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RESEARCH LETTER

Comparison of SARS-CoV-2 neutralizing antibody testing of convalescent plasma donations in the Netherlands and England: A pilot study

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Since the early stages of COVID-19 pandemic, convalescent plasma has been considered as a treatment option for SARS-CoV-2 infected patients.[1] Convalescent plasma treatment was previously shown to reduce mortality in patients with SARS-CoV infection and severe influenza.[2] The effectiveness of such therapy as a treatment for SARS-CoV,[3] MERS-CoV,[4] and subsequently SARS-CoV-2 infections[5-7] has been linked to high levels of virus neutralizing antibodies present in plasma. For these reasons, convalescent plasma collections from individuals with past SARS-CoV-2 infection were commenced in many European countries already in April 2020. When comparing the presence of neutralizing antibodies in convalescent plasma donations collected in the Netherlands and England, it was noted that neutralizing antibody titers in convalescent plasma were generally lower in the Netherlands than in England. From the first 103 donations tested for neutralizing antibodies in the Netherlands, only 14 donations had neutralizing antibody titers higher than 1:100 (14%). In comparison, a statistically higher proportion of donations collected in England between 22 April and 12 May contained neutralizing antibody titers higher than 1:100 (146/447; 33%[8]; χ^2 statistical test, $P < .001$). In order to determine if testing was a reason for this observed difference, a panel of 15 convalescent plasma samples was exchanged for testing. Both countries used live virus neutralization assays for testing of convalescent plasma donations.

The convalescent plasma panel included six samples collected in the Netherlands and nine samples collected in England. They were tested in the Netherlands using a previously described SARS-CoV-2 specific virus neutralization test protocol with minor modifications.[9,10] In England, SARS-CoV-2 specific neutralizing antibody levels were measured using a modification of the WHO influenza microneutralization method.[8,11] All work was undertaken in biosafety level 3 (BSL-3) laboratories. Both assays were performed in 96-well plates, and a serial dilution of heat-inactivated serum or plasma samples (56°C for 30 minutes) was first incubated with a standardized amount of SARS-CoV-2. Both laboratories used the same amount of virus (100 $TCID_{50}$), which had been quantified by determining the virus concentration at which at least 50% of the infected cells display

cytopathic effect (CPE), but the virus strains used were different (HCoV-19/Netherlands/ZuidHolland_10004/2020 in the Netherlands [EVAg cat.nr. 014V-03968] and England/02/2020 in England). Sequence comparison demonstrated that HCoV-19/Netherlands/ZuidHolland_10004/2020 strain differed by a single amino acid in the spike protein (D614G) from England/02/2020 and the Wuhan-Hu-1 prototype strains. All incubations were done at 35°C in the Netherlands based on their experience that any respiratory virus replicated to higher titers at 35°C than at the traditionally used 37°C. An incubation temperature of 37°C was used in England. After a 1-hour incubation, a suspension of Vero-E6 cells (African green monkey cells; total of 2×10^4 cells per well) was added and plates were further incubated at the given temperatures. In the Netherlands, plates were incubated for 3 days, and the 50% neutralization titer was defined as the highest serum dilution that protected more than or equal to 50% of cells from CPE. In England, plates were fixed, and in-cell SARS-CoV-2 nucleoprotein (NP) expression was determined by ELISA at 22 hours after inoculation. The virus neutralizing antibody titer was determined as the serum concentration that inhibited at least 50% SARS-CoV-2 NP expression. We used Chi-square test to compare neutralizing antibody content in convalescent plasma, linear regression to investigate the correlation between the neutralization titers determined by two laboratories, and Mann-Whitney *U*-test to assess the difference in these measurements, using SPSS version 26. *P* values <.05 were considered statistically significant.

Signed written informed consent was obtained from each donor at the time of donation. The study was approved by National Blood Supply Committee for Audit and Research Ethics.

Assay titers for the panel samples were comparable between both laboratories despite using the different incubation times and temperatures as well as differences in the methods used for determining the end point neutralizing antibody titer (based either on NP expression measured by ELISA or CPE recorded manually) (Figure 1). Despite the scaling difference in absolute quantitation, there was a good correlation between neutralization titers determined in samples studied in the Netherlands and England ($R^2 = 0.797$; $P < .001$ by linear regression, Figure 2). Nevertheless, neutralizing antibody titers obtained for convalescent plasma donations in the Netherlands were systematically lower than those measured in England (mean value 63%; $P = .003$ in Mann-Whitney *U*-test).

Traditionally live virus neutralizing antibody testing has been performed either using the plaque reduction neutralization titer (PRNT) and micro-neutralization format. Both are difficult to standardize as they require the use of live virus and cells, while international standards are currently lacking. Although the methods presented here were different, they provided very comparable neutralization titers with the panel samples, thereby excluding technical causes for the systematically lower neutralizing antibody titers observed for the Dutch plasma. A possible explanation for the observed differences might be the differences in the collections. In the Netherlands, individuals with past SARS-CoV-2 infection were invited to donate convalescent plasma 14 days after their recovery, whereas in England they cannot donate until 28 days.[12] It is hence likely that in England their antibody response has time to mature, and hence higher neutralizing antibody titers are observed. Other factors known to be associated with higher neutralizing antibody titers in convalescent plasma donors include male gender, older age, and hospitalization due to SARS-CoV-2 infection.[13] Further studies exploring the different convalescent plasma donor populations and their association to neutralizing antibody levels should be considered.

An interesting aspect is the spike mutation D614G present in the virus isolate used in the Netherlands. This mutation became dominant

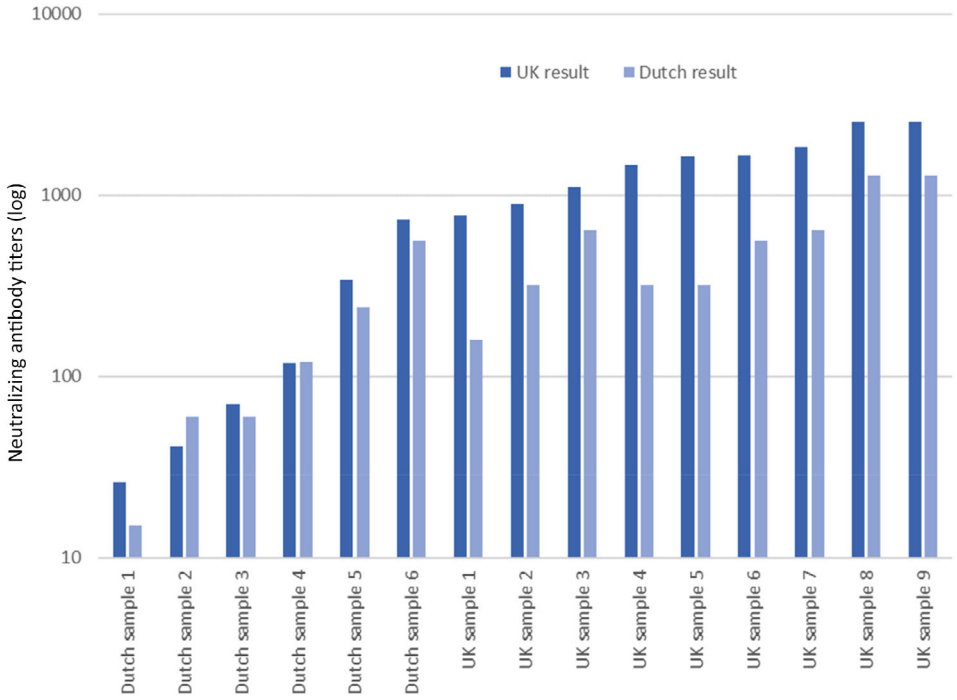

FIGURE 1 Neutralising antibody titers for 6 Dutch convalescent plasma donations and 9 English donations as determined in the public health reference laboratories in the Netherlands (light blue) and England (dark blue)

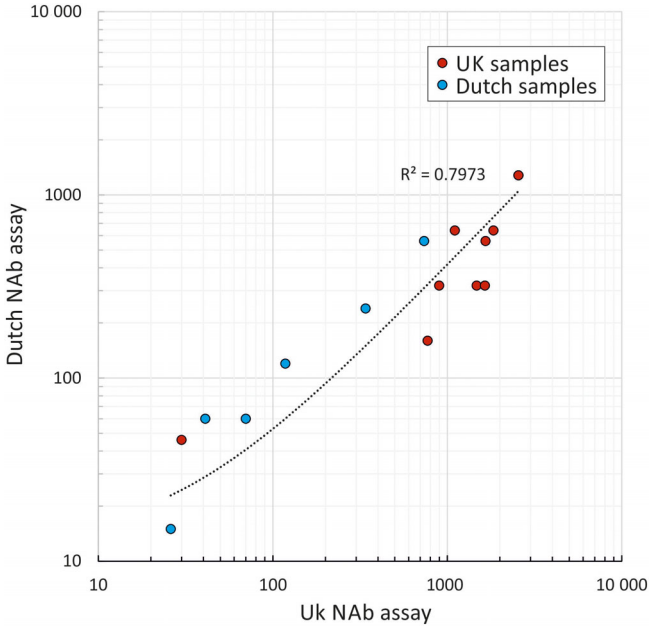

FIGURE 2 Comparison of neutralizing antibody titers determined in the Netherlands (y-axis) and England (x-axis) for 15 samples, from which six were obtained from Dutch convalescent plasma donors and nine from English donors

in SARS-CoV-2 genome during the early stages of pandemic[14] and has been associated with increasing replication in lung tissues.[15] It has also been used to artificially enhance the infectious pseudoparticle production without obviously changing its antigenic properties.[16] Further calibration of neutralization antibody titer measurement can be achieved by using internal and external standards. These include the use of WHO International Standards for SARS-CoV-2 antibodies, which will allow the antibody titer comparison between different laboratories and over time.[17]

Until the beginning of the pandemic, neutralizing antibody testing was mostly utilized as a research tool. However, it should also be recognized as a technically simple and rapidly deployable assay for serological screening in case of newly emerging infections providing the virus isolate and the BSL-3 facilities with trained personnel are available. The use of convalescent plasma therapy has created a new purpose for neutralizing antibody assays in the identification of high titer donations for maximal therapeutic efficacy. It may also support prioritizing the use of convalescent plasma to those patients who do not harbor neutralizing antibodies as they will most likely benefit from it.[18] The widespread adoption of a range of in-house assays that may often be methodologically dissimilar has since created the secondary problem of data comparability and the development of guidelines to standardize neutralizing antibody assay quantitation and sensitivity.[7,19] In conclusion, we demonstrate a good comparability of neutralizing antibody data obtained in the Netherlands and England despite slight differences in the methodologies used and exclude the laboratory technic as a cause for the systematically lower neutralizing antibody titers observed in the Dutch plasma. Further comparative evaluations will help to standardize testing of plasma donations across Europe and beyond.

AUTHOR CONTRIBUTIONS

Conceptualization: Heli Harvala, Maria Zambon, and Ellen van der Schoot

Laboratory work—neutralizing antibody testing: Robin Gopal, Monika Patel, Marieke Hoogerwerf, Boris Hogema

Laboratory work—organizing the panel: Abigail Lamikanra, Ruther Ploeg, Heli Harvala, Ellen van der Schoot, and David Roberts

Formal Analysis: Robin Gopal, Maria Zambon, Hans Zaaijer, Chantal Reusken, Ellen van der Schoot, Johan Reimerink, David Roberts, and Heli Harvala

Writing—original draft: Heli Harvala

Writing—review and editing: All authors

All authors have read and approved the final version of the manuscript. Corresponding author, Dr Heli Harvala, had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis. No specific funding was obtained for this study. No conflicts of interest have been identified or declared by any of the authors.

The lead author, Dr Heli Harvala, affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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