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Original Article

Descriptive evaluation of antibody responses to severe acute respiratory coronavirus virus 2 (SARS-CoV-2) infection in plasma and gingival crevicular fluid in a nursing home cohort—Arkansas, June–August 2020

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Abstract

Objective: To characterize and compare severe acute respiratory coronavirus virus 2 (SARS-CoV-2)–specific immune responses in plasma and gingival crevicular fluid (GCF) from nursing home residents during and after natural infection.

Design: Prospective cohort.

Setting: Nursing home.

Participants: SARS-CoV-2–infected nursing home residents.

Methods: A convenience sample of 14 SARS-CoV-2–infected nursing home residents, enrolled 4–13 days after real-time reverse transcription polymerase chain reaction diagnosis, were followed for 42 days. After diagnosis, plasma SARS-CoV-2–specific pan-Immunoglobulin (Ig), IgG, IgA, IgM, and neutralizing antibodies were measured at 5 time points, and GCF SARS-CoV-2–specific IgG and IgA were measured at 4 time points.

Results: All participants demonstrated immune responses to SARS-CoV-2 infection. Among 12 phlebotomized participants, plasma was positive for pan-Ig and IgG in all 12 participants. Neutralizing antibodies were positive in 11 participants; IgM was positive in 10 participants, and IgA was positive in 9 participants. Among 14 participants with GCF specimens, GCF was positive for IgG in 13 participants and for IgA in 12 participants. Immunoglobulin responses in plasma and GCF had similar kinetics; median times to peak antibody response were similar across specimen types (4 weeks for IgG; 3 weeks for IgA). Participants with pan-Ig, IgG, and IgA detected in plasma and GCF IgG remained positive throughout this evaluation, 46–55 days after diagnosis. All participants were viral-culture negative by the first detection of antibodies.

Conclusions: Nursing home residents had detectable SARS-CoV-2 antibodies in plasma and GCF after infection. Kinetics of antibodies detected in GCF mirrored those from plasma. Noninvasive GCF may be useful for detecting and monitoring immunologic responses in populations unable or unwilling to be phlebotomized.

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Nursing home populations are at elevated risk for coronavirus disease 2019 (COVID-19) outbreaks due to rapid transmission in congregate living facilities.[1] Infection with severe acute respiratory coronavirus virus 2 (SARS-CoV-2), the virus that causes COVID-19, is associated with high risk of severe disease and death

among nursing home residents due to increased age and the prevalence of comorbidities.[2,3]

Currently, data are limited on whether nursing home residents develop and maintain robust antibody responses to SARS-CoV-2 infection, the duration of these responses, and how antibody responses relate to host and viral dynamics. Plasma antibodies are important indicators of the humoral immune response and may be indicative of protection against SARS-CoV-2 reinfection and disease. Previous SARS-CoV-2 seroepidemiologic studies have focused on systemic serologic responses in healthcare personnel, hospitalized patients, and first responders[4,5]; however, literature describing the kinetics of SARS-CoV-2 antibody responses to infection in nursing home residents is limited.[6,7]

Secreted mucosal and plasma-derived antibodies found in gingival crevicular fluid (GCF) serve as the first line of defense against infection in the oral cavity.[8] Previous studies have evaluated the utility of these antibodies for use in monitoring population immunity to SARS-CoV-2 infection in outpatients[9] and children;[10] however, data comparing the presence and kinetics of postinfection SARS-CoV-2–specific antibodies in the oral cavity to those detected systemically are limited.[10–12]

To characterize and compare antibodies detected in plasma and GCF from nursing home residents during and after natural infection, we measured plasma anti–SARS-CoV-2 spike pan-immunoglobulin (Ig), IgG, IgA, IgM, and neutralizing antibodies and GCF anti–SARS-CoV-2 spike IgG and IgA antibodies in a cohort of nursing home residents infected during a COVID-19 outbreak.

Methods

We enrolled a prospective cohort of SARS-CoV-2 infected nursing home residents identified during serial point-prevalence surveys from a single nursing home experiencing a COVID-19 outbreak during June–August 2020, as described previously.[13,14] Participation was voluntary, and residents were eligible for enrollment if they were cognitively capable of providing verbal and written informed consent. This activity was reviewed by the US Centers for Disease Control and Prevention (CDC) and was conducted consistent with applicable federal law and CDC policy (eg, 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq.).

Participants with real-time reverse transcription polymerase chain reaction (RT-PCR)–confirmed SARS-CoV-2 infection and >1 blood or >1 GCF specimen were included in this evaluation. All participants were enrolled within 15 days of their first SARS-CoV-2–positive RT-PCR result and were followed for 42 days. Visits occurred every 3 days for the first 21 days then weekly through the end of the evaluation (Supplementary Fig. 1). During each visit, collection of oropharyngeal swabs, anterior nasal swabs, and saliva was attempted. These specimens were tested using the CDC 2019-novel coronavirus RT-PCR diagnostic panel.[15] Specimens were considered positive if both N1 and N2 targets had a cycle threshold (Ct) < 40.[13] A composite RT-PCR result was determined among all respiratory and saliva specimens collected for each participant per visit. If any specimen was RT-PCR positive, that participant was recorded as RT-PCR positive; if all specimens were RT-PCR negative, that participant was RT-PCR negative. All respiratory specimens with a RT-PCR Ct ≤34 were submitted for viral culture.[13]

Blood was collected on evaluation days 0, 6, 12, 21, and 42 in K2 EDTA tubes (Hemogard Closure; Franklin Lakes, NJ) with day

0 defined as day of enrollment (4–13 days after the first RT-PCR-positive result). Plasma was analyzed for pan-Ig using a validated enzyme-linked immunosorbent assay (ELISA) against full length prefusion-stabilized SARS-CoV-2 spike protein.[16] Specimens were considered reactive for SARS-CoV-2 antibodies if the background-corrected pan-Ig signal-to-threshold ratio was >1 at a 1:100 dilution, with a threshold optical density (OD) of 0.4. Isotype-specific tests were performed on pan-Ig–reactive specimens using the same ELISA technique, with IgG-, IgA-, or IgM-specific secondary antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, MD). ELISA antibody titers were calculated by performing log2 transformations, spline analysis, and extrapolating the titer at the cutoff of 0.4; titers ≥1:100 were considered positive.

Microneutralization tests were performed on confirmed antibody-reactive plasma specimens using live SARS-CoV-2 USA-WA1/2020.[17] Two-fold serial dilutions ranging from 1:20 to 1:640 were incubated with virus for 30 minutes at 37ºC, and the plasma–virus mixtures were then used to inoculate Vero CCL-81 cells cultured in 96-well plates. After 5 days, cells were fixed and stained with formalin-crystal violet to observe live and dead cells. The neutralizing titer was the highest dilution at which plasma blocked viral infection; titers ≥1:80 were considered positive.

GCF was collected on evaluation days 9, 15, 21, and 42 using the Oracol+ Saliva Collection Device (Malvern Medical Limits, Worcester, UK). Collection was performed >30 minutes after consumption of food or liquids by gently rubbing the swab along the gumline around the entire mouth for 1 minute. GCF specimens with at least 100 μL volume were tested. GCF was inactivated by γ radiation before processing and tested using a validated ELISA against full-length prefusion-stabilized SARS-CoV-2 spike protein.[18] GCF antibodies were reported as a gingival crevicular ratio, defined as SARS-CoV-2 specific Ig per total Ig (ng/100 μg). GCF was first tested for IgA, with the remaining volume used for IgG testing. In total, 51 GCF specimens were tested for IgA and 38 (75%) were tested for IgG.

Measures of central tendency, frequencies, and proportions were calculated using Microsoft Excel software (Microsoft Corporation, Redmond, WA) and SAS version 9.4 software (SAS Institute, Cary, NC). Severe COVID-19 illness was defined as a decrease from baseline oxygen saturation (SpO$_2$) of >3% regardless of whether the participant was on room air or supplemental oxygen.[19] Geometric mean titers were calculated, with titers >1:640 equaling 1:640. Time to antibody detection in plasma and GCF was calculated in days since the first RT-PCR–positive result. Peak measures for plasma (pan-Ig, IgG, IgA, IgM) and GCF (IgG and IgA) antibodies for each participant were categorized by percentile. These categories were based on the relative magnitude of each antibody type from each participant compared with that of other participants in the cohort: <25th percentile (+); 25th–49th percentile (++); 50th–74th percentile (+++); ≥75th percentile (++++). Peak neutralization titers were categorized based on magnitude of response as: ≤1:160 (+); 1:320 (++); 1:640 (+++); >1:640 (++++).

Results

Of 95 total residents, 90 had RT-PCR–confirmed SARS-CoV-2 infection identified between June 9 and July 15, 2020. Among 39 eligible residents, 17 consented to participate and 14 were included in this evaluation. The 3 excluded residents had blood collected only at enrollment and were SARS-CoV-2 antibody

Table 1. Conversion and Persistence of Plasma and Gingival Crevicular Fluid SARS-CoV-2–specific Antibodies in Participants with SARS-CoV-2 Infection (N = 14) in a Nursing Home Cohort—Arkansas, June–August 2020

Antibodies Detected	Participants with Antibodies Detected During Evaluation		Time to Antibody Detection[a] (Days)			Participants with Antibodies Still Detected at End of Follow-Up[b]		
	(N = 12)		(N = 11)[c]			(N = 12)		
Plasma	No.	%	No.	Median (IQR)	Range	No. Tested	No. Positive	%
Pan-Ig	12	100	11	10 (5–17)	5–55	12	12	100
IgG	12	100	11	10 (5–17)	5–55	12	12	100
IgA	9	75	9	11 (10–16)	5–17	9	9	100
IgM	10	83	9	11 (11–16)	5–55	10	8	80
Neutralizing	11	92	10	11 (11–17)	5–55	11	8	73
	(N = 14)		(N = 14)			(N = 11)[d]		
GCF	No.	%	No.	Median (IQR)	Range	No. Tested	No. Positive	%
IgG	13	93	13	19 (14–22)[e]	13–55	9	9	100
IgA	12	86	12	14 (14–19)	13–26	9	7	78

Note. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, real-time reverse-transcription polymerase chain reaction; IQR, interquartile range; GCF; gingival crevicular fluid.
[a]Time to antibody detection was calculated in plasma and GCF as the time from the first positive SARS-CoV-2 RT-PCR result.
[b]46–55 days since first RT-PCR–positive result.
[c]Excludes 1 participant (O) who was hospitalized mid-evaluation and missed blood collections to determine time to antibody detection.
[d]3 participants (A, F, and H) did not have GCF tested on the last day of follow-up due to insufficient volume for testing.
[e]Fewer GCF specimens were tested for IgG due to insufficient volume. 54 GCF specimens were collected. GCF specimens were first tested for IgA (n = 51, 94%). Testing for IgG could proceed if sufficient volume remained; 38 (70%) were tested for IgG. 3 (6%) GCF samples had insufficient volume for testing.

negative; 2 residents did not provide any GCF specimens, and 1 provided a single GCF specimen but with insufficient volume for testing. Among the 14 included participants, all (100%) provided >1 GCF specimen and 12 (86%) provided >1 blood specimen; 2 participants could not be phlebotomized. Deidentified participant codes were first published by Surie et al.[14]

Of the 14 included participants, 8 (57%) were female and 6 (43%) were male; all 14 were White race (Supplementary Table 1 online). The median age of participants was 80 years (range, 58–93 years). All 14 residents (100%) had ≥3 underlying conditions; the most common were cardiovascular disease (n = 13, 93%), nonasthmatic chronic lung disease (n = 6, 43%), and neurologic disorders (n = 6, 43%). Also, 8 participants (57%) were asymptomatic at onset, though all became symptomatic at some point during the evaluation period. Two participants (14%) had severe COVID-19 illness; both were hospitalized for their illness during the evaluation. All 14 participants survived through the end of the evaluation.

All participants demonstrated antibody responses to SARS-CoV-2 infection, as detected in plasma and/or GCF. Among 12 phlebotomized participants, plasma was positive for pan-Ig and IgG in all 12 participants, for IgM in 10 participants (83%), and for IgA in 9 participants (75%). Neutralizing antibodies were found in 11 participants (92%) (Table 1). Geometric mean titers for each isotype, computed across the entire evaluation period, were 3,187 for pan-Ig, 3,807 for IgG, 479 for IgA, 497 for IgM, and 213 for neutralizing antibody (Supplementary Table 2 online). Of 14 participants with GCF tested, IgG or IgA was detected in all: 13 (93%) with IgG and 12 (85%) with IgA. Both participants who could not be phlebotomized (N and A) had GCF with detectable IgG and IgA.

The median times from first RT-PCR–positive result to detection of antibodies were similar for pan-Ig (10 days; interquartile range [IQR], 5–17 days), IgG (10 days; IQR, 5–17 days), IgA

(11 days; IQR, 10–16 days), IgM (11 days; IQR, 11–16 days), and neutralizing antibodies (11 days; IQR, 11–17 days) (Table 1). Participant O was excluded from the time-to-antibody detection calculation due to hospitalization during the evaluation. The median times from first RT-PCR–positive to detectable GCF IgG were 19 days (IQR, 14–22 days) and 14 days (IQR, 14–19 days) for IgA.

Plasma pan-Ig, IgG, and GCF IgG showed similar kinetics, increasing across weeks 1–4 since first RT-PCR–positive result and remaining elevated throughout the evaluation period (Fig. 1). Peak IgG responses were observed in plasma and GCF at a median of 4 weeks since first RT-PCR positive result. Plasma IgA, IgM, and GCF IgA showed peak responses at a median of 3 weeks since first RT-PCR–positive result before decreasing. Cohort isotype distributions were influenced by 1 participant (P) who had high plasma titers of pan-Ig, IgG, IgM, and IgA at week 5 and high GCF responses at week 8.

Antibody persistence was examined at the end of the evaluation, 42 days after enrollment (46–55 days after RT-PCR diagnosis). All (100%) participants with plasma pan-Ig antibodies (12 of 12), IgG antibodies (12 of 12) and IgA antibodies (9 of 9) remained positive for these antibodies, compared to 8 (80%) of 10 with IgM antibodies and 8 (73%) of 11 of those with neutralizing antibodies (Table 1). Not all participants with detectable GCF antibodies had sufficient GCF volume collected for testing at the final time point. Of 9 participants with GCF IgG or IgA detected at any time and tested at the end of the evaluation, all participants (100%) with IgG antibodies remained positive and 7 (78%) with IgA antibodies remained positive.

For each participant, peak antibody levels in plasma and GCF were categorized based on their relative antibody response magnitude compared with the overall cohort (Table 2). Of 6 participants with peak plasma IgG antibodies at or above the median, 5 (83%) had GCF IgG antibodies at or above the median. Of 5 participants

Table 2. Peak Plasma and GCF Antibody Responses in Participants with SARS-CoV-2 Infection (N = 14) in a Nursing Home Cohort — Arkansas, June–August 2020

Participant	Age Category, Years	Plasma Antibody Responses[a]					GCF Antibody Responses[a]	
		Pan-Ig	IgG	IgA	IgM	Neutralizing	IgG	IgA
N[b]	75–84	N/A	N/A	N/A	N/A	N/A	+	+
A[b]	65–74	N/A	N/A	N/A	N/A	N/A	+	++
F	85–94	+	+	−	−	−	−	+
Q	85–94	+	+	−	+++	+	+	−
J	85–94	+	++	+	+	+++	+++	+++
C	75–84	++	+	+++	−	++	+	+++
M	55–64	++	++	+	++	+	++	+
E	75–84	++	++	++	++	++	++	++
O[c,d]	65–74	+++	+++	−	+	+	++	−
I	55–64	+++	+++	++	++++	+++	+++	+++
G	75–84	+++	++++	++++	++++	++++	+++	++++
L	85–94	++++	+++	++++	+++	+++	++++	++
H	65–74	++++	++++	++++	++	+++	++++	++++
P[d]	85–94	++++	++++	+++	++++	++++	++++	++++

Note. GCF; gingival crevicular fluid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; N/A, not applicable.
[a]Peak plasma (pan-Ig, IgG, IgA, IgM) and GCF (IgG and IgA) antibody response magnitude reported as: <25th percentile (+); 25th–49th percentile (++); 50th–74th percentile (+++); ≥75th percentile (++++). Peak neutralization titers were categorized based on magnitude of response as: ≤1:160 (+); 1:320 (++); 1:640 (+++); >1:640 (++++). Participants are presented in ascending order of peak plasma pan-Ig response.
[b]Participants N and A could not be phlebotomized.
[c]Unable to describe time to seroconversion due to hospitalization during the evaluation.
[d]Participants O and P had severe COVID-19 illness, defined as a decrease from baseline of >3% in oxygen saturation (SpO2) regardless of whether the participant was on room air or supplemental oxygen.
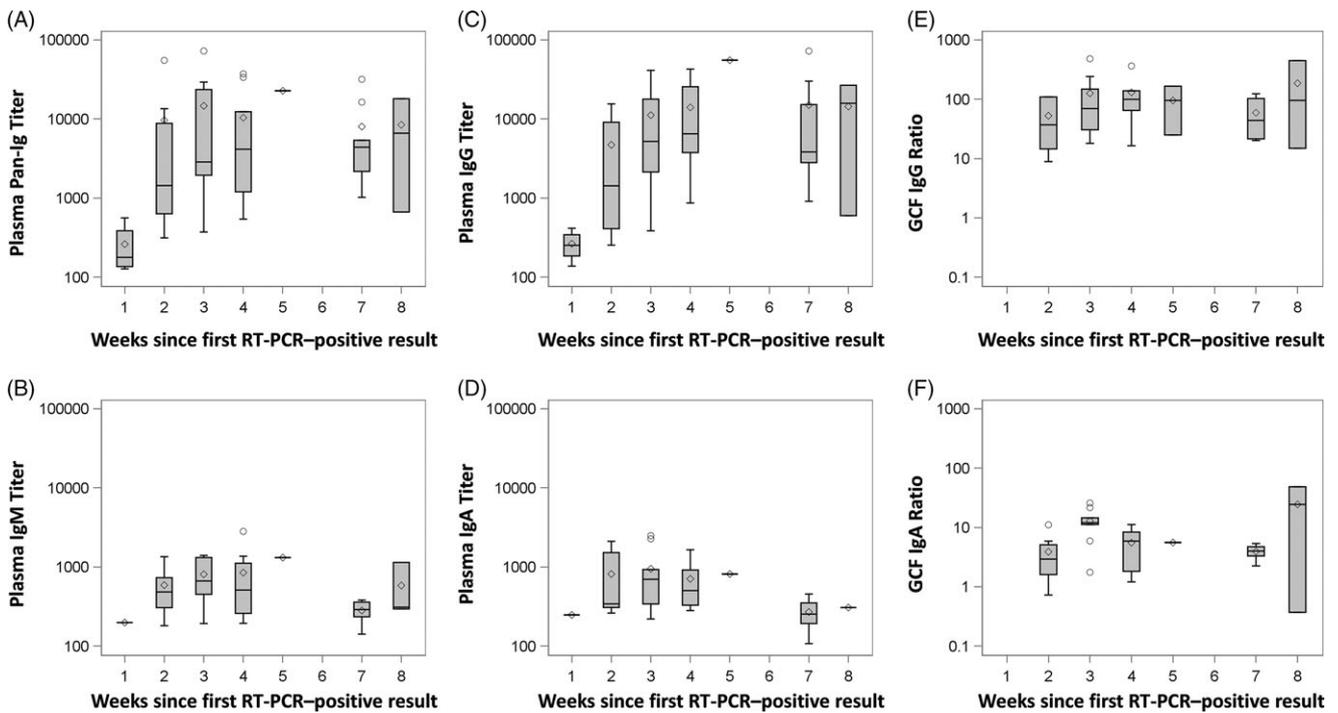
Fig. 1. Distribution of plasma and GCF antibody responses to SARS-CoV-2 infection over time in a nursing home cohort—Arkansas, June–August 2020. Distribution of positive plasma pan-Ig (panel A), IgM (panel B), IgG (panel C), and IgA (panel D) antibody titers by the number of weeks since the first RT-PCR–positive result. Plasma titers ≥1:100 were considered positive. Distribution of positive GCF IgG (panel E) and IgA (panel F) ratios by the number of weeks from the first RT-PCR–positive result. GCF Ig ratios defined as SARS-CoV-2 specific Ig/total Ig are reported in ng/100 µg. The y-axes are plotted in logarithmic scale. Note. GCF, gingival crevicular fluid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, real-time reverse-transcription polymerase chain reaction.

with peak plasma IgA antibodies at or above the median, 4 (80%) had GCF IgA antibodies at or above the median.

Participant P developed some of the highest plasma titers (pan-Ig, IgG, IgM, neutralizing antibodies) and GCF ratios (IgG and IgA antibodies) of any participant in the cohort (Fig. 2). Participant P was 1 of 2 participants with severe COVID-19 illness and was hospitalized twice during the evaluation. Despite having the some of the highest titers overall, participant P was negative for neutralizing antibodies by the end of the evaluation.

Participant O, the second participant with severe COVID-19, was also hospitalized during the evaluation and only had 2 blood collections, at enrollment and the end of the evaluation. Peak plasma pan-Ig and IgG titers at the end of the evaluation were relatively strong (+++), though IgM and neutralizing activity were relatively low (+) and plasma IgA was not detected. Of 3 GCF specimens collected from participant O, we detected IgG antibodies in all, but we detected IgA antibodies in none. Peak GCF IgG levels for participant O were intermediate (++) relative to the cohort.

Participant Q also had no IgA antibodies detected in plasma or GCF. Participant Q was immunocompromised and lacked detectable plasma pan-Ig until the final collection at 55 days after diagnosis. At the end of the evaluation, participant Q had relatively strong plasma IgM (+++) and low plasma IgG and neutralization (+) responses, with no detectable plasma IgA antibodies.

Participant F was the only participant with blood analyzed to have no neutralizing antibodies detected. This participant lacked plasma IgA and IgM antibodies. At 26 days after diagnosis, participant F produced a relatively low (+) GCF IgA response. Their peak plasma pan-Ig and IgG titers were also relatively low (+).

Discussion

All SARS-CoV-2–infected participants in this nursing home cohort had measurable SARS-CoV-2–specific antibodies, and 92% of participants with plasma tested developed neutralizing antibodies. Time to antibody detection was similar amongst pan-Ig and isotypes, detected with a median of 10–11 days since the first RT-PCR–positive result, consistent with other reports.[4,5,20–22] This finding suggests that the temporality of humoral immune responses to SARS-CoV-2 in older adults may be comparable to those in younger cohorts.

Binding plasma and GCF IgG antibodies persisted through the end of the evaluation. Although we did not measure antibody responses beyond 42 days after enrollment, recent publications have shown that SARS-CoV-2 IgG antibodies persist at least 6–11 months after infection.[6,22,23] A recent evaluation in nursing home residents found that 91% of residents sampled had serum SARS-CoV-2–specific IgG antibodies 6 months following diagnosis,[6] which suggests that IgG antibodies in older adults persist in a manner similar to younger cohorts.

Following antibody detection, declines in plasma IgM and IgA were observed in this nursing home cohort. These decreases in IgM and IgA seropositivity by the end of the evaluation are consistent with previous reports.[4,22,24,25] For example, a study of 343 symptomatic SARS-CoV-2 RT-PCR–positive individuals (median age of 59 years) estimated the median time to seroreversion of IgM and IgA was 49 days and 71 days, respectively.[24] Our findings highlight that IgM and IgA responses to SARS-CoV-2 infection in older adults may be short-lived markers of acute infection.

Neutralizing antibodies may offer protection against SARS-CoV-2 reinfection and disease.[26] Similar to our findings, other studies report that most SARS-CoV-2–infected individuals, often

>90%,[4,5,7,22,25] develop neutralizing antibodies. Although comparisons of neutralizing antibody magnitude are difficult without standardized methodologies,[27] our testing used the same microneutralization method employed to analyze specimens from the first 14 SARS-CoV-2–infected patients diagnosed in the United States. In those first 14 patients, who were sampled over a similar time frame from diagnosis as participants in this evaluation, 1 (8%) of 12 patients with neutralizing antibodies reached a titer of 1:640.[22] In our nursing home cohort, 6 (55%) of 11 participants with neutralizing antibodies reached titers ≥1:640, suggesting higher neutralizing antibody responses potentially due to differences in their severity of infection or initial antigen exposure.[4,28,29] Additional studies may determine whether a higher magnitude of neutralizing antibody responses correlates with protection from SARS-CoV-2 reinfection or disease.

Recovery of replication-competent virus, most often using viral culture, suggests that a person is potentially infectious. Following antibody detection in plasma or GCF, replication-competent SARS-CoV-2 was not recovered from any participant in our cohort. These findings are consistent with previous reports[14,22,30] indicating that binding antibodies could have served as an early humoral marker of noninfectivity among these participants. Furthermore, the slightly earlier detection of plasma pan-Ig and IgG antibodies in this evaluation suggests that these isotypes could serve as even timelier markers.

Two participants had notably weak immune responses to SARS-CoV-2 infection. First, participant Q was severely immunocompromised due to ongoing chemotherapy with ibrutinib. Participant Q remained viral-culture positive at 19 days from diagnosis, though replication-competent SARS-CoV-2 is rarely detected >10 days following symptom onset or diagnosis in immunocompetent persons.[31] Plasma and GCF antibody responses, including neutralizing antibodies, were not detected until 55 days after this participant's first RT-PCR–positive result, despite having plasma and GCF collected throughout the evaluation. In immunocompromised participants, in our cohort and in others,[30] binding and neutralizing antibodies were detected later in recovery.

Participant F was the other participant to have a weak immune response to infection, with no detectable plasma IgA, IgM, or neutralizing antibodies and GCF IgA detected at only a single time point. Participant F was older (>90 years) but had relatively few underlying conditions compared to others in the cohort. A recent report suggested the presence of neutralizing antibodies is associated with robust multi-isotype antibody responses.[32] Participant F's low plasma IgG response and lack of detectable plasma IgA and IgM are consistent with this report.

Although antibody response evaluation via serology is standard, options for routine monitoring that are less invasive than phlebotomy are needed. Individuals hesitant of needlesticks may refuse traditional serology,[33] which may prevent immunological surveillance for vaccine responses or developing correlates of protection in populations of interest, such as nursing home residents, children, and persons living in communal settings. Common factors impeding phlebotomy in nursing home populations include blood thinning medications, fragile skin, dehydration, and reduced arm mobility.

Saliva has been proposed for routine immunological monitoring, and GCF contains more highly concentrated plasma-derived IgG antibodies than whole saliva.[33] Using GCF in this evaluation, we found similar kinetic profiles and timelines between plasma and GCF antibodies. Additionally, we were able to describe immune responses detected in GCF in 2 participants who could not be phlebotomized, and we identified a GCF IgA response in a participant
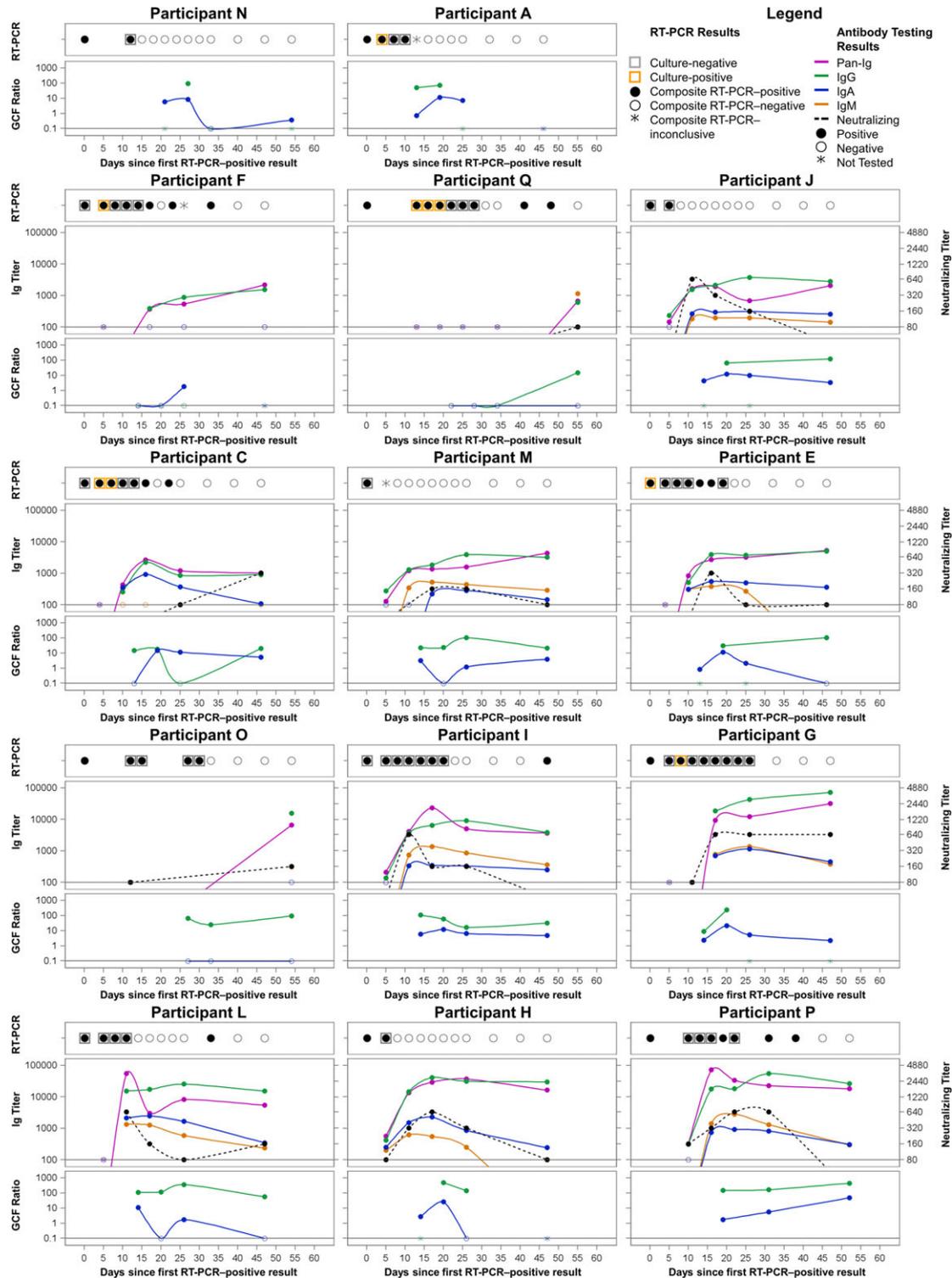
Fig. 2. Plasma and GCF antibody responses in relation to RT-PCR and viral culture results in individual participants with SARS-CoV-2 infections in a nursing home cohort—Arkansas, June–August 2020. (Top panels) Composite RT-PCR results were determined from oropharyngeal, anterior nasal, and saliva specimens collected at each evaluation visit. If any respiratory or saliva specimen obtained from a participant at a given visit was RT-PCR positive, that participant was considered RT-PCR positive on that day. If all respiratory and saliva specimens obtained from a participant at a given visit were RT-PCR negative, that participant was considered RT-PCR negative on that day. Respiratory specimens with a RT-PCR Ct ≤34 were submitted for viral-culture testing. Due to challenges with specimen collection, transport, and processing, RT-PCR results for each specimen type were not always available for each visit. (Middle panels) Plasma pan-Ig (purple), IgG (green), IgA (blue), and IgM (orange) antibodies were considered positive with titers ≥1:100. Neutralization (black dashed) antibodies were considered positive with titers ≥1:80. Filled circles indicate a positive result, open circles indicate negative results, and asterisks indicate specimens were not tested. The y-axis is plotted in logarithmic scale. (Bottom panels) GCF IgG (green) and IgA (blue) antibodies are reported as GCF ratios in ng/100 µg and represent SARS-CoV-2 specific Ig/total Ig. Filled circles indicate a positive result, open circles indicate negative results, and asterisks indicate specimens were not tested. The y-axis is plotted in logarithmic scale. Note. GCF, gingival crevicular fluid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RT-PCR, real-time reverse-transcription polymerase chain reaction; Ct, cycle threshold. All data shown in days since the first RT-PCR positive result.

who did not have plasma IgA detected. These results demonstrate that GCF can be a critical tool for monitoring immune responses in those who are unable or unwilling to be phlebotomized. GCF may also prove to be a more accessible and less invasive specimen for antibody detection and may provide additional opportunities for routine immunological monitoring,[9] including tracking COVID-19 vaccine responses.

This evaluation had several limitations. Due to refusals and severe dementia preventing eligibility, this evaluation was limited to a small cohort, making it difficult to generalize trends to the larger nursing home population. GCF was not collected until day 9 after enrollment, potentially missing earlier antibody detection in GCF. In several GCF specimens, the volume was insufficient for testing both IgA and IgG, thus limiting data on GCF IgG detection. Lastly, plasma and GCF were not collected beyond 46–55 days after diagnosis, limiting analyses of antibody persistence in this cohort.

This longitudinal evaluation of SARS-CoV-2–specific antibody responses in plasma and GCF highlights that nursing home residents developed robust humoral immune responses to natural infection with SARS-CoV-2, which may offer protection against future reinfection and disease. Kinetics of antibodies detected in GCF mirrored those from plasma. Oral fluids, such as GCF, could provide critical, noninvasive specimens for detecting and monitoring immunologic responses in populations unable or unwilling to be phlebotomized, particularly as a means of implementing routine testing or surveillance for immunologic correlates of protection against SARS-CoV-2.

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