Supplement S2:

Supplementary Material & Methods – Virus Neutralisation Assay - DAWn PLASMA TRIAL

Anti-SARS-CoV-2 virus neutralisation titers (NT50) were determined on plasma or serum samples in four Belgian laboratories (Liège University, Institute of Tropical Medicine, Rega Institute Leuven, Sciensano). NT50 titers were determined on donor plasma samples to select COVID-19 convalescent donors eligible for CCP donation for the DAWn plasma trial. NT50 titers were also determined on serum samples of study patients at Day 0 and Day 6 after randomisation. All the virus neutralisation assays (VNA) conducted in this study were performed in BSL3 laboratories in a 96-well plate format, using heat-inactivated plasma or serum samples (30-60 minutes at 56°C).

Methodological details of the protocols applied in the four laboratories are described hereafter.

Liège University (D. Desmecht/ M. Garigliany - Department of Animal Pathology, Liège University, Liège, Belgium)

Two-fold serial dilutions (1/10 to 1/1280) of heat-inactivated serum or plasma samples were mixed vol/vol with 100 TCID50/reaction of SARS-CoV-2 (strain BetaCov/Belgium/Sart-Tilman/2020/1, passage 5), corresponding to final testing dilutions of 1/20 to 1/2560 in DMEM supplemented with 2% foetal bovine serum, 1% antibiotic and 1% antimycotic. Following incubation for 1 h at 37 °C, triplicates of sample plus virus mixtures were transferred in 96-well plates containing confluent monolayer of Vero E6 cells (ATCC CRL-1586) (Wu H-S et al. Taiwan Emerg Inf Dis. 2004; 10: 304–310). Two samples, a negative control and a strong positive control (provided by the Croix-Rouge de Belgique) were tested per plate. This VNA relies on direct cytopathic effect (CPE) observation under light microscopy at day 5 post infection. Dilutions of samples/controls associated with CPE were considered as negative, while the absence of CPE indicated a complete neutralisation of SARS-CoV-2 inoculum and were considered positive for neutralisation. Virus neutralisation titers are reported as the highest dilution of serum that neutralised CPE in 50% of the wells. Serum/plasma specimens with a NT50 titer ≥ 40 are considered to neutralise the virus. *Method as described in Misset et al. BMC Pulm Med. 2020 Dec 7;20(1):317.*

Institute of Tropical Medicine (K.K. Ariën - Virology Unit, Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; University of Antwerp, Antwerp, Belgium)

Serial dilutions (1/33 to 1/1048) of heat-inactivated serum were incubated with 3xTCID100 of SARS-CoV-2 (strain 2019-nCoV-Italy-INMI1, reference 008V-03893, passage 5), corresponding to final testing dilutions of 1/50 to 1/1600 in EMEM supplemented with 2 mM L-glutamine, 100 U/ml - 100 µg/ml of Penicillin-Streptomycin and 2 % foetal bovine serum. Following incubation for 1 h (37 °C / 7 % CO2), 8 replicates of sample/virus mixtures and virus/cell controls were added to Vero cells (18.000 cells/well) in a 96-well plate and incubated for 5 days (37 °C / 7 % CO2). The cytopathic effect (CPE) caused by viral growth was scored microscopically. The Reed-Muench method was used to calculate the neutralising antibody titer that reduced the number of infected wells by 50 % (NT50) (Reed and Muench. Am J Hyg 1938; 27: 493-497), these values were used as a proxy for the neutralising antibody concentration in each sample. *Method as described in Mariën et al. J Virol Methods. 2021 Feb;288:114025. Epub 2020 Nov 20.*

Katholieke Universiteit Leuven (P. Maes - Department of Microbiology, Immunology and Transplantation, Laboratory of Clinical and Epidemiological Virology, KU Leuven, Leuven, Belgium)

Serial dilutions of heat-inactivated serum were incubated with 400 plaque-forming units (pfu) of SARS-CoV-2 (strain SARS-2-CoV/Belgium/GHB-03021/2020, GISAID accession number EPI_ISL_407976, passage 5) in 96-well plates seeded with Vero E6 wells (1 h, 37°C, humidified 5% CO2 atmosphere). Following incubation, a 1% agarose (SeaKem LE agarose, Lonza, Belgium) overlay was added and plates were incubated for 4 days (37°C, humidified 5% CO2 atmosphere). Following overlay with 1% neutral red/1% agarose (24 h, 37°C), plaques were counted. Virus neutralisation titers were reported as a 50% reduction (NT50) in the number of plaques in comparison to a non-neutralising antibody control. *Method as described in Betrains et al. Br J Haematol 2021; 192(6):1100-1105.*

Sciensano (C. Barbezange – Unit Respiratory viruses, Directorate Infectious diseases in humans)

Two-fold serial dilutions (1/40 to 1/5120) of heat-inactivated serum samples were mixed vol/vol with 100 TCID50/reaction of SARS-CoV-2 (hCoV-19/Belgium/S1871/2020, passage 3), corresponding to final testing dilutions of 1/80 to 1/10240 in DMEM supplemented with 2% foetal bovine serum, 100 U/ml - 100 µg/ml of Penicillin-Streptomycin. Following incubation for 1 h at 37 °C (5% CO_2), triplicates of sample plus virus mixtures were transferred in 96-well plates containing a confluent monolayer of Vero E6 cells (ATCC CRL-1586) seeded the day before at 20,000 per well. A negative control (serum from 2017) and a strong positive control (provided by Croix-Rouge de Belgique) were tested in parallel in each experimental run. This VNA relies on the staining of infected cells using SARS-CoV-2 specific antibodies and was adapted from Okba et al. (Emerg Infect Dis 2020; 26(7):1478-1488) and from Amanat et al. (Curr Protoc Microbiol. 2020; 58(1):e108). More specifically, two days post-infection, cells are fixed with 4% paraformaldehyde, permeabilised with PBS supplemented with 0.2% Triton X-100, incubated with diluted mouse monoclonal anti-SARS-CoV/SARS-CoV-2 Nucleocapsid antibody (Bio-Connect, 40143-MM08), incubated with diluted goat anti-mouse IgG HRP conjugated secondary antibody (Biorad, 172-1011) and revealed with a precipitate forming 3,3',5,5'-tetramethylbenzidine substrate (True Blue; VWR, KPLI50-78-02). Visual evaluation of individual wells is performed and wells with >50% of blue area are scored as positive for the virus. The Reed-Muench method was used to calculate the neutralising antibody titer that reduced the number of infected wells by 50 % (NT50). Samples with a NT50 titer ≥ 80 are considered as positive for neutralising antibodies against the virus.