

High Prevalence of Asymptomatic COVID-19 Infection in Hemodialysis Patients Detected Using Serologic Screening

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ABSTRACT

Background Strategies to minimize the risk of transmission and acquisition of COVID-19 infection in patients with ESKD receiving in-center hemodialysis have been rapidly implemented across the globe. Despite these interventions, confirmed COVID-19 infection rates have been high in the United Kingdom. Prevalence of asymptomatic disease in an adult hemodialysis population has not been reported. Also, to our knowledge, the development of humoral response to SARS-CoV-2 has not been previously reported in this population. Although serologic testing does not provide information on the infectivity of patients, seroprevalence studies may enable investigation of exposure within dialysis units and hence, assessment of current screening strategies.

Methods To investigate the seroprevalence of SARS-CoV-2 antibodies in a hemodialysis population, we used the Abbott IgG assay with the Architect system to test serum samples from 356 patients receiving in-center hemodialysis for SARS-CoV-2 antibodies.

Results Of 356 patients, 121 had been symptomatic when screened before a dialysis session and received an RT-PCR test; 79 (22.2% of the total study population) tested positive for COVID-19. Serologic testing of all 356 patients found 129 (36.2%) who tested positive for SARS-CoV-2 antibodies. Only two patients with PCR-confirmed infection did not seroconvert. Of the 129 patients with SARS-CoV-2 antibodies, 52 (40.3%) had asymptomatic disease or undetected disease by PCR testing alone.

Conclusions We found a high seroprevalence of SARS-CoV-2 antibodies in patients receiving in-center hemodialysis. Serologic evidence of previous infection in asymptomatic or PCR-negative patients suggests that current diagnostic screening strategies may be limited in their ability to detect acute infection.

JASN 31: 1969–1975, 2020. doi: <https://doi.org/10.1681/ASN.2020060827>

Strategies to minimize the risk of transmission and acquisition of coronavirus disease 2019 (COVID-19) infection in

patients with ESKD receiving in-center hemodialysis (ICHHD) have been rapidly implemented across the globe.^{1–3} Despite

interventions, infection rates in the United Kingdom have been high, with over 2000 cases of COVID-19 infection confirmed in patients on ICHD and a reported mortality rate of 22%.^{4,5}

Case ascertainment to enable early isolation has been a key component of preventative measures performed in dialysis centers. National guidance supporting the routine screening of patients for symptoms prior to each dialysis session was issued in March 2020 in the United Kingdom, with confirmatory diagnostic testing as indicated.³ The current gold standard diagnostic test for acute infection is identifying viral RNA with real-time RT-PCR of isolates from upper respiratory tract swabs using

Received June 15, 2020. Accepted July 3, 2020.

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Published online ahead of print. Publication date available at www.jasn.org.

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oligonucleotides directed to nucleocapsid or viral RNA-dependent RNA polymerase genes.^{6,7} However, there are several potential limitations to the use of nucleic acid tests in diagnosing COVID-19 in this selective setting; these include the potential for false negative testing, which may be linked to inadequate nasopharyngeal sampling, and the inability to diagnose pre- or asymptomatic infection.^{8,9}

Although serologic testing does not provide information on the infectivity of patients, seroprevalence studies may enable investigation of exposure within dialysis units and hence, assessment of current screening strategies. There are several commercial tests available for the detection of IgM and IgG against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including lateral flow immunoassays, ELISAs, fluorescence immunoassays, chemiluminescence assays, and pseudovirus neutralization assays.^{10–13} Assays detect antibodies against either the SARS-CoV-2 spike glycoprotein, a unique region of the virus that mediates viral entry to cells *via* the Angiotensin converting enzyme-2 (ACE-2) receptor, or the nucleocapsid protein.^{10,14} In the United Kingdom, several serologic tests have now been approved for use by Public Health England (PHE). One, manufactured by Abbott, is a two-step chemiluminescent microparticle immunoassay using a nucleocapsid-protein antigen and detecting IgG antibodies. This assay is reported to have 93.9% sensitivity at ≥ 14 days after positive PCR testing in patients with confirmed SARS-CoV-2 infection and 100% specificity using historic control sera, including from a small subset with confirmed seasonal coronavirus infection.¹⁵

To our knowledge, the development of humoral response to SARS-CoV-2 in adult patients with ESKD receiving hemodialysis has not been previously reported. In this study, we investigated the seroprevalence of SARS-CoV-2 in a large hemodialysis cohort of patients who have been managed per United Kingdom national guidelines on dialysis provision in the context of COVID-19.³

METHODS

Patient Selection

In total, 376 patients receiving ICHD within two units affiliated with Imperial College Renal and Transplant Centre between April 27 and May 7, 2020 were included. Clinical and routine pathology data were obtained from electronic records and dialysis records. All pathology data from each patient were analyzed over a 12-week period from February 24, 2020.

The study was approved by the Health Research Authority, Research Ethics Committee (reference: 20/WA/0123—The Impact of COVID-19 on Patients with Renal Disease and Immunosuppressed Patients).

Diagnosis of COVID-19 Infection

Infection with SARS-CoV-2 was confirmed through real-time RT-PCR assay of nasopharyngeal swab specimens following either routine screening or acute presentation. RT-PCR was carried out as per PHE guidelines using certification-marked assays with primers directed to the nucleocapsid or RNA-dependent RNA polymerase genes.¹⁶ Routine screening of patients for the development of symptoms or a fever occurred prior to each hemodialysis session from March 9. Patients found to be positive received dialysis in an isolated unit for a period of 14 days.

SARS-CoV-2 Antibody Detection

Sera from all patients were tested using the Abbott SARS-CoV-2 IgG assay using an Architect system by staff working in the Department of Infection and Immunity, North West London Pathology NHS Trust. The assay is an automated two-step chemiluminescent microparticle immunoassay in which patient samples are incubated with SARS-CoV-2 antigen-coated paramagnetic microparticles followed by anti-human IgG acridinium-labeled conjugate to generate a chemiluminescent reaction. The index (sample/control) is calculated by comparing relative light units in the sample to the calibrator relative light units. Samples were interpreted as positive or negative according

Significance Statement

Strategies to limit acquisition and transmission of SARS-CoV-2 infection in patients with ESKD receiving in-center hemodialysis have been implemented globally. Despite these measures, acute SARS-CoV-2 infection rates confirmed by RT-PCR testing have been high in the United Kingdom. The seroprevalence rate in an in-center hemodialysis adult population has not been reported previously. In a study of 356 patients receiving in-center hemodialysis, the authors report a 36.3% seroprevalence rate. They also found that 40.3% of patients with IgG SARS-CoV-2 antibodies had either asymptomatic infection or undetected disease by PCR testing alone. These findings reveal limitations of current diagnostic screening strategies for active SARS-CoV-2 infection using PCR testing of individuals screened for symptoms prior to dialysis sessions. Effective screening is likely to require a hybrid strategy of PCR and serologic testing.

to the manufacturer's instructions, with a cutoff index value of 1.4.¹⁵

Statistical Analyses

Statistical and graphical analyses were performed with MedCalc v19.2.1. The two-sided level of significance was set at $P=0.05$. Normally distributed variables were compared with *t* test, and nonparametric data were compared with the Mann-Whitney test. Fisher exact tests or chi-squared tests were used for proportional assessments.

RESULTS

Serologic Testing Confirms High Rates of SARS-CoV-2 Infection in Patients Receiving ICHD and Identifies Patients with Asymptomatic or PCR-Negative Disease

Of the 376 patients included in the study, 20 were excluded as they did not or were unable to give consent. As shown in Figure 1, 121 patients were tested for COVID-19 by PCR, and 79 of 121 (65.3%) were positive. This represents 79 of 356 (22.2%) total study population. Using serology testing, 129 of 356 (36.2%) patients tested positive for SARS-CoV-2

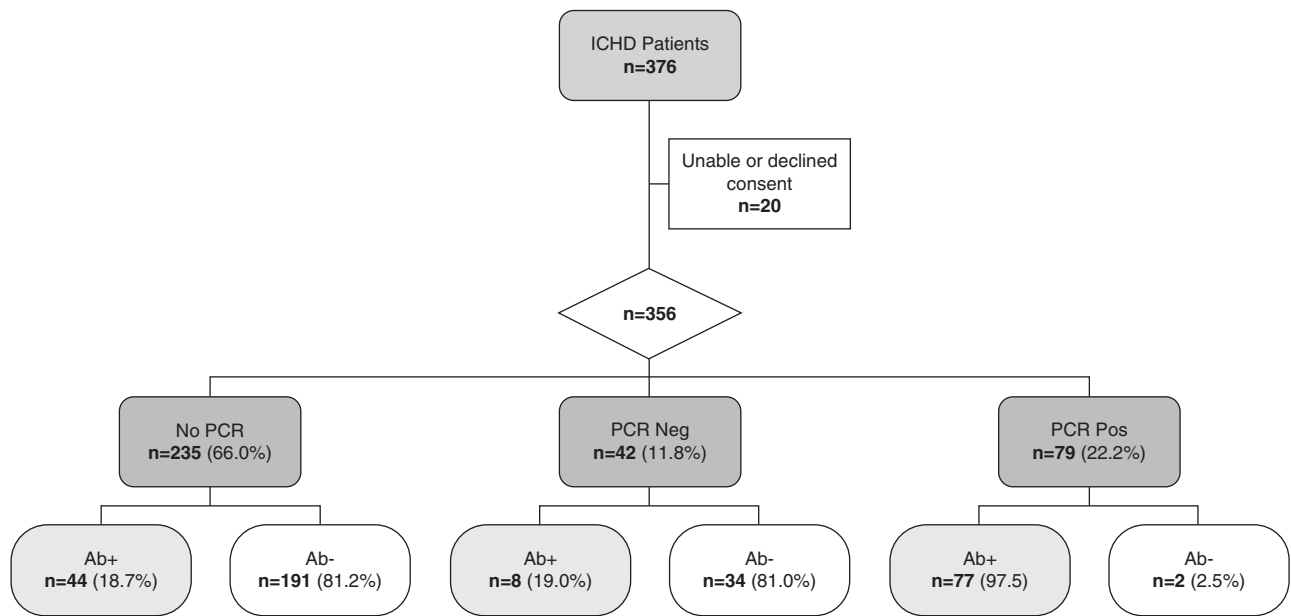


Figure 1. Study flow diagram. Three hundred seventy-six patients were eligible for inclusion in the study and 356 were included in analysis. Flow chart indicates the number of patients in each group by PCR and antibody status. Neg, negative; Pos, positive.

antibodies. Only 3 of 356 (0.84%) patients had a borderline antibody result that was within $\pm 20\%$ of the cutoff index for a positive result (Figure 2A). Unit A had a significantly higher prevalence of proven infection than unit B, both by PCR (42 of 98 [42.9%] and 37 of 258 [14.3%] PCR-positive cases, respectively; $P < 0.001$) and serologic testing (51 of 98 [52.0%] and 78 of 258 [30.2%] patients testing positive for antibodies, respectively; $P < 0.001$).

Across both units, 77 of 79 (97.8%) PCR-positive patients had detectable antibodies at a mean time of 34 ± 6.4 days after PCR testing. In patients who were PCR tested, there was moderate correlation between the magnitude of the antibody test result and time from PCR testing to antibody testing ($r = 0.30$; $P < 0.001$) (Figure 2B). The two PCR-positive patients without antibodies were tested on days 18 and 23 after PCR testing, and both had received significant immunosuppression previously. One patient has lupus nephritis and is still receiving maintenance prednisolone, whereas the other patient had ESKD secondary to antglomerular basement membrane disease. Forty-one of 227 (18.1%) antibody-negative patients and 14 of 129 (10.9%) antibody-positive patients were receiving

maintenance immunosuppression therapy ($P = 0.07$).

Of the 42 patients who were tested but PCR negative, 8 of 42 (19.0%) had SARS-CoV-2 antibodies. This was not due to differences in time from PCR to antibody testing, as the median time between tests was 23 (14–35) days in those who were antibody negative and 22 (14–34) days in those who were antibody positive ($P = 0.94$).

Asymptomatic SARS-CoV-2 Infection Is Common in ICHD Units

In total, 235 patients were asymptomatic during the study period and thus, did not have a PCR test. Of these, 44 of 235 (18.7%) had SARS-CoV-2 antibodies, which was no different from the antibody detection rate in the PCR-negative patients ($P = 0.96$). Of note, asymptomatic infection rates were the same in units A and B (9 of 56 [16.1%] and 41 of 219 [18.7%], respectively; $P = 0.64$).

Laboratory Features May Distinguish Patients with PCR-Proven Infection

We then analyzed clinical and laboratory characteristics of the entire cohort in order to identify associations of proven (PCR positive), suspected (PCR negative),

and asymptomatic (PCR not done) patients, as shown in Table 1. Patients who were tested but PCR negative were younger than patients not tested ($P = 0.04$). There were no other differences in baseline demographics between the groups. Analysis of the nadir lymphocyte count, peak C-reactive protein (CRP), and peak ferritin levels between the three groups showed that PCR-positive patients had a significantly lower lymphocyte count ($P < 0.001$), higher CRP ($P < 0.001$), and higher ferritin levels ($P < 0.001$) compared with asymptomatic patients. When compared with asymptomatic patients, PCR-negative patients had a significantly higher CRP ($P < 0.001$) and higher ferritin level ($P = 0.03$).

Laboratory Features May Distinguish Patients with Serologically Proven Infection but Not in Those Who Were Previously PCR Negative or Asymptomatic

Overall, there was no difference in sex, age, ethnicity, cause of ESKD, proportion receiving immunosuppression, or time on dialysis between patients who tested positive or negative for SARS-CoV-2 antibodies (Table 2). Analysis of pathology results showed that the nadir lymphocyte counts were lower, whereas

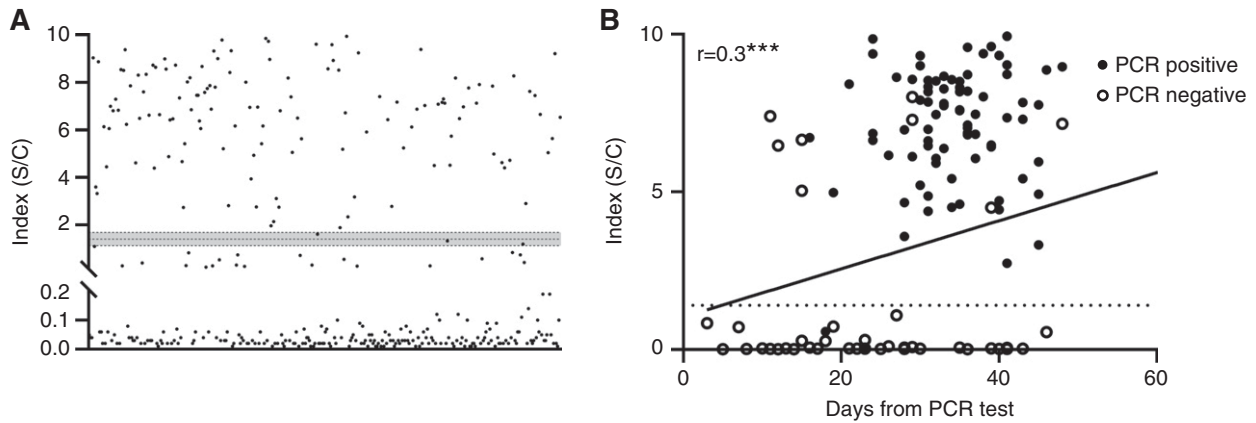


Figure 2. Raw data from SARS-CoV-2 IgG chemiluminescent microparticle immunoassay showing correlation between days from PCR testing and index result. (A) Scatterplot of the index value (sample/control [S/C]) relative light units for each sample tested. The manufacturer’s cutoff for a positive value (1.4) ±20% is indicated by dashed lines. (B) In the group of patients who were PCR tested, there is moderate positive correlation between the index value of antibody test and time from antibody testing to PCR testing. Statistical analysis performed using Spearman correlation. ****P*<0.001.

peak CRP and ferritin levels were higher, in antibody-positive versus antibody-negative patients (Table 2).

However, when specifically considering PCR-negative patients, there was no

difference between the patients who were antibody positive compared with those who were antibody-negative in terms of sex, age, ethnicity, proportion receiving immunosuppression, or cause

of ESKD (Table 3). There was also no difference in either lymphocyte count or CRP at the time of PCR test between the antibody-positive and antibody-negative patients. Likewise, there was

Table 1. Patient characteristics by swab status

Variable	No Swab ^a (%), n=235	PCR Negative (%), n=42	PCR Negative/No Swab <i>P</i> Value	PCR Positive (%), n=79	PCR Positive/No Swab <i>P</i> Value
Sex					
Women	84 (35.7)	20 (47.6)	0.27	26 (32.9)	0.62
Men	151 (64.2)	22 (52.3)		53 (67.1)	
Age (median), yr	68 (54–73)	62 (51–74)	0.04 ^c	65 (54–73)	0.69
Ethnicity					
Black	29 (12.3)	8 (19.0)	0.49	9 (11.4)	0.27
White	62 (26.4)	9 (21.4)		19 (24.1)	
Indoasian	97 (41.2)	19 (45.2)		38 (48.1)	
Other	47 (20.0)	6 (14.2)		13 (16.5)	
Cause of ESKD					
APKD	16 (6.8)	1 (2.3)	0.36	2 (2.5)	0.43
Diabetic nephropathy	85 (36.1)	17 (40.5)		32 (40.5)	
GN	43 (18.2)	6 (14.2)		12 (15.2)	
Other	35 (14.9)	9 (21.4)		5 (6.3)	
Unknown	47 (20.0)	8 (19.0)		25 (31.6)	
Urologic	9 (3.8)	1 (2.3)		3 (3.8)	
Time at ESKD (median), yr	2.2 (0.9–4.1)	1.6 (0.7–2.7)	0.10	1.6 (0.9–2.9)	0.13
Unit					
A	43 (18.2)	13 (30.9)	0.09	42 (53.2)	0.001 ^c
B	192 (81.7)	29 (69.0)		37 (46.8)	
Lymphocyte nadir ^b (median), 10 ⁹ /L	1.2 (0.9–1.6)	1.0 (0.8–1.4)	0.24	1.0 (0.7–1.3)	<0.001 ^c
CRP peak ^b (median), mg/L	7.5 (2.8–19.4)	31.1 (12.4–95.7)	<0.001 ^c	53.4 (19.3–112.0)	<0.001 ^c
Ferritin peak ^b (median), μg/L	404 (298–534)	495 (344–597)	0.03 ^c	817 (529–1532)	<0.001 ^c
Immunosuppressed, yes	43 (18.3)	4 (9.5)	0.19	8 (10.1)	0.11

APKD, autosomal dominant polycystic kidney disease.

^aReference group.

^bOf all values over the 12-week observation period.

^c*P*<0.05.

Table 2. Patient characteristics by SARS-CoV-2 antibody status

Variable	Antibody Positive (%), n=129	Antibody Negative (%), n=227	P Value
Sex			
Women	47 (36.4)	83 (36.6)	0.98
Men	82 (63.6)	144 (63.4)	
Age (median), yr	65 (55–73)	68 (57–77)	0.05 ^a
Ethnicity			
Black	18 (14.0)	28 (12.3)	0.36
White	29 (22.5)	61 (26.9)	
Indoasian	60 (46.5)	94 (41.4)	
Other	22 (17.1)	44 (19.4)	
Cause of ESKD			
APKD	6 (4.7)	13 (5.7)	0.90
Diabetic nephropathy	48 (37.2)	86 (37.9)	
GN	19 (14.7)	42 (18.5)	
Other	12 (9.3)	37 (16.3)	
Unknown	38 (29.5)	42 (18.5)	
Urologic	6 (4.7)	7 (3.1)	
Time at ESKD (median), yr	1.7 (0.9–3.2)	2.2 (0.9–4.0)	0.18
Unit			
A	51 (39.5)	47 (20.7)	<0.001 ^a
B	78 (60.5)	180 (79.3)	
Lymphocyte nadir (median), 10 ⁹ /L	1.1 (0.7–1.4)	1.2 (0.9–1.5)	0.01 ^a
CRP peak (median), mg/L	32.7 (7.1–87.9)	9.8 (3.6–24.5)	<0.001 ^a
Ferritin peak (median), μg/L	610 (426–1044)	417 (299–545)	<0.001 ^a
Immunosuppressed, yes	14 (10.9)	41 (18.0)	0.07

APKD, autosomal dominant polycystic kidney disease.

^aP<0.05.

no difference in patient characteristics between those who were antibody-positive and antibody-negative in the cohort of patients who were not tested by PCR (Supplemental Table 1).

DISCUSSION

In this study, we report an overall SARS-CoV-2 seroprevalence of 36.2% (129 of 356) in patients receiving ICHD; 40.3% (52 of 129) of total cases were found in asymptomatic patients or patients with undetected disease who were screened but had a negative PCR result. None of these patients would have been isolated, which means that they may have been a source of infection transmission for a period within the dialysis centers. Although the use of personal protective equipment would have limited this risk, these results highlight the limited utility of current diagnostic screening strategies within dialysis cohorts.

Importantly, we found that the sensitivity of the Abbott assay in patients on hemodialysis with PCR-proven disease is equivalent to that reported in other populations. Only 2 of 79 patients with PCR-positive disease did not have detectable antibodies, and both patients had a history of significant immunosuppression, which may have affected the immune response. Overall, 15.4% of patients were receiving baseline immunosuppression, and there was a trend to greater rates of current immunosuppression use in those who were antibody negative compared with those who were antibody positive, although this was not statistically significant. The use of this and other serologic assays in patients with a greater burden of immunosuppression, such as a functioning renal transplant or for GN, warrants further investigation.

Seroprevalence rates will vary depending on geographical location, the population being studied, and timing of analysis.¹⁷ In London, a seroprevalence

of 14.5% was found in blood donors in the first week of June 2020, which was comparatively higher than in other parts of the United Kingdom.¹⁷ It is recognized that health care workers have had high rates of COVID-19 infection, with a recent report suggesting an overall seroprevalence of 45.3% among health care workers in one London hospital.¹⁸ We have yet to study the seroprevalence within our dialysis staff; however, a recent report from our unit has shown a correlation between staff-reported illness and rates of RT-PCR–confirmed patient infection.⁵ Therefore, a seroprevalence of 36.2% seen in this study is likely to be in keeping with rates expected within a high-risk cohort in London. The high disease prevalence seen in our dialysis population highlights the risk of exposure to these patients because they attend routine dialysis sessions, thus rendering them unable to shield or socially distance from each other or health care workers. Further evidence to support high seroprevalence rates in patients receiving ICHD outside our center can be taken from a small study of 13 pediatric patients on hemodialysis, which reported a seroconversion rate at 3 weeks postexposure of 23% in patients and 44% in health care workers, with the majority remaining asymptomatic. Of interest, results from our study show that, although the overall seroprevalence was different between the two centers, asymptomatic rates were the same ($P=0.64$). As such, further consideration of patient-level characteristics associated with asymptomatic disease would be beneficial and may help identify patients who require bespoke screening strategies.

Using seroprevalence data, this study has also enabled analysis of routine dialysis blood as a mechanism to help identify patients on hemodialysis with SARS-CoV-2 infection. There were significant differences in biochemical parameters between patients who were PCR positive and those who were negative or not swabbed, with higher peak CRP and ferritin levels and lower nadir lymphocyte counts in the PCR-positive group. This correlates with the results of a previous study, which showed that

Table 3. Patient characteristics by antibody status in PCR-negative patients

Variable	PCR Negative, Ab– (%), n=34	PCR Negative, Ab+, n=8	P Value
Sex			
Women	15 (44.1)	5 (62.5)	0.45
Men	19 (55.9)	3 (37.5)	
Age (median), yr	62 (52–75)	64 (58–74)	0.93
Ethnicity			
Black	6 (17.6)	2 (25.0)	0.17
White	9 (26.5)	0	
Indoasian	15 (44.1)	4 (50.0)	
Other	4 (11.7)	2 (25.0)	
Cause of ESKD			
APKD	1 (2.6)	0	0.54
Diabetic nephropathy	12 (35.3)	5 (62.5)	
GN	5 (14.7)	1 (12.5)	
Other	7 (20.6)	2 (25.0)	
Unknown	8 (23.5)	0 (8.3)	
Urologic	1 (2.9)	0	
Time at ESKD (median), yr	1.5 (0.4–2.9)	1.7 (1.1–3.5)	0.67
Unit			
A	12 (35.3)	1 (12.5)	0.40
B	22 (64.7)	7 (87.5)	
Lymphocyte count (median)	0.9 (0.8–1.4)	1.2 (0.7–2.8)	0.41
CRP (median)	27.9 (11.9–94.6)	66.2 (8.1–144.5)	0.57
CXR			
No CXR	20 (58.8)	5 (62.5)	0.05
No abnormalities	10 (29.4)	0	
COVID-19 abnormalities	4 (11.8)	3 (37.5)	
Immunosuppressed, yes	4 (11.8)	0	0.80

APKD, autosomal dominant polycystic kidney disease; CXR, chest X-ray.

serum ferritin levels are higher in patients with infection confirmed by PCR alone.¹⁹ There were no differences in pathology results between PCR-negative, antibody-positive patients and PCR-negative, antibody-negative patients, with the latter group likely having had an alternative, infectious etiology for their symptoms that prompted testing.

Although it is not yet known whether patients who have developed antibody responses to SARS-CoV-2 are protected from reinfection, data are emerging to suggest that acquired immunity with primary SARS-CoV-2 offers protection from re-exposure.²⁰ As such, antibody testing may help stratify patients at risk should another peak of infection occur. Although further longitudinal studies are required to determine the longevity of antibody responses, serial monitoring for antibodies may enable population-level assessment of infection rates within centers over time. This could help further

identify risks and therefore, interventions to minimize disease transmission within dialysis centers. In addition, seroprevalence of health care workers within dialysis centers may also enable appropriate assignment of staff to try and limit bidirectional transmission of infection.

This study has several limitations, which include lack of clarity of the timing of infection. Screening at our center started on March 9, with the first confirmed case on March 13, 2020. Although we are unable to confirm undiagnosed infection prior to this date, it is unlikely that the large proportion of patients with asymptomatic disease we detected were all infected prior to the implementation of the national screening guidelines. The results, therefore, support the statement that our current screening strategy is limited in its ability to identify all active infections.

To conclude, we believe that this is the first study to report on SARS-CoV-2

seroprevalence within an adult hemodialysis population. We have shown that seroprevalence is high and that asymptomatic disease in patients on dialysis is common. It is likely that effective screening strategies within hemodialysis populations need to consist of both PCR and antibody screening. As our understanding of the immune response to SARS-CoV-2 infection expands, seroprevalence data within the hemodialysis population are likely to transition from a screening tool to assessment of patient-level immunity, which will need to be acquired *via* vaccination in infection-naïve patients as the disease becomes endemic.

DISCLOSURES

M. Griffith reports an educational grant from Vifor Pharmaceuticals for £400 to attend the American Society of Nephrology 2019, outside the submitted work. All remaining authors have nothing to disclose.

FUNDING

This research is supported by the National Institute for Health Research (NIHR) Imperial Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London.

ACKNOWLEDGMENTS

The authors thank the West London Kidney Patient Association, all of the patients and staff at Imperial College Healthcare NHS Trust (the Imperial College Healthcare NHS Trust renal COVID-19 group and dialysis staff, in particular Ms. Claire Edwards and Mr. Colin Smith), and staff within the North West London Pathology laboratories (Dr. Mary Guckian and Mr. Graham Pickard). The authors are also grateful for the support from Hari and Rachna Murgai, and Milan and Rishi Khosla.

Dr. Liz Lightstone reports grants from Roche, outside the submitted work.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at <http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2020060827/-/DCSupplemental>.

Supplemental Table 1. Comparison of characteristics in antibody-positive and -negative patients without a PCR test.

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Table S1. Comparison of characteristics in antibody positive and negative patients without a PCR test

Variable		No swab, Ab- N=191 (%)	No swab, Ab+ N=44 (%)	p value
Gender	Female	67 (35.0)	17 (38.6)	0.73
	Male	124 (64.9)	27 (61.3)	
Age	Years (median)	69 (59-78)	66 (58-75)	0.25
Ethnicity	Black	22 (11.5)	7 (15.9)	0.84
	Caucasian	51 (26.4)	11 (25.0)	
	Indoasian	78 (40.8)	19 (43.2)	
	Other	40 (20.9)	7 (15.9)	
Cause of ESKD	APKD	12 (6.3)	4 (9.0)	0.12
	Diabetic nephropathy	74 (38.7)	11 (25.0)	
	Glomerulonephritis	35 (18.3)	8 (18.1)	
	Other	30 (15.7)	5 (11.3)	
	Unknown	34 (17.8)	13 (29.5)	
	Urological	6 (3.1)	3 (6.8)	
Time at ESKD	Years (median)	2.2 (0.92-4.2)	1.6 (0.94-4.2)	0.82
Unit	A	35 (18.3)	8 (18.2)	1.00
	B	156 (81.6)	38 (86.3)	
Lymphocyte Nadir	Median (10⁹/l)	1.2 (0.9-1.6)	1.1 (0.9-1.5)	0.52
CRP Peak	Median (mg/l)	7.7 (2.9-20.7)	7.2 (2.6-12.0)	0.87
Ferritin Peak	Median (µg/l)	391 (294-545)	431 (324-532)	0.57
Immunosuppressed	Yes	36 (18.8)	7 (15.9)	0.83