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Development of a SARS-CoV-2 Total Antibody Assay and the Dynamics of Antibody Response over Time in Hospitalized and Nonhospitalized Patients with COVID-19

Erik H. Vogelzang,*,†,1 Floris C. Loeff,‡,§,1 Ninotska I. L. Derksen,‡ Simone Kruithof,‡ Pleuni Ooijevaar-de Heer,‡ Gerard van Mierlo,‡ Federica Linty,¶ Juk Yee Mok,‖ Wim van Esch,‖ Sanne de Bruin,# Alexander P. J. Vlaar,# Amsterdam University Medical Center COVID-19 Biobank Study Group,2 Bart Seppen,* Maureen Leeuw,* Anne J. G. van Oudheusden, Anton G. M. Buiting,** Kin Ki Jim,†,†† Hans Vrielink,‡‡ Francis Swaneveld,‡‡ Gestur Vidarsson,¶ C. Ellen van der Schoot,¶ Peter C. Wever,†† Wentao Li,§§ Frank van Kuppeveld,§§ Jean-Luc Murk,** Berend Jan Bosch,§§ Gerrit-Jan Wolbink,*,‡,¶¶ and Theo Rispens‡**

Severe acute respiratory syndrome coronavirus (SARS-CoV)-2 infections often cause only mild disease that may evoke relatively low Ab titers compared with patients admitted to hospitals. Generally, total Ab bridging assays combine good sensitivity with high specificity. Therefore, we developed sensitive total Ab bridging assays for detection of SARS-CoV-2 Abs to the receptor-binding domain (RBD) and nucleocapsid protein in addition to conventional isotype-specific assays. Ab kinetics was assessed in PCR-confirmed, hospitalized coronavirus disease 2019 (COVID-19) patients (*n* = 41) and three populations of patients with COVID-19 symptoms not requiring hospital admission: PCR-confirmed convalescent plasmapheresis donors (*n* = 182), PCR-confirmed hospital care workers (*n* = 47), and a group of longitudinally sampled symptomatic individuals highly suspect of COVID-19 (*n* = 14). In nonhospitalized patients, the Ab response to RBD is weaker but follows similar kinetics, as has been observed in hospitalized patients. Across populations, the RBD bridging assay identified most patients correctly as seropositive. In 11/14 of the COVID-19–suspect cases, seroconversion in the RBD bridging assay could be demonstrated before day 12; nucleocapsid protein Abs emerged less consistently. Furthermore, we demonstrated the feasibility of finger-prick sampling for Ab detection against SARS-CoV-2 using these assays. In conclusion, the developed bridging assays reliably detect SARS-CoV-2 Abs in hospitalized and nonhospitalized patients and are therefore well suited to conduct seroprevalence studies. ***The Journal of Immunology*, 2020, 205: 3491–3499.**

In December 2019, the first cases of atypical pneumonia were reported in China (1). The causative agent was a β-coronavirus; now known as severe acute respiratory syndrome coronavirus (SARS-CoV)-2. SARS-CoV-2 is related to the human SARS-CoV-1 and bat CoV RaTG13 (2). In the following months, an increase in RT-PCR–confirmed cases of coronavirus disease 2019 (COVID-19) were reported in various countries, resulting in the World Health Organization declaring a pandemic. RT-PCR–based diagnostics

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G.-J.W. and T.R. conceived of the study; E.H.V., F.C.L., B.S., B.J.B., G.-J.W., and T.R. were involved in study design and organization; N.I.L.D., S.K., P.O.-d.H., G.v.M., F.L., J.Y.M., and W.L. conducted experiments; E.H.V., F.C.L., and T.R. analyzed data; E.H.V., F.C.L., G.V., G.-J.W., and T.R. wrote the paper; W.v.E., S.d.B., A.P.J.V., M.L., A.J.G.v.O., A.G.M.B., K.K.J., H.V., F.S., C.E.v.d.S., P.C.W., F.v.K., and J.-L.M. provided critical revision of the paper; and T.R. supervised the study.

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The online version of this article contains supplemental material.

Abbreviations used in this article: COVID-19, coronavirus disease 2019; HCoV, human coronavirus; HCW, healthcare worker; HD, healthy donor; IQR, interquartile range; N, nucleocapsid; nOD, normalized OD; NP, N protein; NP-Ab, NP bridging assay; PBS-T, PBS supplemented with 0.02% polysorbate-20; PTG, PBS-T supplemented with 0.3% gelatin; RBD, receptor-binding domain; RBD-Ab, RBD bridging assay; RT, room temperature; S, spike; SARS-CoV, severe acute respiratory syndrome coronavirus.

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requires active virus shedding. As such, many COVID-19 patients that were not hospitalized remain undiagnosed, and the true extent of SARS-CoV-2 infections is unquestionably higher than initially reported (3, 4). Abs that emerge during infection cannot be detected during the early stages of infection but will remain present long after the infection has cleared. Therefore, assays that measure these Abs are expected to provide more insight into the proportion of individuals that have been infected. Several seroprevalence studies that have since been conducted indeed confirm a larger extent of SARS-CoV-2 infections than initially reported (Ref. 5, 6, and E. Slot, B.M. Hogema, C.B.E.M. Reusken, J.H. Reimerink, M. Molier, J.H.M. Karregat, J. IJlst, V.M.J. Novotný, R.A.W. van Lier, and H.L. Zaaijer, manuscript posted on Research Square, DOI: 10.21203/rs.3.rs-25862/v1). However, insight into the dynamics and variability of the Ab response in nonhospitalized patient populations with only mild symptoms remains to be elucidated. Furthermore, the suitability of various assays to assess these—presumably partly rather weak—responses, is a point of concern.

The coronavirus comprises four common structural proteins; the spike (S), envelope, membrane, and nucleocapsid (N) proteins (7, 8). Previous studies on SARS-CoV-1 identified the S protein as a major Ag, and Abs against the S protein were demonstrated to have neutralizing capacity (9–13). The S protein and more specifically the receptor-binding domain (RBD) in subunit S1 in SARS-CoV-2 is reported to be an immunodominant epitope and has been demonstrated to have negligible cross-reactivity with human coronaviruses (HCoV) HKU1, 229E, NL63, and OC43 or Middle East Respiratory Syndrome coronavirus and only to SARS-CoV-1 (14). The N protein (NP) in SARS-CoV-1 is also immunogenic, eliciting an Ab response (15, 16). Studies on SARS-CoV-1 and SARS-CoV-2 reported that Abs against the NP appeared earlier than those directed at the S protein (Refs. 17–20 and L. Grzelak, S. Temmam, C. Planchais, C. Demeret, C. Huon, F. Guivel, I. Staropoli, M. Chazal, J. Dufloo, D. Planas, et al., manuscript posted on medRxiv, DOI: 10.1101/2020.04.21.20068858). Furthermore, in SARS-CoV-2, immunodominance of the N Ag in COVID-19 patients was described (A. Hachim, N. Kavian, C.A. Cohen, A.W.H. Chin, D.K.W. Chu, C.K.P. Mok, O.T.Y. Tsang, Y.C. Yeung, R.A.P.M. Perera, L.L.M. Poon, et al., manuscript posted on medRxiv, DOI: 10.1101/2020.04.30.20085670). Implementing validated serological assays will provide much-needed information on transmission, the ascertainment of the true infection rate, corresponding mortality and morbidity, and the immune response. Also, it will allow a better understanding of the dynamics of SARS-CoV-2 in the general population.

Various studies describe (commercially available) assays and report their various sensitivity or specificity, which is not always in accordance to the manufacturer's product sheet. Also, these studies have mostly focused on hospitalized patients (21–24). Because other studies described an association between disease severity and level and/or longevity of the Ab response for SARS-CoV-2 and Middle East Respiratory Syndrome coronavirus, a

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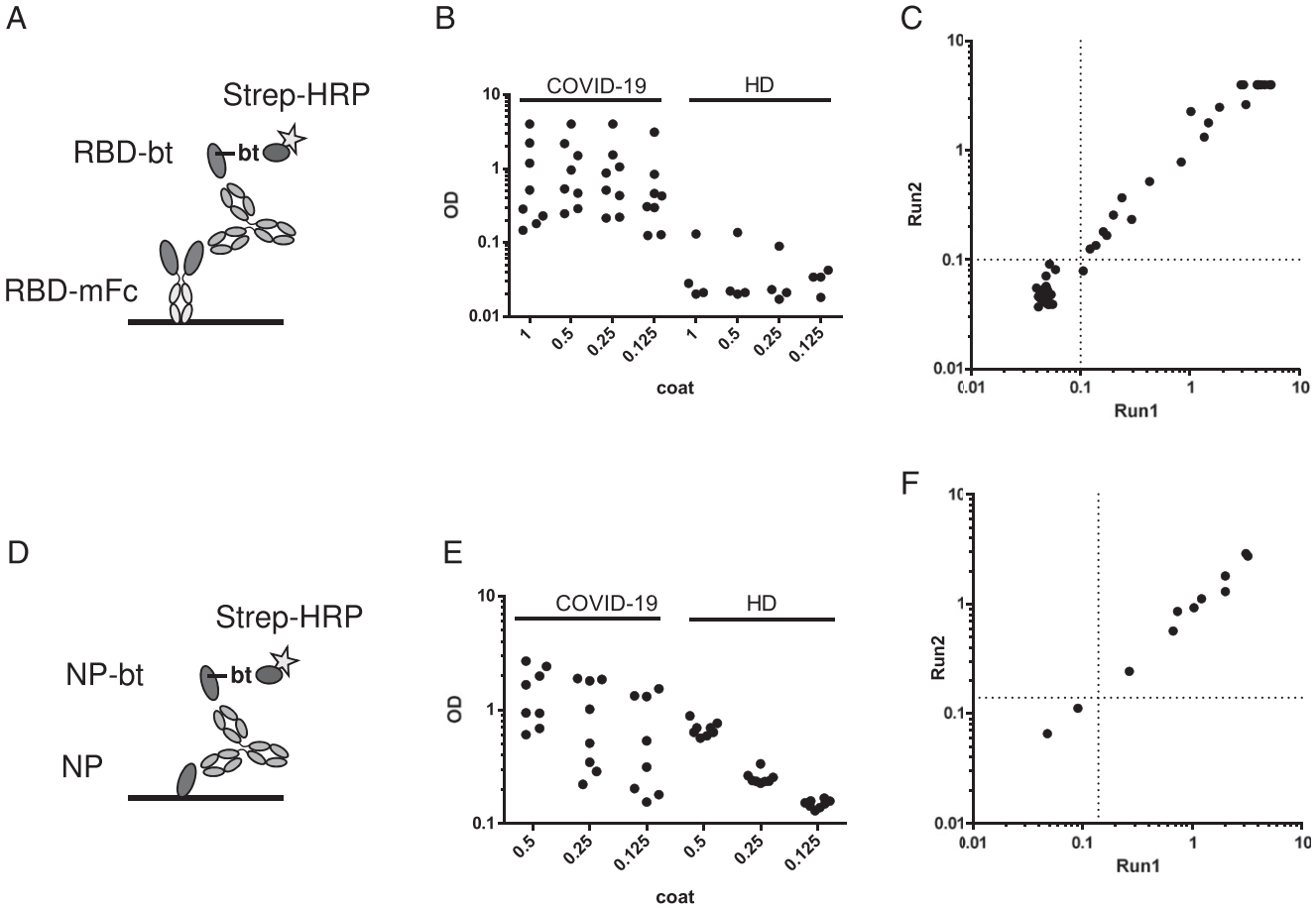

FIGURE 1. Setup of total Ab bridging assays for (**A**) RBD and (**D**) NP Ag. (**B** and **E**) Impact of coating density on signals obtained from sera of selected COVID-19 patients and preoutbreak controls for (B) RBD and (E) NP. (**C** and **F**) Correlation between two independent runs for (C) the RBD-Ab, and (F) the NP-Ab.

weaker immune response in an out of hospital population is expected, which makes such a population more relevant for evaluating serological assays (14, 25–27). In this study, we describe the development of a serological bridging assay for the detection of Abs against the RBD and NP of SARS-CoV-2. Bridging assays, or double-Ag sandwich assay, have the advantage of being able to detect Abs regardless of isotype, which potentially makes these assays maximally sensitive for seroprevalence studies. In this study, we used these assays, together with isotype-specific assays, to assess the Ab response in several hospitalized and nonhospitalized COVID-19 patient populations. In particular, we describe the early Ab response to SARS-CoV-2 in nonhospitalized individuals highly suspect of COVID-19 during the symptomatic stages of disease.

Materials and Methods

Samples and patients

Serum or plasma samples obtained from RT-PCR–positive, SARS-CoV-2 hospitalized (mean age 45 y, SD 13, and 37% was female) and nonhospitalized recovered adult patients (mean age 50 y, SD 8, and 16% was female) (n = 182) who underwent plasmapheresis at Sanquin, the national blood bank, Amsterdam, the Netherlands, were included.

Also, serum samples from hospitalized, SARS-CoV-2, RT-PCR–confirmed patients (n = 41) at the Jeroen Bosch Hospital, Den Bosch, the Netherlands (median age 72 y, interquartile range [IQR] 61–78 y, and 26% was female), and Amsterdam University Medical Center, Location Academic Medical Center, Amsterdam, the Netherlands (median age 60 y, IQR 55–63 y, and 41% was female), were included.

From the Elisabeth-TweeSteden Hospital, Tilburg, the Netherlands, serum samples of SARS-CoV-2, RT-PCR–confirmed healthcare workers (HCWs) were obtained (n = 47) at various time points (median age 49 y, IQR 43–56 y, and 94% was female). None of the HCWs were admitted to the hospital for COVID-19.

From the Amsterdam Rheumatology and Immunology Center, Location Reade, the Netherlands, serum samples were obtained at regular intervals in a group of otherwise healthy volunteers, which based on recent travel to an endemic area abroad, were highly suspect for COVID-19 (n = 7) and household contacts (n = 2) of this group that did not go abroad. Moreover, serum samples were collected from a SARS-CoV-2, RT-PCR–positive volunteer and close contacts, all with no comorbidities (n = 5). All 14 volunteers had symptoms not requiring hospital admission. Samples were collected at regular intervals after the onset of potential COVID-19 disease symptoms. The average age of participants was 31 y (SD 4.2), 50% were female, and none reported relevant comorbidities. See Supplemental Table I for additional clinical characteristics.

Negative control serum samples were included from healthy blood donors collected by Sanquin (n = 587). The negative control samples had been collected from the Dutch population before mid-January 2020, thus prior to the first reported SARS-CoV-2 case in the Netherlands (February 27, 2020).

This study has been conducted in accordance with the ethical principles set out in the declaration of Helsinki and all participants provided written informed consent, if applicable. Approval was obtained from the Medical Ethics Committees from the Academic Medical Center, VU University Medical Center, and Elisabeth-TweeSteden Hospital.

Development of SARS-CoV-2 RBD and NP-based bridging assays

The RBD-mFc and RBD-ST proteins were produced as described by Okba et al. (14). The NP was produced in HEK cells with HAVT20 leader peptide, 10× His-tag and a BirA tag as described by Dekkers et al. (28). MaxiSorp microtiter plates (Thermo Fisher Scientific) were coated with 100 μl/well 0.125 μg/ml RBD-mFc or NP in PBS overnight at 4°C. Plates were washed five times with PBS supplemented with 0.02% polysorbate-20 (PBS-T). Samples were diluted 10-fold in PBS-T supplemented with 0.3% gelatin (PTG) and incubated on the plate (100 μl) for 1 h at room temperature (RT) while shaking. After washing five times with PBS-T, 100 μl/well biotinylated RBD-ST (EZ-Link Sulfo-NHS-LC-Biotin; Thermo Fisher Scientific) or biotinylated NP [via the BirA tag as described in (28)] was added at 0.5 or 0.015 μg/ml, respectively, in PTG and incubated for 1 h at RT, followed by incubation for 30 min with streptavidin–poly-HRP (Sanquin). Plates were washed five times with PBS-T, and 100 μl of tetramethylbenzidine substrate (100 μg/ml) and 0.003% (v/v) hydrogen peroxide (Merck) in 0.11 M sodium acetate buffer (pH 5.5) was added to each well. A total of 100 μl of 0.2 M H_2SO_4 (Merck) was added to stop the reaction. Absorbance was measured at 450 and 540 nm. The difference was used to evaluate Ab binding. OD values were normalized to readings of a reference serum pool that was included on each plate and reported as normalized OD (nOD).

SARS-CoV-2 RBD and NP isotype-specific assays

MaxiSorp microtiter plates were coated with 1.0 μg/ml RBD-ST or NP or 4.0 μg/ml monoclonal mouse anti-human IgM (MH15; Sanquin) in PBS overnight at 4°C. Following washing with PBS-T, samples were diluted 100-fold/200-fold in PTG and incubated for 1 h at RT. After washing, 0.5 μg/ml HRP-conjugated monoclonal mouse anti-human IgG (MH16; Sanquin) or anti-human IgA (MH14; Sanquin) was added, diluted in PTG, and incubated for 1 h. Following enzymatic conversion of tetramethylbenzidine substrate, absorbance was measured at 450 and 540 nm, and the difference was used to evaluate Ab binding as described above. For IgM, the assay was finished using biotinylated RBD-ST as described above for the bridging assay.

Data processing and analysis

Statistical analysis was carried out using GraphPad Prism 7.

Results

Total Ab assays

Total Ab bridging assays depend on the multivalency of Abs of any isotype to generate a bridge between Ag for capture as well as detection. Therefore, Ag coating density is generally a critical parameter. We set up bridging assays for RBD and NP Ags and optimized Ag coating density to obtain optimal differentiation between specific and nonspecific signals using a small set of sera from COVID-19 patients and preoutbreak healthy donors (HD), see Fig. 1. A good reproducibility between runs was observed (Fig. 1). After an initial evaluation of 80 preoutbreak samples, we arrived at a cutoff value of 0.1 nOD, anticipated to provide ~99% specificity, which was validated using larger panels (see below). Subsequent samples were measured, retested if initially positive (>0.1 nOD), and only considered positive if the repeated measurement was also >0.1 nOD. Analogously, a cutoff value of 0.14 nOD was set for the NP bridging assay (NP-Ab).

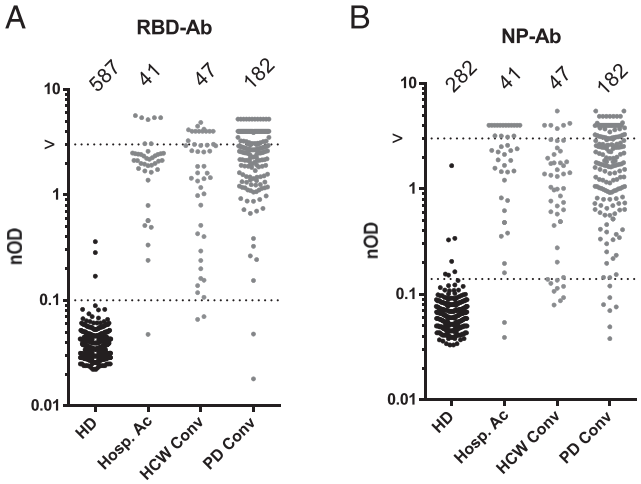

FIGURE 2. Total Ab titers measured in different patient populations. Total Ab titers against the RBD (**A**) and NP (**B**) measured in different patient populations: preoutbreak HD, hospitalized active-disease patients (Hosp. Ac), nonhospitalized, convalescent HCW (HCW Conv), and convalescent plasmapheresis donors (PD Conv). Samples below the lower dotted line are considered negative, whereas the upper dotted line indicates upper boundary of dynamic range.

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Seroprevalence in different study populations

We assessed the performance for accurately monitoring seroprevalence with the RBD bridging assay (RBD-Ab) and NP-Ab in several different study populations of COVID-19 patients that were all confirmed by PCR, as well as preoutbreak HD. Only three (0.5%) positive samples were detected in preoutbreak HD ($n = 587$) using the RBD-Ab, whereas seven (2.5%) positive samples were detected using the NP-Ab (Fig. 2). In 40 out of 41 (97.5%) and 39 out of 41 (95.1%) hospitalized patients with active, RT-PCR–confirmed COVID-19, we detected Abs against the RBD or NP, respectively. In PCR-confirmed nonhospitalized convalescent HCW ($n = 47$) Abs against RBD or NP were detected in 45 (95.7%) and 39 (83.0%) samples, respectively. Abs against RBD were detected in 180 (98.9%) and for the NP in 175 (96.2%) of PCR-confirmed convalescent plasmaphereses donors ($n = 182$), which consists both of patients with and without prior hospitalization because of COVID-19. Except for one sample, all samples negative in the RBD-Ab were also negative in the NP-Ab (see below). The above-described seroprevalences demonstrated a good overall sensitivity and specificity of the RBD-Ab (98.1 and 99.5%) and somewhat less for the NP-Ab (93.7 and 97.5%).

Ab response at different time points

To study the effect of time on the seroprevalence and Ab levels determined by using the RBD-Ab and the NP-Ab, we stratified the samples of the nonhospitalized cases (plasmapheresis donors and HCWs) for different days since symptom onset (information available for $n = 167$; Fig. 3). As comparison, in addition to the hospitalized patients with active disease (~8–23 d after symptom onset), convalescent cases that were previously admitted to the hospital are also included ($n = 27$; ~20–66 after symptom onset). There is an increase in nOD over time for the RBD-Ab in hospitalized cases and a similar, nonsignificant trend in nonhospitalized cases. However, there is no apparent effect of time after onset of symptoms on the detection of Abs in the RBD-Ab or NP-Ab. Because many samples yield signals in plateau, we retested convalescent plasma samples in the RBD-Ab at a higher dilution (1:100) to obtain more insight in the dynamics of the Ab response. A significant difference in signals is observed between hospitalized and nonhospitalized cases. Furthermore, in the nonhospitalized patients, signals rise over time (Supplemental Fig. 1).

To obtain insight into the dynamics of the different isotypes, we also measured Abs using assays for the detection of IgM, IgG, and IgA (Fig. 3). For the Ab response to the RBD, there is a (nonsignificant) trend toward declining IgM and IgA levels as time progresses in both hospitalized and nonhospitalized patients. Hospitalized patients have significantly higher IgG levels later in time. In nonhospitalized patients, the IgG response rises slowly over time and gains prominence relative to other isotypes at later time points (Supplemental Fig. 2), in line with expectations.

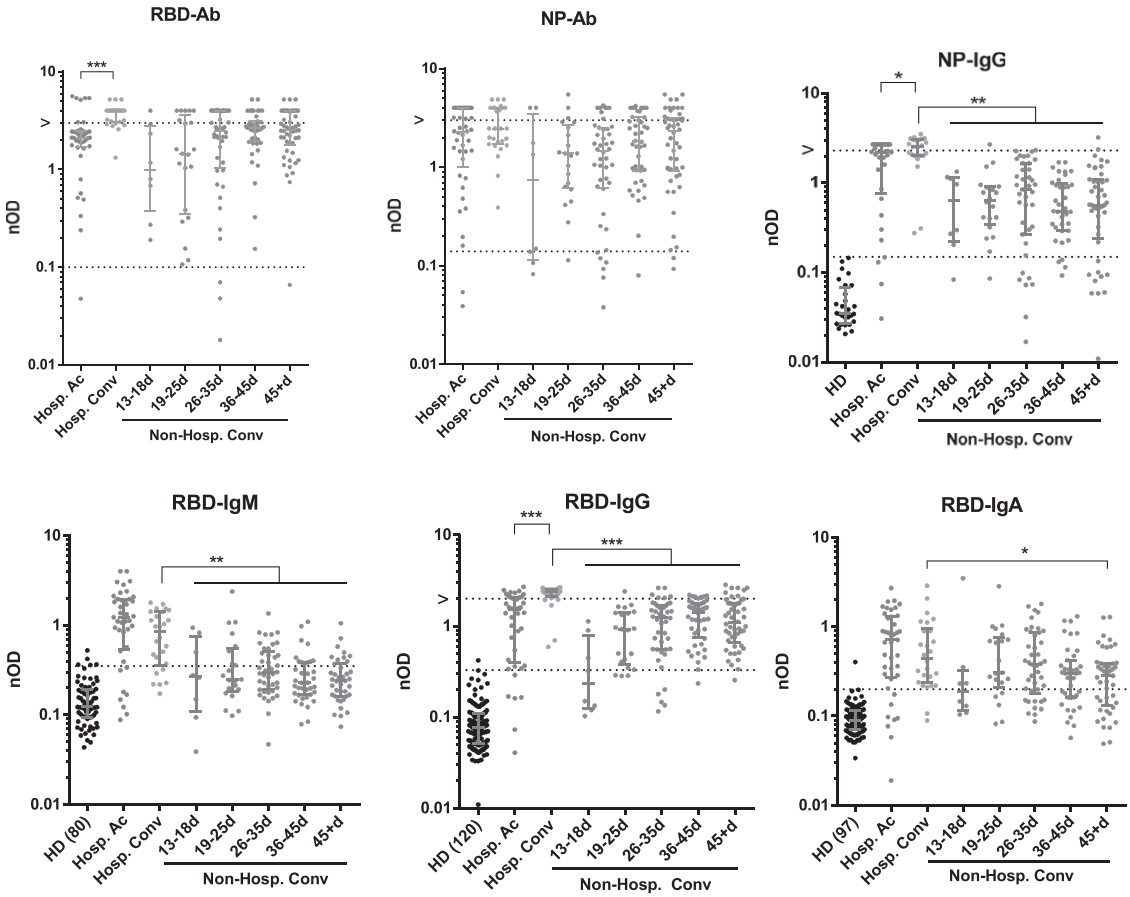

FIGURE 3. Ab titers and isotypes measured in different patient populations and at various time points. Total and isotype-specific Ab titers against the RBD and NP were measured in different patient populations: hospitalized patients with active disease (Hosp. Ac; 8–23 d after symptom onset; $n = 41$) or hospitalized convalescent patients (Hosp. Conv; 20–66 d after symptom onset; $n = 27$), and nonhospitalized convalescent individuals (Non-Hosp. Conv; $n = 167$). For the latter group, on the x-axes, the number of days after symptom onset are indicated. For the isotype-specific assays, signals measured in preoutbreak HD are also indicated (for total Ab, see Fig. 2). Dotted lines indicate ~95% (IgM) and 98% (IgG and IgA) of the negative controls. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, tests performed: Hosp. Ac versus Hosp. Conv, Mann–Whitney U; Hosp. Conv versus Non-Hosp. Conv, Kruskal–Wallis test/Dunn multiple comparisons test.

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At least up to 60 d after symptom onset, substantial amounts of IgG to the RBD can be detected in this population. In comparison with hospitalized convalescent patients, the IgG response in nonhospitalized patients is significantly weaker, as shown in Fig. 3. A similarly weaker IgG response is also observed against the NP.

Ab kinetics in nonhospitalized patients at early time points

To obtain a better insight into the Ab response against SARS-CoV-2 and thus the seroprevalence in a specific population, we tested sequentially collected sera from a group of nonhospitalized and otherwise healthy individuals who were highly suspected of having contracted COVID-19 (Reade patient cohort; Fig. 4). Day 3 after symptom onset, samples were available for two individuals and were negative in both the bridging assays as well as in the IgM, IgG, and IgA assays. Within 12 d after symptom onset, Abs against the RBD were detected in 11 out of 14 (78.6%) patients as compared with 8 out of 14 (53.1%) against NP Abs, as shown in Fig. 4, using the bridging assays. At day 60, one of the previously negative samples became positive in both bridging assays.

Fig. 4 shows the Ab kinetics for IgM, IgG, and IgA. Most patients (n = 9) seroconverted for IgG within 15 d after symptom onset, although three patients seroconverted later. The slow appearance of IgG Abs to RBD is clearly seen in this population. Interestingly, at 2 mo, IgG anti-RBD appears to be declining again, suggesting a lack of sustained overall anti-RBD response in this group of patients. The Ab response to NP is not detectable in most patients around day 10 both in the NP-Ab and NP-IgG assays and remains weak in many patients at later time points.

Comparison of assay formats

The RBD-Ab identifies positive patients most reliably, with few false-positive cases. Sensitivity and/or specificity could theoretically be enhanced by combining two or more assays. When comparing the RBD-Ab with the NP-Ab, we observe that for HD, none of the samples were positive in both assays, as shown in Fig. 5. Requiring both assays to be positive would therefore result in enhanced specificity, albeit with reduced sensitivity. Also, for the RBD-IgG assay, it appears that negative control samples either have elevated signals in either this assay or the RBD-Ab, but not both (Fig. 6). There is a moderately strong correlation between RBD-Ab and RBD-IgG (Spearman $r = 0.69$; $p < 0.0001$) across the populations; whereas the correlation between RBD-Ab and NP-Ab is moderate ($r = 0.58$; $p < 0.0001$).

Finger-prick sampling

A relatively low volume of serum is needed for the developed assay to assess the Ab response, therefore making it well suited for finger-prick sampling. A small number of samples (n = 11) was obtained by finger-prick sampling rather than obtained by venipuncture. Abs were detected by the RBD-Ab and compared with a matching venipuncture draw (Fig. 7). The results suggest that a finger prick is an equivalent method for drawing blood when using this assay to detect Abs against the RBD.

Discussion

In this study, we describe the development of sensitive serological bridging assays based on the RBD and NP to detect Abs against SARS-CoV-2. Assay performance was evaluated in serum samples of different patient populations (hospitalized and nonhospitalized), and the RBD-Ab was found to best discriminate between cases and preoutbreak controls across different time points, especially in individuals with COVID-19 symptoms who did not require hospital admission. We also show the early Ab kinetics in a group of nonhospitalized individuals highly suspected of having contacted COVID-19 during their symptomatic phase. Finally, we demonstrate the feasibility of using finger-prick sampling for the detection of Abs against SARS-CoV-2.

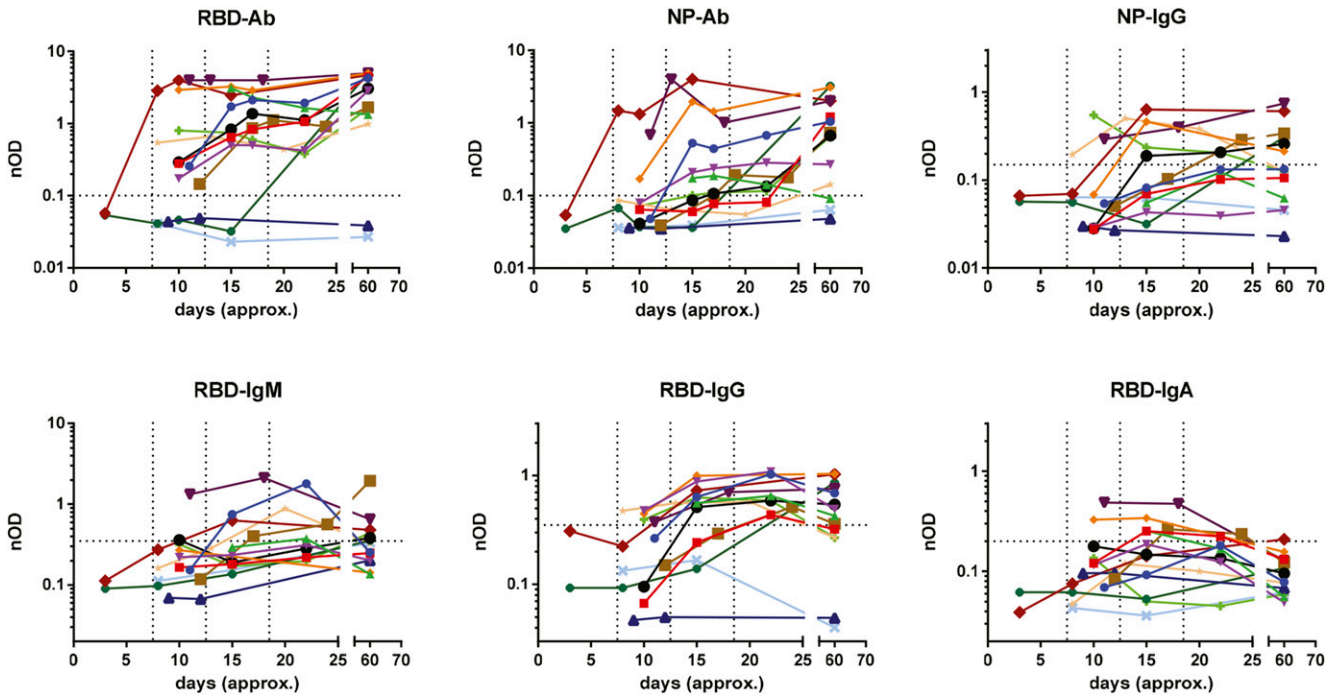

FIGURE 4. Kinetics of Ab response against SARS-CoV-2. In 14 nonhospitalized patients with mild symptoms and a high likelihood (one SARS-CoV-2 RT-PCR confirmed) of SARS-CoV-2 infection, we tested the Ab response against the RBD-Ab, NP-Ab, NP-IgG, RDB-IgM, RBD-IgG, and RBD-IgA. The x-axes show the number of days after symptom onset. The horizontal dotted line is the cutoff; samples below this line are considered negative. The vertical dotted lines represent from left to right 8, 13, and 19 d after symptom onset.

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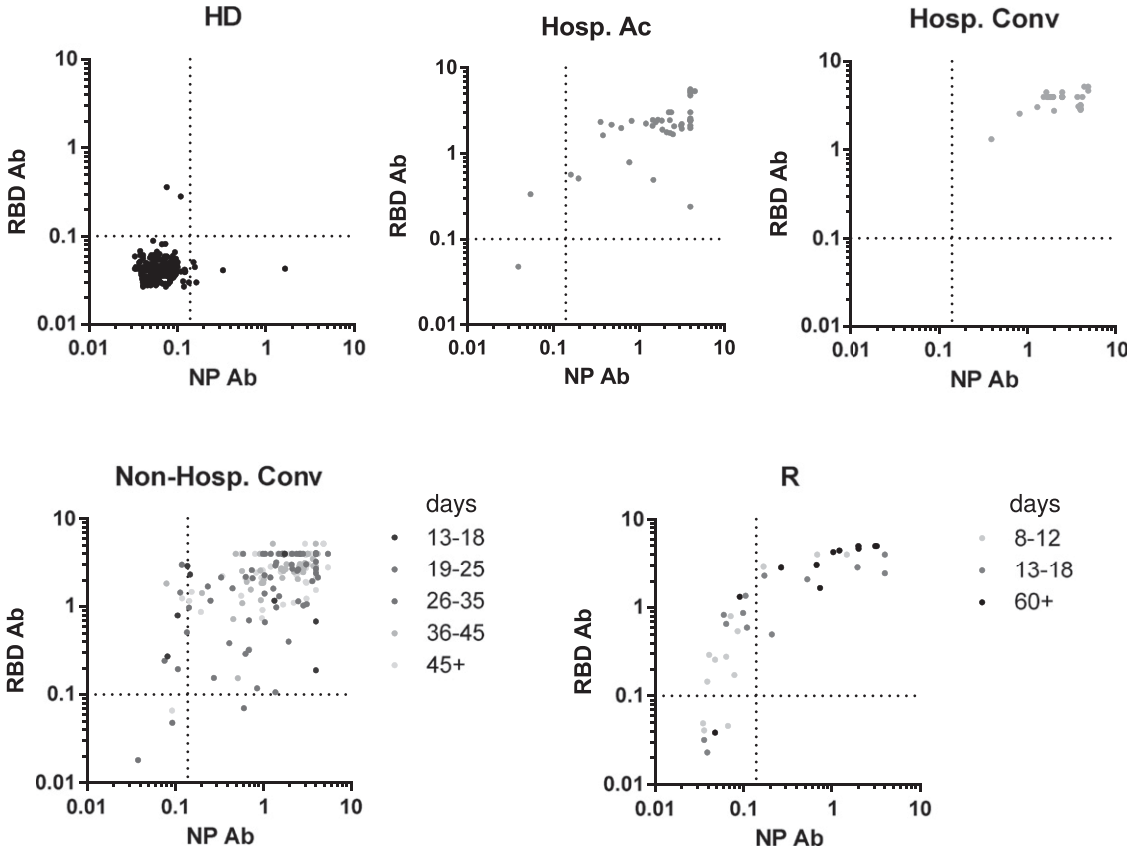

FIGURE 5. Anti-NP and RBD immune responses compared. Abs detected with the NP-Ab and the RBD-Ab plotted on the *x*–*y*-axes for different patients groups: preoutbreak HD, hospitalized patients with active disease (Hosp. Ac) or hospitalized convalescent patients (Hosp. Conv), nonhospitalized convalescent individuals (Non-Hosp. Conv), and the Reade patient cohort (R); stratified according to days after symptom onset as indicated. Dotted lines indicate the cutoff for both assays.

In recent studies, seroconversion of SARS-CoV-2–infected, hospitalized patients usually occurred between 5 and 14 d (14, 19, 20, 29, 30). Our study demonstrated a similar result in nonhospitalized patients. Because an adequate Ab response requires time to develop, false-negative results will occur depending on the timing of sampling independent of the type of assay used, thereby influencing seroprevalence study results. In one patient followed over time, seroconversion occurred after day 15. Although this patient went to work (not healthcare related) and did groceries, other public measures regarding SARS-CoV-2 were followed, and this is probably a late seroconversion.

In the nonhospitalized patients, the isotype-specific Ab response to RBD follows a pattern similar to hospitalized patients (37), with a relatively late appearance of IgG Abs. Although the IgG levels are significantly lower in the nonhospitalized population, these Abs nevertheless are present in readily detectable amounts up to 60 d after symptom onset. The long-term persistence of these responses remains to be investigated.

Bridging assay, or homologous double-Ag sandwich immunoassays, have found firm ground in the assessment of (weak) immune responses against biopharmaceutical drugs (31, 32) but are as yet relatively uncommon for measurement of Abs to viral Ags. Nevertheless, a commercially available assay using a similar format for SARS-CoV-2 has been described (R. Lassaunière, A. Frische, Z.B. Harboe, A.C.Y. Nielsen, A. Fomsgaard, K.A. Krogfelt, and C.S. Jørgensen, manuscript posted on medRxiv, DOI: 10.1101/2020.04.09.20056325). Advantages of this assay type include the ability to detect Abs of any isotype [with the exception of IgG4 (33), which is unlikely to be of relevance in the context of an antiviral immune response] and a generally low background. Disadvantages include the lower responsiveness to low-affinity Abs. In general, the assay described in this study is robust in detecting SARS-CoV-2 Abs in patients with symptoms not requiring hospital admittance. This will aid in determining the true prevalence of COVID-19. The use of this developed assay could, therefore, lead to a better understanding of the dynamics of SARS-CoV-2 infections in the population because it is assumed that a large proportion of COVID-19 patients have mild symptoms and are not hospitalized.

In prior publications, mainly in hospitalized patients, the Ab response against the NP appeared earlier than that against the S protein (Refs. 17–20, L. Grzelak et al., manuscript posted on medRxiv, DOI: 10.1101/2020.04.21.20068858, and A. Hachim et al., manuscript posted on medRxiv, DOI: 10.1101/2020.04.30.20085670). Similarly, a study in SARS-CoV-1 described that the IgG immune response is more frequently directed against the NP compared with the S protein (34). Moreover, in a SARS-CoV-2 study, it was found that most intensive care unit patients had a higher N-IgG than S-IgG titer compared with non–intensive care unit–hospitalized patients (35). Other studies demonstrated or suggested an association between disease severity and level and/or longevity of the Ab response (14, 25–27). Our results suggest that the Ab response against the NP of SARS-CoV-2 needs more time to develop compared with the Ab response against the RBD in patients with mild symptoms and is overall more variable. Our finding regarding the NP may, therefore, be (partially) based on the difference in immune response based on disease severity (e.g., hospitalized and nonhospitalized). However, the discrepancy

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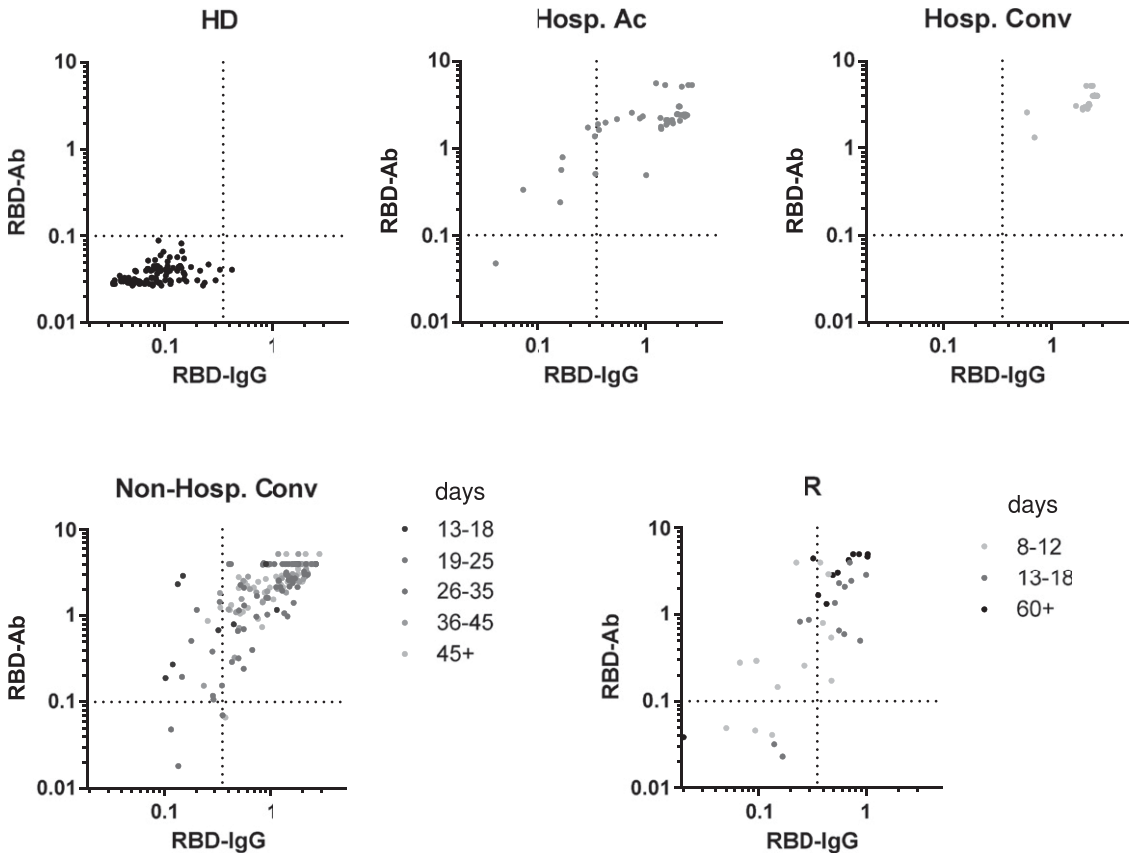

FIGURE 6. Comparison between the RBD-Ab and RBD-IgG assay. Comparison of the detection of Abs with the RBD-Ab and RBD-IgG assay plotted on the *x*–*y*-axes for different patient groups; stratified according to days after symptom onset as indicated. Dotted lines indicate the cutoff for both assays.

between our finding and that of previous research needs to be confirmed in a larger sample size because other factors such as the timing of samples and difference between assay types can also influence this.

Because preoutbreak HD samples that tested positive in the RBD-Ab were negative in the NP-Ab and vice versa, there is a rationale for performing both assays, which would consequently result in enhanced specificity. The trade-off will however be a somewhat diminished sensitivity, as can be judged from Fig. 5. A similar pattern is seen for the RBD-Ab compared with the RBD-IgG assay. In another study, combining different Ags has also been proposed to optimize the sensitivity and specificity of a SARS-CoV-2 serological assay (A. Hachim et al., manuscript posted on medRxiv, DOI: 10.1101/2020.04.30.20085670). Two-tier testing strategies are regularly implemented for other infectious diseases such as Lyme disease or syphilis.

In a recent study describing a SARS-CoV-2 transmission model, a range of transmission scenarios were examined, and it was suggested that "serological testing is required to understand the extent and duration of immunity to SARS-CoV-2 which will help determine the post-pandemic dynamics of the virus" (36). Determining the true extent of the total incidence of SARS-CoV-2 by serological testing will optimize these transmission models and could aid in determining which public health measures are needed and their timing to maintain control of the SARS-CoV-2 pandemic. To assess this during periods of widespread transmission and when nonessential travel is prohibited, we developed a reliable serological assay that needs a low volume of serum and is therefore well suited to use for blood obtained by finger prick. In general, measurement of specific Abs can be reliably (and semi-quantitatively) carried out using collection of finger prick blood instead of venipunctures, as demonstrated by a number of head-to-head studies (37–40).

There are some limitations to this study. Further investigation of cross-reactivity for the different coronaviruses may be necessary because the RBD of SARS-CoV-2 may show cross-reactivity with other HCoV and, in particular, SARS-CoV-1. However, we anticipate that this will not alter our findings because of the limited number of cases with SARS-CoV-1 in Europe (41). By including a large population of healthy controls, the risk of having missed substantial cross-reactivity against other HCoV is also limited; especially because Abs against HCoV are detected in the majority of the population (>90% for all four HCoV) (42, 43). Finally, we did not compare the assay to a neutralizing Ab assay that could be of added value in patients undergoing plasmapheresis. However, several studies have demonstrated that assays that measure binding Abs of IgG isotype correlate with neutralizing Ab titers as measured in vitro, and we observe a good correlation between the bridging ELISA and the IgG anti-RBD assay (Supplemental Fig. 1) (Refs. 14, 22, and F. Wu, A. Wang, M. Liu, Q. Wang, J. Chen, S. Xia, Y. Ling, Y. Zhang, J. Xun, L. Lu, et al., manuscript posted on medRxiv, DOI: 10.1101/2020.03.30.20047365, and 44).

Furthermore, although this study provides an insight into the Ab response in the early phase of infection in nonhospitalized patients, the number of cases is still modest. Our study describes the early Ab kinetics in a population based on probable exposure and not based on symptoms or hospitalized patients who are RT-PCR positive. Yet, this provides an unbiased view of the performance of the developed serological assay and the Ab response in patients with mild SARS-CoV-2 infection.

In summary, this study demonstrates the use of a sensitive bridging assay to study seroprevalence in nonhospitalized COVID-19 patients

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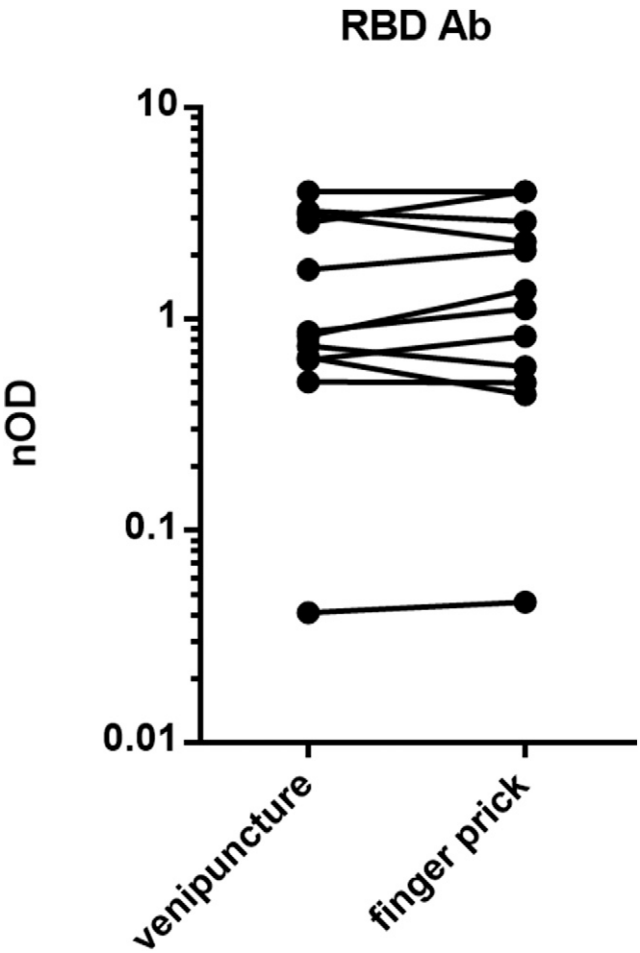

FIGURE 7. Comparison of venipuncture and finger prick. For the RBD-Ab, we compared the Ab titers measured in 11 serum samples obtained from a venipuncture and that were obtained by finger prick.

and provides insight into the early kinetics of the Ab response to SARS-CoV-2 in this population.

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Disclosures

The authors have no financial conflicts of interest.

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