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Convalescent plasma donors show enhanced cross-reactive neutralising antibody response to antigenic variants of SARS-CoV-2 following immunisation

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Short title: Neutralising antibody responses in vaccinated donors

Abstract

Background. The therapeutic benefit of convalescent plasma (CP) therapy to treat COVID-19 may derive from neutralising antibodies (nAbs) to SARS-CoV-2. To investigate effects of antigenic variation on neutralisation potency of CP, we compared nAb titres against prototype and recently emerging strains of SARS-CoV-2, including Delta and Omicron, in CP donors previously infected with SARS-CoV-2 before and after immunisation.

Methods and Materials. Samples were assayed from previously SARS-CoV-2 infected donors before (n=17) and after one (n=43) or two (n=71) doses of Astra-Zeneca or Pfizer vaccinations. Ab titres against Wuhan/wild type (WT), Alpha, Beta and Delta SARS-CoV-2 strains were determined by live virus microneutralization assay while titres to Omicron used a focus reduction neutralisation test. Anti-spike antibody was assayed by Elecsys anti-SARS-CoV-2 quantitative spike assay (Roche).

Results. Unvaccinated donors showed a geometric mean titre (GMT) of 148 against WT, 80 against Alpha but mostly failed to neutralise Beta, Delta and Omicron strains. Contrastingly, high GMTs were observed in vaccinated donors against all SARS-CoV-2 strains after one vaccine dose (WT:703; Alpha:692; Beta:187; Delta:215; Omicron:434). By ROC analysis, reactivity in the Roche quantitative Elecsys spike assay of 20,000 U/ml was highly predictive of donations with nAb titres of ≥1:640 against Delta (90% sensitivity; 97% specificity) and ≥1:320 against Omicron (89% sensitivity; 81% specifciity)

Discussion. Vaccination of previously infected CP donors induced high levels of broadly neutralising antibodies against circulating antigenic variants of SARS-CoV-2. High titre donations could be reliably identified by automated quantitative anti-spike antibody assay, enabling large-scale pre- selection of high-titre convalescent plasma.

Keywords

SARS-CoV-2; COVID-19; Convalescent plasma; Antibody neutralisation; Antigenic variants; Vaccination; Omicron; Delta

Introduction

During the pandemic, SARS-CoV-2 has continued to rapidly evolve to evade immune responses, with many variants displaying multiple mutations in the spike gene that have been shown to reduce their susceptibility to neutralising antibodies[1]. These antigenic changes potentially contribute to immune evasion[2] and may abrogate neutralisation by monoclonal antibodies (mAbs) recently deployed as immunotherapy[3-5]. Convalescent plasma has been shown to be an effective and affordable treatment if given soon after infection or in immunocompromised patients[6,7], although its efficacy may be conditioned by the ability of anti-SARS-CoV-2 antibodies to effectively neutralise currently circulating strains[8,9]. We investigated whether individuals with previous SARS-CoV-2 infection who have been subsequently immunised represent an effective source of high-titre cross-reactive nAb for immunotherapy. Such plasma is urgently needed to assess its treatment efficacy for immunocompromised individuals infected with recently emerging Delta and Omicron antigenic variants of SARS-CoV-2.

Methods

Convalescent plasma collections in England. A cohort of registered NHS Blood and Transplant (NHSBT) convalescent donors with a suspected or laboratory confirmed SARS-CoV-2 infection and subsequently known to be immunised were invited to the current study (Table S1; Suppl.Data). SARS-CoV-2 type was inferred based on their first convalescent plasma donation date - WT: April-December 2020; Alpha: January-March 2021 (based on https://www.gisaid.org). We targeted donors who had evidence of a moderate level of anti-SARS-CoV-2 antibodies prior to immunisation (see below)[10]. A patient information leaflet was given for those interested to join this study and an appointment for blood sample in the NHSBT Birmingham donor centre was provided after signed

consent was received. Up to two blood samples were collected a minimum of 28 days after the immunisation from these individuals who had previously donated COVID-19 convalescent plasma. An archive sample of their previous donation given prior to vaccination was also obtained when available.

Ethical approval. Approval for this study was received from the West Midlands Solihull Research Ethics Committee, UK (REC reference: 21/WM/0082, IRAS project ID: 296926).

Study participants. A total of 131 samples were obtained from 94 convalescent donors originally infected with SARS-CoV-2; 80 of them had moderate anti-SARS-CoV-2 antibody levels in the IgG EUROimmun assay targeting the spike S1 domain (Perkin Elmer; S/Co ratio 1-5.99) and 14 with higher antibody levels (S/Co ratio >5.99) prior to immunisation. A total of 17 convalescent samples were taken from donors prior to their immunisation, and 114 samples taken from donors after receiving one or two doses of AstraZeneca or Pfizer vaccines (43 samples taken 33 to 79 days after the first dose and 71 samples 29 to 140 days after the second dose). Most donors received AstraZeneca vaccine (87/94, 93%), while the remainder were immunised with the Pfizer vaccine (both vaccines based on WT sequence). All three samples were available for 5 donors whereas two samples were available for 27 donors and one post-immunisation sample for 62 donors, from which most provided sample after second dose of vaccine (n=49). All samples were taken before the third booster dose was introduced. Donors were aged between 21 and 65 years (mean age 50 years), and most were males (75/94, 80%).

Detection of neutralising antibodies. The presence of SARS-CoV-2 nAbs in plasma samples were determined using a live virus microneutralization assay (MNA) with WT (England-2), Alpha (B.1.1.7), Beta (B.1.351) and Delta (B.1.617.2) strains as previously described [8]. A selection of samples (n=33) were tested for nAb to Omicron (B.1.1.529) using focus reduction neutralisation test (FRNT). This

was necessitated by the absence of cytopathology in cells infected with Omicron, preventing the use of a traditional MNA[11]. However, to ensure nAb titres produced by the two assays were comparable, we assayed 33 samples in MNA and FRNT assays against WT SARS-CoV-2 and calibrated results by a regression analysis of nAb titres (Figure S2; Suppl. Data). These samples were selected to provide a mixture of samples obtained pre- and post-vaccine and a range of Nab titres. Nab titres were comparable in two assay formats, necessitating only minor correction using the formula MNA = (FRNT – 0.159) / 1.071 when comparing assay results.

All samples were assayed by Elecsys anti-SARS-CoV-2 quantitative spike assay using a WT receptor binding domain recombinant protein as antigen (Roche, London, UK).

Statistical analysis was performed using SPSS software, version 28.

Results

Neutralising antibody response after natural infection and vaccination. A total of 131 samples were obtained from 94 convalescent donors originally infected most likely with either WT (n=74) or Alpha variants of SARS-CoV-2 (n=20). Neutralising antibody against WT (geometric mean titre[GMT] 1:148, range < 1:20 - 1:1280) and Alpha (GMT 1:80, < 1:20 - 1:1280) variants were significantly higher than to Beta (GMT 1:29, < 1:20 - 1:160) and Delta (GMT 1:23, < 1:20 - 1:160) variants (n=17, $p < 0.001$, Figure 1A). Neutralising antibodies quantified by FRNT against WT (GMT 1:207, <1:20 - 1:534) were significantly higher than those measured against Omicron (GMT 1:23, <1:20 - 1:230) (n=6, $p < 0.001$, Figure 1B).

The largest increase in the nAb titres were seen after first dose of vaccine (WT 4.8-fold; Alpha 8.7-fold; Beta 6.5-fold and Delta 9.5-fold increase in MNA; n=43, Figure 1A); levels in sequential samples are shown in Fig. 2). Similarly, 17.5-fold and 18.9-fold increases following immunisation were observed against WT and Omicron strains respectively in the FRNT (Figure 1B). Actual nAb levels varied, with highest levels measured after first dose of vaccine against WT or Alpha with GMTs of 1:703 (1:160 - 1:5120) and 1:692 (1:80 - 1:5120) respectively compared to GMTs of 1:215 (< 1:20 - 1280) against Delta and 1:187 (< 1:20 - 1280) against Beta. The FRNT titres measured against the Omicron were similarly reduced when compared to WT(GMT 1:434 and 1:3637, respectively). Titres did not change significantly after the second dose of vaccine (Figs. 1, 2). No significant differences in neutralising antibody titres were seen between donors who received Astra Zeneca or Pfizer vaccine (data not shown).

Predicting samples most suitable for convalescent plasma therapy using binding antibody titres

Virus neutralisation titres and reactivity in Roche Elecsys spike quantitative antibody assays were compared using a total of 131 samples obtained from convalescent plasma donors (Fig. 3A; Roche result was not available for three samples). Antibody titres measured by the Roche Elecsys spike total antibody assay were significantly associated with nAb titres against each SARS-CoV-2 strain in samples obtained from vaccinated donors; this was evident also with Omicron despite testing of smaller number of samples. However, the lower levels of nAbs detected in samples from pre-vaccinated donors showed little (WT; p = 0.04) to no (Alpha, Beta, Delta and Omicron) significant association with reactivity in the Elecsys assay. Furthermore, infection induced relatively weaker reactivity in the Elecsys spike assay than would be predicted from neutralising titres. Vaccination appears therefore to induce a much greater proportion of immunoluminescence-detected antigen-reactive antibodies and a qualitatively different antibody response.

A neutralising antibody titre of 1:640 against Delta was selected as a likely therapeutic threshold for the use of convalescent plasma in patients infected with this strain, or possible subsequent variants. That level of nAbs should allow a titre of more than 1:100 to be achieved in an average recipient, based on the dilution of 500-560 ml of CP (2 x 250-280 ml plasma donations) into a plasma volume of around 2.5 – 3 litres in the recipient. These levels have been shown to protect also against reinfection in a non-human primate model for SARS-CoV-2[12]. The optimal cut-off value in the Roche Elecsys assay in terms of specificity and sensitivity for predicting samples with ≥1:640 nAb titres to be used for plasma selection was determined by receiver operating characteristic (ROC) analysis (21 samples with titres ≥1:640; 110 samples <1:640). Antibody titres between 10,000 and 35,000 U/ml obtained by the Roche assay were selected as potential cut-off values for sensitivity and specificity analysis (Fig. 3B); a titre of 20,000 U/ml correctly identified 88% of donations (19/21) above 1:640, whereas 97% of donations below this nAb threshold were classified correctly as below 1:640 (107/110). A further analysis of the Roche Elecsys assay's ability to predict donations with a normalised nAb levels of >1:640 and >1:320 against Omicron were similarly evaluated by ROC analysis. While the precision of this analysis would benefit from larger numbers, a level of 20,000 U/ml in the Roche assay in those with previous infection followed by vaccination shows a sensitivity of 89% and specificity of 81% for predicting units with titres >1:320.

Based on these calculations, and in the absence of scalable nAb test for donation screening, we propose that convalescent plasma donations could be selected with a minimum antibody level in the Roche Elecsys spike total assay.

Discussion

In England, NHSBT collected convalescent plasma from individuals with confirmed or suspected SARS-CoV-2 infection at least 28 days after the resolution of their symptoms between 22 April 2020 and 18 March 2021. Donations containing a minimum nAb titre of 1:100 were provided for two

clinical trials based on Euroimmun IgG testing but collections were stopped as the analysis of trial results did not show overall benefit for hospitalised patients[13,14] However, the results were suggestive of possible benefit in the immunocompromised patient group inviting further trials with high-titre plasma in this particular subgroup.

The study findings demonstrate that vaccinated convalescent donors develop high levels of cross-reactive neutralising antibodies against WT and newly emerging virus variants. This is important as new SARS-CoV-2 variants continue to emerge and may lead to reduction in the neutralisation capacity of collected convalescent plasma. In the study sample of 94 convalescent plasma donors inititally infected with either WT or Alpha variant, 21 also developed high levels of nAb against Beta, Delta and Omicron variants after the first vaccine dose. This data is consistent with other recently published evidence indicating that a single dose of mRNA SARS-CoV-2 vaccine in individuals who have had a previous natural infection will indeed elicit higher antibody titres than measured in vaccinated individuals who have not been infected by SARS-CoV-2[15-17]. Recently published studies show that a single dose of mRNA vaccine boosts pre-existing immunity of individuals towards new SARS-CoV-2 variants, including Omicron, that they have not been previously infected with[17-19].

Plasma from vaccinated convalescent donors produce high levels of cross-reactive nAb against newly emerging variants; these have potential therapeutic value for COVID-19 and their polyclonal nature may be advantageous compared to monoclonal antibodies therapies that have substantially lost their neutralisation capacity against antigenic variants of SARS-CoV-2. While the FRNT had to be used to quantify nAbs to Omicron, MNA and FRNT assays determined comparable titres for WT virus (Fig. S1; Suppl. Data), although it is possible in principle that this relationship may differ for other SARS-CoV-2 strains. Further cross-antibody comparisons will be required to address this conclusively.

Finally, we have shown that these post-vacccinated high-titre donors with cross-reactive antibodies can be readily identified by automated quantitative spike assay; this provides the means for scalable, rapid and large scale prospective collection of high titre donations against SARS-CoV-2 strains circulating to date from previously infected and subsequently vaccinated individuals.

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References

1. World Health Organisation. *Classification of Omicron (B.1.1.529): SARS-CoV-2 variant of concern [monograph on the internet]*. 2021. Available from: https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern
2. Liu L, Iketani S, Guo Y, Chan JF, Wang M, Liu L, Luo Y, Chu H, Huang Y, Nair MS, Yu J, Chik KK, Yuen TT, Yoon C, To KK, Chen H, Yin MT, Sobieszczyk ME, Huang Y, Wang HH, Sheng Z, Yuen KY, Ho DD. Striking Antibody Evasion Manifested by the Omicron Variant of SARS-CoV-2. Nature 2021.
3. Cameroni E, Bowen JE, Rosen LE, Saliba C, Zepeda SK, Culap K, Pinto D, VanBlargan LA, De Marco A, di Iulio J, Zatta F, Kaiser H, Noack J, Farhat N, Czudnochowski N, Havenar-Daughton C, Sprouse KR, Dillen JR, Powell AE, Chen A, Maher C, Yin L, Sun D, Soriaga L, Bassi J, Silacci-Fregni C, Gustafsson C, Franko NM, Logue J, Iqbal NT, Mazzitelli I, Geffner J, Grifantini R, Chu H, Gori A, Riva A, Giannini O, Ceschi A, Ferrari P, Cippà PE, Franzetti-Pellanda A, Garzoni C, Halfmann PJ, Kawaoka Y, Hebner C, Purcell LA, Piccoli L, Pizzuto MS, Walls AC, Diamond MS, Telenti A, Virgin HW, Lanzavecchia A, Snell G, Veesler D, Corti D. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. Nature 2021.
4. Planas D, Saunders N, Maes P, Guivel-Benhassine F, Planchais C, Buchrieser J, Bolland WH, Porrot F, Staropoli I, Lemoine F, Péré H, Veyer D, Puech J, Rodary J, Baele G, Dellicour S, Raymenants J, Gorissen S, Geenen C, Vanmechelen B, Wawina-Bokalanga T, Martí-Carreras J, Cuypers L, Sève A, Hocqueloux L, Prazuck T, Rey F, Simon-Loriere E, Bruel T, Mouquet H, André E, Schwartz O. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature 2021.
5. Wilhelm A, Widera M, Grikscheit K, Toptan T, Schenk B, Pallas C, Metzler M, Kohmer N, Hoehl S, Helfritz FA, Wolf T, Goetsch U, Ciesek S. Reduced Neutralization of SARS-CoV-2 Omicron Variant by Vaccine Sera and Monoclonal Antibodies. medRxiv 2021: 2021.12.07.21267432.
6. Libster R, Pérez Marc G, Wappner D, Coviello S, Bianchi A, Braem V, Esteban I, Caballero MT, Wood C, Berrueta M, Rondan A, Lescano G, Cruz P, Ritou Y, Fernández Viña V, Álvarez Paggi D, Esperante S, Ferreti A, Ofman G, Ciganda Á, Rodriguez R, Lantos J, Valentini R, Itcovici N, Hintze A, Oyarvide ML, Etchegaray C, Neira A, Name I, Alfonso J, López Castelo R, Caruso G, Rapelius S, Alvez F, Etchenique F, Dimase F, Alvarez D, Aranda SS, Sánchez Yanotti C, De Luca J, Jares Baglivo S, Laudanno S, Nowogrodzki F, Larrea R, Silveyra M, Leberzstein G, Debonis A, Molinos J, González M, Perez E, Kreplak N, Pastor Argüello S, Gibbons L, Althabe F, Bergel E,

Polack FP. Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. N Engl J Med 2021.

7. Salazar E, Christensen PA, Graviss EA, Nguyen DT, Castillo B, Chen J, Lopez BV, Eagar TN, Yi X, Zhao P, Rogers J, Shehabeldin A, Joseph D, Masud F, Leveque C, Olsen RJ, Bernard DW, Gollihar J, Musser JM. Significantly Decreased Mortality in a Large Cohort of Coronavirus Disease 2019 (COVID-19) Patients Transfused Early with Convalescent Plasma Containing High-Titer Anti-Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Spike Protein IgG. Am J Pathol 2021;**191**: 90-107.
8. Nguyen D, Simmonds P, Steenhuis M, Wouters E, Desmecht D, Garigliany M, Romano M, Barbezange C, Maes P, Van Holm B, Mendoza J, Oyonarte S, Fomsgaard A, Lassaunière R, Zusinaite E, Resman Rus K, Avšič-Županc T, Reimerink JH, Brouwer F, Hoogerwerf M, Reusken CB, Grodeland G, Le Cam S, Gallian P, Amroun A, Brisbarre N, Martinaud C, Leparc Goffart I, Schrezenmeier H, Feys HB, van der Schoot CE, Harvala H. SARS-CoV-2 neutralising antibody testing in Europe: towards harmonisation of neutralising antibody titres for better use of convalescent plasma and comparability of trial data. Euro Surveill 2021;**26**.
9. Nguyen D, Xiao J, Simmonds P, Lamikanra A, Odon V, Ratcliff J, Townsend A, Roberts DJ, Harvala H. Effects of SARS-CoV-2 strain variation on virus neutralisation titres: therapeutic use of convalescent plasma. J Infect Dis 2021.
10. Harvala H, Mehew J, Robb ML, Ijaz S, Dicks S, Patel M, Watkins N, Simmonds P, Brooks T, Johnson R, Gopal R, Roberts DJ, Zambon M. Convalescent plasma treatment for SARS-CoV-2 infection: analysis of the first 436 donors in England, 22 April to 12 May 2020. Euro Surveill 2020;**25**.
11. Bewley KR, Coombes NS, Gagnon L, McInroy L, Baker N, Shaik I, St-Jean JR, St-Amant N, Buttigieg KR, Humphries HE, Godwin KJ, Brunt E, Allen L, Leung S, Brown PJ, Penn EJ, Thomas K, Kulnis G, Hallis B, Carroll M, Funnell S, Charlton S. Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. Nat Protoc 2021;**16**: 3114-40.
12. Deng W, Bao L, Liu J, Xiao C, Liu J, Xue J, Lv Q, Qi F, Gao H, Yu P, Xu Y, Qu Y, Li F, Xiang Z, Yu H, Gong S, Liu M, Wang G, Wang S, Song Z, Liu Y, Zhao W, Han Y, Zhao L, Liu X, Wei Q, Qin C. Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. Science 2020;**369**: 818-23.
13. Convalescent plasma in patients admitted to hospital with COVID-19 (RECOVERY): a randomised controlled, open-label, platform trial. Lancet 2021;**397**: 2049-59.

14. Estcourt LJ, Turgeon AF, McQuilten ZK, McVerry BJ, Al-Beidh F, Annane D, Arabi YM, Arnold DM, Beane A, Bégin P, van Bentum-Puijk W, Berry LR, Bhimani Z, Birchall JE, Bonten MJM, Bradbury CA, Brunkhorst FM, Buxton M, Callum JL, Chassé M, Cheng AC, Cove ME, Daly J, Derde L, Detry MA, De Jong M, Evans A, Fergusson DA, Fish M, Fitzgerald M, Foley C, Goossens H, Gordon AC, Gosbell IB, Green C, Haniffa R, Harvala H, Higgins AM, Hills TE, Hoad VC, Horvat C, Huang DT, Hudson CL, Ichihara N, Laing E, Lamikanra AA, Lamontagne F, Lawler PR, Linstrum K, Litton E, Lorenzi E, MacLennan S, Marshall J, McAuley DF, McDyer JF, McGlothlin A, McGuinness S, Miflin G, Montgomery S, Mouncey PR, Murthy S, Nichol A, Parke R, Parker JC, Priddee N, Purcell DFJ, Reyes LF, Richardson P, Robitaille N, Rowan KM, Rynne J, Saito H, Santos M, Saunders CT, Serpa Neto A, Seymour CW, Silversides JA, Tinmouth AA, Triulzi DJ, Turner AM, van de Veerdonk F, Walsh TS, Wood EM, Berry S, Lewis RJ, Menon DK, McArthur C, Zarychanski R, Angus DC, Webb SA, Roberts DJ, Shankar-Hari M. Effect of Convalescent Plasma on Organ Support-Free Days in Critically Ill Patients With COVID-19: A Randomized Clinical Trial. Jama 2021;**326**: 1690-702.

15. Saadat S, Rikhtegaran Tehrani Z, Logue J, Newman M, Frieman MB, Harris AD, Sajadi MM. Binding and Neutralization Antibody Titers After a Single Vaccine Dose in Health Care Workers Previously Infected With SARS-CoV-2. Jama 2021;**325**: 1467-9.

16. Reynolds CJ, Gibbons JM, Pade C, Lin KM, Sandoval DM, Pieper F, Butler DK, Liu S, Otter AD, Joy G, Menacho K, Fontana M, Smit A, Kele B, Cutino-Moguel T, Maini MK, Noursadeghi M, Brooks T, Semper A, Manisty C, Treibel TA, Moon JC, McKnight Á, Altmann DM, Boyton RJ. Heterologous infection and vaccination shapes immunity against SARS-CoV-2 variants. Science 2022;**375**: 183-92.

17. Carreño JM, Alshammary H, Tcheou J, Singh G, Raskin A, Kawabata H, Sominsky L, Clark J, Adelsberg DC, Bielak D, Gonzalez-Reiche AS, Dambrauskas N, Vigdorovich V, Srivastava K, Sather DN, Sordillo EM, Bajic G, van Bakel H, Simon V, Krammer F. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. Nature 2021.

18. Stamatatos L, Czartoski J, Wan YH, Homad LJ, Rubin V, Glantz H, Neradilek M, Seydoux E, Jennewein MF, MacCamy AJ, Feng J, Mize G, De Rosa SC, Finzi A, Lemos MP, Cohen KW, Moodie Z, McElrath MJ, McGuire AT. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. Science 2021.

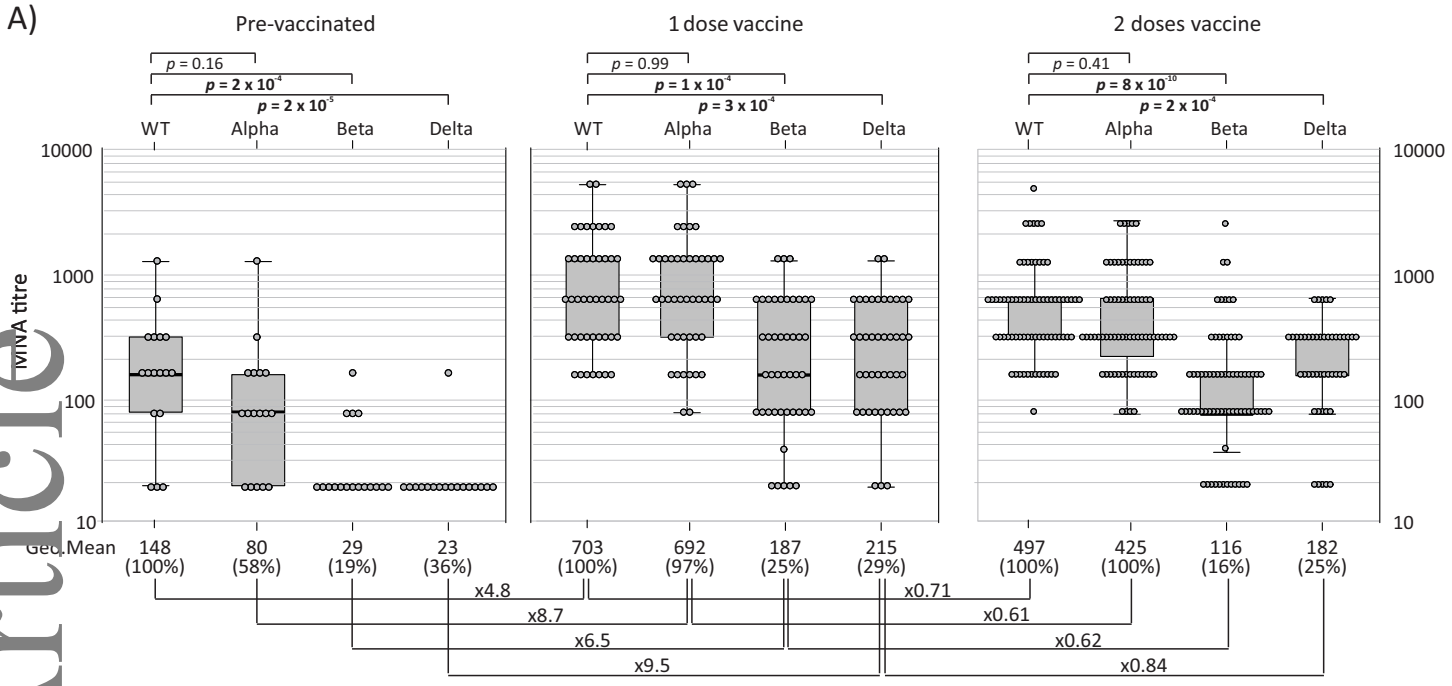
19. Rössler A, Riepler L, Bante D, von Laer D, Kimpel J. SARS-CoV-2 Omicron Variant Neutralization in Serum from Vaccinated and Convalescent Persons. N Engl J Med 2022.

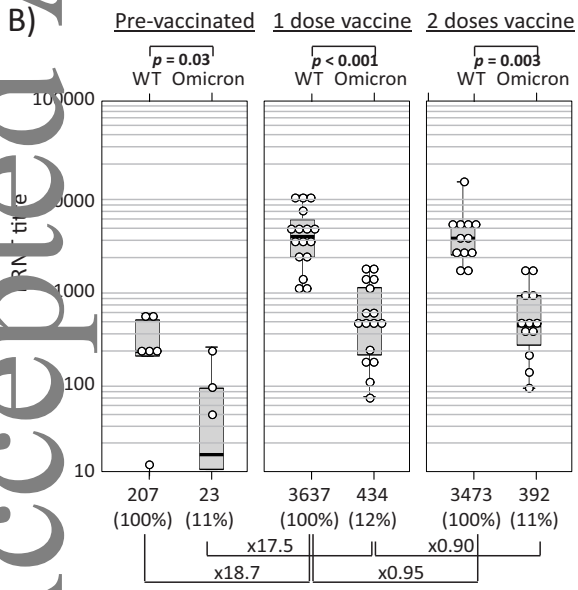
Figure Legends

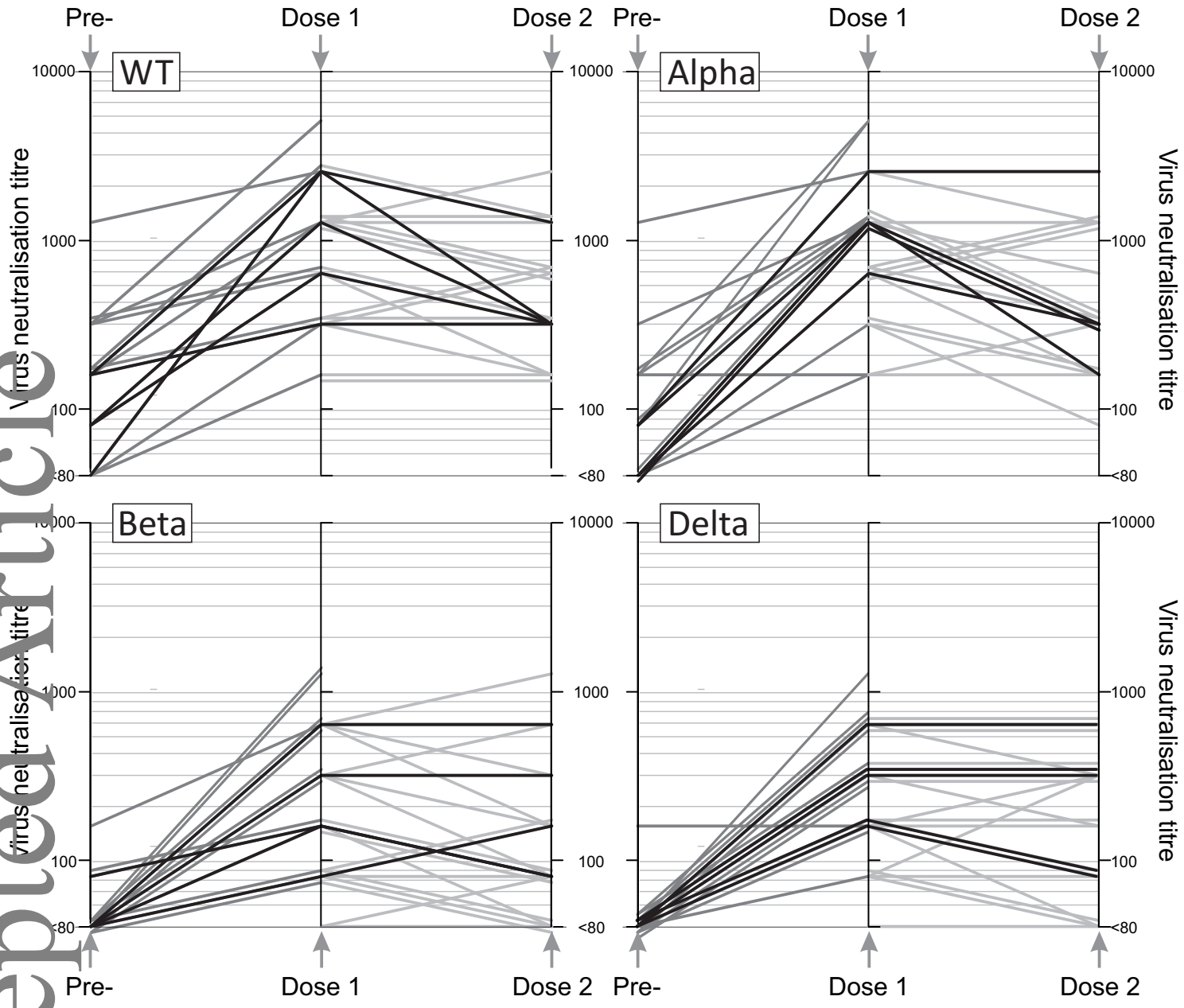
Figure 1. Comparison of neutralising antibody titres against each SARS-CoV-2 strain in plasma from infected, prevaccinated individuals with those receiving 1 or 2 doses of vaccine obtained by microneutralisation assay (A) and focus reduction neutralisation assay (B). Median values of reactivity and fold change from reactivity to the WT strain are shown under each graph. Further comparisons of fold-changes in reactivity after immunsiation with 1 or 2 vaccine doses are shown along links. Statistical comparisons of antibody levels induced by different SARS-CoV-2 strains used the Spearman rank correlation test (*p* values <0.05 shown in bold).

Figure 2. Time course of neutralising antibody levels to each SARS-CoV-2 strain in sequential samples from pre- and post-vaccinated subjects (time intervals and totals listed in Table S1; Suppl. Data); Grey lines indicate results from subjects with samples collected pre- and post-single vaccine does, or between dose1 and dose 2 vaccinations.

Figure 3. (A) Associations between neutralising antibody titres to each SARS-CoV-2 strain by microneutralisation assay (x-axis panels) and in the Roche Elecsys assay (y-axis). Samples collected pre-vaccination and after 1 or 2 vaccination doses are plotted separately (see key). Neutralising antibody titre values have been jittered by ±1.2 fold to avoid overlapping points. Datapoints Lines of best fit of log transformed values from both assays were separately plotted for samples collected pre- and post-vaccination; R^2 and *p* values shown. **(B)** Receiver operating characteristic (ROC) analysis to evaluate the predictive value of the Roche S quantitative assay for neutralising antibody titres of 1:640 and 1:320 against Delta and Omicron. The sensitivity and specificity of chosen Roche antibody levels to predict high titre convalescent donations containing a minimum neutralising antibody titre of 1:640 or 1:320 against Delta and Omicron variants is tabulated to the right.







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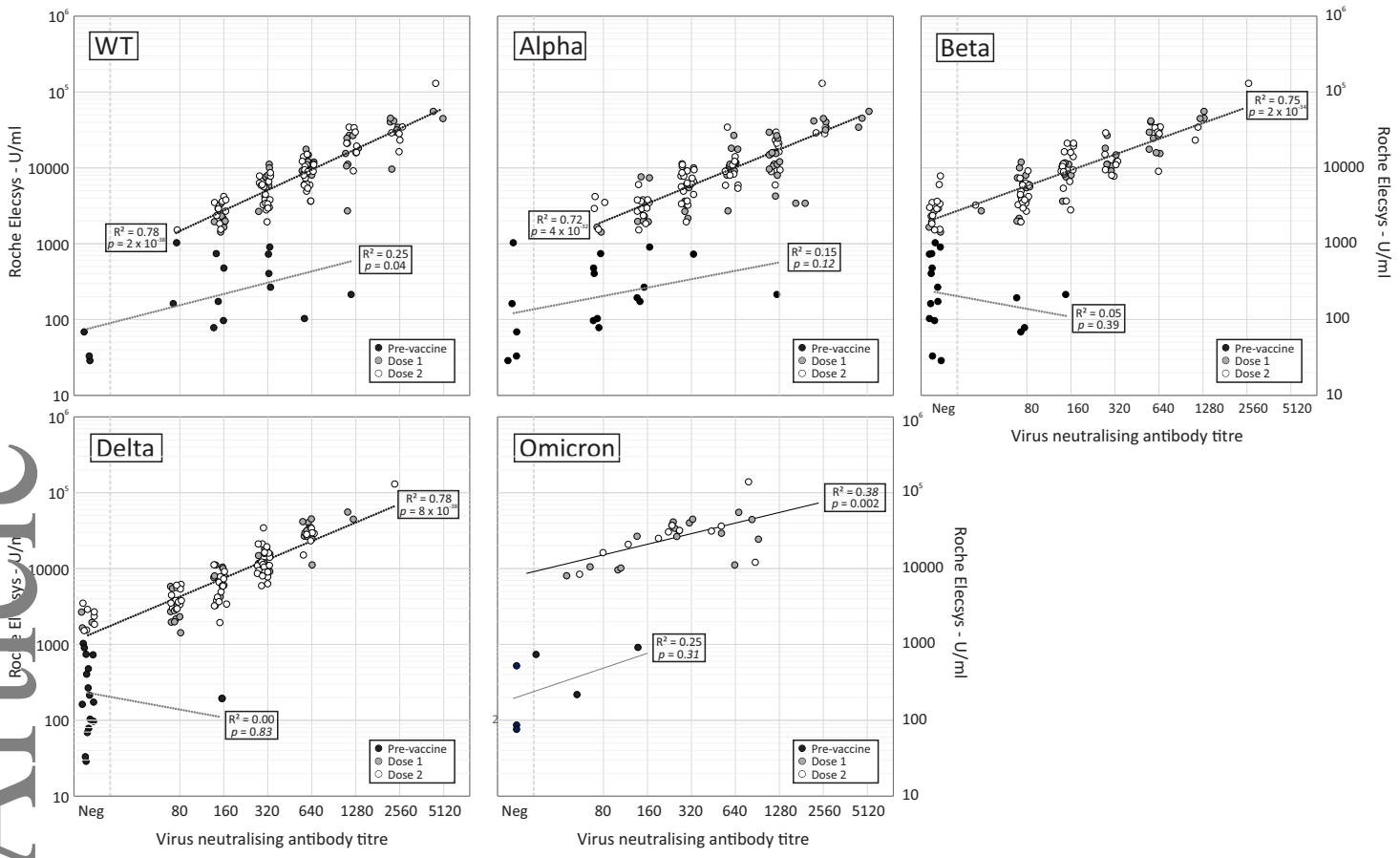

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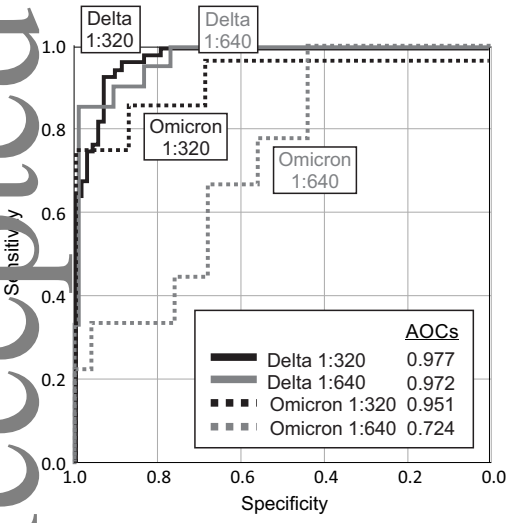

		NAb Titre prediction			
		>1:640		>1:320	
Virus	Roche U/ml	Sensitivity	Specificity	Sensitivity	Specificity
Delta	15,000	90%	91%	50%	100%
	20,000	86%	99%	38%	100%
	25,000	76%	99%	30%	100%
Omicron	15,000	89%	48%	89%	75%
	20,000	89%	52%	89%	81%
	25,000	83%	56%	83%	87%