hospitalized patients have the drawback of using this antibody therapy at a late stage of disease when it is less likely to be effective, when most patients have developed an endogenous antibody response and/or when viral replication does not dominate. Moreover, some of these studies did not measure antibody titres or used CCP that contained low titres.

Table 5 (I). RCTs of CCP in hospitalized immunocompetent patients

Sample size (Rand.)	Days from symptoms to randomization (median)	Baseline 10-point WHO score	Control arm	CCP volume IgG titer nAbs titer	Efficacy results	RCT identifier Reference
49 (1:1)	<3 (from hosp)	5 - 7	SOC	400 ml >1.25 (ELISA) NA	Reduction in duration of infection by 4 days (p<0.05) Signal of benefit in survival (4.8% vs 28.6%, p>0.05)	Rasheed et al., 2020[120]
80 (1:1)	13	5 - 6	SOC	400 ml NA NA	No reduction of mortality (HR 0.67, p=0.34)	Ray et al., 2022[121]
103* (1:1)	30	5 - 7	SOC	200 ml ≥1:640 ≥1:40	No difference in clinical improvement at day 28 (HR 1.4, p=0.26) or mortality (15.7% vs 24%, OR 0.59, p=0.3) Benefit in clinical improvement in severe COVID-19 (HR 2.15, p=0.03)[¥]	Li et al., 2020[98]
86* (1:1)	10	5 - 7	SOC	300 ml 15.08 (median) 1:160 (PRNT50) (median)	No reduction of mortality (14% vs 26%, OR 0.47, p>0.05)	**ConCOVID** Gharbharan et al., 2021[122]
464 (1:1)	6	4 - 5	SOC	400 ml NA 1:40 (median)	No reduction of progression to severe disease or mortality (19% vs 18%, RR 1.04, p>0.05)	**PLACID** Agarwal et al., 2020[123]
350 (1:1)	6	4 - 5	SOC	200-300 ml (MB, riboflavin, psoralen) 8.2 (median) (VITROS) 1:157 (ID50) (median)	No difference in survival at day 14 (HR 0.46, p=0.09) Benefit in preventing progression or death at 28 days (p=0.02)[¥]	**ConPlas-19** Avendaño-Solà et al., 2021[124]
58 (1:1)	6	4 - 6	Early vs late CCP	400 ml ≥1:100 (median) ≥1:160	No reduction of mortality at day 30 (32.1% vs 33.3%, OR 0.95, p>0.999)	Balcells et al., 2021[125]
333 (2:1)	8	5 - 6	Normal saline + SOC	500 ml 1:3200 (median) 1:300 (IC50) (median)	No difference in clinical outcomes in ordinal scale (OR 0.83, p=0.46) No reduction of mortality (10.96% vs 11.43%)	**PlasmAr** Simonovich et al., 2021[126]
40 (1:1)	NA	4 - 5	SOC	400 ml (MB) NA NA	No reduction of mortality at day 28 (RR 0.67, p=0.72)	AlQahtani et al., 2021[127]

Table 5 (II). RCTs of CCP in hospitalized immunocompetent patients

Sample size (Rand.)	Days from symptoms to randomization (median)	Baseline 10-point WHO score	Control arm	CCP volume IgG titer nAbs titer	Efficacy results	RCT identifier Reference
11,558** (1:1)	9	4 - 7	SOC	275±75ml (median) (81% 2 units from 2 donors; 12% 1 unit) IgG ≥6 (EUROIMMUN)	No reduction of mortality at day 28 (24% vs 24%, RR 1.00, *p*=0.95) No differences in mortality in baseline seronegative¥	**RECOVERY** Abani et al., 2021[117]
940** (2:1)	8	4 - 6	SOC	500 ml NA 1:250	No reduction of intubation or death at day 30 (32.4% vs 28%, RR 1.16, *p*=0.18)	**CONCOR-1** Bégin et al., 2021[118]
2,011**	≤3 (from ICU)	5 - 6	SOC	550 ± 150 ml NA ≥1:80 – 1:160	No difference in organ support-free days, mortality (37.3% vs 38.4%), or median number of days alive at day 21	**REMAP-CAP** Abdelhady et al., 2021[119]
223 (2:1)	9	4 - 7	Non-CP + SOC	200 - 250 ≥1:400 1:160 (median)	No improvement in clinical status at day 28 (OR 1.5, *p*=0.18) Reduction of mortality at day 28 (12.6% vs 24.6%, OR 0.44, *p*=0.034)	O'Donell et al., 2021[128]
190 (2:1)	12	6 - 7	SOC + IVIG 1.5 mg/dl	400 ml NA NA	No reduction of mortality at day 28 (46.2% vs 43%, *p*=0.75)	Gonzalez et al., 2021[129]
74* (4:1)	9	NA	Non-CP + SOP	400 ml NA 1:526 (median)	No difference in ventilator-free days (*p*=0.86) or mortality at day 28 (27% vs 33%, *p*=0.63)	Bennett-Guerrero., 2021[130]
105 (1:1)	7	4 - 7	SOC	850 ml NA 1:160 (PRNT50)	No difference in survival (43.4% vs 32.7%, *p*=0.32) Benefit with higher nAbs¥	**CAPSID** Körper et al., 2021[131]
160 (1:1)	10	5 - 7	SOC	600 ml NA >1:80	No difference in clinical improvement on day 28 (61.3% vs 65%) or mortality at day 28	**PLACOVID** Sekine et al., 2022[132]

Table 5 (III). RCTs of CCP in hospitalized immunocompetent patients

Sample size (Rand.)	Days from symptoms to randomization (median)	Baseline 10-point WHO score	Control arm	CCP volume IgG titer nAbs titer	Efficacy results	RCT identifier Reference
320 (2:1)	7	4 - 5	SOC	884 ml NA ≥1:320	No difference in mechanical ventilation (OR 0.99, *p*=0.98)	**DAWN-plasma** Devos et al., 2022[133]
31** (1:1)	<4	5	SOC	600 – 750 ml NA 1:116	No difference in median time of oxygen treatment among survivors (11 vs 7, *p*=0.4), or mortality (*p*=0.64).	**COP20** Holm et al., 2021[134]
80 (1:1)	6	4 - 5	SOC	400 -500 ml NA NA	Significant benefit by clinical severity score (*p*=0.04) and 28-day mortality (26% vs 5%, *p*=0.01).	**PennCCP2** Bar et al., 2021[135]
941 (1:1)	7	5	SOC	250 ml >12 (Ortho VITROS) (after Jan 2021) 1:93	No improvement in WHO ordinal scale at day 14 (OR 0.94, P[cOR<1]=72%) Benefit in participants enrolled after Jan 2021, receiving higher titer CCP P[cOR<1]=93%)[¥]	**CONTAIN** Ortigoza et al., 2021[136]
110* (1:2)	9	6 - 9	SOC	1,800 ml NA 1:120	No reduction in mortality at day 30 (22% vs 25%, or 0.84, *p*=0.81), requirement of invase ventilation, and duration of hospital stay	De Santis et al, 2022[137]
136*[Σ] (1:1)	7	4 -7	SOC	476 - 674 ml NA >1:80	No clinical improvement in the overall study population (HR 1.29; *p*=0.21) Shortened median time to improvement (HR 2.5, *p*=0.003) and improved survival (HR 0.28, *p*=0.042) in patients with cancer[¥]	Denkinger et al., 2022[138]

Legend: Rand.: randomization; CCP: COVID-19 convalescent plasma; IgG: immunoglobulin G; nAbs: neutralizing antibodies; RCT: randomized clinical trial; SOC: standard of care; ml: millilitres; NA: not available; HR: hazard ratio; OR: odds ratio; PRNT: plaque reduction neutralization test; RR: risk ratio; MB: methylene-blue; ID50: inhibitory dilution 50%; IVIG: intravenous immunoglobulin; non-CP: non-convalescent plasma.

*Early termination; **Stopped for futility; [¥]Sensitivity analysis

[Σ]Four risk groups were included: cancer (pre-existing or concurrent haematological cancer and/or receiving active cancer therapy for any cancer within the past 24 months) (n 56), immunosuppression (chronic immunosuppression, either pharmacological or due

to underlying diseases not meeting criteria for cancer) (n 16), laboratory-based risk factors (lymphopenia and/or elevated D-dimers) (n 36), advanced age (>75 years) (n 26)

Colour legend: Green: positive efficacy results in primary outcome(s); yellow: positive efficacy results in secondary outcome(s) or in subanalysis; red: negative efficacy results.

3.1.5.2. Non-hospitalized patients (outpatients) that are immunocompetent

No evidence on the efficacy of CCP for non-hospitalized patients with COVID-19 had been reported before the design and implementation of the first study included in this thesis, which was a RCT evaluating the efficacy of high-titre CCP for the early treatment of COVID-19 outpatients (COnV-ert trial).

By the time of writing this thesis report, five well-designed RCT (including the COnV-ert trial) have been reported evaluating the efficacy of CCP as early treatment in outpatients, prior to the emergence of the Omicron variants. One trial has evaluated the efficacy of CCP as post-exposure prophylaxis (PEP). Data from these trials are conflicting and summarized in **Table 6**.

Two RCT assessing the early treatment with CCP for outpatients with COVID-19 were published during the implementation of the COnV-ert trial and before its publication. The first trial, conducted in Argentina and published in February 2021, included 160 COVID-19 outpatients, aged 75 years and older.[139] CCP with high IgG titres (>1:1000) and administered within 72 hours of symptom onset was associated with a lower likelihood of progression to severe disease. The second RCT, which was conducted in the US and published in November 2021, included 511 high-risk outpatients with COVID-19 attending emergency rooms. CCP showed no benefit in preventing disease progression to severe disease when given at a median of 4 days after symptom onset.[140] The first study included in this thesis (COnV-ert trial), a RCT evaluating high-titre CCP for the treatment of COVID-19 outpatients, showed no reduction of hospitalization or viral load with the treatment of methylene blue-treated CCP with high titres of antibodies. Two more RCT were published after the publication of the COnV-ert trial. First, the CSSC-004 trial, which was conducted in the US and included 1,225 non-hospitalized adult (≥18 years)

patients with COVID-19 within 8 days from symptoms onset.[141] In this very large trial, the administration of CCP with high titres of IgG antibodies (>1:4860) was associated with a 54% relative risk reduction of disease progression leading to hospitalization. Second, a trial conducted in the Netherlands included 421 COVID-19 outpatients and found a reduction of disease progression that was not statistically significant.[142]

Differences in patient populations included, the placebo used (saline or non-convalescent plasma), and CCP manufacturing and testing methods may have contributed to the mixed outcomes. The emergence of SARS-CoV-2 variants and the widespread of vaccination further complicate the assessment of benefit from the use of CCP. The results of all these studies and their implications will be discussed in further detail in the discussion section, along with the results from the articles included in this thesis.

Table 6. RCTs of CCP in non-hospitalized immunocompetent patients

Sample size (Rand.)	Days from symptoms to randomization (median)	Population	Control arm	CCP volume IgG titer nAbs titer	Efficacy results	RCT identifier Reference
160* (1:1)	39.6 hours	≥75 years or 65-74 years + RF	Normal saline + SOC	250 ml >1:1000 NA	Reduction of severe respiratory disease (16% vs 31%; RR 0.52, *p*=0.03)	Libster et al., 2021[139]
511** (1:1)	4	≥50 years or ≥1 RF At emergency department	Normal saline (colored) + SOC	250 ml NA 1:641 ID50	No reduction of disease progression (30% vs 31.9%, RD 1.9, posterior probability of superiority of CCP 0.68) No reduction of disease progression excluding patients hospitalized during index visit (posterior probability of superiority of CCP 0.93)***	**C3PO** Korley et al., 2021[140]
376* (1:1)	4.4	≥50 years	Normal saline + SOC	200-300 ml (MB) ≥6 (EUROIMMUN) 1:1379 (ID50) 1:342 (IU/ml) (median)	No reduction of hospitalization at day 28 (12% vs 11%, RR 1.05, *p*=0-76) No reduction of viral load at day 7	**COnV-ert**[Σ] Alemany et al., 2021
1225* (1:1)	6	≥18 years	Non-CP	250 ml 1:14,580 ≥8 IU/ml	Reduction in hospitalization (2.9% vs 6.3%, RR 0.46, *p*=0.05)	**CSSC-004** Sullivan et al., 2022[141]
421* (1:1)	5	≥50 years and ≥1 RF	Non-CP	300 ml NA 1:386 IU/ml (median)	No improved disease (OR 0.86, 0.59-1.22) No reduction of hospitalization (4.8% vs 8.6%, HR 0.61, 0.28-1.34)	**COV-Early** Gharbharan et al., 2022[142]
180* (1:1)	PEP Exposed within 120 hs of infusion (median 2 days)	≥18 years Close contacts	Non-CP	1 unit >1:320 NA	No reduction in infection (14.8% vs 14.9%, RD 0.01, *p*=0.42) or symptomatic disease rate (7.4% vs 8%, RD 0.012) Not powered no show reduction in hospitalization	**CSSC-001** Shmuel Shoham et al., 2023[143]

Legend: Rand.: randomization; CCP: COVID-19 convalescent plasma; IgG: immunoglobulin G; nAbs: neutralizing antibodies; RCT: randomized clinical trial; RF: risk factors; SOC: standard of care; ml: milliliters; NA: not available; RR: risk ratio; ID50: inhibitory dilution 50%; MB: methylene-blue; IU: international units; non-CP: non-convalescent plasma; OR: odds ratio.
*Early termination; **Stopped for futility; ¥Sensitivity analysis; ΣThis trial is part of this thesis

Colour legend: Green: positive efficacy results in primary outcome(s); yellow: positive efficacy results in secondary outcome(s) or in subanalysis; red: negative efficacy results.

3.1.6. Meta-analyses

Several meta-analyses including observational studies and RCT, and data from both hospitalized and nonhopitalized patients have been published. First meta-analyses using data from observational studies were generally in favour of CCP, especially when infused early in the course of disease and with high antibody titres.[144] Conversely, most meta-analyses including data from RCT have found no benefit in the reduction of mortality, use of invasive mechanical ventilation, and hospital discharge of CCP in hospitalized immunocompetent patients and inconclusive evidence in nonhospitalized patients.[145–148] Other meta-analyses performing subgroup analysis have shown some beneficial effects of CCP.[149] Despite the general trend of RCT-based meta-analyses showing no effect of CCP, some authors have suggested that these conclusions might be biased by the strong statistical contribution of large studies like the RECOVERY trial (nearly 12,000 participants) showing no effect of CCP in particular populations of severely ill patients.[150]

3.1.7. Evidence in immunocompromised patients

Evidence supporting the use of CCP for the treatment of COVID-19 in patients who are immunocompromised is limited. No randomized, adequately powered trials have evaluated the efficacy of CCP for the treatment of these patients. However, there is a physiologic rationale for the use of antibody therapies in immunocompromised patients, who are at risk of (1) having reduced antibody responses to SARS-CoV-2 infection and COVID-19 vaccination, (2) prolonged SARS-CoV-2 replication, and (3) severe COVID-19 outcomes. Furthermore, several reports of clinical improvement in immunocompromised patients treated with CCP support potential efficacy in this population and suggest a longer potential therapeutic window than in immunocompetent patients.

First, evidence from case series and retrospective case-control studies have suggested a potential benefit of CCP in immunocompromised patients,[151–158] even administered in the era of the omicron variant.[159] Second, some subgroup analyses generated from RCT that included hospitalized patients who are immunocompromised also suggest a potential benefit of CCP in this population.[138] Third, a recent systematic review and meta-analysis showed an association between CCP use and a mortality benefit in hospitalized immunocompromised patients with COVID-19 (RR 0.63, 95% CI 0.50 to 0.79), with a high level of concordance between individual studies.[160]

3.1.8. Adverse events

According to the available evidence from several observational studies and RCTs reported to date, serious adverse reactions associated to CCP are infrequent and consistent with the risk associated with plasma infusions for other indications. These adverse events include the risk of transfusion-transmitted infections (e.g., HIV, hepatitis B, hepatitis C), which is currently low due to standard screening for blood-borne pathogens.

The most common adverse reactions associated with CCP therapy are local reactions, mild allergic reactions and febrile nonhemolytic reactions, and -less frequently- anaphylactic reactions. Other infrequent adverse reactions include transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), haemolytic reactions and thromboembolic events. Hypothermia, metabolic complications, and post-transfusion purpura have also been described.[161]

A theoretical risk of antibody-dependent enhancement (ADE) of SARS-CoV-2 infection is an additional risk of CCP transfusion. ADE is an exaggerated inflammatory response triggered by non-neutralizing antibodies, sometimes developed during a prior infection with a different viral serotype, that enhances viral cellular entry, exacerbating the severity of symptoms. During ADE, an antibody molecule binds a viral particle through its Fab region, while the antibody Fc region interacts with the Fc receptor (FcR) on the surface of host cells, leading to the formation of a virus-antibody-FcR complex for endocytosis. ADE can occur in several viral diseases, such as Dengue virus and Zika virus. The theoretical concern for coronaviruses relies on antibodies to one type of coronavirus, which could enhance infection to another viral strain, which would be a concern specially when using antibody-based therapies with low-titres of SARS-CoV-2 antibodies. Most

available evidence from the use of CCP for COVID-19 suggests ADE does not occur. However, in the CONCOR-1 trial, higher levels of full transmembrane spike IgG were associated with worse outcomes, suggesting that the use of CCP with nonfunctional anti-SARS-CoV-2 antibodies may be harmful.[118] Moreover, a subgroup analysis in the REMAP-CAP trial showed potential harm in patients who received CCP transfusions more than 7 days after being hospitalized.[119]

3.1.9. US Expanded Access Program and international guidelines

In early April 2020, with no effective available treatments and the aim to fill an urgent need to provide patient expanded access to therapies with a potential benefit, the National Expanded Access Program (EAP) in the US for CCP was initiated. The objectives of the program, sponsored by the Mayo Clinic, were to provide access to CCP for hospitalized patients and to further assess the safety of this therapy.[162] Compassionate use of CCP was extended to other countries globally. The robust evidence on the safety of CCP in hospitalized patients obtained from EAP led the US Food and Drug Administration (FDA) to issue an Emergency Use Authorization (EUA) for CCP for the treatment of hospitalized patients with COVID-19 in August 2020.[163]

Based on cumulative evidence indicating lack of efficacy of low-titre CCP in immunocompetent hospitalized patients, on February 2021, the US FDA amended the EAP and limited the use to only high-titre CCP (**Table 7**) for the treatment of hospitalized patients early in the disease course or patients with impaired humoral immunity who cannot produce an adequate antibody response to control SARS-CoV-2 replication.[164] High-titre CCP was defined on the basis of correlation with a reference standard, the Broad Institute live-virus, 5-dilution VNT, as a 50% inhibitory dilution (ID50) of 1:250 or more.[165] On December 2021, the EAP was limited to the use of CCP with high-titres of anti-SARS-CoV-2 antibodies for the treatment of COVID-19 in nonhospitalized or hospitalized patients who had immunosuppressive disease or were receiving immunosuppressive treatment.[166] Basing its recommendations on RCT meta-analyses, also in December 2021, WHO guidelines recommended against the use of CCP, except in the context of randomized clinical trials with severely ill patients.[167] On May 2022, the EAP for CCP ended. Currently, NIH and IDSA guidelines recommend against CCP for immunocompetent hospitalized patients, and report insufficient evidence to recommend

either for or against CCP for immunocompetent non-hospitalized patients and immunocompromised patients.

Table 7. FDA definition for high-titre CCP and acceptable tests

Manufacturer	Assay	Qualifying Result	Date of Listing under the EUA
Abbott	AdviseDx SARSCoV-2 IgG II (ARCHITECT and Alinity i)	≥ 1280 AU/mL	December 28, 2021
Diasorin	LIAISON SARS-CoV-2 TrimericS IgG	≥ 87 AU/mL	December 28, 2021
EUROIMMUN	Anti-SARS-CoV-2 S1 Curve ELISA (IgG)	>55 RU/mL	February 9, 2022
GenScript	cPass SARS-CoV-2 Neutralization Antibody Detection Kit	Inhibition ≥ 80%	December 28, 2021
Kantaro	COVID-SeroKlir, Kantaro SemiQuantitative SARS-CoV-2 IgG Antibody Kit	Spike ELISA > 69 AU/mL	December 28, 2021
Ortho	VITROS Anti-SARS-CoV-2 IgG Quantitative Reagent Pack	>200 BAU/mL	December 28, 2021
Roche	Elecsys Anti-SARS-CoV-2 S	> 210 U/mL	December 28, 2021

Table from Convalescent Plasma EUA Letter of Authorization 128282021 [166]

3.2. Hyperimmune immunoglobulins

3.2.1. Overview

COVID-19 hyperimmune immunoglobulin (hIG), a drug product manufactured from plasma pooled from multiple donors who have recovered from COVID-19, has also been proposed for the treatment and prophylaxis of COVID-19. hIG products contain polyclonal IgG antibodies at higher concentrations than CCP. Moreover, these titres are standardized in a specific volume which is typically small (1 mL to 200 mL for various diseases). hIG products manufactured from plasma of healthy donors vaccinated with COVID-19 vaccines have also emerged as potential candidates, with even higher neutralization content.

3.2.2. Mechanism of action

The main mechanism for the clinical benefit of hIG, as well as for CCP, is through viral neutralization. COVID-19 hIG shows high-affinity binding to the spike protein, the RBD, the N-terminal domain of the S protein, and the nucleocapsid protein of SARS-CoV-2, and blocks RBD binding to ACE2.[168] hIG possesses other antiviral properties that have been described for CCP. These include Fc functions, mediated by the interaction of Fc with cellular Fc receptors, such as antibody-dependent cellular phagocytosis (ADCP), antibody-dependent cellular cytotoxicity (ADCC), and complement-mediated cytotoxicity.[169]

3.2.3. Preclinical *in vitro* and animal model studies

COVID-19 hIVIG from convalescent donors and from vaccinated donors have shown a potent broad-spectrum *in-vitro* neutralization against SARS-CoV-2 variants,[169] including delta and omicron.

Preclinical studies in hamster model have shown significant reduction in viral replication in the lower respiratory tract when hamsters are given a single dose of COVID-19 hIVIG (400 mg/kg) two days after challenge with SARS-CoV-2.[170] *In-vivo* studies in mouse model show significant reduced weight loss, lung viral loads, and lung pathological injury of COVID-HIG (prepared from plasma of healthy donors vaccinated with Sinopharm COVID-19 vaccine) used as prophylaxis and treatment.[168]

3.2.4. Randomized clinical trials

No evidence evaluating the efficacy of hIG for COVID-19 had been reported before the design and implementation of the studies included in this thesis. Currently, clinical data on the efficacy of hIG for COVID-19 are limited to five clinical trials, with different immunoglobulin products used in different populations. Results from these trials are mixed and summarized in **Table 8**.

Two trials used hyperimmune intravenous immunoglobulins (hIVIG) at different doses for the treatment of hospitalized patients and did not find statistically significant clinical benefits and reduction of mortality. One trial included 18 severely immunocompromised hospitalized COVID-19 patients and showed a reduction of severe COVID-19 associated with the administration of hIVIG. Interestingly, 61% of the participants included were fully vaccinated. The fourth trial assessed the efficacy of EpAbs INM005, an antibody product consisting in equine polyclonal antibodies (purified F(ab')2 fragments obtained from horses immunized with the RBD domain of the viral spike protein), in hospitalized patients with moderate and severe COVID-19 pneumonia. Results did not show benefits in the overall population, although sensitive analyses showed a beneficial effect in severe patients and in patients who were seronegative at baseline. The last study is the GC2010 trial, which is included in this thesis.

Table 8. RCTs of hyperimmune immunoglobulins for COVID-19

Sample size (Rand)	Population	Days from symptoms to randomization (median)	Control arm	IG Volume Dose IgG/nAbs titers	Efficacy results	RCT identifier Reference
50 (4:1)	Hospitalized Severe or critical COVID-19	8	SOC	hIVIG 150, 200, 250, 300 mg/kg NA >10 COI (ECLIA, IgG)	No significant reduction in mortality on day 28 (25% vs 60%, *p*>0.05) Significant reduction in severe COVID-19 (*p*=0.002)[¥]	Ali et al., 2021[139]
593 (1:1)	Hospitalized Without end-organ failure	8	Normal saline + SOC	hIVIG 400 mg/kg 40g NA	No significant improvement of clinical status on day 28 (seven-category ordinal scale) (OR 1.06, *p*=0.72)	**ITAC** Polizzotto et al., 2022[140]
18 (1:1)	Hospitalized Severely immunocompromised[┼] 61% fully vaccinated	9	IVIG 150 ml 15g	hIVIG 150 ml 15g 900 IU/ml (VNT50, nAbs)	Reduction of severe COVID-19 (20% vs 88%, *p*=0.015) Potential benefit in mortality (0% vs 38%)	Huygens et al., 2023[171]
245 (1:1)	Hospitalized Moderate and severe COVID-19 pneumonia	6	Matching placebo	EpAbs INM005 200 ml IV 4 mg/kg 1:20,000 (nAbs)	No difference in improvement in ≥2 categories in WHO clinical scale on day 28 (RD 5.28%, *p*=0.15) Beneficial effect in severe patients and those with no baseline antibodies[¥]	Lopardo et al., 2021[172]
461* (1:1:1)	Asymptomatic SARS-CoV-2 infection	3.1 (from positive test)	Normal saline	C19-IG20% SC hIG 10 ml 1 and 2 g 1:13,510 (ID50) (mean against alpha VOC)	No difference in proportion of participants who remained asymptomatic on day 14 (59.9% vs 64.7% vs 63.5%, *p*=0.53 and *p*=0.85)	**GC2010** Alemany et al., 2023[Σ]

Legend: Rand.: randomization; IG: immunoglobulin IgG: immunoglobulin G; nAbs: neutralizing antibodies; RCT: randomized clinical trial; SOC: standard of care; hIVIG: hyperimmune intravenous immunoglobulin; NA: not available; COI: cut-off index; ECLIA: electrochemiluminescence immunoassay; OR: odds ratio; IU: international units; VNT50: virus neutralization titre 50%; EpAbs: equine polyclonal antibodies; IV: intravenous; VOC: variant of concern.
*Early termination; ¥Sensitivity analysis; ΣThis trial is part of this thesis.

┼6 B cell-depleted patients with hematologic malignancies, 9 solid organ transplant recipients, 1 B cell-depleted patient with autoimmune disease, 1 patient with congenital B-cell deficiency, 1 patient with acquired B-cell deficiency

Colour legend: Green: positive efficacy results in primary outcome(s); yellow: positive efficacy results in secondary outcome(s) or in subanalysis; red: negative efficacy results.

3.2.5. Adverse events

Most frequent adverse events reported are mild and self-limiting, and include local infusion reactions (infection site pain, puncture site pain and erythema), vasovagal syndrome, fever, chills, and headache. No severe allergic reactions or anaphylaxis and thromboembolic events related to hIG IV and SC infusion have been reported.[171–174]

3.3. Specific anti-SARS-CoV-2 monoclonal antibodies

3.3.1. Overview

Several neutralizing monoclonal antibodies (mAbs) that target the SARS-CoV-2 spike protein have been developed, as an alternative approach for passive immunotherapy. Following the emergence of SARS-CoV-2, mAbs targeting the spike receptor binding domain (RBD) were rapidly isolated from humanized mice and from peripheral B cells of recovered patients.

3.3.2. Mechanism of action

The clinical efficacy of mAbs in viral infections is mediated through direct binding to free virus particles and neutralization, thus blocking viral entry into host cells.[175,176] mAbs might also bind to viral antigens expressed on the surface of infected cells and stimulate antibody-dependent phagocytosis and cytotoxicity via the crystallizable fragment portion of the antibody.[177] Effector functions might have a role especially when given as treatment but not as prophylaxis.

Some COVID-19 mAb products are a combination of mAbs, which bind to non-overlapping epitopes, with the aim to minimize the potential loss of antiviral activity associated with immunity escape of viral variants.

3.3.3. Preclinical *in vitro* and animal model studies

Anti-SARS-CoV-2 mAbs have shown very potent SARS-CoV-2 specific neutralization activity *in-vitro.*[178]

mAbs have also shown *in-vivo* efficacy in several studies in both therapeutic and prophylactic settings in mouse models and non-human primate models, with decreases in viral load and lung pathology.[178–181]

3.3.4. Randomized clinical trials

No evidence on the efficacy of mAbs for COVID-19 had been reported before the design and implementation of the two RCT included in this thesis, which evaluate the efficacy of high-titre CCP and hIG for the early treatment and prevention of COVID-19. Currently, five anti-SARS-CoV-2 mAb products have been developed and have shown clinical

benefits for COVID-19: bamlanivimab plus etesevimab, casirivimab plus imdevimab, sotrovimab, bebtelovimab and tixagevimab plus cilgavimab.

mAbs were used initially for hospitalized patients and then for outpatients and for prophylaxis. Better results were observed for pre- and post-exposure prophylaxis and in outpatients, especially when those at high risk of disease progression were recruited to increase the cost-effectiveness of the procedure. Moreover, some mAb products have also shown clinical benefit as treatment for selected groups of hospitalized patients (i.e., without detectable anti-SARS-CoV-2 antibodies). The evidence from these RCT is summarized in **Table 9**.

Based on the reported evidence, four anti-SARS-CoV-2 mAb products (bamlanivimab plus etesevimab, casirivimab plus imdevimab, sotrovimab, and bebtelovimab) received EUA from the FDA for early therapy in outpatients at high risk of disease progression and one mAb product (Evusheld) as COVID-19 PrEP. Interestingly, mAbs were administered to vaccinated individuals even when no RCT were conducted after vaccine coverage was high, and no conclusive evidence supported their efficacy in these settings. Moreover, all these RCT were conducted before the widespread circulation of the omicron VOC.

Table 9 (I). RCTs of monoclonal antibodies for COVID-19

Sample size (Rand)	Population	Days from symptoms to rand. (median)	Control arm	mAb Dose Adm. route	Efficacy results	RCT identifier Reference
769 (2:1)	**Nonhospitalized** Mild to moderate COVID-19 High-risk patients†	4	Normal saline	**Bamlanivimab plus Etesevimab** 700/1400mg IV	Reduction of COVID-19 related hospitalization or death on day 29 (0.8% vs 5.8%, change of -5.0%, p<0.001) Reduction in viral load (p<0.0001)	**BLAZE-1** Dougan et al., 2022[182]
1035 (1:1)	**Nonhospitalized** Mild to moderate COVID-19 High-risk patients†	4	Normal saline	**Bamlanivimab plus Etesevimab** 2800+2800mg IV	Reduction of COVID-19 related hospitalization or death on day 29 (2.1% vs 7%, RR 0.7, p<0.001) Reduction in viral load (p<0.0001)	**BLAZE-1** Dougan et al., 2021[183]
314** (1:1)	**Hospitalized** Without end-organ failure	7	Matching placebo	**Bamlanivimab** 7000 mg IV	No differences in sustained recovery on day 90 (54% vs 50%, OR 0.85, p=0.45)	**ACTIV-3 / TICO** Study group., 2021[184]
714 (1:1:1)	**Nonhospitalized** Mild to moderate COVID-19 No risk factors for severe COVID-19	4.7	BEB+BAM+ETE 175+700+1400 mg Placebo IV	**Bebtelovimab** 175 mg IV	No difference of COVID-19 related hospitalization or death on day 29 compared to placebo (3% vs 2%, p>0.05)	**BLAZE-4** Dougan et al., 2022[185]
1355 (1:1:1)	**Nonhospitalized** Mild to moderate COVID-19 High-risk patients†	3	Normal saline	**Casirivimab plus imdevimab (REGEN-COV)** 600+600 mg 1200+1200 mg IV	Reduction of COVID-19 related hospitalization or death on day 29 (1% vs 1.3% vs 4.6%, p<0.001) Reduction in viral load (p<0.005)	Weinreich et al., 2021[186]
9785 (1:1)	**Hospitalized**	7 - 9	SOC	**Casirivimab plus imdevimab (REGEN-COV)** 250 ml 4+4 g IV	Reduction of all-cause mortality on day 28 (24% vs 30%, RR 0.79, p=0.0009) in baseline seronegative patients	**RECOVERY** Abani et al., 2022[187]

Table 9 (II). RCTs of monoclonal antibodies for COVID-19

Sample size (Rand)	Population	Days from symptoms to rand. (median)	Control arm	mAb Dose Adm. route	Efficacy results	RCT identifier Reference
2475 (1:1)	**PEP** Household contacts	<96 hs after contact	Matching placebo sc	**Casirivimab plus imdevimab (REGEN-COV)** 1200 mg SC	Reduction of symptomatic SARS-CoV-2 infection (1.5% vs 7.8%, RRR 81.4%, *p*=0.001)	O'Brien et al., 2021[188]
1057 (1:1)	**Nonhospitalized** Mild to moderate COVID-19 High-risk patients┼	3 - 5	Normal saline	**Sotrovimab (Xevudy)** 500 mg IV	Reduction of COVID-19 related hospitalization or death on day 29 (1% vs 6%, RR 0.21, *p*<0.001)	**COMET-ICE** Gupta et al., 2023[189]
5973 (2:1)	**PrEP** Adults with increased risk of an inadequate response to vaccination, increased risk of exposure, or both	NA	Normal saline IM	**Tixagevimab plus cilgavimab (Evulsheld)** 300 mg IM	Reduction of symptomatic COVID-19 up to 183 days (0.2% VS 1%, RRR 76.7%, *p*<0.001)	**PROVENT** Levin et al., 2022[190]
1014 (1:1)	**Nonhospitalized** Mild to moderate COVID-19	4.9	Normal saline	**Tixagevimab plus cilgavimab (Evulsheld)** 600 mg (300 + 300 mg) IM	Reduction of severe COVID-19 or death on day 29 (4% vs 9%, RRR 50.5%, *p*=0.0096)	**TACKLE** Montgomery et al., 2022[191]
1455 (1:1)	**Hospitalized**	8	Normal saline + SOC	**Tixagevimab plus cilgavimab (Evulsheld)** 600 mg (300 + 300 mg) IV	No difference in time to sustained recovery up to 90 days (89% vs 86%, RR 1.08, *p*=0.21). Reduction of mortality (9% vs 12%, HR 0.7, *p*=0.032)	**ACTIVE-3 / TICO** Ginde et al., 2022[192]

Legend: Rand.: randomization; mAb: monoclonal antibody; adm. Route: administration route; RCT: randomized clinical trial; IV: intravenous; OR: odds ratio; SOC: standard of care; PEP: post-exposure prophylaxis; SC: subcutaneous; RRR: relative risk reduction; RR: risk ratio; BEB+BAM+ETE: bebtelovimab + bamlanivimab + etesevimab; PrEP: pre-exposure prophylaxis; NA: not available; IM: intramuscular.

**Terminated for futility; ¥Sensitivity analysis; ┼High risk patients: at least one risk factor for progressing to severe COVID-19 and/or hospitalization

Colour legend: Green: positive efficacy results in primary outcome(s); yellow: positive efficacy results in secondary outcome(s) or in subanalysis; red: negative efficacy results.

3.3.5. Adverse events

Most common adverse events reported were non-severe and self-limiting, and included diarrhoea and nausea, injection-site reactions, headache, chills, and bronchospasm. Serious adverse events occurred very rarely.

3.3.6. Resistance to mAbs

In November 2021, with the emergence of omicron (BA.1) VOC, with high number of spike mutations and deletions, most clinically approved mAbs showed a massive reduction on their neutralizing activity and only sotrovimab and bebtelovimab retained *in-vitro* efficacy. Later, after BA.2 sublineage emerged, sotrovimab also lost its efficacy. Evusheld was effective against BA.2, but lost efficacy against BA.2.11 and B1.4/5 (with L452R/Q substitution).[193] All licensed mAbs show no efficacy *in-vitro* against BQ.1/B1.1.1 (B1.5) and XBB/XBB.1/XBB.1.5. Thus, authorizations have been restricted for all mAbs and international guidelines recommend currently against their use in settings with predominance of these circulating variants.

4. The thesis in the context of the rapidly evolving field

This thesis is based on work done during the COVID-19 pandemic, as a response to a global public health emergency. The projects included in this thesis were conceptualized, designed, and started between June and December 2020, with the aim of finding treatments for outpatients to reduce the progression, severity, and fatality of COVID-19. At that time, the only treatment that had shown clinical benefit for COVID-19 was dexamethasone, when administered to hospitalized patients. Passive immunotherapy had shown benefits in preclinical studies and observational studies, and only one RCT had reported signals of efficacy of passive immunotherapy (only CCP) in hospitalized individuals. No data regarding efficacy of CP, hIG or mAbs had been reported in outpatients. Most of the current evidence was generated during the recruitment and after the publication of the studies contained in this thesis. The trials and their protocols were adjusted and appropriately amended according to the newly generated evidence, when needed.

This thesis dissertation includes four articles:

The first article reports the results from a randomized clinical trial (COnV-ert trial) assessing the efficacy of early treatment with high-titer CCP (methylene blue-treated) for COVID-19 outpatients. This trial was designed and coordinated by Fundació Lluita contra les Infeccions and Hospital Universitari Germans Trias i Pujol (Badalona, Spain) in collaboration with IrsiCaixa, Banc de Sang i Teixits de Catalunya, ISGlobal, Hospital de Bellvitge, Hospital Sant Bernabé de Berga, ICS Metropolitana Nord and ICS Catalunya Central. The trial was funded by Fundació Lluita contra les Infeccions (FLI) and Grifols.

The second article (the COMPILEhome study) is a meta-analysis of individual participant data of two randomized clinical trials on CCP for COVID-19 outpatients (COnV-ert trial and COV-Early trial). It was a collaboration between Fundació Lluita contra les Infeccions / Hospital Universitari Germans Trias i Pujol and Erasmus MC University Medical Center (Rotterdam, The Netherlands).

The third article reports the results of a meta-analysis of individual participant data that included all international RCT that had reported efficacy of CCP in COVID-19 outpatients (including COnV-ert trial). It was a collaboration between all research teams that had designed and coordinated these RCTs, led by the Johns Hopkins Bloomberg School of Public Health (Baltimore, Maryland, USA).

The fourth article reports the results from a RCT (GC2010 trial) assessing the efficacy of the subcutaneous 20% hyperimmune immunoglobulin C19-IG20% in asymptomatic SARS-CoV-2 infected individuals. The trial was designed and coordinated by Fundació Lluita contra les Infeccions and Hospital Universitari Germans Trias i Pujol (Badalona, Spain) and Grifols, in collaboration with ISGlobal, ICS Metropolitana Nord, ICS Barcelona Ciutat, ICS Metropolitana Sud, ICS Catalunya Central and Gerencia Asistencial de Atención Primaria Madrid. GC2010 trial was funded by Grifols.

Hypothesis

Overall hypothesis:

Passive immunotherapy, including COVID-19 convalescent plasma and hyperimmune immunoglobulins, is efficacious for treating and preventing COVID-19 when administered early in the course of SARS-CoV-2 infection.

This general hypothesis can be structured in the following specific hypotheses:

Hypothesis 1: A single intravenous infusion of high-titre COVID-19 convalescent plasma is efficacious in preventing hospitalization by day 28 among adult outpatients with confirmed symptomatic SARS-CoV-2 infection within 9 days from the onset of symptoms.

This hypothesis will be addressed in the first, second, and third articles.

Hypothesis 2: A subcutaneous infusion of 1g and 2g of 20% hyperimmune immunoglobulin C19-IG20% is efficacious in preventing the progression to symptomatic COVID-19 among early asymptomatic adults with confirmed SARS-CoV-2 infection within 5 days from diagnosis.

This hypothesis will be addressed in the fourth article.

Objectives

The overall objective of this thesis was to assess the efficacy of passive immunotherapy (convalescent plasma and hyperimmune immunoglobulins) in treating and preventing COVID-19.

This overarching objective has been structured into the following specific objectives, defined according to the PICO principles of evidence-based medicine.[194]

Objective 1: To assess the efficacy of a single intravenous infusion of high-titre COVID-19 convalescent plasma (as defined by FDA) in preventing hospitalization by day 28 among adult outpatients with confirmed symptomatic SARS-CoV-2 infection, regardless of comorbidities, within 9 days from the onset of symptoms, as compared to placebo.

Objective 2: To assess the efficacy of a subcutaneous infusion of 1g and 2g of 20% hyperimmune immunoglobulin C19-IG20% in preventing the progression to symptomatic COVID-19 among early asymptomatic adults with confirmed SARS-CoV-2 infection, regardless of comorbidities, within 5 days from diagnosis, as compared to placebo.

Material, methods, and results

Study 1

High-titre methylene blue-treated convalescent plasma as an early treatment for outpatients with COVID-19: a randomised, placebo-controlled trial.

Alemany A*, Millat-Martinez P*, Corbacho-Monné M, Malchair P, Ouchi D, Ruiz-Comellas A, Ramírez-Morros A, Rodríguez Codina J, Amado Simon R, Videla S, Costes G, Capdevila-Jáuregui M, Torrano-Soler P, San José A, Bonet Papell G, Puig J, Otero A, Ruibal Suarez JC, Zarauza Pellejero A, Llopis Roca F, Rodriguez Cortez O, Garcia Garcia V, Vidal-Alaball J, Millan A, Contreras E, Grifols JR, Ancochea À, Galvan-Femenia I, Piccolo Ferreira F, Bonet M, Cantoni J, Prat N, Ara J, Forcada Arcarons A, Farré M, Pradenas E, Blanco J, Àngel Rodriguez-Arias M, Fernández Rivas G, Marks M, Bassat Q, Blanco I, Baro B, Clotet B, Mitjà O; CONV-ERT Group.

Lancet Respir Med. *2022 Mar;10(3):278-288.* **[IF: 102.642]**

High-titre methylene blue-treated convalescent plasma as an early treatment for outpatients with COVID-19: a randomised, placebo-controlled trial

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See Comment page 226

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Summary

Background Convalescent plasma has been proposed as an early treatment to interrupt the progression of early COVID-19 to severe disease, but there is little definitive evidence. We aimed to assess whether early treatment with convalescent plasma reduces the risk of hospitalisation and reduces SARS-CoV-2 viral load among outpatients with COVID-19.

Methods We did a multicentre, double-blind, randomised, placebo-controlled trial in four health-care centres in Catalonia, Spain. Adult outpatients aged 50 years or older with the onset of mild COVID-19 symptoms 7 days or less before randomisation were eligible for enrolment. Participants were randomly assigned (1:1) to receive one intravenous infusion of either 250–300 mL of ABO-compatible high anti-SARS-CoV-2 IgG titres (EUROIMMUN ratio ≥6) methylene blue-treated convalescent plasma (experimental group) or 250 mL of sterile 0·9% saline solution (control). Randomisation was done with the use of a central web-based system with concealment of the trial group assignment and no stratification. To preserve masking, we used opaque tubular bags that covered the investigational product and the infusion catheter. The coprimary endpoints were the incidence of hospitalisation within 28 days from baseline and the mean change in viral load (in log_{10} copies per mL) in nasopharyngeal swabs from baseline to day 7. The trial was stopped early following a data safety monitoring board recommendation because more than 85% of the target population had received a COVID-19 vaccine. Primary efficacy analyses were done in the intention-to-treat population, safety was assessed in all patients who received the investigational product. This study is registered with ClinicalTrials.gov, NCT04621123.

Findings Between Nov 10, 2020, and July 28, 2021, we assessed 909 patients with confirmed COVID-19 for inclusion in the trial, 376 of whom were eligible and were randomly assigned to treatment (convalescent plasma n=188 [serum antibody-negative n=160]; placebo n=188 [serum antibody-negative n=166]). Median age was 56 years (IQR 52–62) and the mean symptom duration was 4·4 days (SD 1·4) before random assignment. In the intention-to-treat population, hospitalisation within 28 days from baseline occurred in 22 (12%) participants who received convalescent plasma versus 21 (11%) who received placebo (relative risk 1·05 [95% CI 0·78 to 1·41]). The mean change in viral load from baseline to day 7 was –2·41 log_{10} copies per mL (SD 1·32) with convalescent plasma and –2·32 log_{10} copies per mL (1·43) with placebo (crude difference –0·10 log_{10} copies per mL [95% CI –0·35 to 0·15]). One participant with mild COVID-19 developed a thromboembolic event 7 days after convalescent plasma infusion, which was reported as a serious adverse event possibly related to COVID-19 or to the experimental intervention.

Interpretation Methylene blue-treated convalescent plasma did not prevent progression from mild to severe illness and did not reduce viral load in outpatients with COVID-19. Therefore, formal recommendations to support the use of convalescent plasma in outpatients with COVID-19 cannot be concluded.

Funding Grifols, Crowdfunding campaign YoMeCorono.

Introduction

Immunotherapies that administer antibodies directly to the patient are classified as passive immunotherapies, as opposed to active immunotherapy that aims to stimulate the host's immune response. Passive immunotherapies, including the use of convalescent plasma (obtained from donors who have recovered from infection) and monoclonal antibodies targeting specific epitopes, have

Research in context

Evidence before this study

We searched PubMed and medRxiv databases from March 1, 2020, to Aug 20, 2021, with no language restrictions, for randomised trials or meta-analyses of trials evaluating the effect of convalescent plasma in patients with COVID-19. We used the terms ("COVID-19", "COVID", "SARS-CoV-2", or "Coronavirus") AND ("convalescent plasma", "passive immunization", "passive immunotherapy", "plasma therapy"), and 13 trials and one meta-analysis were identified. 11 trials, including one with more than 10 000 participants enrolled, included hospitalised patients with severe or critical COVID-19. In hospitalised patients with COVID-19, convalescent plasma was not associated with a reduction in mortality or with benefits in other clinical outcomes. Only two trials included patients with COVID-19 who had not been admitted to hospital. Both trials were placebo-controlled and enrolled a total of 671 randomly assigned patients. The first trial was published in February, 2021, and included 160 older adults (aged ≥75 years) within 72 h after the onset of mild COVID-19 symptoms. In this Argentinian trial, early administration of convalescent plasma reduced the proportion of patients progressing to severe respiratory disease from 25 (31%) of 80 patients in the placebo group to 13 (16%) of 80 in the convalescent plasma group. The second trial (SIREN-C3PO), published in August, 2021, included 511 participants with non-severe COVID-19 recruited at an emergency room. The trial showed no benefit of treatment with convalescent plasma in preventing hospitalisation (81 [32%] of 254 had disease progression or hospitalisation in the placebo group *vs* 77 [30%] of 257 in the convalescent plasma group). Convalescent plasma was administered in the first week after symptom onset, with a median time of 4 days (IQR 2–5), and the patients were either aged 50 years and older or had one or more risk factors. Criticism was raised regarding the fact that 16% of patients were admitted in the index visit.

Added value of this study

We found that compared with placebo, high-titre convalescent plasma did not reduce hospitalisation up to day 28 after random assignment and did not reduce viral load at day 7 when administered to outpatients aged 50 years and older with COVID-19 with less than 7 days from symptom onset. Our results are consistent with evidence reported from the SIREN-C3PO trial of convalescent plasma in outpatients with COVID-19. Our trial is important not only for replication, but also because it addresses some of the limitations of the SIREN-C3PO trial. Unlike SIREN-C3PO, our participants were not recruited in emergency room departments and, therefore, probably presented with milder earlier symptoms. We assessed the antibody serum status in patients at enrolment and we confirmed the absence of efficacy of the early treatment with convalescent plasma in serum antibody-negative patients, who represented most of our cohort. Moreover, we confirmed the neutralising activity of plasma units against the common circulating SARS-CoV-2 variants during recruitment, and plasma units were near-sourced, reducing the risk of efficacy being affected by antigenic shifts in viral strains from regional differences. In addition, plasma was characterised and the median titre of SARS-CoV-2 neutralising antibodies administered was very high (median 50% inhibitory dilution for original virus strain Wuhan-Hu-1 lineage B was 1:1379 [IQR 1:602–1:2801] and for alpha [B.1.1.7] variant was 1:943 [1:428–1:2236]).

Implications of all the available evidence

The results on the efficacy of convalescent plasma generated to date do not allow a formal recommendation to support its use in outpatients with COVID-19. Our results suggest that methylene blue-treated convalescent plasma does not prevent progression from mild to severe illness and does not reduce viral load in outpatients with COVID-19. The findings of our study need to be taken with caution due to a possible reduced activity of plasma collected during former COVID-19 waves against the alpha variant and the potential effect of methylene blue inactivation on the observed efficacy, as well as in the context of early termination of the study.

emerged as candidates for preventing severe illness when administered early after COVID-19 onset.[1,2] To date, various anti-SARS-CoV-2 monoclonal antibodies have shown efficacy in reducing the combined rates of hospitalisation and death in outpatients with early, mild disease, and a small benefit in reducing death rates among seronegative patients who are admitted to hospital.[2–6] The US Food and Drug Administration has issued the Emergency Use Authorization[5] for monoclonal antibodies in patients with mild to moderate COVID-19 who are at high risk of progression to severe COVID-19. However, the high cost and complexity of monoclonal antibody production is a challenge to the widespread global use of this strategy, and concern has arisen regarding how these antibodies will respond to emerging SARS-CoV-2 variants.[7] For instance, the new omicron variant (B.1.1.529) of concern is resistant against almost all licensed monoclonals.[8,9]

Convalescent plasma, the traditional approach to passive immunotherapy, has yielded promising results in other viral respiratory infections.[10] Compared with monoclonal antibodies, convalescent plasma has the drawback of lacking standardisation in dose, affinity, and specificity of antibodies, which might lead to varying neutralising activity in different plasma units. The overall dose of specific antibodies is generally lower in convalescent plasma, although convalescent plasma has the potential advantage of a broader antiviral activity than monoclonal antibody therapy. However, randomised controlled trials involving patients admitted to hospital (severe disease) with COVID-19 have found no survival benefit with convalescent plasma treatment.[11–22] The

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See Online for appendix

results of one recent randomised controlled trial (SIREN-C3P0) of convalescent plasma in 511 high-risk outpatients with COVID-19 showed no benefit in preventing disease progression from mild to severe disease when given at a median of 4 days (IQR 2–5) after symptom onset.[23] However, in this trial, patients were recruited at emergency rooms and were, therefore, likely to present with moderate-to-severe symptoms. Moreover, 25 (16%) of 158 patients who met the primary outcome of disease progression within 15 days after randomisation were ultimately admitted to hospital during the index visit (at baseline). Additionally, serological tests were not done at enrolment, and benefit of convalescent plasma is most likely in seronegative individuals. In addition, plasma units were sourced more than 150 miles (>240 km) from plasma recipients, which might affect efficacy if they are derived from donors infected with different strains of SARS-CoV-2.[24] A smaller randomised trial done in Argentina in 160 outpatients, aged 75 years and older and treated within 72 h of symptom onset (mild disease), found that high-titre convalescent plasma was associated with a lower likelihood of progression to severe disease (relative risk [RR] 0·52 [95% CI 0·29–0·94]).[25]

More conclusive information on convalescent plasma efficacy in outpatients is required. In this randomised controlled trial, we investigated whether near-sourced, high-titre convalescent plasma, administered within 7 days after symptom onset, would prevent hospital admission or reduce SARS-CoV-2 viral load in outpatients with mild-to-moderate COVID-19.

Methods

Study design

The CONV-ERT study was a multicentre, double-blind, randomised, placebo-controlled trial to assess the efficacy of convalescent plasma in preventing severe COVID-19 in patients infected with SARS-CoV-2 with mild and moderate illness. The trial was done at four health-care centres providing universal health care to a catchment population of 3·9 million people in Catalonia, Spain (appendix p 3).

The study was done according to the Helsinki Declaration of the World Medical Association. The study protocol was approved by the Ethics Committee at Hospital Germans Trias i Pujol (number PI 20-313) and the institutional review boards of participating centres. The study was supervised by an independent data and safety monitoring board.

Participants

To be eligible for participation, patients had to be aged 50 years or older and non-hospitalised (not admitted to hospital) with mild-to-moderate COVID-19. All patients had to have a confirmed SARS-CoV-2 infection, with a positive PCR or validated antigen rapid test result received no more than 5 days before randomisation, and symptom onset no more than 7 days before randomisation. Mild and moderate COVID-19 were defined according to international guidelines:[26] patients with fever, cough, sore throat, malaise, headache, and muscle pain were considered to have mild COVID-19, whereas evidence of lower respiratory disease by clinical assessment or imaging and a saturation of oxygen 94% or more on room air was considered moderate COVID-19. Patients were excluded if they had severe COVID-19 or required hospitalisation for any cause, a history of a previous SARS-CoV-2 infection, received one or two doses of a COVID-19 vaccine, contraindications to the investigational product, increased thrombotic risk, history of clinically significantly abnormal liver function (eg, Child-Pugh C), or chronic kidney disease stage 4 or worse. We excluded patients who were pregnant, breastfeeding, or planning a pregnancy during the study period. Further details on the eligibility criteria are listed in the trial protocol (appendix p 21).

We identified study participants from two sources: (1) active screening of laboratory-confirmed new infections at study sites and (2) individuals who voluntarily registered on an institutional website launched by the sponsor and the Catalan Institute of Health. Investigators contacted participants by telephone or in person to inform them about the study, invite participation, and check their eligibility. We scheduled eligible participants for a baseline visit, done either at hospital or at home by the hospital domiciliary homecare unit, during which written informed consent was obtained, and eligibility confirmed.

Randomisation and masking

We used a central web-based randomisation system with allocation concealment and no stratification to randomly assign participants (1:1) to receive convalescent plasma or placebo. Study researchers confirmed eligibility of participants and contacted an independent technician based at the central blood bank (Banc de Sang i Teixits de Catalunya, Barcelona, Spain), with no information about the participant, who used the web-based system to assign participants to the trial groups. Blood bank staff masked the investigational products with opaque tubular bags that covered the entire unit of plasma or saline solution and the infusion catheter. Finally, an unmasked study nurse, who was not involved in patient follow-up, administered the investigational product. All participants and other investigators (including all personnel involved in patient follow-up, laboratory staff, and statisticians) were masked to treatment allocation. Random assignment and infusion were always done on the same day.

Unmasking was permitted only if a clinical emergency occurred during or immediately after the infusion or an unexpected severe adverse event occurred during follow-up. Only the principal investigator was allowed to unmask individual study participants using a specific command in the electronic case report form.

Procedures

Participants received one intravenous infusion of either 250–300 mL of ABO-compatible high-titre methylene blue-treated convalescent plasma (experimental group) or 250 mL of sterile 0·9% saline solution (control group). For participants with a bodyweight of less than 45 kg, dosing of intervention (ABO-compatible high-titre methylene blue-treated convalescent plasma or 0·9% saline solution) was bodyweight adjusted to 5 mL/kg. All patients also received standard medical treatment. The study convalescent plasma units were sourced from the central blood bank located 12 km or less from the two largest study sites, and 90 km or less from all study sites (appendix pp 3, 12). Plasma was selected after screening for high anti-SARS-CoV-2 IgG titres with ELISA (EUROIMMUN ratio ≥6), according to international guidelines.[27] After transfusion, we further characterised plasma post hoc with a pseudovirus-based neutralising antibody assay that used a spike from the virus lineage Wuhan-Hu-1.[28] The plasmid SARS-CoV-2.SctΔ19 was generated (GeneArt) from the full protein sequence of the original Wuhan-Hu-1 lineage B SARS-CoV-2 spike (Genbank MN908947.3) with a deletion of the last 19 amino acids in C-terminal. The sequence was human-codon optimised and inserted into pcDNA3.1(+). To assess the neutralising activity against the alpha variant (B.1.1.7), post hoc we repeated the neutralising antibody testing using an alpha-variant pseudotyped virus.[28] Also, to assess the effect of methylene blue treatment on neutralising antibodies, we compared the neutralising activity of stored biospecimens from the donor (ie, before methylene blue treatment) and that of the plasma unit (ie, after methylene blue treatment) in a subset of participants. To establish calibrating factors for conversion of ID50 geometric mean titres into IU/mL, we used a panel of plasma samples developed and distributed by the National Institute for Biological Standards and Control (UK, number 20/136). For the purpose of data analysis, neutralising results were used to define high-titre convalescent plasma with a threshold of 50% inhibitory dilution (ID50) of more than 1:250 (equivalent to more than 60 IU/mL; details are provided in the appendix p 5).

Patients were asked to complete a symptom inventory every day for 14 days after random assignment by means of an electronic form. In-person follow-up visits were scheduled on days 7 and 28 at participants' residence, or at the hospital if the participant was hospitalised. Additionally, we contacted study participants by telephone on days 3, 14, and 60 to assess their clinical status. WHO Clinical Progression Scale score (range 0–10) was determined at each study visit (appendix p 7). During follow-up visits, we obtained blood samples (at baseline and day 7) to assess anti-SARS-CoV-2 serum antibodies and inflammatory biomarkers, and nasopharyngeal swabs (at baseline and days 7 and 28) for quantification of SARS-CoV-2 viral load. We used a structured electronic case report form to record data.

Serum antibody status of all enrolled participants was prospectively characterised from baseline samples by chemiluminescence immunoassay in a fully automated platform (LIAISON XL, DiaSorin, Vercelli, Italy). Patients were designated serum antibody-negative if they were negative for both of the following antibodies: IgG anti-SARS-CoV-2 trimeric spike glycoprotein (DiaSorin, Vercelli, Italy) and IgM anti-SARS-CoV-2 S1-RBD (DiaSorin, Vercelli, Italy; appendix p 6). Viral load was determined by real-time quantitative RT-PCR in a single step with the Allplex 2019-nCoV assay (Werfen, Hospitalet de Llobregat, Spain) on the CFX96 instrument (BIO-RAD, Hercules, CA, USA). For absolute quantification, a standard curve was built using 2-fold serial dilutions of a SARS-CoV2 plasmid RNA of known concentration (Amplirun Coronavirus RNA Control, catalogue reference MBC090, Vircell Microbiologists, Granada, Spain). Study samples were run in parallel to the set of prequantified samples covering all thermal cycles used in the analysis. The viral load was extrapolated from the standard curve using the corresponding cycle threshold values in the *RdRP* gene results (appendix p 6). We tested biomarkers with most evidence as predictors for severe COVID-19 at baseline and on day 7, including D-dimer, ferritin,

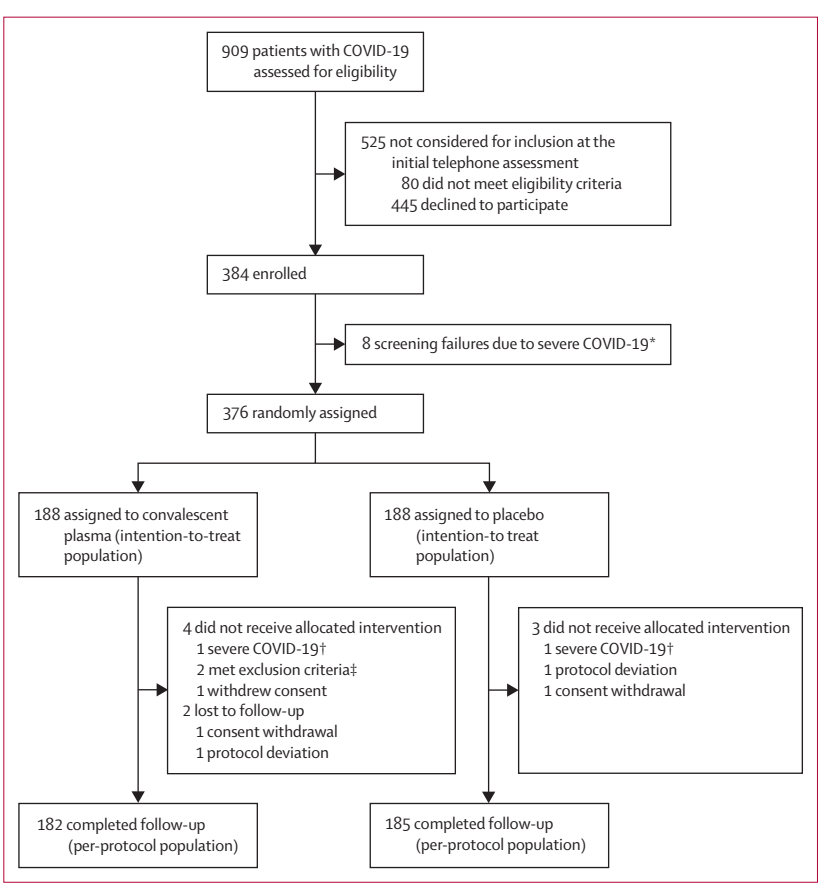

Figure 1: **Trial profile**

*Worsening of clinical status before random assignment. †Worsening of clinical status before infusion. ‡1 history of chronic kidney disease stage 4 or worse and 1 pre-existing condition with increased risk of thrombosis.

interleukin (IL)-6, lymphocytes, C-reactive protein, and prealbumin.[29]

Outcomes

We defined two coprimary outcomes regarding treatment efficacy. First, the clinical outcome was the incidence of hospitalisation within 28 days from baseline. Second, the virological outcome was the mean change in viral load (in $\log_{10}$ copies per mL) in nasopharyngeal swabs from baseline to day 7.

Prespecified secondary outcomes were time to complete symptom resolution, change in the 10-point WHO Clinical Progression Scale score[30] within the 60 days following infusion, change from baseline in inflammatory biomarkers on day 7 of follow-up, mean change in viral load in nasopharyngeal swabs at day 28, death rate, titres of neutralising antibodies against SARS-CoV-2 in plasma of a subgroup of participants at day 7, and rate of adverse events. Details of all secondary outcomes are included in the study protocol (appendix p 21), including three prespecified outcomes that will be reported elsewhere as they were related to ancillary substudies.

Safety was assessed as the proportion of patients with adverse events that occurred or worsened during the follow-up period. Adverse events were assessed for severity and causality. The safety population included all patients who received the investigational product.

	Convalescent plasma group (n=188)	Placebo group (n=188)
Demographics		
Age, years	56 (52–62)	56 (53–63)
Women	83 (44%)	90 (48%)
Men	105 (56%)	98 (52%)
Body-mass index, kg/m²	27·9 (4·5)	27·6 (4·5)
Primary coexisting risk factors for severe COVID-19		
At least one risk factor	137 (73%)	141 (75%)
Smoker	94 (50%)	97 (52%)
Obesity	51 (27%)	45 (24%)
Cardiovascular disease	14 (7%)	9 (5%)
Lung disease (chronic obstructive pulmonary disease, asthma, or both)	17 (9%)	16 (9%)
Diabetes	20 (11%)	19 (10%)
Chronic renal failure	3 (2%)	3 (2%)
Immune-compromised	6 (3%)	3 (2%)
COVID-19 duration		
Days from symptoms onset to random assignment*	4·4 (1·4; 185)	4·4 (1·4; 187)
Days from positive test† to random assignment	2·8 (1·0; 185)	2·7 (1·1; 187)
COVID-19 severity		
Mild COVID-19	183 (97%)	183 (97%)
Moderate COVID-19	5 (3%)	5 (3%)
Serum IgM and IgG antibody status‡		
Negative	160/183 (87%)	166/186 (89%)
Positive	23/183 (13%)	20/186 (11%)
Laboratory parameters§		
D-dimer, ng/mL	325 (250–516; 181)	355 (250–513; 180)
Ferritin, ng/mL	222·0 (106·8–410·0; 184)	223·5 (107·8–368·3; 184)
IL-6, pg/mL	5·1 (3·1–12·9; 186)	5·1 (2·8–10·9; 185)
Lymphocytes, $\times 10^9$ cells per L	1·2 (1·0–1·6; 188)	1·2 (0·9–1·6; 188)
C-reactive protein, mg/L	5·5 (2·3–14·1; 187)	5·4 (2·5–12·5; 186)
Prealbumin, mg/dL	27·0 (20·9–38·8; 182)	27·5 (22·0–47·2; 178)

Data are median (IQR), n (%), mean (SD), mean (SD; N), n/N (%), or median (IQR; N). IL=interleukin. *Random assignment and infusion were always done on the same day. †Positive PCR or validated antigen rapid test result for SARS-CoV-2 infection. ‡Patients were designated serum antibody-negative if they were negative for both of the following antibodies: IgG anti-SARS-CoV-2 trimeric spike glycoprotein, and IgM anti-SARS-CoV-2 S1-RBD. §Laboratory reference ranges: D-dimer 0–500 ng/mL; ferritin 30·0–400·0 ng/mL; IL-6 0·0–6·4 pg/mL; lymphocytes 1·2–3·5 $\times 10^9$ cells per L; C-reactive protein 0·0–5·0 mg/L; prealbumin 20·0–40·0 mg/dL.

Table 1: **Baseline characteristics**

Statistical analysis

We estimated that a sample size of 474 participants (237 per group) would provide 80% power to detect a 50% reduction in hospitalisation incidence by day 28,[31] assuming an expected rate of hospitalisation of 15%, at a significance level of $\alpha=0{\cdot}05$, and allowing a 5% loss to follow-up. Approximately 150 participants per group were required to have 80% power to detect a difference of $0{\cdot}5$ $\log_{10}$ copies per mL in the mean reduction of SARS-CoV-2 viral load at a two-sided significance level of $\alpha=0{\cdot}05$, assuming an expected overall SD of $1{\cdot}5$. A $0{\cdot}5$ $\log_{10}$ copies per mL difference in reduction was chosen to represent the minimal threshold for a biologically relevant change for our analyses. On May 28, 2021, despite the sample size not being reached, the data and safety monitoring board recommended halting recruitment to the trial because more than 85% of the target population had received SARS-CoV-2 vaccination.

Primary efficacy analyses were done in the intention-to-treat population. Hospitalisation rate was compared between groups using the RR obtained by fitting a generalised estimating equation log-binomial model that accounted for clustering (centre of recruitment). To determine whether the estimator was significantly different from zero, we used the Wald test on the robust SE from the fitter treatment effect coefficient. Virological efficacy was determined by comparing the mean reduction of the viral load from baseline to days 7 and 28. The mean reduction of viral load (in $\log_{10}$ scale) was compared by fitting linear mixed-effect models using the centre of recruitment and the individual as nested random effects (cluster or individual) in the intercept to adjust for intra-individual and intra-cluster correlation. According to available evidence on factors influencing the successful treatment of COVID-19, prespecified analyses of the primary outcomes were done in subgroups (as an interaction term with the treatment) defined by participant's baseline antibody serum status (IgG or IgM anti-SARS-CoV-2 positive and negative), duration of illness (≤3 days and >3 days), and according to the neutralisation activity of the plasma received

(ID50 >1:250 and ID50 ≤1:250). Prespecified sensitivity analysis of the primary outcomes were done in the per-protocol population.

The days to complete resolution of symptoms were analysed using Kaplan-Meier survival functions and hazard ratios (HRs) obtained by fitting Cox proportional hazards regression models based on the assumptions of proportional risks. The Kaplan-Meier curves were compared using the log-rank test. The mean reduction of the 10-point WHO Clinical Progression Scale score was compared by fitting linear mixed-effect models. The median values of laboratory parameters at day 7 were compared between treatment groups by means of the non-parametric Wilcoxon-Mann-Whitney test. Death rate and adverse events rate were compared between groups using the RR obtained according to Deeks and Higgins.[32] Comparison of median of neutralising antibody titres against SARS-CoV-2 in plasma of a subgroup of participants at day 7 was done by Wilcoxon matched-pairs signed rank test.

Post-hoc comparison of median titres of neutralising antibody of alpha variant versus original virus was done by Wilcoxon matched-pairs signed rank test.

All analyses were done with the R statistical package (version 4.1 or higher) with a significance level of 0·05. We did not adjust the type I error for multiplicity because we considered that both coprimary endpoints individually must show statistically significant treatment benefit.

This study is registered with ClinicalTrials.gov, NCT04621123.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Nov 10, 2020, and July 28, 2021, we assessed 909 patients with confirmed COVID-19 for eligibility. The recruitment and follow-up of study participants are shown in figure 1. 525 (58%) of 909 screened patients did not meet the selection criteria or declined to participate and were therefore not enrolled. Additionally, eight (2%) of 384 consented participants were not randomly assigned to intervention and were excluded from the intention-to-treat analysis because of screening failure. Therefore, 376 participants were randomly assigned (convalescent plasma n=188; placebo n=188). All 376 participants were included in the intention-to-treat analysis.

Baseline demographics and clinical characteristics were similar in the convalescent plasma group and the placebo group (table 1). The median age of the participants was 56 years (IQR 52–62), 173 (46%) were women, 203 (54%) were men, and 278 (74%) had at least one risk factor for progression to severe COVID-19 related to coexisting conditions. The mean time from symptom onset to random assignment was 4·4 days (SD 1·4). Overall, 366 (97%) of 376 patients had mild COVID-19. Baseline serum antibody status was negative in 326 (88%) of 369 patients for whom results were available. The mean viral load from the nasopharyngeal swab at baseline was 6·7 $\log_{10}$ copies per mL (SD 1·6) for the convalescent plasma group and 6·8 $\log_{10}$ copies per mL (1·4) for the placebo group.

Of the 148 units of methylene blue-treated convalescent plasma with available neutralising antibody titres, 132 (89%) had a SARS-CoV-2 neutralising ID50 of more than 1:250. The median ID50 was 1:1379 (IQR 1:602–1:2801 [equivalent to median 1:342 IU/mL, IQR 1:147–1:705]) for the original virus (Wuhan-Hu-1 [Genbank MN908947.3]; appendix p 8). Distribution of neutralising antibody titres against Wuhan-Hu-1 and the alpha variant pseudovirus in a subset of 40 samples showed a decrease of 1·33-fold (median ID50 1:1256 [IQR 1:709–1:2712] against Wuhan-Hu-1 and median ID50 1:943 [1:428–1:2236] against the alpha variant; p=0·0032; appendix p 9). Neutralising activity titres to Wuhan-Hu-1 remained unchanged after methylene blue treatment (median ID50 1:1256 [IQR 1:709–1:2712] before treatment *vs* 1:1287 [1:349–1:3333] after treatment;

	Convalescent plasma group	Placebo group	RR or crude difference (95% CI)	p value
Clinical primary end point: hospitalisation within 28 days of random assignment				
Overall population	22/188 (12%)	21/188 (11%)	RR 1·05 (0·78 to 1·41)	0·76
Subgroups according to serostatus at baseline*				
Negative baseline serum antibody status	20/160 (13%)	19/166 (11%)	RR 1·09 (0·83 to 1·44)	0·54
Positive baseline serum antibody status	2/23 (9%)	2/20 (10%)	RR 0·87 (0·20 to 3·88)	0·86
Subgroups according to duration of illness†				
≤3 days	4/49 (8%)	6/52 (12%)	RR 0·83 (0·56 to 1·25)	0·37
>3 days	18/136 (13%)	15/135 (11%)	RR 1·19 (0·89 to 1·60)	0·24
Subgroups according to plasma neutralisation activity‡				
ID50 >1:250§	13/132 (10%)	21/188 (11%)	RR 0·88 (0·70 to 1·12)	0·30
ID50 ≤1:250	2/16 (13%)	21/188 (11%)	RR 1·12 (0·77 to 1·63)	0·56
Virological primary and secondary endpoints: change in viral load from baseline¶‖				
Overall population				
Day 7	−2·41 (1·32; 174)	−2·32 (1·43; 174)	−0·10 (−0·35 to 0·15)	0·42
Day 28	−3·86 (1·56; 180)	−4·00 (1·45; 172)	0·12 (−0·17 to 0·40)	0·33
Subgroups according to serostatus at baseline*				
Negative baseline serum antibody status				
Day 7	−2·54 (1·31; 149)	−2·35 (1·43; 155)	−0·19 (−0·45 to 0·07)	0·16
Day 28	−4·12 (1·35; 154)	−4·10 (1·37; 154)	−0·02 (−0·28 to 0·25)	0·89
Positive baseline serum antibody status				
Day 7	−1·45 (1·19; 21)	−1·85 (1·42; 17)	0·29 (−0·54 to 1·12)	0·49
Day 28	−1·91 (1·60; 22)	−2·97 (1·87; 16)	0·86 (−0·20 to 1·91)	0·11

Data are n/N (%) or mean (SD; N) except where otherwise stated. ID50=50% inhibitory dilution. RR=relative risk. *Seven of 376 participants did not have baseline serological test. †Four of 376 participants did not have records on duration of illness. ‡40 of 188 participants in the convalescent plasma group did not have a plasma neutralisation activity test. §ID50 value of 1:250 is equivalent to 60 IU/mL (appendix p 5). ¶28 of 376 participants did not have nasal swab collected on day 7. ‖24 of 376 participants did not have nasal swab collected on day 28.

Table 2: **Coprimary endpoints and virological secondary endpoint in the intention-to-treat population**

p=0·32; appendix p 9). Convalescent plasma donations were collected at a time when the B1, B1.1, and B1.177 variants of the SARS-CoV-2 virus were predominant in Catalonia (April, 2020, to January, 2021), and all trial participants were recruited during the second wave (largely B1.177, October, 2020, to January, 2021) and third wave (largely the alpha variant, February–May, 2021; appendix p 10) of the COVID-19 pandemic. The plasma units were sourced 12 km or less from the two largest study sites that recruited 174 (93%) of the participants in the convalescent plasma group, and 90 km or less from all study sites (appendix p 12).

For the clinical primary outcome, there was no significant difference in hospitalisation up to day 28 between the two groups (table 2). Hospitalisations occurred in 22 (12%) of 188 participants in the convalescent plasma group and 21 (11%) of 188 participants in the placebo group (RR 1·05 [95% CI 0·78–1·41]). According to the log-binomial regression model, age, body-mass index, lymphocytes, and ferritin were independently associated with the hospitalisation event (appendix p 14). In prespecified subgroup analyses according to the patients' baseline serum antibody status, duration of illness, and neutralisation activity of the convalescent plasma, hospitalisation rates were not significantly different between groups (table 2).

For the coprimary virological outcome, the mean difference in viral load from baseline to day 7 was –2·41 log_{10} copies per mL (SD 1·32) in the convalescent plasma group and –2·32 log_{10} copies per mL (1·43) in the placebo group (crude difference –0·10 log_{10} copies per mL [95% CI –0·35 to 0·15]; table 2, figure 2). The analysis of the reduction of the viral load followed a similar trend at day 28: –3·86 log_{10} copies per mL (SD 1·56) in the convalescent plasma group versus –4·00 log_{10} copies per mL (1·45) in the placebo group (crude difference 0·12 log_{10} copies per mL [95% CI –0·17 to 0·40]). Results for the virological outcomes from the subgroup analyses according to the patients' baseline serum antibody status were not significantly different between groups (table 2). Primary outcomes in the per protocol population are shown in the appendix (p 15).

In the analysis of the secondary outcome of median time from random assignment to the resolution of COVID-19 symptoms, there was no significant difference between the convalescent plasma group (12·0 days [IQR 6·0–21·3]) and the placebo group (12·0 days [6·0–22·0]; HR 1·05 [95% CI 0·85–1·30]; appendix p 16). The proportional hazard assumption of the Cox regression of the risk over time was satisfied (Schoenfeld test p=0·81; appendix p 17). There were no differences between the groups in the secondary endpoint of change in the 10-point WHO Clinical Progression Scale score within the 60 days following infusion (appendix p 18). Two (1%) of 188 convalescent plasma recipients and four (2%) of 188 placebo recipients required mechanical ventilation (reached ordinal score ≥7). No participants in the convalescent plasma group died, whereas two (1%) participants in the placebo group died (RR 0·20 [95% CI 0·01–4·14]).

There were no significant differences in inflammatory parameters between the groups at day 7 of follow-up, except a minor difference in IL-6 with no clinical significance (figure 3).

Levels of neutralising antibodies at day 7 after infusion, measured in a subcohort of 125 (33%) of 376 participants, did not differ between the convalescent plasma group (n=67; median ID50 1:1017 [IQR 1:296–1:2501]) and the placebo group (n=58; median ID50 1:989 [1:424–1:2321]; appendix p 13).

32 treatment-related adverse events were reported, in 24 (13%) of 188 patients in the convalescent plasma

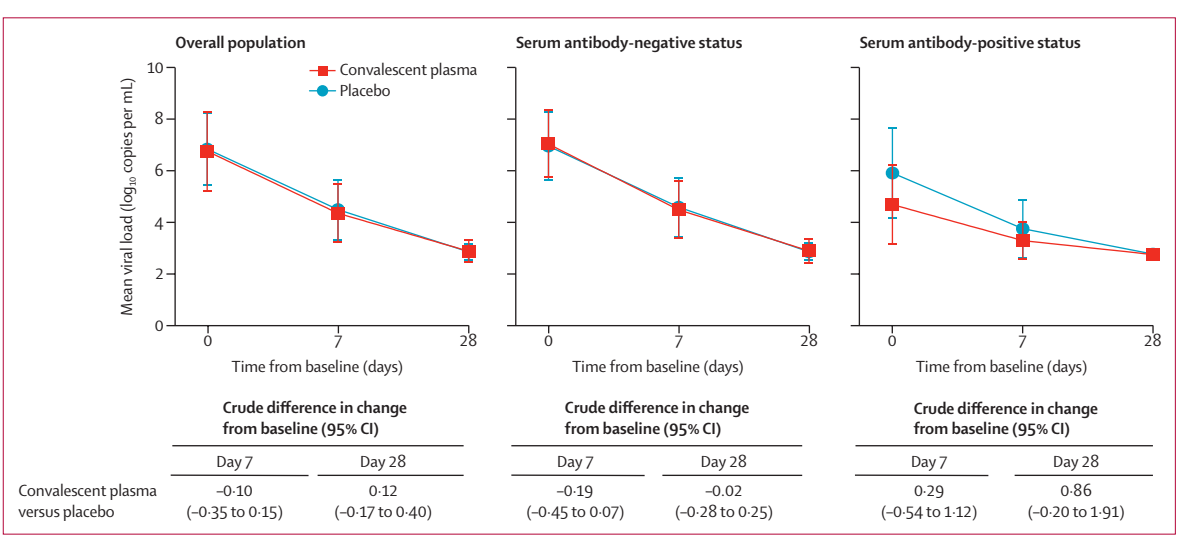

Figure 2: **Viral load change from baseline to day 7 and day 28**
Comparison of the mean reduction of the viral load between treatment groups was done using a linear mixed-effect model. Error bars are 95% CIs.

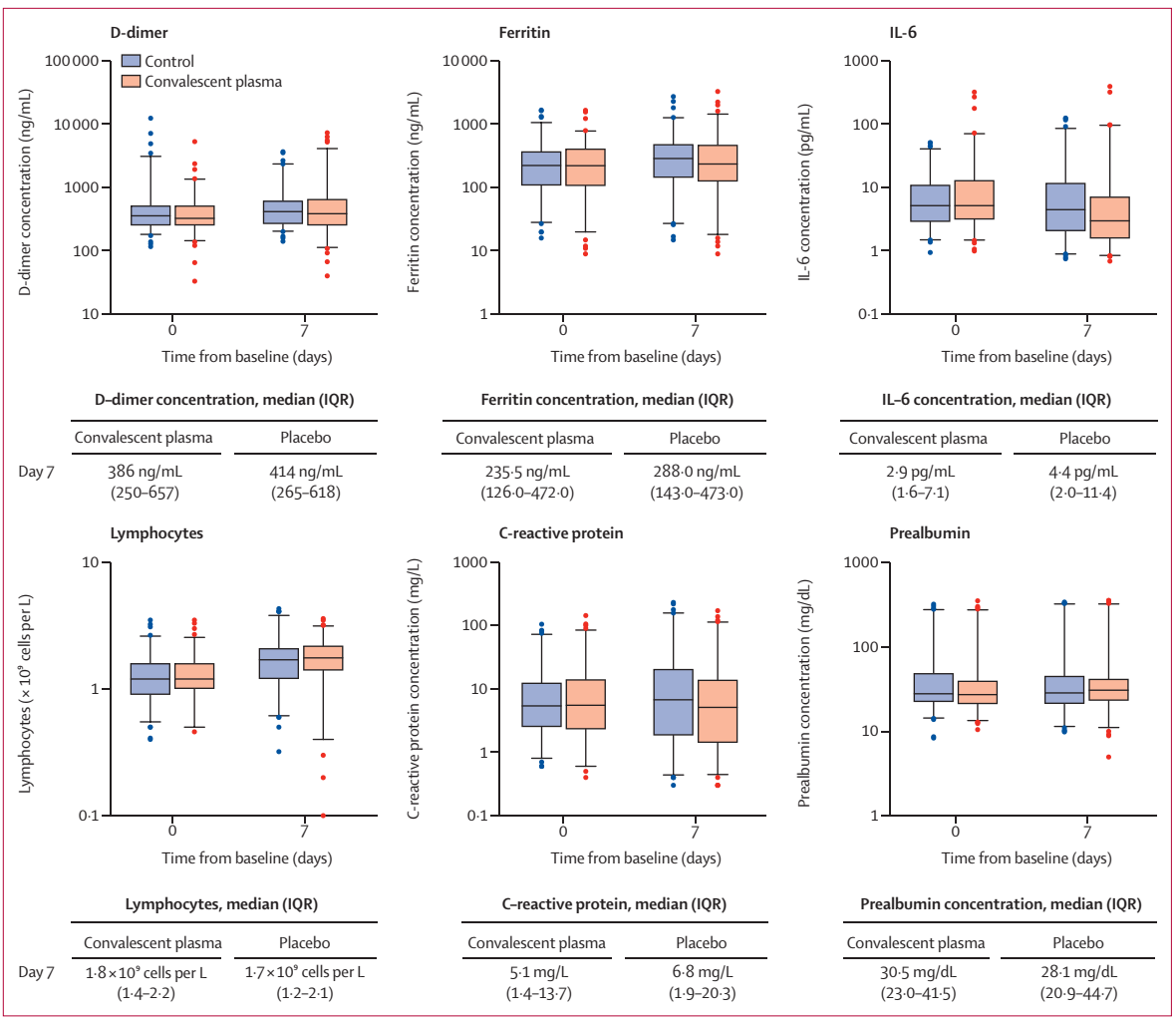

Figure 3: **Inflammatory parameters on day 7**

Box plots indicate median (middle line) and IQR (box), 2·5th and 97·5th percentile (whiskers), and outliers (single points). Difference (Wilcox test p value) between median value of the convalescent plasma group compared with the median value of the placebo group: D-dimer p=0·23; ferritin p=0·26; IL-6 p=0·0042; lymphocyte count p=0·084; C-reactive protein p=0·052; prealbumin p=0·41. Laboratory reference ranges: D-dimer 0–500 ng/mL; ferritin 30·0–400·0 ng/mL; IL-6 0·0–6·4 pg/mL; lymphocytes 1·2–3·5 × 10⁹ cells per L; C-reactive protein 0·0–5·0 mg/L; prealbumin 20·0–40·0 mg/dL. IL=interleukin.

group and eight (4%) of 188 patients in the placebo group (RR 3·00 [95% CI 1·38–6·51]). The most common treatment-related adverse events reported were mild allergic reactions, fever, and local reactions (appendix p 19). One participant with mild COVID-19 signs and symptoms developed a thromboembolic event 7 days after convalescent plasma infusion, which was reported as a serious adverse event possibly related to COVID-19 or to the experimental intervention.

Discussion

In this randomised double-blind trial of high-titre methylene blue-treated convalescent plasma for adult patients aged 50 years and older who had mild to moderate COVID-19 for a week or less, we found that patients receiving convalescent plasma had no better clinical or virological outcomes than those who received a placebo infusion. There was also no evidence of benefit in the convalescent plasma group for any of our secondary endpoints nor in any of our prespecified subgroup analyses.

Our data indicate no significant difference in the proportion of participants who had to be hospitalised within 28 days of entering the trial (RR 1·05 [95% CI 0·78–1·41]). This absence of effect was also observed in the subgroup of serum-antibody-negative patients, who were the majority of our cohort and among whom benefit of other passive immunotherapy such as monoclonal antibodies is predicted to be the highest.[2] Moreover, convalescent plasma did not enhance reduction of viral load in the nasopharynx 7 and 28 days after the intervention.

Previous randomised trials have reported either partial benefits[21,22] or failure[11–20] of convalescent plasma to

improve any relevant outcome in patients admitted to hospital with COVID-19 or patients recruited at emergency rooms.[23] The only evidence of a potential benefit of convalescent plasma in the outpatient setting comes from a smaller randomised trial done in Argentina with a study population more similar than the other randomised trials to ours.[25] The main differences between that trial and ours include an earlier convalescent plasma administration timing (mean time since onset of symptoms 39·6 h [SD 13·9] *vs* 4·4 days [1·4]) and the selection of older patients (mean age 77 years [SD 8·5] *vs* 58 years [8]) in the Argentinian trial.

Several limitations of our clinical trial should be mentioned. A major limitation is that the data safety and monitoring board recommended to terminate the trial early because more than 85% of the population aged 50 years or older were fully vaccinated in Spain (and those who were not were unlikely to participate in a clinical trial), and because monoclonal antibodies became available for outpatients who were at high risk of progression to severe COVID-19. The trial was therefore underpowered. Vaccination was one of the exclusion criteria of our trial but it does not necessarily preclude the use of convalescent plasma in real life, especially considering the immunity conferred by vaccines wanes over time.

Moreover, we need to consider a number of factors that might reduce the efficacy of convalescent plasma, including the clinical time course when therapy is administered, the dose, the affinity of antibodies, and the effect of plasma pathogen inactivation procedures on immunoglobulin function.

First, we enrolled participants up to 7 days from symptom onset and we cannot rule out the potential efficacy if treatment was started earlier. Nonetheless, the fact that 326 (88%) of 369 patients were SARS-CoV-2 IgM and IgG negative at the time of inclusion confirms that they were recruited before the endogenous immune response was initiated.

Second, patients in our trial received a single high-titre plasma unit. Although this approach was similar to other outpatient trials,[23,25] higher plasma volumes are typically administered in patients who have been admitted to hospital for COVID-19. We acknowledge that higher doses might be needed in early stages, when pathology is driven by infection as opposed to inflammation. Our data do not directly address whether higher doses of convalescent plasma or titres of neutralising antibodies would be efficacious. To better understand the kinetics of antibodies in the recipients, we measured neutralisation antibodies 7 days after infusion in the peripheral blood of participants, and we found no differences between the convalescent plasma and placebo groups. It is likely that by 7 days after enrolment, endogenous antibody response will have reached high levels.[33] An earlier comparison of neutralising antibody concentrations between the placebo and intervention groups on days 2–3 after infusion might have provided a better insight into the pharmacokinetics of antibodies delivered.

Third, antigenic shifts, due to discrepancy between donor and recipient infecting variants, might have affected efficacy. Convalescent plasma units for this trial were collected during a wave sustained by SARS-CoV-2 variants (B.1, B.1.1, and B.1.177), which also dominated during the first half of the recruitment period but were different to the one (alpha variant) dominating in the second half. To assess plasma neutralisation activity, we first used a pseudoviral neutralisation assay that used a spike from an original virus lineage (Wuhan-Hu-1), and then repeated testing with an alpha pseudotyped virus. We observed a 1·33-fold decrease in neutralising activity against the alpha variant compared with Wuhan-Hu-1. This finding is in line with previous reports of a 1·5-fold to 3·0-fold decrease in neutralising activity (appendix p 11). The negative results of our study could be partly influenced by a reduction of efficacy of antibodies due to differences in viral variants of donors and recipients. Of note, most previous laboratory studies did not show a statistically significant reduction in neutralising activity against the alpha variant of concern, whereas the reduction was larger and statistically significant for the beta (B.1.351) and delta (B.1.617.2) variants of concern (appendix p 11). To a lesser extent, antigen shifts in viral strains is expected to be region dependent.[24] In our study, plasma units were sourced 12 km or less from the two largest study sites that recruited more than 90% of study participants.

Finally, studies focusing on the effect of methylene blue on SARS-CoV-2 neutralisation have produced mixed results. Methylene blue is a method of pathogen inactivation for plasma that is widely used in some countries in Europe. A study from Russia showed that some units of plasma lost neutralising activity with methylene blue inactivation,[34] whereas other studies found no difference.[35,36] We analysed the neutralising activity of stored donor samples (ie, before methylene blue treatment) compared with the plasma unit (ie, after methylene blue treatment) in a subgroup of plasma units and we found no differences in neutralising antibody titres (median ID50 1:1256 [IQR 1:709–1:2712] *vs* 1:1287 [1:349–1:3333]; p=0·32). Although we observed preserved neutralising activity after methylene blue treatment, we could not evaluate the potential risk of damage to the Fc-region of the immunoglobulins. Fc-dependent functions have important antimicrobial effects, including phagocytosis, complement activation, and antibody-dependent cellular toxicity.[37] Previous studies suggest that the main driver of clinical benefit from convalescent plasma units relies on their neutralising antibody content,[38] and that the cell receptor binding capacity of the Fc-region is preserved after methylene blue treatment.[36] Still, a concern remains that the dye might react with the glycosylation domain and affect Fc-region functionality and thus the overall response.[39]

The relatively low cost and straightforward production of convalescent plasma have resulted in its widespread use for patients with COVID-19. Our analysis builds on previous data[23] suggesting that COVID-19 convalescent plasma does not prevent progression from mild to severe illness in non-hospitalised participants and that convalescent plasma does not reduce viral load. Taking together all the results on the efficacy of convalescent plasma generated to date, formal recommendations to support its use in outpatients with COVID-19 cannot be concluded. The findings of this study need to be taken with caution due to limitations related to a possible reduced activity of plasma collected during former waves against the alpha variant and the potential effect on efficacy of methylene blue inactivation, as well as in the context of the early termination of the trial.

Contributors

OM, AA, PM-M, MC-M, BB, SV, AM, J-RG, and JB conceived and designed the study. All authors acquired, analysed, and interpreted the data. DO, FPF, and IG-F did the statistical analysis. OM, AA, and PM-M drafted the manuscript. All authors critically revised the manuscript for important intellectual content. All authors were responsible for the final decision to submit for publication. All authors have seen and approved the manuscript. AM, PM-M, DO, IG-F, FPF, and OM had full access to all of the data.

Declaration of interests

We declare no competing interests.

Data sharing

Individual participant data that underlie the results reported in this article, after de-identification (text, tables, figures, and appendices) are available from the corresponding author on reasonable request.

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Study 2

Prospective individual patient data meta-analysis of two randomized trials on convalescent plasma for COVID-19 outpatients.

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Prospective individual patient data meta-analysis of two randomized trials on convalescent plasma for COVID-19 outpatients

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Data on convalescent plasma (CP) treatment in COVID-19 outpatients are scarce. We aimed to assess whether CP administered during the first week of symptoms reduced the disease progression or risk of hospitalization of outpatients. Two multicenter, double-blind randomized trials (NCT04621123, NCT04589949) were merged with data pooling starting when <20% of recruitment target was achieved. A Bayesian-adaptive individual patient data meta-analysis was implemented. Outpatients aged ≥50 years and symptomatic for ≤7days were included. The intervention consisted of 200–300mL of CP with a predefined minimum level of antibodies. Primary endpoints were a 5-point disease severity scale and a composite of hospitalization or death by 28 days. Amongst the 797 patients included, 390 received CP and 392 placebo; they had a median age of 58 years, 1 comorbidity, 5 days symptoms and 93% had negative IgG antibody-test. Seventy-four patients were hospitalized, 6 required mechanical ventilation and 3 died. The odds ratio (OR) of CP for improved disease severity scale was 0.936 (credible interval (CI) 0.667–1.311); OR for hospitalization or death was 0.919 (CI 0.592–1.416). CP effect on hospital admission or death was largest in patients with ≤5 days of symptoms (OR 0.658, 95%CI 0.394–1.085). CP did not decrease the time to full symptom resolution.

Trial registration: Clinicaltrials.gov NCT04621123 and NCT04589949. Registration: NCT04621123 and NCT04589949 on https://www.clinicaltrials.gov

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The unprecedented pace and amount of research on the pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to the availability of mortality-reducing therapies within a year after the start of the coronavirus disease 2019 (COVID-19) pandemic[1–3]. For non-hospitalized COVID-19 patients, only anti-SARS-CoV-2 monoclonal antibodies have emerged as a treatment that reduces hospital admission but only when given in the first week of illness. However, they are typically unavailable to middle and low-income countries[4–7].

Convalescent plasma (CP) from COVID-19 recovered patients contains polyclonal anti-SARS-CoV-2 antibodies, can be collected in large quantities at relatively low costs and was used as a therapeutic strategy in previous viral outbreaks[8,9]. So far, randomized trials were unable to generate convincing evidence in support of CP for hospitalized patients with COVID-19[10–18]. However, because an autologous SARS-CoV-2 antibody response typically precedes hospital admission, CP is more likely to be beneficial when it is administered very early after symptom onset[19]. Indeed, the only evidence from a randomized trial in favor of CP for COVID-19 comes from a small study in which elderly outpatients received CP in the first 72 h after symptom onset[20]. In a more recent trial, CP did not reduce the risk of disease progression of COVID-19 in patients with early disease (≤7 days). However, in this trial, patients were recruited at emergency rooms and were, therefore, more likely to manifest severe symptoms[21]. This approach resulted in a trial profile of patients with moderate or late-stage disease, opposed to what was intended in the design. Hence, whether early treatment with CP improves the outcome of outpatients with COVID-19 remains an important question.

As soon as effective vaccines against COVID-19 became available in high-income countries, they were prioritized for individuals at higher risk for a poorer COVID-19 outcome. Because studies on CP for outpatients with COVID-19 focus on these high-risk populations as well, a high vaccination uptake will reduce the number of COVID-19 patients eligible for these studies. More importantly, the risk for a severe outcome will be small when patients become infected despite vaccination. Therefore, we anticipated that vaccination would slow down recruitment, reduce the number of events in the recruited patients and result in individual studies being underpowered. In light of the uncertainty for achieving recruitment goals, real-time pooling of individual patient data from ongoing clinical trials was proposed as a tool for providing timely data to respond to the public health crisis[22]. With this in mind, we initiated the COntinuous Monitoring of Pooled International trials of convaLEscent plasma for COVID-19 patients at home Consortium ($COMPILE_{home}$), which provided a platform to pool individual patient data continuously and in real-time from randomized clinical trials (RCTs) on CP for outpatients with COVID-19[22]. This $COMPILE_{home}$ consortium prospectively pooled and monitored the data from 2 double-blind RCTs, the CoV-Early (NCT04589949) and the COnV-ert (NCT04621123) studies, to assess the effectiveness of high-titer CP for COVID-19 outpatients.

Results

Trials profile. The search for trials resulted in 35 identified studies, thirty-one of which did not meet the selection criteria of the consortium (Supplementary Fig. 1). Of the four remaining studies, one study team opted to abstain from pooling data while another never responded to repeated emails and calls, resulting in two trials included in the pooled analysis: The COnV-ert study (NCT04621123) and the CoV-Early study (NCT04589949). The COnV-ert study received approval from the Institutional Review Board of the Hospital Germans Trias I Pujol (reference PI 20-313) and the CoV-Early study received approval from the Institutional Review Board of the Erasmus Medical Center Rotterdam (reference MEC-2020-0682). Briefly, the COnV-ert study randomized outpatients at 4 sites in Catalunya (Spain) aged ≥50 years with ≤7 days of symptoms to one unit (200–300 mL) of CP or sterile 0.9% saline solution, both covered with opaque tubular bags for blinding investigators and patients. The COnV-ert study joined the consortium when 65 of 474 planned patients were enrolled. CoV-Early enrolled outpatients at 10 sites aged ≥50 years with ≤7 days of symptoms and at least one additional risk factor for severe COVID-19 to receive either one unit (300 mL) of CP or non-convalescent plasma (donated before 01/2020) masked to investigators and patients. It had randomized 150 of the 690 planned patients when they joined the consortium. Details about the allocation concealment, blinding and selection of CP donors in both trials can be found in Supplementary Table 2 and the study protocols.

The COnV-ert study selected the CP after being screened for high anti-SARS-CoV-2 IgG titers with ELISA (EUROIMMUN ratio ≥6), according to guidelines, and supplied by the regional blood bank (Banc de Sang i Teixits de Catalunya—BST); and the CoV-Early study selected the convalescent plasma based on a plaque reduction neutralization test (PRNT) 50 titer of 1:160 or higher. The two trials used a different assay to measure the titer of SARS-CoV-2 neutralizing antibodies. Therefore, a panel of 15 plasma samples was provided for comparison by the Support-E consortium, aimed at harmonizing CP evaluation in Europe[23]. These results confirmed the linearity of both assays and allowed conversion of all neutralizing antibody titers into international units (IU/mL). The median neutralizing antibody titer in the plasma units was 1:386 (IQR 1:233–1:707) IU/mL, which is twice the median titer we previously observed in Dutch CP donors[19]. More details are described in the online Supplementary Data (page 13), in Supplementary Tables 1, 2 and in the individual study protocols.

Study patients and recruitment. Between November 2020 and July 2021, the CoV-Early and COnV-ert study teams contacted approximately 4450 outpatients with a positive SARS-CoV-2 PCR or an antigen test. The majority of exclusions occurred for one of the following reasons: few remaining or clearly improving symptoms, no comorbidities, >7 days of symptoms, unable to come to study site or declined to participate. The online supplement provides more information about the recruitment procedures of each trial.

The rapid uptake of COVID-19 vaccination in Europe, which significantly affected recruitment rate in both studies (Supplementary Fig. 2) and the authorization of specific anti-SARS-CoV-2 monoclonal antibodies for high-risk outpatients resulted in early trial termination (COnV-ert on 8th of June and CoV-Early on 13th of July 2021) following recommendations of their DSMBs. By that time, 797 participants had been enrolled and 782 of them had received the allocated intervention and could be pooled for the analysis (Fig. 1).

Patients included in the analysis had a median age of 58 years (IQR 53–64), a median of 5 days (IQR 4–6) from symptom onset, and a median of 1 comorbidity (IQR 0-2). According to the baseline assessment, 688 patients (93%) had a negative result for serum IgG anti-SARS-CoV-2 S-protein, and 21 had completed their COVID-19 vaccination. 14 participants had received one of 2 doses of a mRNA vaccine at the time of inclusion. Baseline characteristics were comparable between both study arms (Table 1).

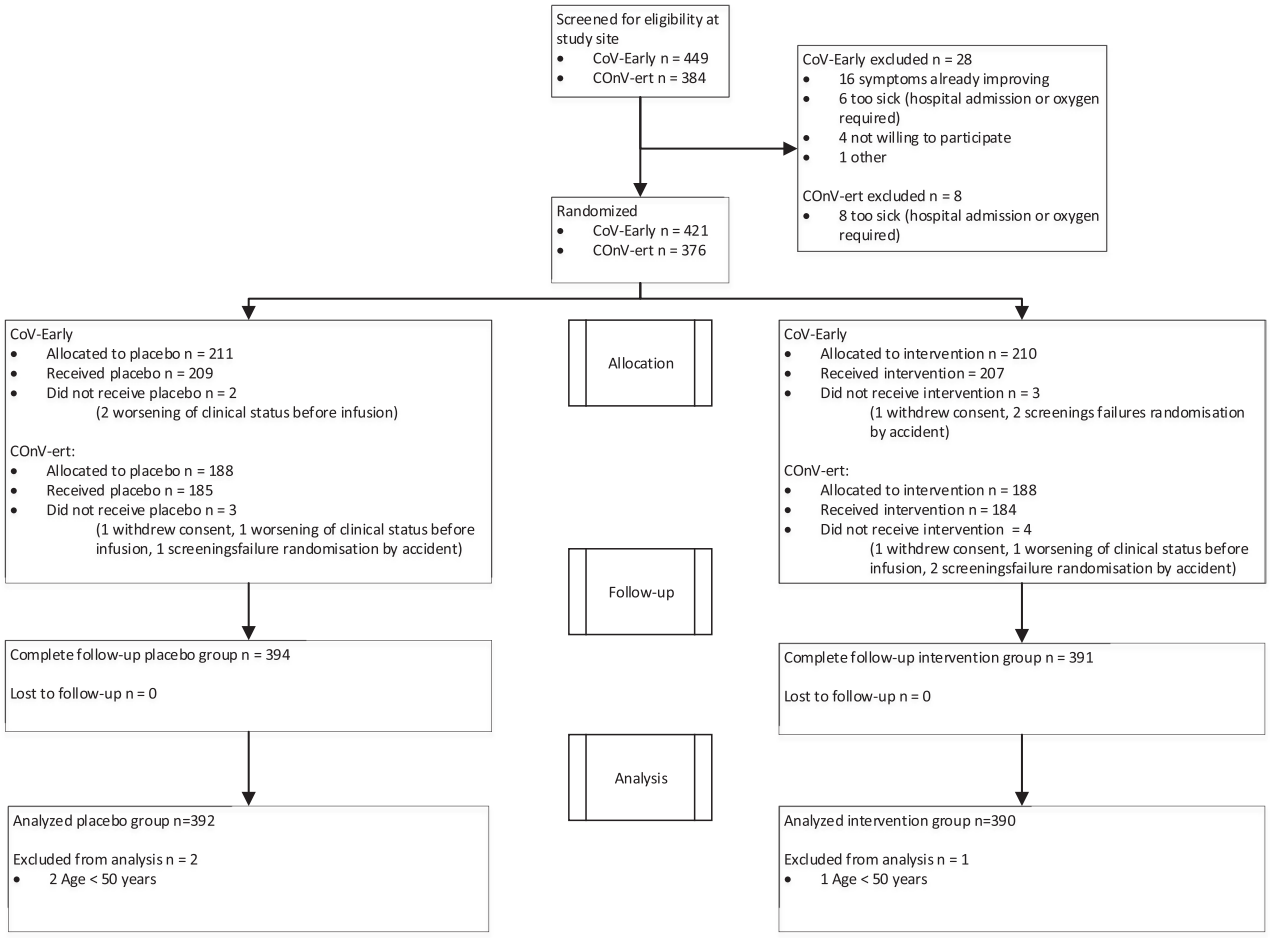

Fig. 1 CONSORT flow diagram. Figure shows the CONSORT flow diagram of the COMPILEhome patients. 833 patients were screened at a study site and 782 were included for analysis.

Table 1 Baseline characteristics.

Characteristic	Total ($n = 782$)	CP[a] ($n = 390$)	Control ($n = 392$)
Male sex—no. (%)	522 (66.8%)	267 (68.5%)	255 (65.1%)
Age—median (IQR)	58 (53-64)	58 (53-64)	58 (54-65)
50-60 y	428	222	206
61-70 y	217	103	114
>70 y	82	36	46
O_2 saturation—median (IQR)[b]	97 (96-98)	97 (96-98)	97 (96-98)
Severe immunodeficiency—no. (%)	13 (1.7%)	5 (1.3%)	8 (2.1%)
Number of comorbidities—median (IQR)[c]	1 (0-2)	1 (0-2)	1 (0-2)
0	225	111	114
1	349	171	178
2-3	192	100	92
>3	15	7	8
Days since first symptoms—median (IQR)	5 (4-6)	5 (4-6)	5 (4-6)
Positive antibody status at baseline—no. (%)	53 (7.0%)	28 (7.7%)	24 (6.4%)

[a]Convalescent plasma.
[b]Baseline oxygen saturation without supplementary oxygen.
[c]Obesity, cardiac disease, lung disease, neurological disease, diabetes, chronic renal failure, cancer and/or liver disease. See the Supplementary Appendix for additional details of the comorbidities.

Primary endpoints. Table 2 and Fig. 2 show the distribution of patients across the five categories of the disease severity scale. The overall estimated OR for patients treated with CP was 0.936 (posterior mean, 95% credible interval 0.667–1.311) with a 64.9% posterior probability of benefit (OR <1). Hospital admission or death occurred in 34 of 390 (8.7%) patients treated with CP and in 40 of 392 (10.2%) patients in the control arm with an OR of 0.919 (posterior mean, 95% credible interval 0.592–1.416) and a 64.3% posterior probability of benefit. Although being included in the COnV-ert trial was associated with a poorer overall outcome,

Table 2 Distribution of the outcome of the patients in the 28 days after inclusion across the 5-points disease severity scale.

Worst disease severity score	Total ($n = 782$)	CP[a] ($n = 390$)	Control ($n = 392$)
Recovered before day 8 after transfusion—no. (%)[b]	143 (18.3%)	74 (19.0%)	69 (17.6%)
Continued symptoms after day 7—no. (%)[c]	565 (72.3%)	282 (72.3%)	283 (72.2%)
Admitted to hospital but no invasive ventilation needed—no. (%)	65 (8.3%)	31 (7.9%)	34 (8.7%)
Admitted to hospital and invasive ventilation needed—no. (%)	6 (0.8%)	2 (0.5%)	4 (1.0%)
Death—no. (%)	3 (0.4%)	1 (0.3%)	2 (0.5%)

[a]Convalescent plasma.
[b]Recovered with no symptoms related to COVID-19 within 7 days after inclusion.
[c]Continued symptoms attributable to COVID-19.

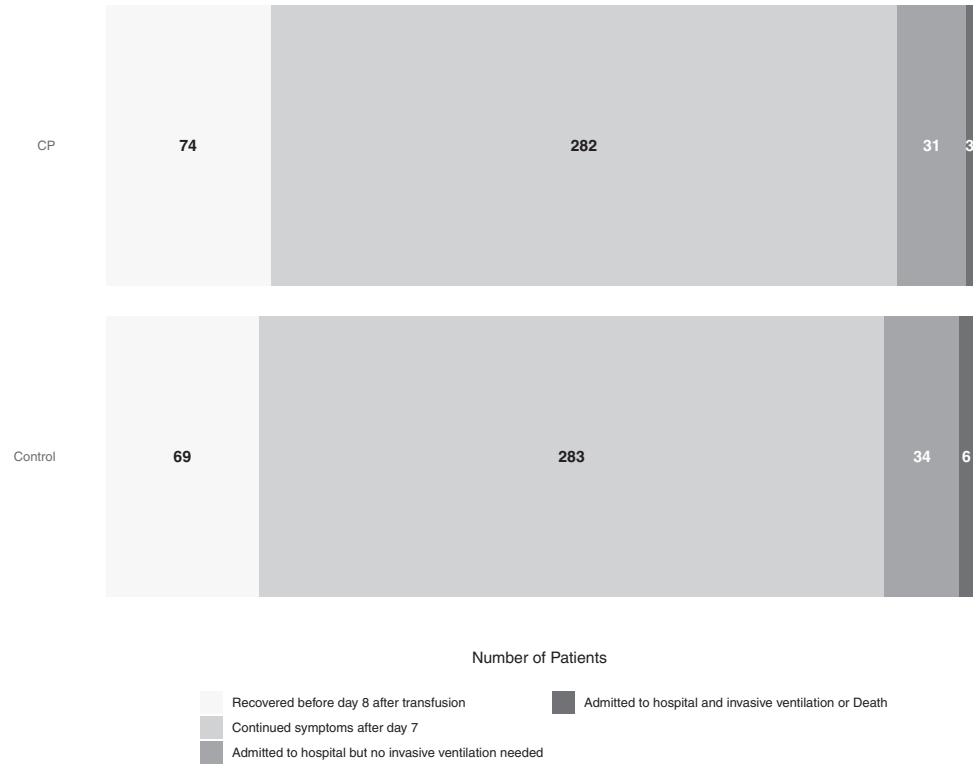

Fig. 2 Distribution for COVID-19 severity at 28 days. CP Convalescent plasma. Figure shows the distribution of the outcome of the patients in the 28 days after inclusion across the 5-point disease severity scale: 1 = recovered before day 8 after transfusion, 2 = continued symptoms after day 7, 3 = hospital admission, 4 = invasive ventilation, 5 = death. Moving from lighter to darker shading represents increasing scores on the severity scale. The darker shade includes point 4 and 5 of the scale (invasive ventilation or death).

the effect of CP was similar in both trials. This increased risk for patients in COnV-ert was independent of age, sex, and the number of comorbidities. The results of all covariates included in the primary analysis can be found in the online supplement (Supplementary Figs. 5, 6).

Secondary endpoints. No differences between CP and control patients regarding time to complete resolution of COVID-19 symptoms was seen (log-rank $p = 0.66$, Fig. 3). The effect size of CP on the binary outcome of hospital admission or death was larger in patients with ≤5 days of symptoms (OR 0.658, 95% CI 0.394-1.085) compared to those with >5 days (OR 1.427, 95% CI 0.789–2.580) and comparable results were observed for the ordinal outcome (OR 0.720, 95% CI 0.486–1.064 in the early treated group, Supplementary Figs. 7, 8).

Finally, the ORs for patients who received CP with neutralizing antibody titers above or below the median titer were nearly identical (Supplementary Fig. 9). Also, no notable difference was observed when patients with IgG anti-SARS-CoV-2 antibodies detected at baseline were excluded (OR 0.880, 95% CI 0.590–1.310 for the binary outcome, OR 0.892, 95% CI 0.643–1.236 for ordinal outcome, Supplementary Figs. 7, 8).

Safety. The intervention was well-tolerated. 89 serious adverse events (SAE) were reported, 4 were considered related to the plasma transfusion (3 in the control arm). Three patients could leave the hospital <24 h after transfusion while the fourth was hospitalized for 5 days 1 week after the CP transfusion and diagnosed with thrombophlebitis at the infusion site and a pulmonary embolism (Table 3).

Discussion

In this analysis of 782 patients with COVID-19 randomized to high-titer CP or placebo within 7 days of disease onset, treatment with CP did not prevent COVID-19 progression, hospitalization, or other clinical outcomes. Our results agree with those by Korley

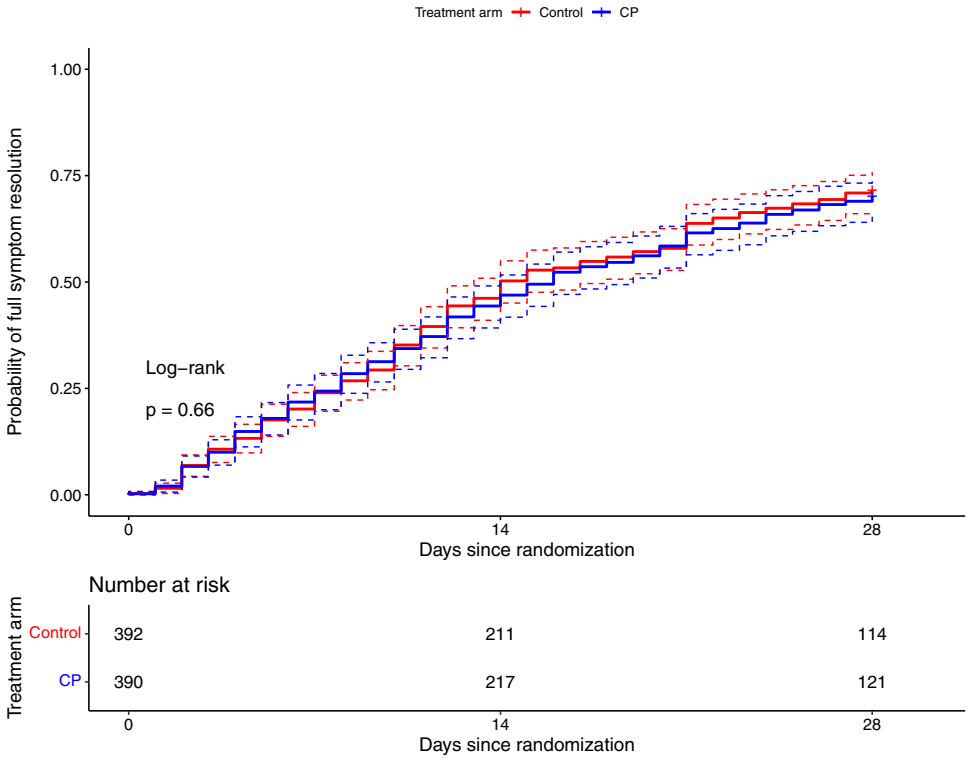

Fig. 3 Time to full symptom resolution up to day 28 (end of follow-up). CP Convalescent plasma. Log-rank test $p = 0.66$. The dotted error bars represent the 95% CI.

Table 3 Serious adverse events[a].

SAE category	Total	CP[b]	Control
(Prolongation of) hospital admission—no.[c]	80	37	43
Death—no.	3	1	2
Serious transfusion related adverse event—no.[d]	4	1	3
Life threatening transfusion reaction—no.[e]	2	0	2
Other AE	2	1	1

[a]Serious adverse events (SAE) were registered in all patients that signed the informed consent form ($n = 797$) regardless of being transfused or not.
[b]Convalescent plasma.
[c]When a patient is hospitalized more than once, each admission is counted separately.
[d]Any transfusion reaction associated with a plasma transfusion that was considered as a SAE.
[e]2 patients with anaphylaxis very soon after discharge that required urgent therapy by paramedics.

et al. in patients of the same age and symptom duration but with probably more severe symptoms as they were recruited at emergency rooms in the USA[21]. These findings differ from those of a smaller trial that used CP within 72 h of symptom onset in much older patients (≥75 years)[20]. We explored signs of efficacy in various subgroups most likely to benefit from CP. The only subgroup in our study that we found that could potentially benefit from CP was the subgroup with ≤5 days from the onset of symptoms (OR 0.70, CI 0.47–1.03). The potential effect of CP when administered early after disease onset has been suggested by other authors[24], and could explain the results reported by Libster et al. study[20]. However, in our study this was a secondary endpoint and the confidence interval was wide, so confirmation in other studies is needed. Regarding the safety parameters of this strategy, our study shows no major concerns, with only four SAEs related to the plasma infusion; these findings are in line with those described in previous studies[25].

Our study has several strengths. It is the largest of its kind, studying the effect of CP for high-risk outpatients with COVID-19 early after initiation of symptoms. The fact that 93% of all patients were SARS-CoV-2 antibody negative at the time of inclusion confirms that they were recruited in the early stage of the disease. Pooling of the data from both studies was pre-planned and initiated before any interim analyses were performed and when both studies were early in their recruitment. Both teams remained fully blinded as the (interim) analyses were done by an unblinded statistical team that shared the results with the DSMB on a regular basis. The COMPILEhome study used the same primary endpoint as the CoV-Early trial, and therefore we did not perform a separate sample size calculation. As our assumptions about the outcome across the ordinal scale were somewhat different than anticipated in the original sample size calculation (fewer hospitalizations and deaths in particular), we repeated the calculation of the effect size that our study was powered for post-hoc. This showed that our study still had 80% power to detect an odds ratio of 0.65 for the primary endpoint, very close to the original power calculation. We, therefore, consider our results methodologically sound.

Several limitations should be mentioned. Although we only included patients aged ≥50, and most of them also had

comorbidities, the hospital admission and death rates were relatively low at 9.5%. Therefore, the study was not powered to exclude a small overall treatment effect on these endpoints. However, administering CP to infectious and symptomatic outpatients is complex and labor-intensive. Hence, we think that CP's clinical role is significantly diminished if unable to establish something greater than "a small effect" because it ceases to be practical. The contribution of the individual comorbidities to COVID-19 risk in our study should be interpreted cautiously because, owing to the lack of consensus regarding the relative relevance of each of them, we summed them in a non-weighted fashion. As vaccination uptake progressed in patients aged 50 or older and monoclonal antibody-based therapy with proven effectiveness in high-risk outpatients became available, the recruitment dropped dramatically as of June 2021. This resulted in the recommendation by the individual and COMPILE$_{home}$ DSMBs that further enrollment was unlikely to change the results, and both studies were discontinued. Regarding the advent of the SARS-CoV-2 variants that may be less susceptible to antibodies induced by the original SARS-CoV-2 virus or the alpha variant, it is reassuring that >95% of the patients in both countries were included at a time when the delta variant was still rare (<5%) (Supplementary Figs. 3, 4). The last limitation of our study (and all studies on CP for COVID-19 so far) is the lack of a proper phase 2 dose-finding study. In a recent study, we administered 600 mL of CP to 25 SARS-CoV-2 antibody-negative B-cell depleted patients diagnosed with COVID-19[26]. While all seroconverted immediately after transfusion, the median virus neutralization titer only rose to 1:40. This is 4 times lower than the median titer in immunocompetent convalescent COVID-19 patients and up to 100 times lower than titers observed after treatment with monoclonal antibodies[7,19]. Therefore, we postulate that the range of neutralizing antibody titers present in the 200–300 mL of plasma we used may well have been too low. That underdosing may partially explain our findings is also suggested by a study in which human CP with a neutralizing antibody titer of 1:320 did not prevent disease in hamsters while a titer of 1:2560 did[27]. Hence, we recommend that any future study on CP for COVID-19 should use donors at the upper extreme end of antibody titers (e.g., >1:2560 IU). Although, this was virtually impossible in 2020, this should no longer be difficult now as plasma donors recently vaccinated or boosted with a mRNA SARS-CoV-2 vaccine can be selected.

Last but not least, a recent preprint publication by Sullivan et al. described the results of the CSSC04 study in which 1181 outpatients received one unit of convalescent or control plasma. In this trial, CP lowered the risk of hospital admission or death from 6.3 to 2.9%, $p = 0.004$[28]. Therefore, the limited impact on hospital admission or death in our study should be interpreted in the context of this trial.

In conclusion, treatment of COVID-19 with CP in the first 7 days after symptom onset did not improve the outcome. Proper dose-finding studies should be conducted, preferentially in patients with ≤5 days of symptoms before future phase 3 studies on CP are initiated.

Methods

Overview of study design and research partners. Beginning in November 2020, we systematically searched for RCTs recruiting outpatients that compared treatment with CP with a blinded or unblinded control arm in the European (https://www.clinicaltrialsregister.eu/) and American (www.clinicaltrials.gov) trial register. Search terms were convalescent plasma, COVID-19, phase 2 or phase 3, adult, and recruiting or not recruiting. Studies were selected if they were RCTs on outpatients, if their inclusion criteria were confined to patients who had symptoms less than 7 days, and if they had a planned sample size of at least 100 participants of age 50 or older. Investigators of qualifying trials were contacted and informed about COMPILE$_{home}$ and invited to collaborate in the study.

The full COMPILE$_{home}$ protocol is available as an online supplement. The study was designed as a Bayesian-adaptive individual patient data meta-analysis of ongoing clinical trials. Prior to the start of pooling, the study teams agreed upon a minimal set of data required to analyze the primary and secondary endpoints was agreed upon by the study teams. Each trial provided updated data every 6 weeks. The pooled data were monitored by 2 unblinded statisticians and a data and safety monitoring board (DSMB) every 6 weeks using a pre-established stopping guideline for efficacy. At each interim analysis, a posterior distribution of the treatment effect was estimated.

Study patients and selection criteria. Although the exact inclusion and exclusion criteria could vary across the trials, all the subjects had to fulfill the following criteria; (1) Participant of a trial that joined the COMPILE$_{home}$ consortium, (2) Confirmed COVID-19 diagnosis by a diagnostic PCR or antigen test of <7 days, (3) Neither hospitalized nor at the emergency room department of a hospital before or at the time of randomization, (4) Symptomatic with illness onset ≤7 days at the time of screening for the study defined by a physician with a complete clinical history, and (5) Age 50 or older. Trials had to be approved by the institutional review boards, and competent authorities of the countries involved, and all patients gave written informed consent.

Intervention. To qualify for COMPILE$_{home}$, participants randomly assigned to the experimental group had to receive an infusion of ABO-compatible CP with high antibody titers as determined via a semiquantitative antibody test against the spike protein or a virus neutralization assay. Only trials in which the participants were masked for the intervention were included.

Outcomes. Two primary efficacy outcome variables were selected. The first primary endpoint incorporated the speed of recovery as well as the risk of hospital admission, ICU admission or death in a 5-point ordinal scale. It was defined as the highest score on a 5-point ordinal disease severity scale within the 28 days after randomization. A patient scored 1 if he/she recovered quickly (i.e., fully recovered within seven days after transfusion), 2 when continued symptoms attributable to COVID-19 were present on day seven, 3 when admission to a hospital was required at any point within 28 days, 4 when invasive ventilation was required at any point within 28 days, and 5 when the patient had died at any point within 28 days. This means that the best outcome (ordinal scale score of 1) is given when a patient is fully recovered before day 8 and was never hospitalized nor died in the 28 days after transfusion, while a patient who recovered after day 7 but was never hospitalized nor died in the 28 days scored a 2 on the scale. The second primary endpoint was the occurrence of hospitalization or death within 28 days. Secondary endpoints were time to full symptom resolution (assessed by the blinded study team during a telephone contact on day 7, day 14, and day 28) and the safety of CP in outpatients with COVID-19. Pre-planned subgroup analyses assessed the efficacy of the 2 primary outcomes in the following subgroups: (1) days since disease onset (1–5 or >5 days), (2) level of neutralizing antibody anti-SARS-CoV-2 titers in transfused plasma, and (3) Negative serum anti-SARS-CoV-2 IgG status (Trimeric Spike antibody test, Liaison, Diasorin, Saluggia, Italy).

Statistical analysis. The first primary endpoint was analyzed with a Bayesian proportional odds model with normally distributed priors. The model included a main treatment effect shared among the trials (using a skeptical (i.e., conservative) standard deviation of 0.4), main trial effects (using standard deviation 0.5 for the prior distribution), and trial by treatment interactions (using a standard deviation of 0.14 for the prior distribution). The following covariates were included with a standard deviation of 0.5 for the prior distribution of their effects: age, sex, number of comorbidities (0–9), oxygen saturation at baseline (in %), immunocompromised state (Y/N) and duration of time (in days) since COVID-19 symptom onset (Supplementary Table 1). The second primary endpoint was analyzed with a Bayesian logistic model with a similar specification.

The use of the Bayesian framework and stopping rules enables continuous monitoring of the accrued data, and allowed for real-time decisions without penalties for multiple data looks associated with the classic frequentist approach. The results of each interim analysis were reported to the unblinded DSMB. The process and pre- specified thresholds for efficacy are described in detail in the protocol. The full statistical analysis plan is available as an online supplement.

The number of studies and patients included in COMPILE$_{home}$ was not restricted and there was no pre-determined minimum or maximum sample size. The monitoring was planned to continue until the DSMB determined that there was sufficient evidence to recommend stopping the study. This situation could be achieved when the predefined stopping thresholds signaled efficacy or when the included studies had finished enrollment or any future recruitment was very unlikely to change the conclusion.

Ethical approval. The study was reviewed and approved by the institutional review boards of the Erasmus University Medical Center. The study was done according to the Helsinki Declaration of the World Medical Association. Written informed consent was obtained from every patient or legal representative. The COMPILE-home DSMB consisted of the chair (an infectious diseases physician), the

unblinded statisticians from the individual studies and another infectious diseases specialist. They reviewed the pooled dataset on a regular basis as described in the COMPILEhome study protocol and recommended the study team regarding the further conduct of the study. Findings are reported according to the CONSORT (Consolidated Standards of Reporting Trials) statement. For the COnV-ert study, the protocol was approved by the Ethics Committee at Hospital Germans Trias i Pujol (number PI 20-313) and the institutional review boards of participating centers. For the CoV-Early study, the protocol was approved by the medical ethical review board of the Erasmus Medical Center (METC-2020-0682).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The source data and generated data are available in the Supplementary Data files.

Code availability

The codes generated during the current study are available from the corresponding author on reasonable request.

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Author contributions

B.J.A.R. conducted the search for studies that could potentially enter the COMPILEhome consortium. B.J.A.R. and OM agreed on the terms for the COMPILEhome consortium. B.J.A.R., O.M., P.M.M., A.G., A.A., C.R., M.C.M., B.B., A.T., and J.J.Z. conceived and designed the COnV-ert and CoV-Early studies. The COMPILEhome protocol was written by B.J.A.R. and G.P. G.P. and NvG wrote the statistical analysis plan and did the statistical analysis. P.M.M., A.G., A.A., C.R., C.G., G.P., N.v.G., C.J., G.G., F.S., E.v.d.S., M.C.M., D.O., F.P.F., P.M., S.V., V.G.G., A.R.C., A.R.M., J.R.C., R.A.S., J.R.G., J.B., I.B., J.A., Q.B., B.C., B.B., A.T., J.J.Z., O.M., B.J.A.R. acquired and interpreted the data. P.M.M., A.G., A.A., O.M., G.P., and B.J.A.R. drafted the paper. P.M.M., A.G., A.A., C.R., C.G., G.P., N.v.G., C.J., G.G., F.S., E.v.d.S., M.C.M., D.O., F.P.F., P.M., S.V., V.G.G., A.R.C., A.R.M., J.R.C., R.A.S., J.R.G., J.B., I.B., J.A., Q.B., B.C., B.B., A.T., J.J.Z., O.M., B.J.A.R. critically revised the paper for important intellectual content. O.M. and B.J.A.R. were responsible for the final decision to submit for publication. All authors have seen and approved the paper.

Competing interests

The authors declare no competing interests.

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Study 3

Coronavirus Disease 2019 Convalescent Plasma Outpatient Therapy to Prevent Outpatient Hospitalization: A Meta-Analysis of Individual Participant Data From 5 Randomized Trials.

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MAJOR ARTICLE

Coronavirus Disease 2019 Convalescent Plasma Outpatient Therapy to Prevent Outpatient Hospitalization: A Meta-Analysis of Individual Participant Data From 5 Randomized Trials

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(See the Editorial Commentary by Shoham on pages 2087–9.)

Background. Outpatient monoclonal antibodies are no longer effective and antiviral treatments for coronavirus disease 2019 (COVID-19) disease remain largely unavailable in many countries worldwide. Although treatment with COVID-19 convalescent plasma (CCP) is promising, clinical trials among outpatients have shown mixed results.

Methods. We conducted an individual participant data meta-analysis from outpatient trials to assess the overall risk reduction for all-cause hospitalizations by day 28 in transfused participants. Relevant trials were identified by searching Medline, Embase, medRxiv, World Health Organization COVID-19 Research Database, Cochrane Library, and Web of Science from January 2020 to September 2022.

Results. Five included studies from 4 countries enrolled and transfused 2620 adult patients. Comorbidities were present in 1795 (69%). The virus neutralizing antibody dilutional titer levels ranged from 8 to 14 580 in diverse assays. One hundred sixty of 1315 (12.2%) control patients were hospitalized, versus 111 of 1305 (8.5%) CCP-treated patients, yielding a 3.7% (95% confidence interval [CI], 1.3%–6.0%; $P = .001$) absolute risk reduction and 30.1% relative risk reduction for all-cause hospitalization. The hospitalization reduction was greatest in those with both early transfusion and high titer with a 7.6% absolute risk reduction (95% CI, 4.0%–11.1%; $P = .0001$) accompanied by at 51.4% relative risk reduction. No significant reduction in hospitalization was seen with treatment >5 days after symptom onset or in those receiving CCP with antibody titers below the median titer.

Conclusions. Among outpatients with COVID-19, treatment with CCP reduced the rate of all-cause hospitalization and may be most effective when given within 5 days of symptom onset and when antibody titer is higher.

Keywords. SARS-CoV-2; COVID-19 convalescent plasma; hospitalization; therapy; COVID-19.

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The coronavirus disease 2019 (COVID-19) pandemic is responsible for an estimated 18 million excess deaths through 2021 including >1 million in the United States [1]. Despite widespread vaccination in high- and middle-income countries, new variant outbreaks, including the December 2022 outbreak in China, continue to fuel economic disruptions and increased hospitalizations [2]. Novel vaccines and treatments against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been developed, tested, and deployed in record time, yet most arrived too late to benefit the millions of people who died in the pandemic's first year [1]. Three years into the COVID-19 pandemic, it remains unclear how we can respond faster and more effectively to the next pandemic [3, 4].

Antibodies to the SARS-CoV-2 virus, whether induced by vaccination or infused as monoclonal antibodies (mAbs) or polyclonal convalescent plasma, have been shown to reduce the risk of COVID-19–related hospitalization and death, but only convalescent plasma is likely to be both available and affordable for the majority of the world in the early days of the next viral pandemic [5]. COVID-19 convalescent plasma (CCP) was first administered to a hospitalized patient in China in January 2020 [6] and in the United States in March 2020 [7]. Meanwhile, mAbs to prevent hospitalization [8, 9] and vaccines [10, 11] to prevent symptomatic infection, hospitalization, or death were not available until December 2020. By that time, more than 79 million cases of COVID-19 and 1.7 million deaths had been reported worldwide [12]. Effective oral drug therapy for outpatient use was not available until December 2021 [13]. While safe and effective oral agents against SARS-CoV-2 are ideal to prevent COVID-19 hospitalizations, this solution remains unavailable to many patients worldwide due to high costs [14, 15], with effectiveness threatened at any time by new resistant variants.

Escape spike protein mutations leading to acquired resistance during treatment with a single mAb have been repeatedly described in immunocompromised patients [16, 17]. The rapid rise of variants with spike protein mutations has created a dilemma in mAb development, as pharmaceutical companies weigh the high development cost against short-lived utility [18]. Now that all authorized mAbs are no longer effective against recent omicron variants like BQ.1.1 [19–21], CCP, which can be continuously updated from regionally circulating variants, remains an important therapeutic option, especially for severely immunocompromised and other high-risk patients [22, 23].

Most initial randomized controlled trials (RCTs) of CCP were conducted in patients already hospitalized with COVID-19, largely due to the convenience of conducting research in this population. Later in the pandemic, RCTs of CCP targeting outpatients were designed to determine whether early CCP treatment could prevent hospitalization, though few had sufficient power on their own to measure this outcome. Our objective in this study was to conduct an individual patient meta-analysis of all available RCTs of CCP in adult COVID-19 outpatients to determine whether early CCP therapy reduces hospitalization.

METHODS

This study followed the guidelines provided in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement [24].

Objectives

This review aimed to find, assess, and synthesize all RCTs that assessed the efficacy of CCP in preventing all-cause hospitalization among outpatients with confirmed symptomatic SARS-CoV-2 infection.

Eligibility, Search Strategy, RCT Selection, and Data Extraction and Quality

Our PICO (population, intervention, comparator, and outcome) included the following: population = adult (≥18 years) COVID-19 outpatients (not hospitalized at time of transfusion with CCP or placebo) regardless of risk factors; intervention = intravenous CCP transfusion, qualified by antibody titer; comparators = control (nonconvalescent plasma or normal saline); and outcome = all-cause hospitalization within 28 days of transfusion. We used a modified intention-to-treat (mITT) analysis, which included patients for whom transfusion with CCP or placebo was initiated (though not necessarily completed). For 1 study in Argentina, patients meeting prespecified hypoxic respiratory criteria were sometimes admitted to a specific unit within their long-term care facilities, which provided hospital-level care, to avoid overcrowding hospitals. For purposes of trial eligibility, we considered these admissions to be hospitalizations. Only English-language documents were reviewed.

A literature search was performed independently by 2 authors (Y. F., D. J. S.). The Medline, Embase, medRxiv, Cochrane Library, World Health Organization COVID-19 Research Database, and Web of Science were searched for all RCTs as of 30 September 2022. Search strategies were designed with terms related to CCP and COVID-19 (Supplementary Figure 1). All RCTs were included that met the eligibility criteria above. We contacted the corresponding authors for each of the included trials and asked them to contribute data and serve as coauthors for the prepared manuscript.

The investigators for each RCT provided the following data elements: trial design characteristics, descriptions of the intervention and control groups, baseline characteristics of the patients (including underlying comorbidities and days after symptom onset), CCP characteristics (eg, antibody titers), hospitalizations, enrollment period, target enrollment, number of

enrollments, number of transfusions, and trial locations. Data not provided in the published reports were collected from the authors.

A risk of bias assessment was independently performed by COVID-19 Network Meta-Analysis [25, 26].

Statistical Analysis

Primary and secondary analyses were done in the mITT population including all randomized participants who received the intervention (CCP or control). The primary outcome used for analysis was all-cause hospitalization within 28 days of transfusion, and the secondary outcome was all-cause hospitalization among those patients admitted to hospitals >24 hours after transfusion. Two subgroup analyses were performed: (1) the reduction in hospital admission for patients with ≤5 versus >5 days of symptoms at the time of intervention; and (2) the reduction in hospitalizations for patients receiving CCP with antibody titers above the median SARS-CoV-2 antibody titer value for each individual RCT versus those receiving CCP not above the median.

Descriptive analysis included the country in which the study was conducted, patient demographics, days since symptom onset, plasma donor antibody levels, and high-risk comorbidities. Box plots were used for visualization and comparison of viral neutralization among studies. Treatment effect was determined using the absolute risk reduction (ARR), relative risk reduction (RRR), and number needed to treat (NNT). Odds ratio (OR), 95% confidence interval (CI), weight of each study (inverse of the variances), heterogeneity (I^2), between-study variance (τ^2), and significance levels were estimated using mixed random-effects models and displayed in forest plots. A funnel plot was used to estimate the risk of publication bias. The significance level for analyses was set at .05. All of the data manipulation and analyses were performed using Excel software and R software (version 4.2.0, R Foundation, Vienna, Austria) and its statistical packages "meta" (version 6.0-0) and "metafor" (version 3.8-1).

RESULTS

Trial Population

A total of 617 studies were identified by our primary search strategy. After screening and exclusion of ineligible studies, 5 RCTs were included (Figure 1). Two were conducted in the United States [27, 28], 2 in Europe [29, 30], and 1 in Argentina [31]. All of the trials were stopped early: 1 due to slow recruitment as COVID-19 cases in the trial region decreased considerably [31], 3 due to rapid uptake of vaccination resulting in substantial reduction in hospital admission rates [27, 29, 30], and 1 due to a finding of futility to detect the planned difference after the second planned interim analysis of the primary outcome analysis [28].

The 5 RCTs enrolled 2693 patients from June 2020 to October 2021 [27–31], and transfusion was initiated in 2620 patients (Table 1). Seventy-three patients were either hospitalized or withdrew from the study after randomization but before transfusion with CCP or placebo could be initiated. These 5 trials varied in terms of their demographic and clinical profiles, including median age, sex distribution, and the prevalence of major risk factors for COVID-19–related hospitalization (Table 1). The target study populations were all COVID-19 outpatient participants regardless of comorbidities (diabetes, cardiovascular, or lung disease) without contraindication to plasma transfusion. Studies also varied somewhat in the timing of the intervention, although 1562 patients (60%) were transfused within 5 days of symptom onset. Overall, only 159 (6%) of all patients were fully vaccinated (defined as 2 messenger RNA doses or 1 adenovirus-vectored dose). We found that the risk of bias was low for the 5 RCTs (Supplementary Table 1). Funnel plot analysis did not suggest a risk of publication bias (Supplementary Figure 2).

Convalescent Plasma

The included studies used diverse assays to qualify and characterize the CCP transfused in study subjects (Supplementary Table 2). There was insufficient residual donor plasma samples available to compare neutralization titers across the different studies using the same assay. Two studies qualified units with 50% viral neutralization dilutional plasma titers >1:160. Two studies qualified with dilutional antibody binding greater than 1000 or 320, while the last measured Euroimmun immunoglobulin G was >6.0 AU. Separate viral neutralization indices, depicted in Supplementary Figure 3, show that COVID-19 serologic studies consortium (CSSC-004) and COVID-19 convalescent plasma-Argentina (CCP-Argentina) had slightly lower viral neutralization metrics, albeit a different viral neutralization assay than clinical-trial of COVID-19 convalescent plasma in outpatients (C3PO), early convalescent plasma therapy (CoV-Early), and convalescent methylene blue treated (MBT) plasma for early treatment (CONV-ERT).

Primary Outcome: Hospitalization

Modified intention-to-treat analysis (Table 2) was performed on patients who received either CCP or control. CSSC-004 added 7 all-cause hospitalizations (4 CCP and 3 control plasma) above reported COVID-19–related hospitalizations and C3PO added 2 participants hospitalized after day 15 but before day 28. Overall, 160 (12.2%) subjects in the control group were hospitalized, compared to 111 (8.5%) in the CCP treatment group, yielding an ARR of 3.7% (95% CI, 1.3%–6.0%), NNT of 27, and RRR of 30.1% (95% CI, 12.0%–44.4%) for all-cause hospitalization (Table 2). The OR for hospitalization was 0.64 (95% CI, .45–.92) in the pooled meta-analysis, and trial heterogeneity was moderate, with an I^2 of 42% (Figure 2). A secondary analysis was conducted excluding those

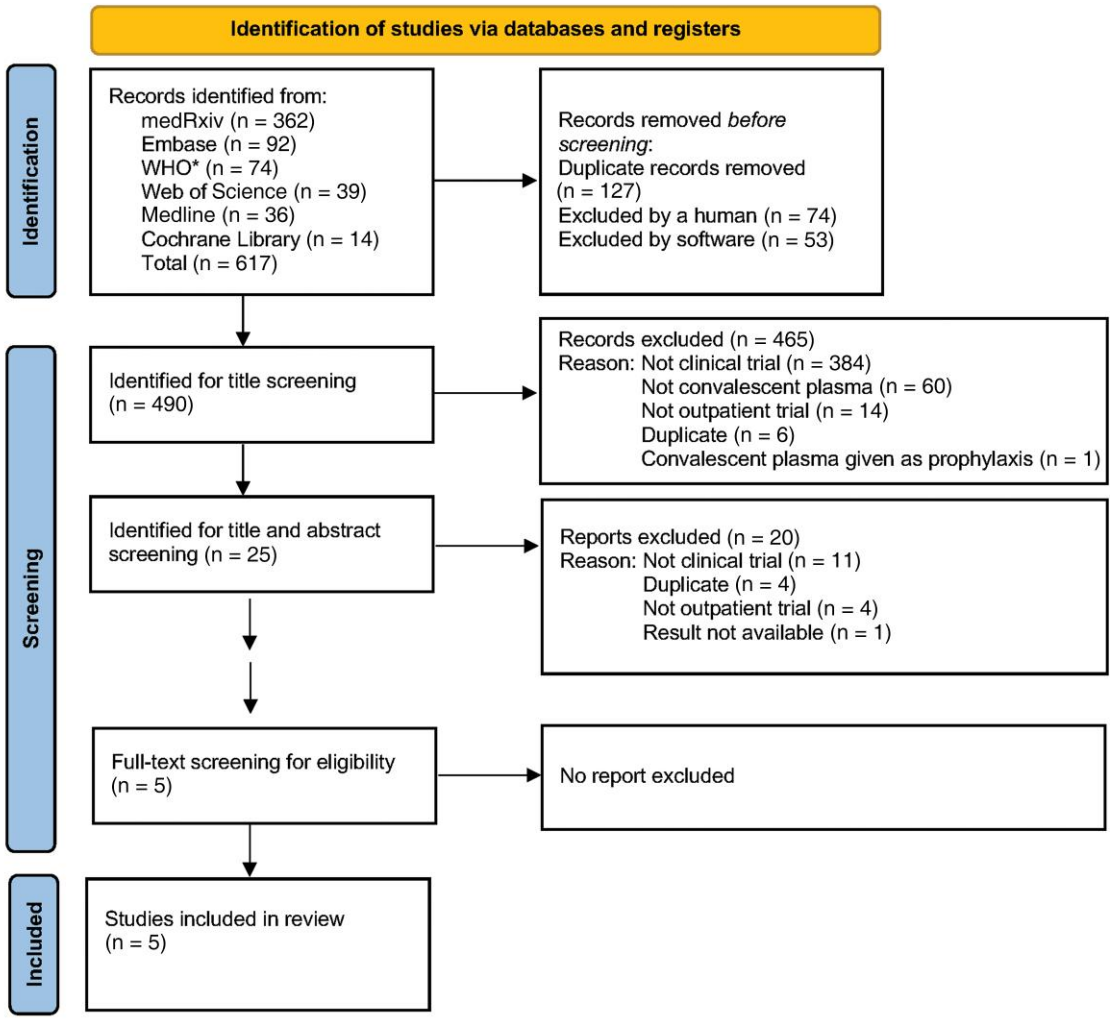

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart. The Medline, Embase, medRxiv, Cochrane Library, World Health Organization (WHO) COVID-19 Research Database, and Web of Science were searched for all randomized controlled trials as of 30 September 2022. Abbreviation: COVID-19, coronavirus disease 2019. *WHO COVID-19 global literature on coronavirus disease.

patients admitted to the hospital within 24 hours of CCP (25 patients) or control (13 patients) transfusion, yielding an ARR of 4.4% (95% CI, 2.2%–6.6%), NNT of 23, and RRR of 39.2% (95% CI, 21.7%–52.8%). The OR for hospitalization was 0.58 (95% CI, .41–.82), and trial heterogeneity was low in this secondary analysis, with an I^2 of 31% (Figure 2).

Subgroup Analyses

Subgroup analyses were performed based upon the timing of CCP transfusion and the SARS-CoV-2 antibody titer level in transfused CCP units. For subjects transfused within 5 days of symptom onset, pooled analysis among all 5 studies indicated an ARR of 5.8% (95% CI, 2.6%–9.0%), NNT of 17, and RRR of 39.5% (95% CI, 19.9%–54.3%) in hospitalizations when compared to control (Table 2 and Figure 3). Study subjects transfused with high antibody titer CCP (defined as equal to or greater than the median neutralization titer for each individual study) had an ARR of 4.8% (95% CI, 2.2%–7.4%), NNT of 21, and RRR of 40.3% (95% CI, 18.8%–56.1%) in hospitalization compared with subjects given the control (Table 2 and Figure 4). Subjects transfused after 6 days of symptoms or with low antibody titer CCP did not show a significant decrease in hospitalization when compared with control (Table 2). The risk reduction in patients receiving high antibody titer CCP and within 5 days of symptom onset was higher for the combined studies at an ARR of 7.6% (95% CI, 4.0%–11.1%), NNT of 13, and RRR of 51.7% (95% CI, 28.3%–67.1%) (Table 2 and Figure 5).

Safety

Due to small numbers, we did not combine severe adverse events related to transfusion in a meta-analysis; however, they were collected for each trial. In CSSC-004, 1 subject experienced a

Table 1. Trial Characteristics

Characteristic	CSSC-004	CCP-Argentina	CONV-ERT	C3PO	CoV-Early	Total
Control arm	Plasma	Saline	Saline	Saline/MVC	Plasma	…
Enrollment period	June 2020 to Oct 2021	June 2020 to Oct 2020	Nov 2020 to July 2021	Aug 2020 to Feb 2021	Nov 2020 to July 2021	…
Trial duration, mo	16	5	9	7	9	46
Variants	614G, Alpha, Beta, Delta	WA-1, D614G	D614G, Alpha	D614G	D614G, Alpha	…
Geography	US	Argentina	Spain	US	Netherlands	…
Target enrollment, No.	1280	210	474	900	690	3554
Enrolled, No.	1225	160	376	511	421	2693
mITT (% of target enrollment)	1181 (92)	154 (73)	369 (78)	500 (55)	416 (60)	2620 (74)
Age, y, median (range)	43 (18–85)	77 (65 to ≥90)	56 (IQR, 52–62)	54 (18–93)	60 (IQR, 55–65)	…
≥1 medical high-risk condition for COVID-19 progression	470 (40)	131 (82)	278 (74)	511 (100)	416 (100)	1806 (68.6)
Enrollment symptom duration for inclusion, d	0–8	0–3	0–7	0–7	0–7	0–8
Symptoms ≤5 d	517 (44)	154 (100)	283 (77)	389 (78)	226 (54)	1569 (60)
Symptoms ≤3 d	168 (14)	154 (100)	101 (27)	240 (48)	52 (13)	715 (27)
Median/mean duration of symptoms, median (mean)	6	3	(4.4)	4	5	…
Total female	675 (57)	98 (64)	169 (46)	265 (53)	93 (22)	1300 (50)
Age >50 y	411 (35)	154 (100)	368 (100)	310 (61)	414 (100)	1657 (63)
Age >65 y	80 (7)	154 (100)	73 (20)	95 (19)	113 (27)	515 (20)
Diabetes	99 (8)	35 (23)	39 (10)	142 (28)	29 (7)	344 (13)
Hypertension	276 (23)	110 (71)	244 (66)	216 (42)	Not reported	846 (38)[a]
Obesity (BMI >30 kg/m^2)	444 (38)	11 (7)	95 (26)	302 (60)	126 (30)	978 (37)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: BMI, body mass index; C3PO, clinical-trial of COVID-19 convalescent plasma in outpatients; CCP-Argentina, COVID-19 convalescent plasma-Argentina; CONV-ERT, convalescent methylene blue treated (MBT) plasma for early treatment; CoV-Early, early convalescent plasma therapy; CSSC-004, COVID-19 serologic studies consortium; COVID-19, coronavirus disease 2019; IQR, interquartile range; mITT, modified intention-to-treat (those transfused); MVC, multivitamin concentrate; US, United States.

[a]Only included 4 reported studies.

transfusion reaction that required cessation of the transfusion [27]. The CCP-Argentina trial did not report any instances of volume overload, allergic reactions, or vasovagal syndromes, but did report 1 case of thrombophlebitis in the control arm. The C3PO authors noted 3 serious transfusion reactions in the CCP arm resulting in steroid or epinephrine administration or hospitalization [28]. The CONV-ERT team communicated no severe adverse events related to transfusion, but 3 vasovagal reactions and mild allergic reactions in 12 of 188 (6.4%) subjects transfused with CCP [30]. A participant with pulmonary embolism was reported 7 days after transfusion. The CoV-Early investigators reported 3 severe adverse events possibly related to plasma transfusion (all with nonconvalescent plasma). Two developed an anaphylactic reaction shortly after receiving plasma for which no hospital admission was required, and 1 patient developed generalized urticaria requiring hospitalization.

DISCUSSION

This meta-analysis of all available RCTs found that early outpatient therapy with CCP in adult patients with COVID-19 was associated with a 30% all-cause hospitalization RRR (NNT, 27) and a 39% RRR (NNT, 23) when excluding patients admitted on the same day as treatment (Table 2). Early treatment with high antibody titer CCP demonstrated a 51% hospitalization RRR (NNT, 13) in all-cause hospitalization among adult patients with COVID-19. Despite differences in the demographics and clinical characteristics of the 5 study populations, overall study heterogeneity was low to moderate, suggesting the appropriateness of combining these studies in a single meta-analysis and broadly generalizing these results. While the effectiveness of early CCP treatment in reducing all-cause hospitalization was less than that of many mAb treatments [32, 33] and antiviral therapies [13, 34], this should be balanced against its increased availability and potential for activity against variant strains of SARS-CoV-2.

Two of the 5 RCTs included in this meta-analysis (CONV-ERT and C3PO) failed to demonstrate a reduction in all-cause hospitalization with CCP, while the other 3 trials (CCP-Argentina, CSSC-004, CoV-Early) all showed approximately 50% reductions in hospitalizations. One potential explanation for the lack of effectiveness for CCP in the CONV-ERT trial is that methylene blue photoinactivation was used for pathogen reduction in transfused units. This might have

Table 2. Overall Numbers and Percentage of Pooled Numbers for Hospitalization and Totals

Study	Total CCP Outcomes	Total CCP	Total Control Outcomes	Total Control	Totals	CCP, %	Control, %	ARR, % (95% CI)	RRR % (95% CI)	Significance Level, *P* Value	NNT Benefit
mITT (all-cause hospitalizations)	111	1305	160	1315	2620	8.5	12.2	3.7 (1.3–6.0)	30.1 (12.0–44.4)	.0011	27
mITT (hospitalizations after 24 h from transfusion)	88	1282	147	1302	2584	6.9	11.3	4.4 (2.2–6.6)	39.2 (21.7–52.8)	.0001	23
Onset ≤5 d	70	787	114	775	1562	8.9	14.7	5.8 (2.6–9.0)	39.5 (19.9–54.3)	.0002	17
Onset ≥6 d	41	518	46	540	1058	7.9	8.5	.6 (−2.7 to 3.9)	7.1 (−39.1 to 37.9)	.3605	166
Donor titer ≥ median	49	687	157	1315	2002	7.1	11.9	4.8 (2.2–7.4)	40.3 (18.8–56.1)	.0004	21
Donor titer below median	62	593	157	1315	1908	10.5	11.9	1.5 (−1.5 to 4.5)	12.4 (−15.6 to 33.7)	.1735	67
High titer AND onset ≤5 d	29	406	114	775	1181	7.1	14.7	7.6 (4.0–11.1)	51.4 (28.3–67.1)	.0001	13
Low titer and onset ≤5 d, high titer and onset >5 d, low titer and onset >5 d	75	836	160	1315	2151	9.0	12.2	3.2 (.6–5.8)	26.3 (4.4–43.2)	.0105	31

Data are presented as No. unless otherwise indicated.

Abbreviations: ARR, absolute risk reduction; CCP, coronavirus disease 2019 convalescent plasma; CI, confidence interval; mITT, modified intention-to-treat; NNT, number needed to treat; RRR, relative risk reduction.

affected the constant regions of antibody function without interfering with the viral neutralization assay [35]. The C3PO trial, unlike the other RCTs, enrolled only patients presenting to the emergency department (ED) with COVID-19, which likely included a more severely ill patient population further along in the inflammatory phase of disease. Indeed, there are often less tangible factors signifying more severe illness that lead a patient to present to the ED rather than to their primary care doctor. This is evidenced by the much larger number of subjects in the C3PO trial (23% of all hospitalizations) who were admitted directly to the hospital from the ED on the same visit in which they were transfused. Eliminating these same-day admissions (as in our secondary analysis) bring the C3PO results in line with those from the other studies and greatly reduces heterogeneity among the 5 studies.

Antibody levels for the transfused CCP used across these 5 trials varied substantially, despite the fact that donors had been selected based upon a minimum antibody level cutoff in each trial. However, different cutoffs were used as well as different antibody tests. Our observation that the reduction in hospital admission was limited to patients receiving CCP with titers above the median concentration level in each of the trials suggests that the CCP selection process was suboptimal. It is likely that more stringent antibody titer criteria for CCP units may further improve the effectiveness of this intervention [36].

Plasma transfusion, unlike the use of antiviral and mAb agents, presents a risk of transfusion reactions, which may vary from easily treatable conditions (eg, urticaria) to life-threatening reactions such as anaphylaxis. Rates of severe adverse reactions, however, appeared to be low in these outpatient trials.

This study does have several important limitations. While CSSC-004 enrolled both COVID-19–vaccinated and unvaccinated individuals, the other RCTs primarily included unvaccinated patients, which limits our ability to analyze the effectiveness of CCP for reducing COVID-19 hospitalization in a primarily vaccinated population. The NNT with CCP may be much higher in a primarily vaccinated population, although this difference may be mitigated by the rise of mutant variants that undermine the effectiveness of vaccines and mAbs. All 5 included studies also ended before meeting their transfusion goals, reducing their individual power to detect a difference in hospitalizations between treatment and control groups, and therefore increasing the need for this meta-analysis.

Our meta-analysis chose to use an mITT analysis, excluding 73 patients who were randomized to a given treatment but did not receive it due to hospitalization or withdrawal prior to transfusion, which could introduce bias. However, this represents <3% of enrolled patients and would be unlikely to significantly affect our results. More importantly, patients and providers did not know of their randomization assignment,

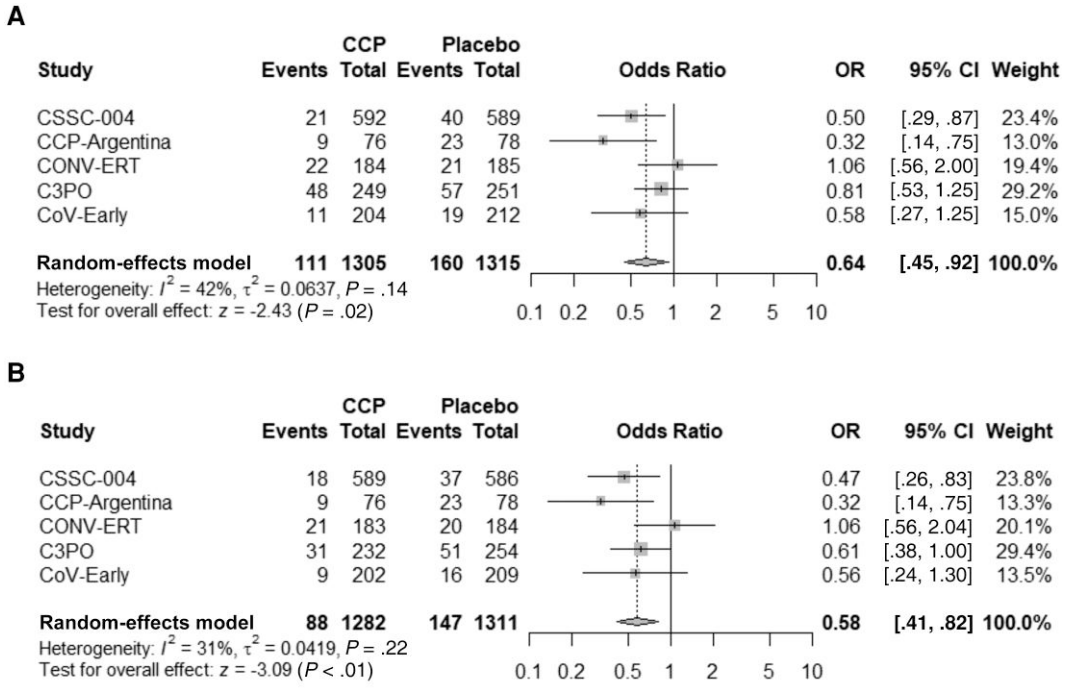

Figure 2. Forest plot of modified intention-to-treat (mITT) analysis (*A*) and of mITT analysis excluding same-day hospital admissions on transfusion day (*B*). Abbreviations: C3PO, clinical-trial of COVID-19 convalescent plasma in outpatients; CCP, coronavirus disease 2019 convalescent plasma; CCP-Argentina, COVID-19 convalescent plasma-Argentina; CI, confidence interval; CONV-ERT, convalescent methylene blue treated (MBT) plasma for early treatment; CoV-Early, early convalescent plasma therapy; CSSC-004, COVID-19 serologic studies consortium; OR, odds ratio.

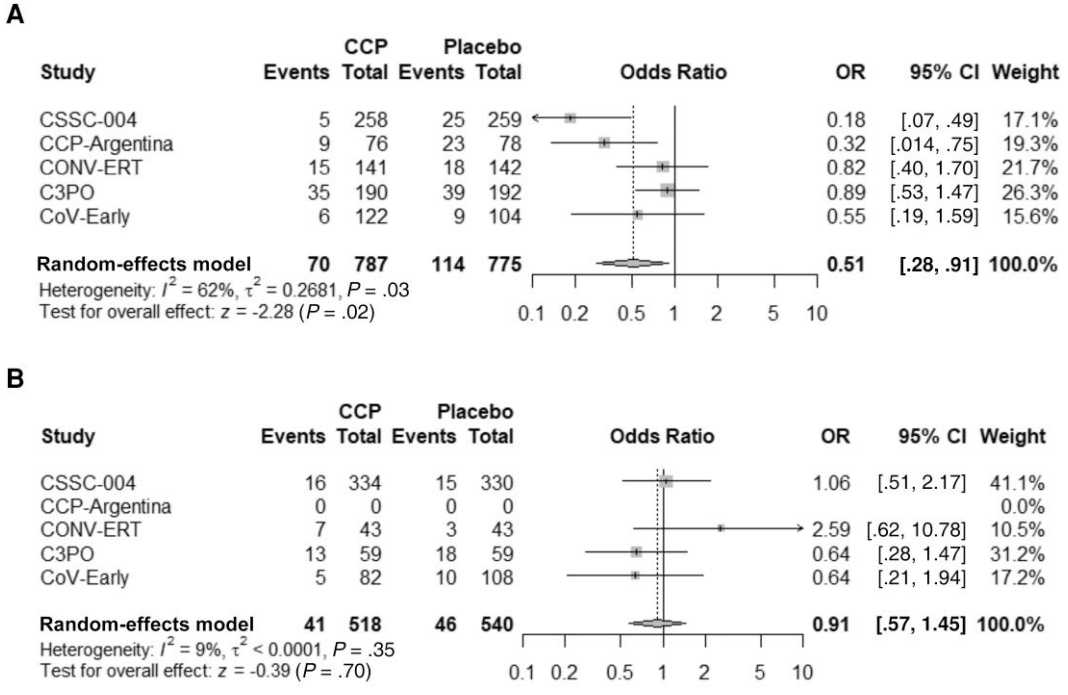

Figure 3. Forest plots of transfusion within 5 days (*A*) or >5 days (*B*). Abbreviations: C3PO, clinical-trial of COVID-19 convalescent plasma in outpatients; CCP, coronavirus disease 2019 convalescent plasma; CCP-Argentina, COVID-19 convalescent plasma-Argentina; CI, confidence interval; CONV-ERT, convalescent methylene blue treated (-MBT) plasma for early treatment; CoV-Early, early convalescent plasma therapy; CSSC-004, COVID-19 serologic studies consortium; OR, odds ratio.

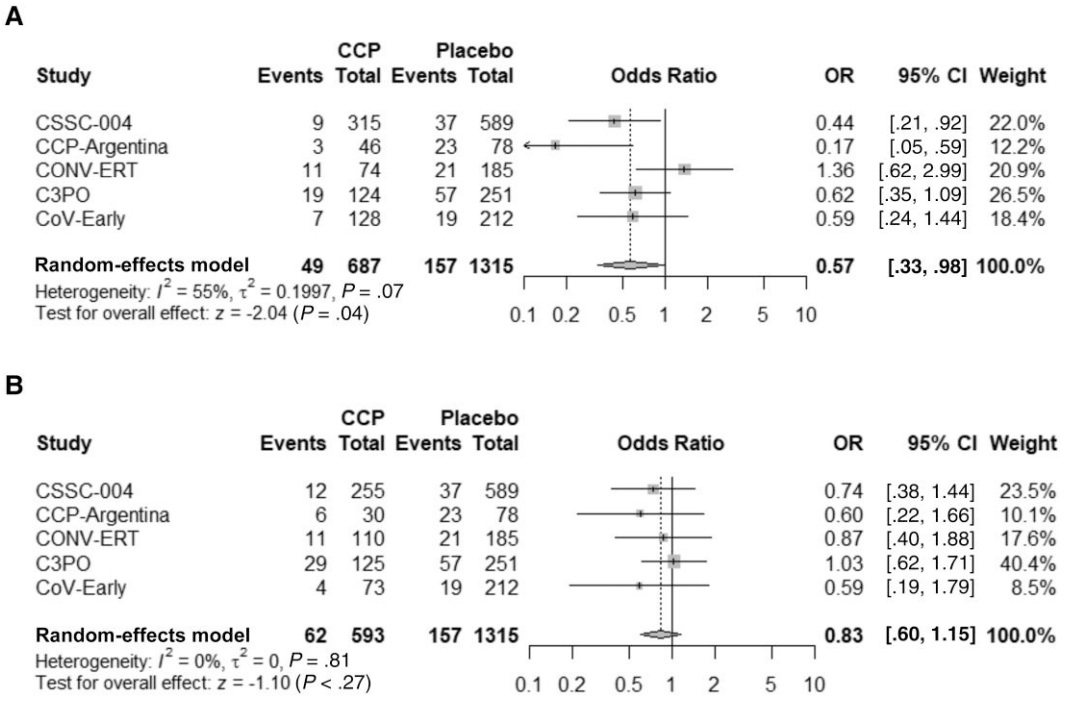

Figure 4. Forest plots of plasma donor antibody levels at or above median titer (*A*) or less than median titer (*B*). Abbreviations: C3PO, clinical-trial of COVID-19 convalescent plasma in outpatients; CCP, coronavirus disease 2019 convalescent plasma; CCP-Argentina, COVID-19 convalescent plasma-Argentina; CI, confidence interval; CONV-ERT, convalescent methylene blue treated (MBT) plasma for early treatment; CoV-Early, early convalescent plasma therapy; CSSC-004, COVID-19 serologic studies consortium; OR, odds ratio.

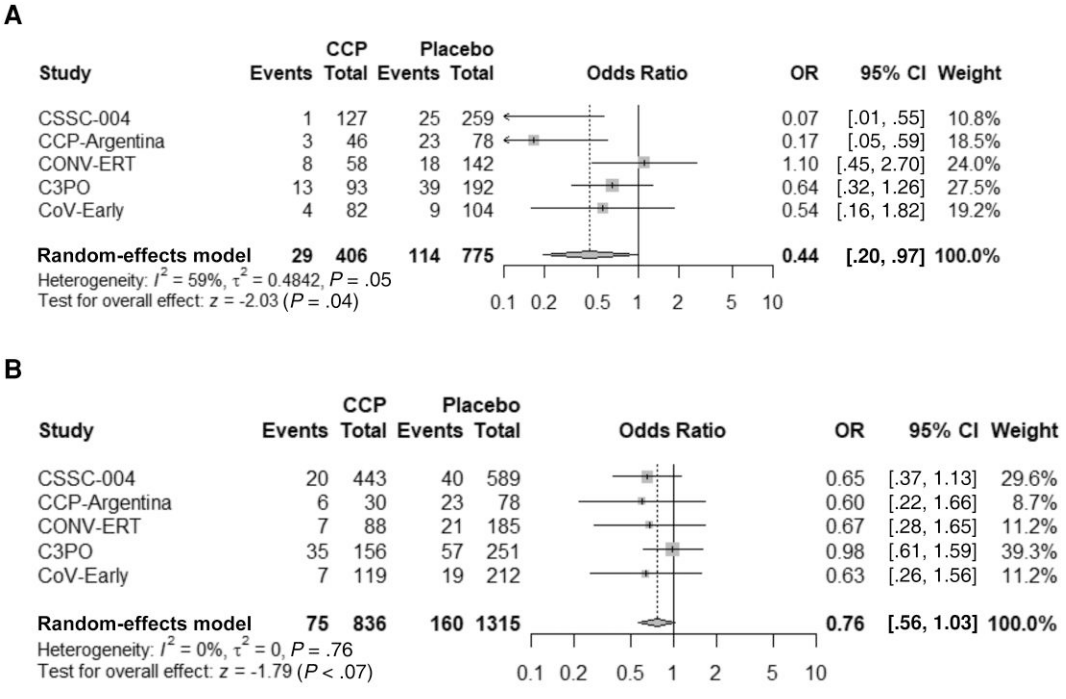

Figure 5. Forest plots of plasma donor antibody levels and early treatment at or above median titer AND transfusion within 5 days (*A*) or total of low titer and onset ≤5 days, high titer and onset over 5 days, low titer and onset over 5 days (*B*). Abbreviations: C3PO, clinical-trial of COVID-19 convalescent plasma in outpatients; CCP, coronavirus disease 2019 convalescent plasma; CCP-Argentina, COVID-19 convalescent plasma-Argentina; CI, confidence interval; CONV-ERT, convalescent methylene blue treated (-MBT) plasma for early treatment; CoV-Early, early convalescent plasma therapy; CSSC-004, COVID-19 serologic studies consortium; OR, odds ratio.

so the risk of bias due to our analysis methodology is low. In the CCP-Argentina study, some patients not actually admitted to a hospital were considered to meet the primary outcome, but these patients did meet standard hospital admission criteria (ie, hypoxia/respiratory distress) and were instead provided with hospital-level care within their long-term care unit. As described above, the actual donor antibody titer levels varied across the 5 RCTs, and the studies used varying assays to measure antibody titer, making it difficult to compare absolute antibody titers across studies. Consequently, we chose to look at median antibody titers within the individual studies as a means of comparing the CCP used in the various RCTs.

Although there are several implementation considerations that could affect the real-world efficacy and sustainability of CCP transfusion programs [37], our pooled meta-analysis including 5 large, rigorously conducted RCTs suggests that high-titer CCP administered early to adult outpatients with COVID-19 significantly reduces the risk of all-cause hospitalizations across a diverse range of demographic and clinical profiles, geographic locations, and transfusion settings. We believe that CCP should be considered as an outpatient treatment option (especially for patients at high-risk for poor outcomes) in settings where mAbs or antivirals are not currently accessible, or when new variants arise that undermine the effectiveness of these interventions. Pandemic preparedness should also incorporate flexible antibody neutralization assay systems for model organisms. Future research should focus on defining the optimal antibody titer and dosage for CCP and evaluating its effectiveness among immunocompromised vaccinated patients. Despite its limitations, CCP has the potential to be an effective, readily available, and highly adaptable intervention for use in both this and future pandemics.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author Contributions. A. C. L. and D. J. S. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: D. J. S., D. F. H., A. C. L. Acquisition, analysis, or interpretation of data: A. C. L., Y. F., M. A. H., J. O., B. R. M., B. P., J. H. P., D. F. H., B. J. A. R., A. G., C. R., J. J. Z., A. A., O. M., V. D.-M., L. J. D., F. K. K., C. W. C., R. L., D. J. S. Drafting of the manuscript: D. J. S., A. C. L., Y. F., M. A. H., J. O., B. R. M., B. P., J. H. P. Critical revision of the manuscript for important intellectual content: A. C. L., Y. F., M. A. H., J. O., B. R. M., B. P., J. H. P., D. F. H., B. J. A. R., A. G., A. A., O. M., V. D.-M., F. K. K., C. W. C., R. L., D. J. S., C. R., J. J. P., P. M.-M. Statistical analysis: J. O., D. J. S., A. C. L. Obtained funding: D. J. S., B. J. A. R., O. M., C. W. C., F. P. Administrative, technical, or material support: C. R., J. J. Z., P. M.-M. Supervision: D. J. S., A. G., A. A., C. W. C., R. L.

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Data sharing. Data are available from individual authors upon request.

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Potential conflicts of interest. R. L. has received fees from Pfizer for serving as subinvestigator of a COVID vaccine trial. F. P. has received fees from Pfizer for serving as a principal investigator for a COVID vaccine trial. D. J. S. is a founder and board member of AliquantumRx with stock options; Hemex Health malaria diagnostics consulting; royalties for malaria diagnostic test control standards from Alere (Binax Inc/D/B/A Inverness Medical); consulting fees for legal malaria case from Mabrey Firm (2019); the following grants or contracts: NIH/NIAID R01AI150763, NIH R21TR001737, NIH R01AI111962, DOD TB210115 (CDMRP Tick-Borne Disease Research Program); the following patents: USP 9642865 (issued 9 May 2017, New angiogenesis inhibitors), USP 7270948 (issued 18 September 2007, Detection of malaria parasites by laser desorption mass spectrometry), Salts and polymorphs of cethromycin for the treatment of disease (pending, application 20210163522), and Macrolide compounds and their use in liver stage malaria and related disease (pending, application PCT/US2015/046665); and participation on 2018 NIAID Safety Monitoring Committee/Independent Safety Monitor Intramural, all outside the submitted work. B. J. A. R. reports advisory board membership for Roche and AstraZeneca on a COVID-19 therapy; membership on a data and safety monitoring board (DSMB) for Exevir; consulting fees from Roche and AstraZeneca; grants or contracts and payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from Gilead Sciences; and support for attending meetings

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Study 4

Subcutaneous anti-COVID-19 hyperimmune immunoglobulin for prevention of disease in asymptomatic individuals with SARS-CoV-2 infection: a double-blind, placebo-controlled, randomised clinical trial.

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Subcutaneous anti-COVID-19 hyperimmune immunoglobulin for prevention of disease in asymptomatic individuals with SARS-CoV-2 infection: a double-blind, placebo-controlled, randomised clinical trial

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Summary

Background **Anti-COVID-19 hyperimmune immunoglobulin (hIG) can provide standardized and controlled antibody content. Data from controlled clinical trials using hIG for the prevention or treatment of COVID-19 outpatients have not been reported. We assessed the safety and efficacy of subcutaneous anti-COVID-19 hyperimmune immunoglobulin 20% (C19-IG20%) compared to placebo in preventing development of symptomatic COVID-19 in asymptomatic individuals with SARS-CoV-2 infection.**

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Methods We did a multicentre, randomized, double-blind, placebo-controlled trial, in asymptomatic unvaccinated adults (≥18 years of age) with confirmed SARS-CoV-2 infection within 5 days between April 28 and December 27, 2021. Participants were randomly assigned (1:1:1) to receive a blinded subcutaneous infusion of 10 mL with 1 g or 2 g of C19-IG20%, or an equivalent volume of saline as placebo. The primary endpoint was the proportion of participants who remained asymptomatic through day 14 after infusion. Secondary endpoints included the proportion of individuals who required oxygen supplementation, any medically attended visit, hospitalisation, or ICU, and viral load reduction and viral clearance in nasopharyngeal swabs. Safety was assessed as the proportion of patients with adverse events. The trial was terminated early due to a lack of potential benefit in the target population in a planned interim analysis conducted in December 2021. ClinicalTrials.gov registry: NCT04847141.

Findings 461 individuals (mean age 39.6 years [SD 12.8]) were randomized and received the intervention within a mean of 3.1 (SD 1.27) days from a positive SARS-CoV-2 test. In the prespecified modified intention-to-treat analysis that included only participants who received a subcutaneous infusion, the primary outcome occurred in 59.9% (91/152) of participants receiving 1 g C19-IG20%, 64.7% (99/153) receiving 2 g, and 63.5% (99/156) receiving placebo (difference in proportions 1 g C19-IG20% vs. placebo, –3.6%; 95% CI -14.6% to 7.3%, $p = 0.53$; 2 g C19-IG20% vs placebo, 1.1%; –9.6% to 11.9%, $p = 0.85$). None of the secondary clinical efficacy endpoints or virological endpoints were significantly different between study groups. Adverse event rate was similar between groups, and no severe or life-threatening adverse events related to investigational product infusion were reported.

Interpretation Our findings suggested that administration of subcutaneous human hyperimmune immunoglobulin C19-IG20% to asymptomatic individuals with SARS-CoV-2 infection was safe but did not prevent development of symptomatic COVID-19.

Funding Grifols.

Keywords: Hyperimmune immunoglobulin; Antibody therapies; COVID-19; SARS-CoV-2; Outpatients; Asymptomatic individuals

Research in context

Evidence before this study

We searched the PubMed database for articles (including preprints) published between April 2020 and October 2022, and reporting results from randomised trials evaluating the effect of hyperimmune immunoglobulins (hIG) for the prophylaxis or treatment of SARS-CoV-2 infected individuals. We used various combinations of the terms "COVID-19", "COVID", "SARS-CoV-2", "Coronavirus", "hyperimmune immunoglobulin", "intravenous immunoglobulin", "hIG", or "hIVIG", "passive immunotherapy", "passive immunization", "plasma therapy", and "clinical trial". The search retrieved only three trials (two pilot studies and an international multicentre study funded by the NIH) evaluating the safety and efficacy of hIG therapies for COVID-19, all of which included only hospitalised patients with COVID-19 and administered intravenous infusion of hIG. No trials were found evaluating the safety and efficacy of hIG therapies in outpatients with SARS-CoV-2 infection.

Added value of this study

This study is the first placebo-controlled randomised clinical trial to report results of anti-COVID-19 hIG as pre-emptive therapy for asymptomatic individuals with confirmed SARS-CoV-2 infection. We found that, compared to placebo, subcutaneous human hyperimmune immunoglobulin C19-IG20% at the dose of either 1 g or 2 g did not reduce the risk of developing symptomatic COVID-19 when administered to asymptomatic individuals with confirmed SARS-CoV-2 infection within 5 days, regardless of risk factors. There was no heterogeneity of treatment effect in efficacy among individuals without endogenous antibodies, nor in any of the other subgroup analyses conducted. There were no significant differences in the safety endpoints, including the proportion of treatment-emergent adverse events and severe adverse events between groups.

Implications of all the available evidence

Our results do not support the use of subcutaneous C19-IG20% in asymptomatic individuals with SARS-CoV-2 infection to prevent symptomatic COVID-19. Our findings indicate that C19-IG20% is safe and well tolerated if administered at the dose of either 1 g or 2 g.

Introduction

Anti-SARS-CoV-2 antibody products have emerged as promising candidates for the treatment and prophylaxis of COVID-19 since the beginning of the pandemic. Five anti-SARS-CoV-2 monoclonal antibody (mAb) products have shown clinical benefit when used to treat COVID-19 outpatients[1–6] and hospitalised patients without detectable antibodies to SARS-CoV-2,[7–9] as well as for pre- and post-exposure prophylaxis.[10–12] However, the efficacy of these mAb therapies can be affected by antigenic shifts of new circulating variants. Currently, all mAbs for the treatment of COVID-19 have shown to be ineffective *in vitro* against the Omicron variant and its subvariants.[13–23] COVID-19 Convalescent Plasma (CCP), an alternative antibody product that contains polyclonal antibodies from donors who have recovered from infection, has proven not to reduce mortality in hospitalised patients.[24–27] CCP has been also tested in outpatients with COVID-19 with mixed results. Positive results were driven by very early administration of CCP (≤5 days after symptoms onset) and high antibody titers.[28–33]

A high-titre and high-concentration antibody preparation can be produced by pooling plasma collected from multiple donors who have recovered from COVID-19, resulting in the so-called anti-COVID-19 hyperimmune immunoglobulin (hIG). The use of hIG preparations has been established for the treatment and prophylaxis of several viral infections, including cytomegalovirus, varicella, rubella, and hepatitis B and A.[34–36] However, clinical data on the use of hIG for COVID-19 are limited to three clinical trials administering the product intravenously to hospitalised patients. The first one, a small single-centre trial of 50 COVID-19 severely or critically ill patients, reported nonsignificant reductions in mortality associated with hIVIG compared to the standard of care.[37] The second trial (ITAC) was an international multicentre study funded by the US National Institute of Health (NIH) that randomized 593 hospitalised COVID-19 patients without end-organ failure to receive either hIVIG or an equivalent volume of saline as placebo in addition to standard clinical care. The trial showed no significant improvement of the clinical status, measured by a seven-category ordinal scale.[38] The third trial showed a reduction in the risk for severe COVID-19 in 18 severely immunocompromised hospitalised patients.[39]

C19-IG20% is a subcutaneous formulation containing 20% human hIG that consists of purified protein from pooled plasma donations, with IgG accounting for at least 98% of the protein. C19-IG20% has some advantages over other anti-SARS-CoV-2 antibody products. First, unlike mAbs, the polyclonal nature of its antibodies could mitigate the immune evasion of emerging viral variants. Second, it contains a standardized and controlled high-titre content of neutralizing antibodies, overcoming the inter-unit variability of CCP. It is also subjected to robust pathogen reduction rendering it virally safe, and it is purified by technologies demonstrated to preserve immunoglobulin neutralization capacity and Fc fragment integrity. Third, unlike most mAbs and other hIG evaluated so far, which need to be administered intravenously, C19-IG20% is available for subcutaneous infusion, allowing easier and faster administration at the primary care level in the outpatient setting. To date, data from controlled clinical trials using hIG products for prophylaxis or treatment of COVID-19 outpatients have not been reported. We evaluated the safety and clinical efficacy of the subcutaneous C19-IG20% in reducing the risk of developing symptomatic COVID-19 in asymptomatic individuals with SARS-CoV-2 confirmed infection.

Methods

Trial design

The GC2010 trial was a multicentre, double-blinded, randomised (1:1:1), parallel group study to assess the safety and efficacy of the anti-COVID-19 hyperimmune immunoglobulin (Human) 20% (C19-IG20%) in preventing symptomatic COVID-19 in asymptomatic outpatients with SARS-CoV-2 infection. The trial was conducted between April 28, 2021, and December 27, 2021, at seven healthcare administrative regions providing universal healthcare to a catchment population of around 12 M people in Spain (Methods S1, Supplementary Appendix).

The study was conducted according to the Helsinki Declaration of the World Medical Association, and the study protocol was approved by the Ethics Committee at Hospital Germans Trias i Pujol (number PI 21-015) and the institutional review boards of the rest of participating centres. All patients provided informed consent before enrolling in the study, which was supervised by an independent data and safety monitoring board. This trial was registered in ClinicalTrials.gov (NCT04847141). The protocol and statistical analysis plan are available in the supplementary materials.

Participants and recruitment

We included asymptomatic individuals aged ≥18 years with laboratory-confirmed SARS-CoV-2 infection within 5 days prior to randomization. SARS-CoV-2 infection was determined by RT-PCR, rapid antigen test, or transcription-mediated amplification (TMA) test. Candidates were considered to be asymptomatic if they had no fever (oral temperature ≥38 °C), cough, shortness of breath, fatigue, anorexia, vomiting/diarrhoea, myalgias, headache, olfactory disorders, or pneumonia at screening. Individuals were excluded from the study if they required hospitalisation for any cause or had an oxygen saturation level (SpO2) of ≤94% on room air, or a National Early Warning Score (NEWS) > 2 points at the baseline visit. Additionally, individuals were

excluded if they had received a complete or incomplete regimen of COVID-19 vaccination, were taking agents with antiviral activity against SARS-CoV-2 and/or convalescent COVID-19 plasma or had contraindications to the investigational product. Female participants who were pregnant, breastfeeding or planning a pregnancy during the study were also excluded. Further details on the eligibility criteria are listed in Methods S2.

Potential eligible participants were identified by searching the database systems for SARS-CoV-2 positive individuals nationwide. The investigators contacted candidates by phone in order to explain the study, invite them to participate, and obtain their oral consent to participate in the screening process. Within 24 h, investigators conducted a baseline visit (day 1) at the home of suitable candidates, during which written informed consent was obtained and eligibility was confirmed.

Randomisation and masking

Participants were randomly assigned using a central web-based randomization system to receive either a 1 g dose of C19-IG20%, a 2 g dose of C19-IG20%, or sterile 0.9% saline solution (placebo). Randomization was stratified by age (<65 years vs. ≥65 years). An unmasked nurse, who was completely independent of the evaluating study team, conducted the randomization after the investigators had confirmed eligibility. The unmasked nurse prepared and administered the blinded investigational product. All participants and investigators were masked to the treatment allocations, including follow-up personnel, laboratory personnel, and statisticians, with the exception of unmasked nurses. The randomization and administration of the investigational product were always conducted on the first day of the study (baseline visit, day 1).

Investigational products and procedures

Both, the investigational product and placebo, were administered with a 10 mL subcutaneous infusion over 10–20 min (1–2 min per mL) on day 1. The investigational product (i.e., C19-IG20%) (prepared and provided by Grifols) was a sterile liquid formulation of immunoglobulin purified from human plasma with high-anti-SARS-CoV-2 antibodies collected from donors recovered from COVID-19 from May 2020 to July 2020. The criteria for the selection of convalescent plasma units were anti-SARS-CoV-2 antibody titre corresponding to ≥10.0 using the Ortho-Vitros method or ≥7.0 using the Architect-Abbott method. The highest dose of 2 g was selected based on the volume that can be safely administered subcutaneously without the need of a peristaltic pump and the maximum lyophilizing capacity of the manufacturer. Further details on the preparation, manufacturing, and characteristics of the C19-IG20% are provided in Methods S3. The neutralizing activity of C19-IG20% was assessed against the virus lineage Wuhan-Hu-1, the alpha (B.1.1.7), beta (B.1.351) and delta (B.1.617.2) VOC (Methods S4), using a pseudovirus neutralization assay, as part of a post-hoc analysis. The distribution of VOC during plasma collection and recruitment periods are shown in Methods S5. Participants were all provided with pulse oximeters and thermometers for daily self-recording of their SpO2 and body temperature at home. In-person follow-up visits were planned for study days 3, 7, 14, and 29 at the participants' residences, or in the hospital if they were hospitalised. Additionally, investigators contacted study participants by telephone on study days 5, 9, and 11 to assess their clinical status, including the development of symptomatic COVID-19, and to record their daily SpO2 and body temperature measurements. We performed a final telephone check on day 60 to assess vital status, hospital admissions, ICU admissions, requirement for invasive mechanical ventilation and adverse events. All collected data were recorded in an electronic case report form.

Nasopharyngeal swabs were obtained for quantification of SARS-CoV-2 viral load on study days 1, 3, 7, 14, and 29. Blood samples were obtained on days 1, 7, and 14 to assess inflammatory biomarkers (D-dimer, ferritin, and C-reactive protein [CRP]), biochemical and haematology parameters (creatinine, albumin, ALT, total bilirubin, LDH, haemoglobin, haematocrit, platelet count, absolute neutrophil and lymphocyte counts, and leukocyte counts) and levels of anti-SARS-CoV-2 antibodies (IgM and IgG).

Viral load was analysed by real-time quantitative RT-PCR in two consecutive steps, viral RNA extraction using QIAmp MinElute Virus Spin kit (Qiagen) and amplification/detection by TaqPath COVID-19 CE-IVD RT-PCR kit (Thermo Fishe Scientific) at a centralized laboratory (Progenika Clinical Diagnostics Laboratory, Progenika Biopharma, a Grifols company, Derio, Spain). For absolute quantification, a standard curve was built using serial dilutions of a SARS-CoV-2 plasmid RNA of known concentration (EVAg), run in parallel to a set of samples covering all thermal cycles used in the analysis (Methods S6). SARS-CoV-2 IgM and IgG antibodies were tested using AESKULISA® SARS-CoV-2 S1 IgG and IgM test (AESKU Enzyme Linked Immunosorbent Assay), processed on the SQII Elisa Analyzer (AESKU), at Progenika Clinical Diagnostics Laboratory under specifications described by provider (Methods S7).

Outcomes

The primary outcome was the proportion of participants who remained asymptomatic through day 14. Symptomatic COVID-19 was defined as fulfilling one of the following four conditions: (1) developing at least two of the following predefined systemic symptoms: fever ≥38 °C, chills, myalgia, headache, sore throat, cough, fatigue that interfered with daily activities, new olfactory or taste disorders, vomiting or diarrhoea; or (2)

experiencing at least one of the following respiratory signs/symptoms: new or worsening shortness of breath or difficulty breathing; or (3) experiencing SpO2 <94% on room air; or (4) having radiographical evidence of pneumonia.

Prespecified secondary clinical outcomes included the proportion of individuals who presented one of the following non-mutually exclusive events: participants who remained in an outpatient setting and maintained SpO2 ≥94% through day 14, and participants who required oxygen supplementation, required any medically attended visit for management or treatment of COVID-19, hospitalisation, or ICU admission through day 29. Time to the onset of COVID-19 symptoms was also analysed.

Secondary virological outcomes included viral load reduction in nasopharyngeal swabs on days 7 and 14 and viral clearance by RT-PCR on days 14 and 29. Other secondary outcomes included change in inflammatory parameters (D-dimer, ferritin, and C-reactive protein [CRP]) from baseline to day 14 of follow-up and change in quantitative anti-SARS-CoV-2 antibodies through day 14.

Safety was assessed by the proportion of patients experiencing treatment-emergent adverse events (TEAEs), defined as the adverse events (AEs) that occurred on or after the time of investigational product administration; and the clinically significant change in key biochemical parameters of organ function/dysfunction (creatinine, albumin, alanine aminotransferase (ALT), total bilirubin, LDH, haemoglobin, haematocrit, platelet count, absolute neutrophil and lymphocyte counts, and leukocyte counts) from baseline to day 14.

Statistical analysis

We estimated that a sample size of 801 (267 cases per arm) would provide the trial with 80% power to detect an increase of 10% in the proportion of asymptomatic participants remaining asymptomatic after treatment, assuming an expected proportion remaining asymptomatic of 80%, at a significance level of $\alpha = 0.025$, and allowing a 10% withdrawal rate.

Primary efficacy analyses were performed on the modified intention-to-treat (m-ITT) population, which included all the randomized participants who received any interventional product infusion. Sensitivity analyses were performed with the intention-to-treat (ITT) population (i.e., all randomized participants) and the per-protocol (PP) population (i.e., participants completing the follow-up without major protocol deviations which might have an impact on the primary efficacy endpoints, and complete at least 80% of the interventional product). Safety was assessed in the safety population, which included all randomized participants who received at least any amount of blinded interventional product infusion.

The baseline characteristics of the study population were summarized descriptively using the number of non-missing observations, mean, standard deviation (SD), median and interquartile range (IQR) for the continuous/quantitative data or absolute and relative frequency counts and percentages for categorical/qualitative data. The primary clinical efficacy endpoint was compared between the two doses of C19-IG20% and placebo using the Cochran-Mantel-Haenszel (CMH) test adjusting for age. Subgroup analyses of the primary clinical efficacy endpoint and analyses of secondary clinical efficacy endpoints were assessed using the Fisher's exact test or Chi-square test.

The secondary efficacy endpoint of change in SARS-CoV-2 viral load ($\log_{10}$ copies/mL) from baseline to day 7 and day 14 was assessed by an analysis of covariance (ANCOVA) with treatment and randomization strata as fixed effects and baseline value as covariate. Time-to-event outcomes were assessed using Kaplan–Meier estimates. Between-arm analysis for other secondary outcomes were done using parametric or non-parametric methods according to its distributions. Non-parametric methods were used for non-normal distributed variables with right-skewed distributions (IgG/IgM variables and laboratory markers) and the assessment of distribution was verified visually. One-way ANOVA or Kruskal–Wallis test for the comparison between all three treatment arms and Student's t or Dunn's tests for the pairwise comparison using Holm's method for p-value correction. All statistical tests were performed in the SAS statistical software under a significance level of 0.05.

Role of the funding source

This study was funded by Grifols. Five authors were employees from Grifols and made substantial contributions to study design (EM), data analysis (YT), and manuscript revision (EM, MT, NC, AS, YT). Other authors, independent from the study funder, were also involved in all of the aforementioned tasks, as described in the authors' contributions disclosure. MT had full access to the data set, as did OM, AA, and DO.

Results

Study setting and patient characteristics

Between 28 April 2021 and 27 December 2021, our team identified and contacted approximately 3000 individuals with confirmed SARS-CoV-2 infection, many of whom presented with COVID-19 symptoms and were therefore not eligible. We screened 555 asymptomatic individuals with confirmed SARS-CoV-2 infection. Fig. 1 summarizes the recruitment and follow-up of study participants. Among 555 individuals screened, 461 met all the selection criteria and received the allocated intervention, thereby being included in the m-ITT analysis: 152 received 1 g C19-IG20%, 153 received 2 g

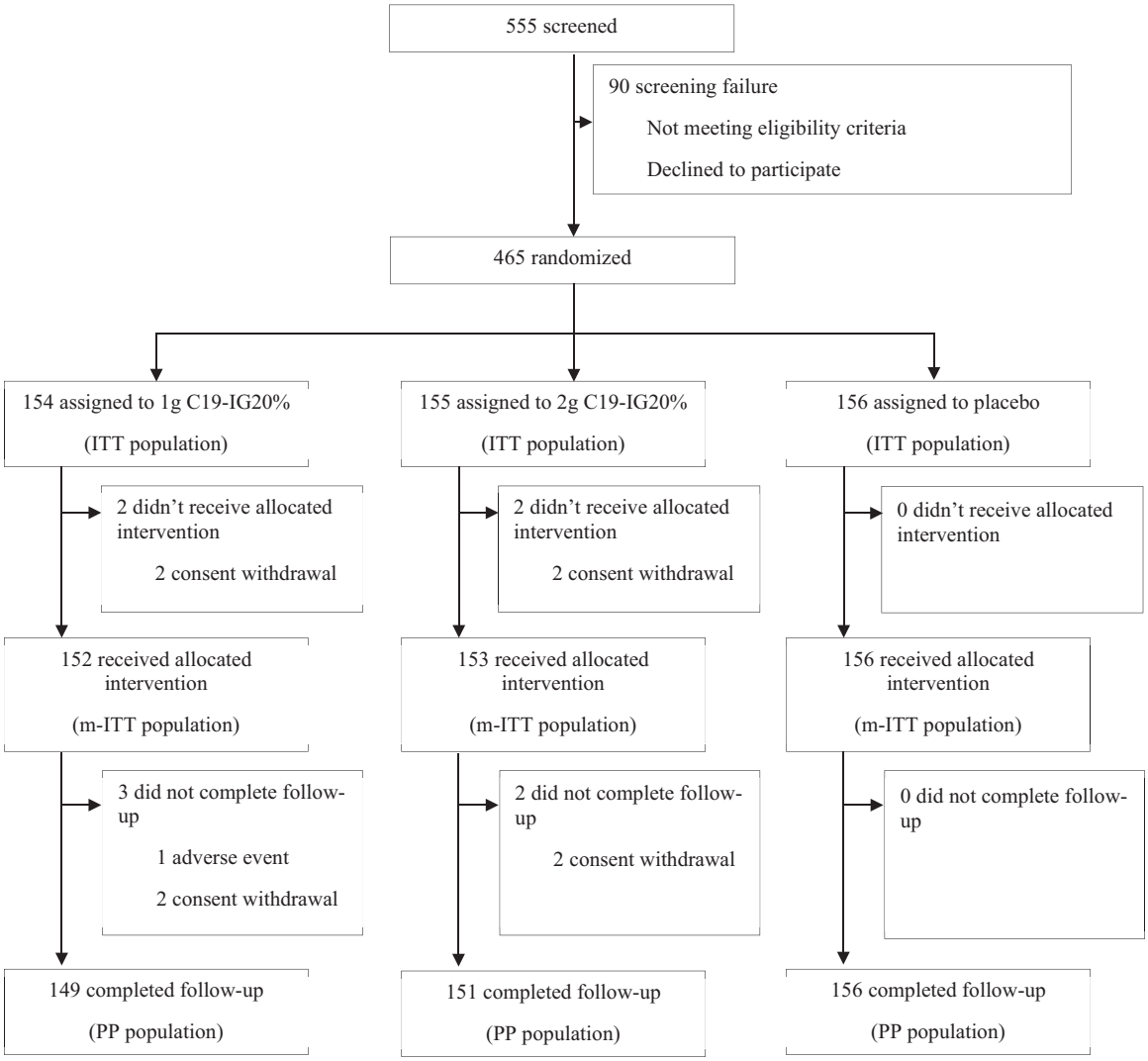

***Fig. 1:* Trial profile**. ITT, intention to treat; m-ITT, modified intention to treat; PP, per protocol.

C19-IG20% and 156 received placebo (Table S1, Supplementary Appendix). All participants included in the m-ITT analysis completed their infusion.

Neutralizing activity of C19-IG20% was evaluated using pseudoviral neutralization assay against the original Wuhan SARS-CoV-2 strain and alpha (B.1.1.7), beta (B.1.351), and delta (B.1.617.2) variants. A 2.4 and 2.9-fold decrease in neutralizing antibody titres was observed against alpha and delta variants, respectively, compared with Wuhan-Hu-1 (geometric mean ID50 13510 for alpha and 11367 for delta vs ID50 32917 for Wuhan-Hu-1) (Methods S4).

The baseline demographic and clinical characteristics were similar in the three groups (Table 1). Overall, study participants had a mean age of 39.6 (SD 12.8) years, 197 (42.7%) of 461 participants were women, and 101 (21.9%) had at least one comorbidity. The mean time from positive SARS-CoV-2 test to random allocation was 3.1 (SD 1.3) days, and the mean time from exposure to random allocation (among 160 of 461 participants in which the potential contact with SARS-CoV-2 could be identified) was 5.6 (SD 2.8) days.

Participants were allocated to a treatment arm and infused (blinded administration) on the same day. Baseline serum antibody results for IgM/IgG were negative for 345 (80%) of the 431 participants for whom results were available. Prior to recruitment, all individuals had a documented positive SARS-CoV-2 test result (either by antigen detection or by DNA detection tests), according to inclusion criteria. However, at baseline, 119 (26%) of 461 participants had a negative SARS-CoV-2 RT-PCR result and 342 (74%) participants had a positive RT-PCR test result. In total, 372 (81%) of the participants had a positive RT-PCR result at any time

	1 g C19-IG20% N = 152	2 g C19-IG20% N = 153	Placebo N = 156
Demographics			
Age, years – mean (SD)	38.8 (12.8)	41.1 (12.4)	38.8 (13.3)
Age group – n (%)			
18–65 years	146 (96.1)	147 (96.1)	149 (95.5)
≥65 years	6 (3.9)	6 (3.9)	7 (4.5)
Women – n (%)	66 (43.4)	62 (40.5)	69 (44.2)
Men – n (%)	86 (56.6)	91 (59.5)	87 (55.8)
BMI (kg/m2) – mean (SD)	26.0 (4.7)	26.4 (5.1)	25.5 (4.4)
SARS-CoV-2 infection characteristics			
Days from positive test[a] to random assignment[b], days – mean (SD, N)	3.1 (1.2, 152)	3.1 (1.4, 153)	3.0 (1.2, 156)
Days from exposure[c] to random assignment[b], days – mean (SD, N)	5.8 (2.8, 56)	5.2 (2.5, 58)	5.7 (3.3, 46)
Comorbidities – n (%)			
At least one comorbidity	35 (23.0)	39 (25.5)	27 (17.3)
Obesity (BMI ≥30 kg/m2)	29 (19.1)	30 (19.6)	18 (11.5)
Diabetes Mellitus	5 (3.3)	11 (7.2)	7 (4.5)
Hypertension	11 (7.2)	11 (7.2)	10 (6.4)
Heart conditions (i.e. heart failure, coronary artery disease, cardiomyopathies)	2 (1.3)	1 (0.7)	1 (0.6)
Chronic Obstructive Pulmonary Disease	0 (0.0)	1 (0.7)	1 (0.6)
Asthma	10 (6.6)	7 (4.6)	9 (5.8)
Chronic kidney disease	1 (0.7)	2 (1.3)	1 (0.6)
History of cancer	3 (2.0)	1 (0.7)	3 (1.9)
Immunocompromised state from solid organ transplant	0 (0.0)	0 (0.0)	0 (0.0)
Serum IgM and IgG antibody status – n (%)			
N[d]	139	146	146
Negative	114 (82)	118 (81)	113 (77)
Positive	25 (18)	28 (19)	33 (23)
Study RT-PCR – n (%)			
Positive at baseline	110 (72)	108 (71)	124 (79)
Negative at baseline	42 (28)	45 (29)	32 (21)
Positive at any time during the study	120 (79)	118 (77)	134 (86)
Positive at baseline and IgM and IgG negative at baseline	77 (51)	80 (52)	83 (53)
Viral load			
Mean Viral Load (SD) in log_{10}	5.8 (2.6)	5.8 (2.6)	6.1 (2.4)
Laboratory parameters – mean (SD)			
N[e]	148	152	153
D-dimer (mg/L)	0.6 (1.5)	0.4 (0.4)	0.6 (2.6)
Ferritin (ug/L)	111.2 (120.2)	115.2 (138.2)	123.7 (163.4)
C-reactive protein (CRP) (mg/dl)	5.8 (10.5)	6.4 (6.7)	5.9 (6.4)

BMI = body mass index; SD = standard deviation. Laboratory reference ranges: D-dimer 0-0.50 mg/L; Ferritin 30.0–400.0 ug/L; C-reactive protein 0.0–1.0 mg/dL. [a]First positive PCR (RT-PCR), NAT or other commercial or public health assay result for SARS-CoV-2 infection. [b]Random assignment and infusion were always done on the same day. [c]Exposure in terms of first potential contact with virus. [d]30/461 participants did not have baseline serological test (13/152 in the 1 g C19-IG20% group; 7/153 in the 2 g C19-IG20% group; and 10/156 in the placebo group). Missing data in this variable can be assumed random. [e]8/461 participants did not have baseline laboratory parameters (4/152 in the 1 g C19-IG20% group; 1/153 in the 2 g C19-IG20% group; and 3/156 in the placebo group). Missing data in this variable can be assumed random.

Table 1: **Baseline characteristics in the modified intention-to-treat population.**

during the study. The mean viral load from the nasopharyngeal swab at baseline was 5.8 (SD 2.6) log_{10} copies per mL in the 1 g C19-IG20% group, 5.8 (SD 2.6) log_{10} copies per mL in the 2 g C19-IG20% group and 6.1 (SD 2.4) log_{10} copies per mL in the placebo group.

Trial enrolment was halted on December 27, 2021, based on the results of a planned interim analysis of all available data on primary and secondary efficacy outcomes, which concluded the lack of potential benefit of the intervention in the target population. This article presents the final and only report analysis after early termination.

Clinical efficacy outcomes

In the modified ITT population, the primary outcome analyses (i.e., the proportion of participants who remained asymptomatic on day 14) did not differ significantly between placebo-treated and C19-IG20%-treated individuals, irrespective of the dose received (Table 2). The primary outcome occurred in 59.9%

	1 g C19-IG20% N = 152	2 g C19-IG20% N = 153	Placebo N = 156	Difference in proportions 1 g C19-IG20% - Placebo (95% CI)	p-value	Difference in proportions 2 g C19-IG20% - Placebo (95% CI)	p-value
Primary clinical efficacy endpoint through day 14							
Remained asymptomatic – n(%)	91 (59.9)	99 (64.7)	99 (63.5)	−3.6 (−14.6 to 7.3)	0.526	1.1 (−9.6 to 11.9)	0.846
Developed ≥2 systemic COVID-19 symptoms[a]	58 (38.2)	54 (35.3)	54 (34.6)	3.6 (−7.3 to 14.5)	0.527	0.8 (−9.9 to 11.5)	0.894
Experienced ≥1 respiratory symptoms[b] (new or worsening shortness of breath, difficulty breathing)	9 (5.9)	8 (5.2)	15 (9.6)	−3.6 (−9.7 to 2.4)	0.245	−4.4 (−10.2 to 1.4)	0.14
Experienced Sp02 < 94% on room air	5 (3.3)	3 (2.0)	3 (1.9)	1.4 (−2.1 to 5.0)	0.439	0.0 (−3.1 to 3.1)	0.988
Had radiographical evidence of pneumonia	6 (3.9)	7 (4.6)	3 (1.9)	2.1 (−1.7 to 5.9)	0.274	2.8 (−1.2 to 6.8)	0.175
Subgroup analyses of the primary clinical efficacy endpoint: remained asymptomatic through day 14							
Positive PCR results at study baseline	59/110 (53.6)	61/108 (56.5)	74/124 (59.7)	−6.1 (−18.7 to 6.9)	0.352	−3.2 (−15.9 to 9.8)	0.623
Positive PCR results at any time during the study	65/120 (54.2)	68/118 (57.6)	84/134 (62.7)	−8.5 (−20.8 to 3.7)	0.169	−5.1 (−17.3 to 7.1)	0.413
Positive IgM/IgG results at baseline	20/25 (80.0)	26/28 (92.9)	28/33 (84.8)	−4.8% (−15% to 24.7%)	0.628	8% (−23.5% to 7.5%)	0.328
Negative IgM/IgG results at baseline	65/114 (57.0)	68/118 (57.6)	66/113 (58.4)	−1.4% (−12.3% to 15.1%)	0.938	0.8% (−12.7% to 14.3%)	1.000
Secondary clinical efficacy endpoints							
Remained in an outpatient setting and maintained Sp02 ≥ 94% through day 14	141/148 (95.3)	140/149 (94.0)	148/154 (96.1)	−0.8 (−6.1 to 4.2)	0.721	−2.1 (−7.8 to 3.1)	0.390
Required oxygen supplementation through day 29	3/152 (2.0)	6/153 (3.9)	2/156 (1.3)	0.7 (−2.9 to 4.6)	0.631	2.6 (−1.2 to 7.3)	0.144
Required ≥1 related medically attended visit through day 29	26/152 (17.1)	29/153 (19.0)	22/156 (14.1)	3 (−5.3 to 11.5)	0.468	4.9 (−3.6 to 13.4)	0.251
Required hospitalisation through day 29	3/152 (2.0)	7/153 (4.6)	3/156 (1.9)	0.1 (−3.8 to 4.0)	0.974	2.7 (−1.6 to 7.5)	0.188
Required ICU admission through day 29	1/152 (0.66)	1/153 (0.65)	1/156 (0.64)	0.02 (−3.05 to 3.12)	0.985	0.01 (−2.99 to 3.07)	0.989

Risk difference for the proportions of subjects between groups using CMH (Cochran-Mantel-Haenszel) method adjusted by age for primary efficacy endpoints and Chi-square for secondary clinical efficacy endpoints. [a]Systemic symptoms: fever (≥38 °C), chills, myalgia, headache, sore throat, cough, fatigue that interfered with daily activities, new olfactory/taste disorder, vomiting/diarrhoea (note that new olfactory/taste disorder and vomiting/diarrhoea only counted as one item of definition). [b]New or worsening shortness of breath or difficulty breathing.

Table 2: **Clinical trial efficacy end points in the modified intention-to-treat population.**

(91/152) of participants receiving 1 g C19-IG20%, 64.7% (99/153) receiving 2 g, and 63.5% (99/156) receiving placebo (difference in proportions: 1 g C19-IG20% vs. placebo, –3.6%; 95% CI -14.6% to 7.3%, p = 0.53; 2 g C19-IG20% vs. placebo, 1.1%; –9.6% to 11.9%, p = 0.85). The most common presentation among symptomatic participants was a combination of two or more systemic COVID-19 symptoms. Only a small proportion of participants experienced ≥1 respiratory symptoms, SpO2 <94%, or had radiographic evidence of pneumonia. The analysis of the primary outcome in the ITT population (Table S2) and the PP population (Table S3) also revealed no significant differences between participants treated with placebo versus those treated with C19-IG20%. We conducted post-hoc analyses of the primary efficacy endpoint in sub-groups according to the study RT-PCR test result (baseline and throughout the follow-up) and serological status of participants at baseline. None of the sub analyses revealed significant differences between groups regarding the primary endpoint (Table 2). Sensitivity analysis of primary efficacy endpoints stratified by age and comorbidities were also performed, finding no differences between groups (Table S4).

Regarding secondary clinical efficacy outcomes, overall, 11 (2.4%) participants required oxygen supplementation at some point during the follow-up, 77 (16.7%) required one or more COVID-19 related medical visits, 13 (2.8%) required hospitalisation and 3 (7.2%) required admission to an ICU. No participants in any treatment group required invasive mechanical ventilation and no participants died during the study. None of these secondary endpoints were significantly different between study groups (Table 2). Time to the onset of COVID-19 symptoms did not show significant differences between groups; 30% of study participants developed symptoms within the first three days after infusion (Fig. S1).

Other efficacy outcomes

Fig. 2 shows the SARS-CoV-2 viral load decay throughout the follow-up period. We found no significant differences between groups regarding the mean difference in viral load from baseline to day 7 (absolute difference 0.10 $\log_{10}$ copies/mL for C19-IG20% 1 g vs. placebo [95% CI -0.24 to 0.44; p = 0.58]; and –0.17 for C19-IG29% 2 g vs. placebo [95% CI -0.52 to 0.17; p = 0.33]) and from baseline to day 14 (0.11 for

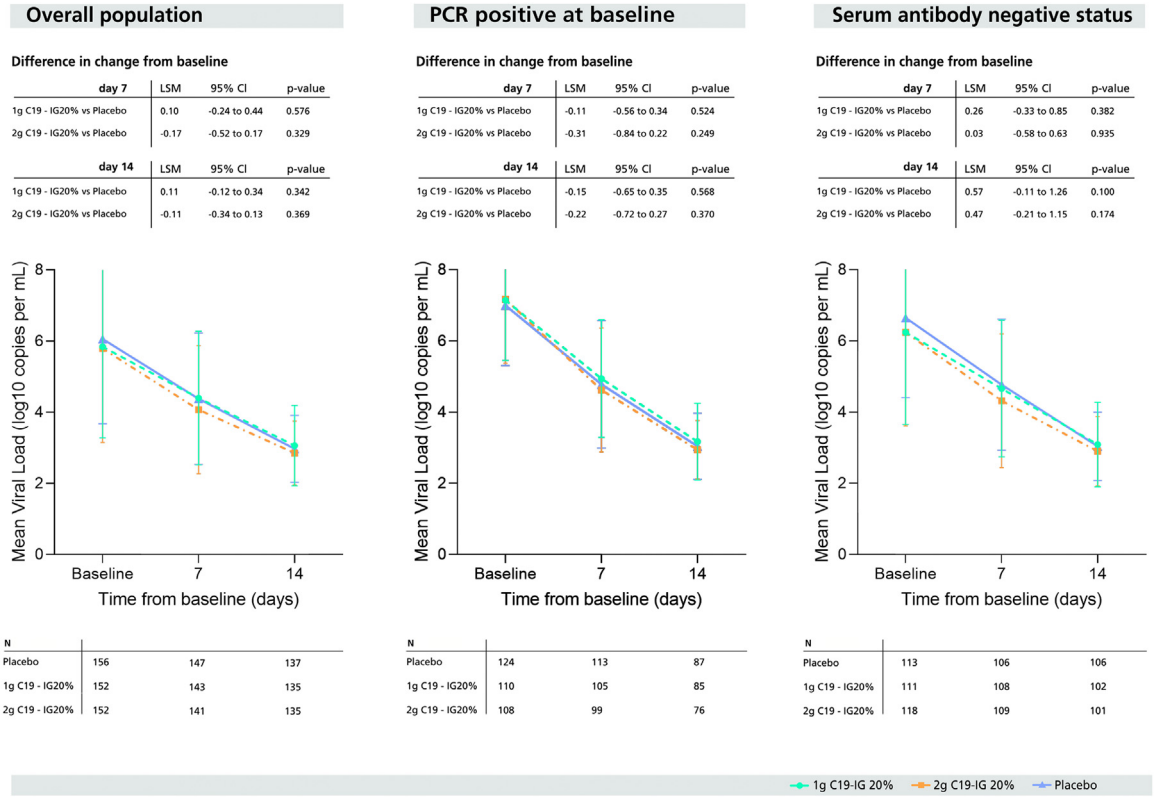

***Fig. 2*: Viral load change over 14 days**. Figure shows the mean viral load (in $\log_{10}$ copies per millilitre) at baseline, day 7 and day 14 in the overall population and in subgroup of PCR positive at baseline and serum antibody negative status (IgM/IgG negative at baseline). Tables on the figure show difference in least-squares means (LSM) of change from baseline to day 7 and day 14 of viral load (in $\log_{10}$ copies per millilitre) for both doses of C19-IG20% compared to placebo in the overall population and in subgroup of PCR positive at baseline and serum antibody negative status. 95% CI for difference in LSM between each of C19-IG20% dose groups (1 g and 2 g) and placebo and the associated p-value were calculated using an ANCOVA model, including the change from baseline value as a dependent variable; treatment group as a fixed effect; and baseline viral load value, age, and gender as covariates.

C19-IGG20% 1 g vs. placebo [95% CI -0.12 to 0.34; $p = 0.34$] and –0.11 for C19-IG29% 2 g vs. placebo [95% CI -0.34 to 0.13; $p = 0.37$]). Likewise, no differences were observed in time to viral clearance, assessed by RT-PCR up to day 29 (Figs. S2 and S3).

Changes in inflammatory parameters, including D-dimer, ferritin, and C-reactive protein (CRP), did not show significant differences between groups from baseline to day 14 of follow-up (Fig. S4). Likewise, the groups did not differ regarding the change in anti-SARS-CoV-2 IgM and IgG throughout the follow-up (Fig. S5).

Safety

Table 3 summarizes the TEAEs that occurred from the time of administration of the investigational product to day 14 of follow-up. A total of 359 TEAEs were reported: 137 TEAEs in 78/152 (51.3%) participants in the C19-IG20% 1 g group, 96 TEAEs in 65/153 (42.5%) in the C19-IG20% 2 g group, and 126 TEAEs in 72/156 (46.2%) in the placebo group, with no differences between treatment groups. Blinded investigators evaluated the 359 TEAEs and determined that 263 (73%) were not related to the investigational product and 10 (2.8%) were definitely related. Regarding the severity of the events, 286 (79.7%) were mild (Grade 1), 61 (17.0%) moderate (Grade 2), 10 (2.8%) severe (Grade 3), and 2 (0.5%) life-threatening (Grade 4). All TEAEs related to the investigational product were mild or moderate in severity. All severe and life-threatening TEAEs were related to COVID-19. No individuals experienced a TEAE leading to death. There were no serious adverse drug reactions reported.

Most common TEAEs were related to COVID-19, including gastrointestinal disorders, arthralgia, headache, cough, and fever. TEAEs related to IP infusion included injection site pain, puncture site pain and erythema, and vasovagal syndrome (Table S5). No severe allergic reactions or anaphylaxis and thromboembolic events were reported.

	1 g C19-IG20% (n = 152)		2 g C19-IG20% (n = 153)		Placebo (n = 156)		Difference in proportions 1 g C19-IG20% - Placebo (95% CI)	p-value	Difference in proportions 2 g C19-IG20% - Placebo (95% CI)	p-value
	Number of subjects[a]	Number of events[b]	Number of subjects[a]	Number of events[b]	Number of subjects[a]	Number of events[b]				
Treatment Emergent Adverse Events (TEAE)										
≥1 TEAE	78 (51.3)	137	65 (42.5)	96	72 (46.2)	126	−5.16% (−7.00% to 17.00%)	0.428	3.67% (−15.00% to 8.00%)	0.593
Relationship to investigational product										
Not related	48 (31.6)	97	43 (28.1)	69	53 (34.0)	97				
Possibly related	26 (17.1)	36	15 (9.8)	20	19 (12.2)	29				
Definitely related	4 (2.6)	4	6 (3.9)	6	0 (0.0)	0				
Severity										
Mild (Grade 1)	57 (37.5)	112	46 (30.1)	72	54 (34.6)	102				
Moderate (Grade2)	17 (11.2)	21	13 (8.5)	18	16 (10.3)	22				
Severe (Grade 3)	3 (2.0)	3	5 (3.3)	5	2 (1.3)	2				
Life threatening (Grade 4)	1 (0.7)	1	1 (0.7)	1	0 (0.0)	0				
Fatal (Grade 5)	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0				
TEAE leading to death	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0				
TEAE leading to study withdrawal	1 (0.7)	1	0 (0.0)	0	1 (0.6)	1				
Treatment Emergent Serious Adverse Events (TE-SAE)										
≥1 TE-SAE	3 (2.0)	3	7 (4.6)	7	3 (1.9)	3	−0.05% (−3.00% to 3.00%)	1	−2.65% (−1.00% to 7.00%)	0.319
COVID-19 pneumonia	3 (2.0)		7 (4.6)		1 (0.6)					
Non-COVID-19 pneumonia	0 (0)		0 (0)		2 (1.3)					

AE = adverse event; IP = investigational product; SAE = serious adverse event; TEAE = treatment-emergent adverse event. Note: Treatment-emergent AEs are AEs that occurred on or after the date/time of IP administration. Percentages were based on the total number of safety subjects in each treatment group (N). [a]At each level of summation (overall, relationship, severity), subjects reporting more than one AE were counted only once using the strongest relationship to study drug and maximum severity. [b]Number of events included all occurrences of AEs.

Table 3: **Safety end points in the safety population.**

13 SAE were reported: 3 (2%) in the C19-IG20% 1 g group, 7 (4.6%) in the C19-IG20% 2 g group, and 3 (1.9%) in the placebo group. All SAEs were related to COVID-19 infection, except for two cases of pneumonia not related to COVID-19.

Change in biochemical and haematological parameters of organ derangement or systemic inflammatory response (i.e., creatinine, albumin, alanine aminotransferase (ALT), total bilirubin, LDH, haemoglobin, haematocrit, platelet count, absolute neutrophil and lymphocyte counts, and leukocyte counts) from baseline to day 14 did not show clinically relevant differences between groups (Table S6 and Fig. S6).

Discussion

Our findings show that early infusion with 1 g or 2 g of C19-IG20%, compared with placebo, did not reduce the risk of developing symptomatic COVID-19 in individuals diagnosed with asymptomatic SARS-COV-2 infection. Furthermore, neither our secondary clinical or virological endpoints nor our prespecified subgroup analyses demonstrated a benefit of this therapy. Safety endpoints, including the proportion of treatment-emergent adverse events (TEAEs) and severe adverse events (SAE) did not differ between the treatment groups and were mainly related to COVID-19. No severe or life-threatening events related to interventional product infusion were reported. Overall, these findings indicate that C19-IG20% at the dose of 1 g and 2 g is safe and well tolerated but did not prevent development of symptomatic COVID-19.

Passive immunotherapies, including hIG, mAbs and CCP, have demonstrated no clinical benefit in reducing the mortality risk in most hospitalised patients with COVID-19, except for selected groups (severely immunocompromised and seronegative).[7–9,24–27,37–39] As opposed to these studies, ours focused on outpatients with a very recent and asymptomatic infection since antibody products are expected to be more beneficial when administered very early in the course of infection. For example, early administration of mAbs and CCP has been demonstrated to have clinical benefits in outpatients with mild and moderate COVID-19.[1,3–6,30–32] Moreover, combinations of mAbs have been shown to reduce the risk of both asymptomatic and symptomatic SARS-CoV-2 infection when administered as pre- and post-exposure prophylaxis (PrEP and PEP).[10–12] Conversely, CCP (tested only as PEP) failed to prevent infection in asymptomatic contacts in a clinical trial conducted in the US.[40]

Our results did not reflect previous findings in the treatment of outpatients with mAbs and CCP, despite the very early administration of immune therapy. On the other hand, our results are consistent with the lack of benefit found for CCP used as PEP, as opposed to the successful use of mAbs (with a much higher content of specific antibodies) in the same context. A possible explanation is that the amount of specific antibodies contained in 10 mL of C19-IG20% may not be sufficient to prevent mild COVID-19 symptoms involving the upper respiratory tract, although it may have been able to prevent progression to severe disease. The combination of casirivimab/imdevimab contains 1.2 g of SARS-CoV-2-specific immunoglobulins, a higher amount than the 200 mg and 400 mg of polyclonal immunoglobulins contained in the 1 g (5 mL) and 2 g (10 mL) doses of C19-IG20%, respectively. Our findings showing no change in viral load in nasopharynx, in contrast with the reduction observed with mAb, also supports this hypothesis. A maximum volume of 10 mL was administered in our clinical trial based on data from previous studies of other subcutaneous hyperimmune immunoglobulin products that are safe and tolerable. Nonetheless, new delivery system designs may enable larger volume subcutaneous infusion viability and tolerability. Intravenous administration could also accept higher volumes; for instance, 400 mg/kg body weight and 3.5 g administered in the ITAC[38] and OTAC (NCT04910269) studies, respectively. However, subcutaneous therapies are more likely to be successfully deployed in community and primary care settings, particularly in countries with limited healthcare systems. Regarding neutralizing activity, plasma for C19-IG20% was collected in the United States from the second half of 2020, before the emergence of alpha variant, while the trial enrolled participants in Spain from April to December 2021, during alpha and delta variants dominance periods. Analyses conducted using pseudoviral neutralization assays identified a two to three fold reduction in neutralizing antibody titres for the circulating variants. In light of this results, we cannot rule out the possibility of clinical efficacy if higher doses of C19-IG20% and/or higher neutralization capacity had been administered.

Our clinical trial has several limitations. Firstly, the trial was terminated early by the data safety monitoring board based on an interim analysis showing no signs of potential benefit to the target population; hence the target sample size was not achieved. However, based on the interim analysis results, including the lack of differences in all pre-defined efficacy endpoints, we do not expect the analysis with the target sample size to yield different conclusions. Second, recruitment was conditioned by the widespread availability of vaccines during the study period, as vaccinated individuals were not eligible to participate. Third, although all participants were required to have a positive test for SARS-CoV-2 to enrol in the study, 26% tested negative by PCR at baseline and 19% at any time during the study. It is possible that some of these asymptomatic participants were diagnosed at the end of their infectious period and viral clearance occurred between diagnosis and randomization. Another explanation might be a false positive diagnostic test, which is more frequent in the context of screening asymptomatic individuals.[41,42] However, the sensitivity analyses on baseline PCR-positive participants did not change the trend of the m-ITT analysis. Regarding key clinical endpoints such as hospitalisation and need for oxygen supplementation, we were unable to draw any strong conclusions due to the low frequency of these events and the relatively small sample size, which limited the statistical power of the analysis. Another limitation is that the study was conducted mainly in unvaccinated patients, most of whom were seronegative at baseline. Also, our trial included 80% of participants with no comorbidities and none of them were immunocompromised. It remains unknown whether this intervention could benefit individuals at higher risk in the absence of other therapies with proven efficacy. Finally, the reduction in neutralization activity of the C19-IG20% against different circulating variants at the time of infusion may have contributed to lack of efficacy, indicating the importance of developing agile production and distribution workflows for hIG therapies, so that they can be administered timely.

On the other hand, several aspects of the methodology and study conduct strengthen and increase the generalizability of our findings. First, this is the first controlled clinical trial to report results of anti-COVID-19 hIG as treatment of ambulatory SARS-CoV-2 infected individuals. The trial included a large and diverse trial population that was enrolled at different sites throughout Spain. In addition, intervention was double-blinded, with a very high percentage of participants receiving the infusion and completing the follow-up. Finally, the main results of our trial regarding clinical efficacy are supported by virological and laboratory endpoints, contributing to more robust conclusions about the potential effect of C19-IG20%.

The results of this trial do not support the use of the subcutaneous human hyperimmune immunoglobulin C19-IG20% at either 1 g or 2 g dose regimen for the prevention of symptomatic COVID-19 in asymptomatic individuals with confirmed SARS-CoV-2 infection. Our findings indicate that C19-IG20% at the dose of 1 g and 2 g is safe and well tolerated. Future studies shall investigate the potential benefits of C19-IG20% and other hIG therapies with higher antibody dose and neutralizing activity in other scenarios, such as prevention of disease progression in outpatients with COVID-19, particularly those that are immunocompromised.

Contributors

OM, EM, AA, PMM, MCM conceived and designed the study. All author acquired, analysed, and interpreted the data. DO, YT did the statistical analysis. AA and OM drafted the manuscript. All authors critically revised the manuscript for important intellectual content. All authors were responsible for the final decision to submit the manuscript for publication. All authors have seen and approved the manuscript. OM, AA, DO, MT had full access to and verified the data.

Data sharing statement

Individual participant data that underlie the results reported in this article, after de-identification (text, tables, figures, and appendices) are available from the corresponding author on reasonable request.

Declaration of interests

EM, MT, NC, AS, and YT were employees from Grifols. The rest of authors declared no conflict of interest.

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Appendix A. Supplementary data

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Discussion

1. Efficacy of high-titre CCP in outpatients

The first objective, regarding the efficacy of high-titre CCP in outpatients, was addressed in three studies: one RCT and two meta-analyses. The first study (COnV-ert trial) was a RCT aimed at assessing the efficacy of 250-300 ml of ABO-compatible high anti-SARS-CoV-2 IgG titres methylene blue-treated CP compared to an equivalent volume of masked saline solution. We did not find differences in hospitalization rates at day 28 and no decrease in mean viral load between study arms. However, the target sample size was not reached because the trial was terminated early, primarily due to recruitment challenged arising from the rapid uptake of vaccination and the approval of effective specific anti-SARS-CoV-2 mAbs. Consequently, a decision was made to collaborate and pool data with other international research groups conducting similar studies for a comprehensive analysis. Studies two and three employed individual pooling data and meta-analyses techniques. Study two (COMPILEhome) included data from two multicentre RCT (COnV-ert and COV-Early) conducted in Spain and the Netherlands, respectively, and showed no differences in improved disease severity and a composite of hospitalization or death by day 28. Study three included data from five multicentre RCTs (COnV-ert, COV-Early, CCP-Argentina, C3PO and CSSC-004) conducted in Spain, the Netherlands, Argentina, and the United States, and showed a 30.1% relative risk reduction for all-cause hospitalization with CCP administration. Moreover, the study found a greatest reduction of hospitalization (51.4% relative risk reduction) in participants who received early transfusion (≤5 days after symptom onset) of CCP with high antibody titres (above the median titre for each individual RCT). This discussion includes an examination of agreements and discrepancies between these studies, as well as a comparison with the existing literature on this therapy for COVID-19 outpatients.

During the first months of the COVID-19 pandemic, when treatment options were limited and no prophylactic therapies were available, CCP was extensively used, often as a compassionate treatment, for hospitalized patients in advanced stages of the disease. [162] Therefore, most initial RCTs of CCP were conducted in hospitalized patients, but no significant survival benefit was reported for CCP in this population.[98,117–119,121–123,125–

[134,137] Importantly, many of these first trials used CCP with unknown or low titres of neutralizing antibodies, in some cases due to unavailable routine assays to determine antibody titres. Later in the pandemic, RCTs started evaluating CCP in non-hospitalized patients and using CCP with high antibody content, following recommendation from FDA guidelines.[164]

Considering the old principles of antibody therapies, CCP would be most effective in the early stages of COVID-19, before patients have developed an endogenous humoral immunity response and when viral replication dominates. However, only five RCTs of early treatment with CCP in non-hospitalized patients have been conducted to date, reporting variable —often disparate— efficacy results.[139–142,195] Furthermore, methodological differences and limitations between these trials do not allow drawing strong conclusions regarding the overall efficacy of this therapy. First, not all trials had sufficient power to measure the primary outcome of hospitalization in the context of early termination. Also, several variables which were defined differently across trials, might have an association with efficacy for CCP. These variables include the populations selected, the disease severity, the timing of administration, the levels and functional aspects of SARS-CoV-2 antibodies, the methods used for CCP testing, the dosage, the CCP origin and its collection timing, the type of placebo used, and the technologies for CCP pathogen inactivation. All these inconsistencies have limited the ability to compare results among trials.[8,150,196]

Aside from RCTs, various meta-analyses have attempted to provide more comprehensive evidence on the efficacy of CCP. Meta-analyses including both non-hospitalized and hospitalized patients with severe disease showed no overall efficacy of CCP.[145–148] Our two meta-analyses of individual participant data, employing individual participant data pooling, have contributed evidence regarding the use of this therapy in outpatients. The first one, which pooled data from two RCTs, showed no ability of CCP to reduce hospitalization or disease progression. Conversely, the second meta-analysis, including data from all RCTs in COVID-19 outpatients, showed efficacy of CCP to reduce all-cause hospitalizations, particularly when administered within the first 5 days following symptom onset. CCP proved to be safe in all studies.

These inconsistencies between efficacy results observed in different studies of outpatients (either RCTs or pooled analyses of individual participant data) may be explained by differences in the methodological approach.

First, study populations varied in terms of demographic, clinical profiles, and baseline severity, including age and prevalence of risk factors for severe disease. Importantly, all trials included non-hospitalized patients with no contraindications to receive a plasma transfusion. Of note, one of the studies (C3PO) enrolled patients presenting to the emergency department (ED) with COVID-19.[140] These patients were still in the first days after symptoms onset, though they had possibly a more severe illness, further along in the inflammatory phase of disease. A prove of that is the higher total hospitalization rate in this study (30%), and the large total number of patients admitted directly to the hospital on the baseline visit (23% of all hospitalized participants). Nevertheless, our meta-analysis (study three) found a low to moderate heterogeneity, confirming the appropriateness of combining these studies and broadly generalizing these results.

Second, the timing of administration was also highly variable between the studies evaluating CCP, even when specifically focusing on those investigating its early infusion for outpatients. In our meta-analysis (study three), all studies infused patients within the first 9 days of symptom onset. However, one of the studies administered CCP in the first 72 hours after symptoms onset (with a median of 39.6 hours),[139] whereas the others had a median time of 4-5 days from first symptoms to infusion. It has been proposed that early antibody administration is more effective at reducing the inoculum and microbial burden in body tissues. In the early phase of infection, the microbial load is smaller, more localized, and easier to contain and be neutralized sufficiently by antibodies within the CCP.[197] A surrogate marker for early stages is the increased proportion of patients lacking antibodies from the endogenous immune response, particularly evident in studies where administration occurred during the early phases following infection.

Third, the levels of antibody titres and the laboratory techniques used to measure them varied highly across the studies, making it difficult to compare absolute antibody titres between trials. Titre determination is essential because neutralizing activity of antibodies in CCP is thought to be the primary mechanism of action for its potential efficacy.[100] In all five studies in outpatients, all donors were selected based on a minimum threshold of

antibody levels according to FDA guidance,[164] however, cutoffs used were different. Thus, the titres of neutralizing antibodies and their binding avidity are probably highly heterogeneous among trials. In addition, a variety of antibody assays were used to assess and characterize the CCP transfused, including viral plaque neutralization tests and surrogate immunoassays for binding antibodies (e.g., ELISA and CLIA), all of which are considered acceptable by the US FDA. Although neutralizing titres generally correlate well with antibodies to the spike receptor binding domain (RBD), there is no perfect correlation.[197] CCP contains a highly variable concentration of IgG and neutralizing antibodies and there is no standardized screening tool for measuring antibody titres, which makes generalization of antibody content and comparison with mixed efficacy results very difficult.

Fourth, volume of plasma infused varied across studies. Efficacy of CCP is associated not only with neutralization titres, but also with volume infused, as it relates to the overall quantity of antibodies administered. Viral neutralization follows a precise stoichiometry, and upon infusion, CCP gets diluted in the bloodstream. For example, when administering 200 ml of antibody product, this gets diluted about ten-fold in approximately 2,500 ml of plasma within the bloodstream.[198] Thus, to induce a two-fold increase in nAb titres in the recipient, the infused dose should be at least ten-fold higher than the one measured in the recipient. High correlation between the predicted SARS-CoV-2 antibody level and the actual SARS-CoV-2 antibody level in the recipient after infusion has been described.[199] All trials in outpatients infused a single unit; however, higher plasma volumes are typically administered in hospitalized COVID-19 patients.[144,149]

Fifth, the origin of CPP and the timing of its collection are also relevant factors that vary among the trials included in the meta-analysis (study three). Efficacy of CCP could potentially be reduced with differences between the infecting variant at the time of plasma collection and the circulating variants at the time of plasma infusion. The match between the donor's polyclonal antibodies and the patient's virus is specifically important for variants with higher number of mutations in the spike protein, such as omicron and its subvariants. Moreover, regional variation in the SARS-CoV-2 virus could also be an important factor in CCP efficacy, although probably to a lesser extent.[200]

Sixth, the control used was not the same across studies. While most studies used saline as control, CSSC-004 and COV-Early studies used non-convalescent plasma.[139,141] Some authors state that these trials using non-convalescent plasma are not placebo-controlled trials but plasma-controlled trials, because the control plasma may have had its own unique biologic effects.[201] It is not clear what is the immunologic effect of non-convalescent plasma containing antibodies to non-SARS-CoV-2 coronavirus strains, such as the human common cold coronavirus (hCCCoV), in persons with COVID-19. A study showed an association between higher magnitudes of hCCCoV and higher and delayed levels of anti-SARS-CoV-2 antibodies, which correlate with greater disease severity.[202] However, CCP could also contain such non-SARS-Cov-2 antibodies. Moreover, hospitalization rates in control groups of both trials were not higher than those in the rest of trials in outpatients, suggesting a minor or inexistent deleterious effect of control plasma.

Seventh, another difference between studies is the methods employed for CCP manufacturing and pathogen inactivation. To reduce the risk of transfusion-transmitted infection, plasma is often either quarantined or alternatively subjected to a pathogen inactivation method, such as methylene blue (MB), riboflavin (RB) or amotosalen (AM). Quarantine time of 3 to 4 months is applied to donors with unknown risk profile in some countries. Methylene blue (MB) is a method for plasma pathogen inactivation that is widely used throughout Europe, including Spain, France, UK, Italy, Belgium, and Austria. MB is a phenothiazine dye, which intercalates into viral nucleic acid and generates reactive oxygen species (ROS) with subsequent illumination with visible light.[203] ROS block viral DNA replication and RNA transcription; but can also impair other proteins, including immunoglobulins.[204] The COnV-ert trial used MB photoinactivation for pathogen reduction in transfused units, following regional blood bank standards. It has been postulated that MB-treatment could have been associated with the negative efficacy results observed in CoNV-ert trial.[205] Evidence on the effects of MB on immunoglobulin function is scarce. On one hand, few studies have shown preservation of neutralizing antibody function of CCP after the use of MB.[206,207] Moreover, comparison between the neutralizing activity of stored biospecimens from the donors (i.e., before MB treatment) with that of the plasma unit (i.e., after MB treatment) in a

subgroup of 40 plasma units infused in the COnV-ert trial was performed, showing that neutralizing antibody titres remained unchanged after MB treatment. On the other hand, the potential risk of damage to the Fc-region of the immunoglobulins could not be evaluated. It is known that the Fc region is large and requires intact glycosylation for function, compared to the region of the antibody that binds antigen, which is relatively small and possibly less vulnerable to direct oxidative damage.[208] Although a study found no effect of MB on the antigen binding capacity and Fc receptor binding capacity of anti-EBV and anti-tetanus toxin IgG,[209] no studies have evaluated the effect of MB on Fc-mediated effector functions of anti-SARS-CoV-2 antibodies. Thus, a concern remains that methylene blue-treatment could have affected Fc-region functionality without interfering with the viral neutralization assay in the COnV-ert trial. Fc might not be so essential when antibody therapies are administered as prophylaxis, but they seem necessary for optimal therapy. Interestingly, a recent ad hoc sub-study of the ConPlas-19 clinical trial, which showed a benefit of CCP in preventing respiratory deterioration or death when administered early in hospitalized COVID-19 patients, found no clinical differences between participants who received CCP treated with MB and CCP treated with other inactivation methods (RB or AM).[210]

All the above-mentioned differences are even more relevant among trials on non-hospitalized and hospitalized patients, with moderate, severe, or critical COVID-19, and might be the cause of the conflicting results and inconclusive evidence regarding CCP use for COVID-19 in the overall population.

Despite these differences, the results from our meta-analysis including all the available data from RCT in COVID-19 outpatients show a significant reduction of the risk of hospitalization with the early treatment of COVID-19 outpatients with high-titre CCP.

2. Efficacy of C19-IG20% for prevention of symptomatic COVID-19

The second objective was to assess the efficacy of the subcutaneous 20% hyperimmune immunoglobulin C19-IG20% in preventing the development of symptomatic COVID-19 in early asymptomatic adults with confirmed SARS-CoV-2 infection within 5 days regardless of comorbidities. This objective was evaluated in the fourth study (GC2010

trial), a multicentre, double-blind, randomized, placebo-controlled clinical trial conducted in Spain, which enrolled 461 asymptomatic unvaccinated adults (≥18 years) with confirmed SARS-CoV-2 infection, who were randomized (1:1:1) to receive a subcutaneous infusion of 10 ml of 1 g or 2 g of C19-IG20% or an equivalent volume of saline solution. The trial reported no differences in the proportion of participants who remained asymptomatic through day 14 after infusion. C19-IG20% was not associated with reduction in disease progression, hospitalization, or viral load.

C19-IG20% is a formulation for subcutaneous infusion with purified protein from pooled COVID-19 convalescent plasma donations, containing 20% human hyperimmune immunoglobulin (hIG), of which 98% is IgG. Unlike CCP, the polyclonal antibodies in hIG are standardized with high titre neutralizing activity, overcoming the inter-unit variability of CCP.

Only four other clinical trials have evaluated the efficacy of hIG for COVID-19, administering different immunoglobulin products to different populations.[171–174] These four RCTs included only hospitalized patients and high volumes (>100 ml) of hIG were infused using the intravenous administration route. Three out of these four trials (including the ITAC study, the largest trial with 593 participants) enrolled unvaccinated immunocompetent individuals and found no statistically significant benefit in reducing mortality rates,[172–174] while another trial that enrolled 18 severely immunocompromised patients, of whom 61% had been fully vaccinated, found a reduction of the rate of severe COVID-19 and a non-statistical reduction of mortality.[171] Unlike these four trials, our study enrolled asymptomatic individuals very early after SARS-CoV-2 infection, following the temporality principle of passive immunotherapy. The aim of the study was to find an efficacious therapy that could be administered easily at home or at the health care level and with a potential high level of acceptance among asymptomatic individuals. Thus, high neutralization capacity CCPs were selected from a pool of donors for the manufacturing of C19-IG20%. This approach reduced the volume required, allowing for a single subcutaneous infusion of 10 ml product without the need for delivery system designs such as pumps. Nevertheless, comparing neutralization capacity among different hIG products is difficult because of several reasons: neutralization titres are not always reported across studies, several neutralization assays are used to quantify antibodies, and

neutralization is affected by antigenic changes in viral variants. For instance, the ITAC study administered 400 mg/kg (40 g approximately) hIVIG intravenously to hospitalized patients,[173] whereas our study, GC2010 trial, administered 10 ml (1 or 2 g) C19-IG20% subcutaneously. These dosages consist of only 10% and 20% polyclonal IgG, respectively, with different neutralization activity, that varies also depending on viral variants. Considering these factors, a plausible hypothesis for the negative outcomes observed in the GC2010 trial is that the principle of a sufficient quantity or dose in antibody therapies might not have been met. This suggests that there is a possibility that higher doses or higher neutralization titres could potentially still exert an effect in reducing the risk of developing mild COVID-19 symptoms, or that may confer a benefit in reducing the risk of severe disease or hospitalization. However, the very low frequency of those clinical endpoints in our trial limited the statistical power to detect differences between the study groups.

In summary, there is limited and inconclusive efficacy evidence to draw recommendations on the use of hIG for the treatment and prevention of COVID-19, despite their safety, which was confirmed in all five RCT. Like for CCP, the efficacy of hIG seems to be associated with high neutralization titres, high doses, and early administration, especially in most at risk-populations such as immunocompromised patients.

3. Passive immunotherapy for the treatment and prevention of COVID-19

Passive immunotherapy has been proposed as one of the most promising therapies for COVID-19. Dozens of randomized clinical trials have evaluated the safety and efficacy of passive immunotherapy for COVID-19 in both hospitalized and non-hospitalized patients across almost all disease severities. Outcomes have varied across studies, with positive or negative results possibly influenced by factors such as the type of passive immunotherapy infused, time of administration after disease onset, and neutralization capacity of the product (defined as titre per volume).

First, mAbs have proven to be highly effective for the treatment of high-risk outpatients and for pre-exposure and post-exposure prophylaxis (PrEP and PEP).[182,183,186,188–190] To a

lesser extent, some benefits have also been reported in selected groups of hospitalized patients (i.e., without detectable anti-SARS-CoV-2 antibodies).[184] Five mAb products have been authorized either for early therapy in outpatients at hight risk of disease progression (including immunocompromised patients) or as PrEP, prior to the advent of the Omicron variant. Compared to CCP and hIG, mAbs have a significant higher specific activity and affinity, with a standardized content. For instance, approved mAb products contain between 0.3 g and 2.4 g of specific anti-SARS-CoV-2 antibodies targeting one or two epitopes. The main concern associated with mAb products is their susceptibility to mutations in the spike protein of viral variants. All licensed mAbs have demonstrated a loss of efficacy *in-vitro* against the Omicron variant and its subvariants (BA.1, BA.2, BQ.1/B1.1.1 and XBB/XBB.1/XBB1.5). As a result, their authorization has been restricted to variants for which they have proven to be effective.[193]

Second, the efficacy of CCP has been assessed in several RCTs. Like mAbs, CCP has shown no significant survival benefits when used for the treatment of immunocompetent hospitalized patients at a late stage of disease.[146,148] Regarding early treatment of outpatients, our meta-analysis of five RCTs has indicated a similar benefit between CCP and mAbs, although there was variability in outcomes observed across individual trials. Contrary to CPP, mAbs have demonstrated consistent efficacy across numerous RCT conducted with a sound methodology and low risk of bias. In the context of PEP, one trial evaluated the efficacy of CCP, and, unlike with mAbs, found no benefit in reducing the rate of infection or symptomatic disease. The main limitations of CCP, compared to mAbs, include the lack of standardized antibody type content and neutralization titers, as well as a lower overall dose of specific antibodies, which are likely contributing factors for the variability in efficacy outcomes. On the other hand, the polyclonal nature of antibodies in CPP confers an advantage in terms of resistance to viral variants. Other important benefits are its affordability and quicker deployment time, which renders CCP readily accessible and adaptable in the context of the emergence of new variants.

Third, in contrast to mAbs and CCP, the efficacy of hIG for COVID-19 has undergone evaluation in a very limited number of studies, and results have been mixed and inconclusive.[171–174] Notably, hIG products have found no benefit in hospitalized immunocompetent patients. The only study that showed a benefit of hIG, was a pilot trial

that included 18 hospitalized severe COVID-19 patients who were immunocompromised.[171] hIG has the advantage over CCP of having a standardized antibody content; however, the dose of specific antibodies and neutralizing capacity is still lower that the one of mAbs. Remarkably, no trials had previously evaluated the efficacy of hIG in COVID-19 outpatients prior to commencement of our trial. The OTAC study (NCT04910269), aimed to assess the efficacy of a 35 ml infusion of hIVIG for the treatment of outpatients, has recently stopped recruitment because the hIVIG used showed reduced activity against BQ.1.1 and substantially reduced activity against XBB, which are the main current circulating variants. Our study GC2010 showed no benefits of the subcutaneous hIG C19-IG29% in reducing the risk of progression from asymptomatic early-stage infection to symptomatic COVID-19 when administered as early therapy. Importantly, no trials have explored the efficacy of hIG for PrEP and PEP.

Limitations

The studies included in this thesis have some limitations that should be mentioned. Firstly, the two RCT we conducted had an early termination due to recruitment slowdown, attributed to the rapid deployment of vaccination campaigns and availability of effective mAbs for key populations that were also the focus of our trials. Consequently, the RCTs were hindered in their power to detect significant differences in primary outcomes among groups. This prompted the undertaking of two meta-analyses of individual participant data for CCP. Second, the overwhelming majority of patients enrolled in the two RCTs and included in the two meta-analyses had not received COVID-19 vaccination. This circumstance limits our capacity to analyse the effectiveness of CCP and hIG for reducing hospitalization in predominantly vaccinated populations and leads to an increase in the number needed to treat (NNT). Third, our trials included a high percentage of participants with no comorbidities and a very low number of immunocompromised individuals. As a results, it remains uncertain whether both interventions could yield benefits for individuals at a higher risk in the absence of other established therapies with proven efficacy. Fourth, all trials were conducted before the emergence of the omicron variant and its subvariants. In this context, while all licensed mAb products exerted an important reduction of disease progression before the emergence of omicron, none of them are recommended for omicron subvariants due to their diminished activity. The use of CCP

or hIG might still have relevance as neutralization studies of omicron subvariants, BQ.1, BQ1.1, XBB, and XBB.1, using sera from individuals infected with omicron or those who received boosters with the bivalent (WA1/BA.5) mRNA vaccine have shown a comparatively lower decline in neutralizing activity *in vitro*.[211]

Strengths

The studies encompassed in this thesis have several notable strengths worth commenting. First, the two RCTs involved a large and diverse trial population enrolled at different sites in Spain. Moreover, most participants received the allocated intervention and completed the follow-up. Second, the trial logistics involved the successful coordination of a large number of centres and investigators, including blood banks, during a pandemic when health care systems were working at a limited capacity. Third, despite the context of the global pandemic with continuous emergence of new evidence related to available therapies and vaccines, the study protocols were adeptly prepared, and updated and amended in a timely manner, facilitating the early generation of relevant evidence. Fourth, the trials included not only clinical efficacy endpoints, but also virological and laboratory endpoints, which contributed to more robust conclusions about the potential effect of both therapies. Fifth, the successful collaboration among international research teams allowed meta-analyses of individual participant data, which require pooling the databases of several studies and provide a more accurate analysis that conventional meta-analyses based on aggregated results only. Finally, all four studies collectively contribute to the existing body of knowledge regarding the use of passive immunotherapy, specifically CCP and hIG, for the management of COVID-19.

4. The way forward regarding passive immunotherapy for COVID-19

In conclusion, both CCP and hIG have proven to be safe therapies. On one hand, CCP should be considered as a valuable option for the early treatment of outpatients, especially for those at high risk of unfavourable outcomes (including immunocompromised individuals), in settings where mAbs or antivirals are not currently accessible or with new variants that have undermined the effectiveness of these interventions. On the other hand, taking together all the results on the efficacy of hIG for COVID-19 generated to date, definitive recommendations in favour of its use cannot be drawn.

Unresolved questions

There are some unresolved and outstanding questions regarding the use of CCP and hIG for COVID-19, that warrant further investigation. First, future research on passive immunotherapy should focus especially on immunocompromised individuals who have been vaccinated. The existing body of evidence from well-designed RCTs regarding the efficacy of both CCP and hIG in this specific population remains limited. Given that this group is at highest risk of inadequate humoral responses to SARS-CoV-2 infection and COVID-19 vaccination, more prolonged SARS-CoV-2 replication, and more severe disease it becomes crucial to develop therapies tailored to their needs. Second, research should also focus on defining the optimal antibody titre and dosage of CCP and hIG. Although there is evidence that a high dose of neutralization antibodies against SARS-CoV-2 is critical for CCP and hIG to be effective, the precise dose or neutralization capacity, expressed as titre per volume, remains unknown. Consequently, there is a need for more evidence stemming from well-structured phase 2 dose-finding studies. Third, stricter antibody titre criteria for CCP and hIG could potentially lead to more consistent clinical efficacy and narrow the dose gap between CCP and mAbs. This could be achieved by obtaining plasma from vaccinated donors infected with more recent variants, which could be used for direct administration as CCP (known as vax-CCP) and/or manufacturing of newer hIG products.[212] Contemporaneous CCP and hIG may also be less susceptible to viral immune escape than mAbs.[213] Fourth, establishing a consensus on the assay to measure antibody titres in CCP is critical to allow cross-study comparisons and to guide the selection of optimal products for clinical use. In general, both plaque reduction neutralization test (PRNT) and surrogate high-throughput serology have a high correlation and should be recommended and used. Achieving standardization of antibody titres could involve the use of the International Standard and IU, to which different assays can be calibrated (IU/ml for neutralizing antibodies and BAU/ml for binding assay formats).[214] Fifth, the effect of methylene blue-treatment on the Fc region of anti-SARS-CoV-2 antibodies is not well understood. Studies evaluating a possible deleterious effect of MB-treatment (approved by the FDA) on effector functions such as Fc-dependent phagocytosis, cell-mediated cytotoxicity, and complement-dependent cytotoxicity are essential to establish a recommendation of its use as a pathogen inactivation method.

5. Lessons learned for the next viral epidemic

Passive immunotherapy, including CP, hIG and mAbs, have been used as potential treatments during viral infectious diseases outbreaks and epidemics, such as Influenza, SARS, MERS, and Ebola.[8] However, the challenges of conducting rigorous RCTs during epidemics have resulted in overall limited evidence on their efficacy. Currently, the use of CP is approved as a first line treatment for Argentine haemorrhagic fever,[36] while a few mAbs are approved for the treatment of Ebola and Respiratory Syncytial virus.[39,45] The COVID-19 pandemic has provided the first comprehensive opportunity to extensively evaluate these therapies with multiple large-scale clinical trials being conducted. These trials have yielded strong evidence. One of the most important lessons learned from the use of passive immunotherapy for COVID-19 is that it is highly effective in preventing disease progression if administered early after infection using antibody therapies with high neutralizing capacity, especially in populations at highest risk of severe disease. This newfound knowledge will be essential to confront new viral epidemics and reduce the possibility of missteps in deploying antibody therapies in the future.

Preparedness for future epidemics requires readily available prophylactic and therapeutic tools for deployment at the first signs of an outbreak. In early phases of an epidemic, passive immunotherapy could become a valuable option while vaccines and antivirals are being developed.[215,216] CP is likely to be the first therapy globally available and affordable in the early stages of a viral,[196] despite facing certain logistical challenges. hIG and mAbs require a longer time for development; although they have been manufactured and deployed at unprecedented speed during the COVID-19 pandemic. In later phases of an epidemic, all three therapies could also offer alternative prophylaxis and early treatment option for populations who are not able to mount an adequate immune response to vaccination, such as immunocompromised individuals. It is important to acknowledge the limitations associated with passive immunotherapy, which include the potential for resistance emergence due to viral variants in viruses with a rapid antigenic variation and the need for parenteral administration. However, these limitations must be weighed against the advantages these therapies bring to the table.

Pandemic preparedness should involve the anticipatory development of clear protocols and guides for the optimal use of passive immunotherapy. Some key elements should be prioritized for the implementation of a successful program employing these therapies. A first key element is the identification of the optimal target patient population. The selection of patients most likely to benefit should be based on clinical grading systems that consider factors beyond the time elapsed since symptom onset or admission to hospital. Another essential issue is antibody testing to identify patients without sufficient levels of endogenous antibodies, using standardized quantitative methods and following a consensus on a threshold for low antibody titre. A second key element is the identification of antibody products with optimal neutralizing capacity. Regarding CP, the selection of optimal CP units requires consensus on dosage (volume and timing), along with establishing a minimum cutoff and criteria for high antibody titre, and the need for rapid development of a "gold standard" quantitative method to estimate antibody content (preferable neutralization tests or validated surrogates). Similarly, hIG products should be manufactured using pooled CP following the same selection criteria. A stockpile of broad-spectrum predeveloped mAbs could potentially shorten the time to deployment of mAb products. A third key element, specifically relevant for CP programs, is the identification of optimal donors. A well-defined strategy should be adopted, including the rapid development of standardized assays for donor screening and the establishment of a donor registry. Importantly, well designed clinical trials should be implemented rapidly and timely, enrolling targeted optimal subpopulations (mainly at earlier disease stages or specific subpopulations at high risk) and following a consensus on a list of key standardized outcomes.[216] Finally, one of the main learning points from the COVID-19 pandemic is the need to ensure global access to prophylactic and therapeutic tools. Facilitating global access to passive immunotherapies is epidemiologically, economically, and socially essential in future epidemics. In this context, extending the availability of these therapies across low- and middle-income countries (LMICs) will require the provision of comprehensive guidance. This involves the development of tailored and regionally relevant approaches for collection and production of CP, along with an equitable distribution of hIG and mAbs.

Conclusions

1. COVID-19 convalescent plasma for the treatment of outpatients has drawn mixed efficacy results in clinical trials, which could be partly attributed to factors such as variable patient populations, timing of administration, convalescent plasma SARS-CoV-2 antibody levels, testing methods for antibody quantification, dosages, origin and time for collection, and pathogen inactivation methods (i.e., methylene blue).
2. According to the overall evidence presented in this thesis, COVID-19 convalescent plasma with high antibody titres should be considered as a valuable option for the early treatment of COVID-19 outpatients who have a high risk of disease progression.
3. The subcutaneous hyperimmune immunoglobulin C19-IG20% at the dose of 1 and 2 g cannot be recommended for use in asymptomatic individuals with SARS-CoV-2 infection to prevent progression to symptomatic COVID-19.
4. There is overall limited evidence regarding the efficacy of hyperimmune immunoglobulins for COVID-19, thus formal recommendations to support their use cannot be concluded.
5. Key learning points from the use of COVID-19 convalescent plasma and hyperimmune immunoglobulins for COVID-19 include the need of an early administration and high neutralization capacity, which currently could be achieved through vaccinated donors with recent infection.
6. Future research endeavours should focus on assessing the efficacy of COVID-19 convalescent plasma and hyperimmune immunoglobulins for COVID-19 following the emergence of the omicron variant, specifically in immunocompromised individuals who have been vaccinated.
7. Additional pivotal considerations for COVID-19 convalescent plasma and hyperimmune immunoglobulins include determining the appropriate dose or neutralization capacity, establishing a consensus on a “gold standard” assay for measuring antibody titres, and assessing the effect of methylene blue treatment on Fc region of anti-SARS-CoV-2 antibodies.

8. Pandemic preparedness should involve the anticipatory development of clear guidance for the optimal use of passive immunotherapy, which should include the identification of the target patient population, the optimal neutralization capacity, and the optimal donors.

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