Detection of SARS-CoV-2 Antibodies in Kidney Transplant Recipients

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Kidney transplant recipients and other patient groups receiving immunosuppression have a poor prognosis following presentation with symptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[1] The immune response to SARS-CoV-2 in an immunocompromised population has not been systematically reported. Recognition that humoral immune responses against common viral infections are blunted in such patients has led to their exclusion from validation studies of serologic assays for SARS-CoV-2.[2,3] In this study, we analyze the seroprevalence of SARS-CoV-2 antibodies in a transplant population. In order to ensure the accuracy of the seroprevalence rate, we also evaluate the performance of different serologic assays within this patient cohort.

We investigated 855 consecutive kidney transplant recipients who attended the phlebotomy service at the Imperial College Renal and Transplant Centre (ICTRC) London in June 2020 for SARS-CoV-2 antibodies. Patient demographics were obtained from the ICRTC transplant registry (Table 1). The study was approved by the Health Research Authority, Research Ethics Committee (reference: 20/WA/0123).

Sera from all patients were tested for the presence of nucleocapsid protein (NP) antibodies using the Abbott SARS-CoV-2 IgG assay on the Abbott Architect system. Samples were interpreted as positive or negative according to the manufacturer's instructions with a cutoff index of 1.4.[4] All samples with an index value of >0.25 (Supplemental Material) were run on a second assay, the Fortress Diagnostics COVID-19 Total Antibody assay, which is a nonquantitative two-step antigen sandwich ELISA that detects total Ig against the receptor binding domain (RBD). Samples are interpreted as positive on the basis of a cutoff value from negative controls assayed on the sample microplate. Samples showing discordant results on the Abbott and Fortress assays were additionally tested using a commercially available lateral flow immunoassay (LFIA; Biomedomics Inc.), which detects both IgM and IgG to the recombinant antigen MK201027 of the RBD.[5] The assay was used as per the manufacturer's instructions, results were assessed by two independent blinded observers, and only IgG results were considered. Serologic samples taken from 85 health care workers (HCWs) with RT-PCR–confirmed infection were used to compare assay performance in an immunocompetent population.

Statistical and graphical analyses were performed with MedCalc v19.2.1. The two-sided level of significance was set at $P<0.05$. The 95% confidence interval (95% CI) of the seroprevalence was calculated from binomial probabilities using Wilson methods. Concordance between assays was analyzed using Cohen κ coefficient of qualitative results.

Sixty-nine of 855 patients tested positive for SARS-CoV-2 IgG using the Abbott assay, giving a seroprevalence of 8.1% (95% CI, 6.4 to 10.1). However, it was noted that 33 of 855 (3.9%) study patients had prior infection confirmed by RT-PCR, of whom 11 of 33 (33.3%) were serologically negative for IgG using the Abbott assay at a median time of testing of 36 (28–58) days postdiagnosis.

To investigate the lack of seroconversion versus inadequate assay sensitivity in an immunocompromised population, we tested samples from 38 transplant recipients (including 33 from our screening cohort) with PCR-confirmed infection across three assays. Patients were tested at a median time of 35 (22–53) days postdiagnosis. All paired historical control samples, which had been taken and stored from our study patients prior to July 2019, were negative for IgG across all three assays. The numbers of patients with antibodies detected by the Abbott, Fortress, and LFIA assays were 26 of 38 (68.4%), 35 of 38 (92.1%), and 31 of 38 (81.6%), respectively (Table 2). Patient characteristics by antibody status are shown in Table 3. Three

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Table 1. Patient characteristics

Variable	Patients, n=855 (%)
Sex	
Men	546 (63.9)
Women	309 (36.1)
Age, yr	
Median	57 (45–66)
Ethnicity	
White	305 (35.7)
Black, Asian, and minority ethnic	550 (64.3)
Cause of ESKD	
ADPKD	82 (9.6)
GN	235 (27.5)
Diabetic nephropathy	199 (23.3)
Urologic	50 (5.8)
Unknown	213 (24.9)
Other	76 (8.9)
Time at ESKD	
Pre-emptive	172 (20.1)
Dialysis dependence	683 (79.9)
Median time, yr	1.7 (0.2–3.5)
Timing post-transplant, yr	
≤1	191 (22.3)
>1	664 (77.7)
Median time	3.7 (1.1–7.8)
Type of graft	
Deceased donor transplant	516 (60.4)
Living donor transplant	276 (32.3)
Simultaneous pancreas-kidney transplant	42 (4.9)
Antibody-incompatible transplant	21 (2.5)
Immunosuppression at diagnosis	
FK monotherapy	512 (59.9)
FK and MMF	187 (21.9)
FK and steroids	44 (5.1)
FK, MMF, and steroids	111 (13.0)
Other	1 (0.1)
Induction agent used	
Alemtuzumab	766 (89.6)
IL-2 receptor blocker	89 (10.4)
History of rejection	
Yes	115 (13.5)
No	740 (86.5)
Transplant number	
First	761 (89.0)
Greater than or equal to second graft	94 (11.0)
Diabetes	
Yes	312 (36.5)
No	543 (63.5)

ADPKD, autosomal dominant polycystic kidney disease; FK, tacrolimus; MMF, mycophenolate mofetil.

Table 2. Test characteristics as determined by sampling historic and current sera from 38 patients who were SARS-CoV-2 RT-PCR positive

Result	Abbott	Fortress Diagnostics	Biomedomics (LFIA)
Historic negative controls			
Positive	0	0	0
Negative	38	38	38
Positive controls			
Positive	26	35	32
Negative	12	3	6
Sensitivity	68.4 (51.3–82.5)	92.1 (78.6–98.3)	84.2 (68.7–94.0)
Specificity	100.0 (92.3–100.0)	100.0 (92.3–100.0)	100.0 (92.3–100.0)
Positive predictive value	100.0	100.0	100.0
Negative predictive value	79.3 (70.6–6.0)	93.9 (83.8–97.8)	88.5 (78.6–94.1)
Accuracy	85.7 (76.4–92.4)	96.4 (89.9–99.3)	92.9 (85.1–97.3)

of 38 (7.9%) patients did not have detectable antibodies on any assay, and these patients may represent true failure to seroconvert.

To compare assay performance in an immunocompetent population, we tested 85 HCWs with RT-PCR–confirmed infection. At a median time of 31 (19–45) days postdiagnosis, three of 85 (3.5%) HCWs had no detectable antibodies by either the Abbott or Fortress assay, and an additional five of 82 (6.1%) HCWs had no antibodies detected by the Abbott assay. The sensitivity values of the Abbott and Fortress assays in HCWs were 90.6% (95% CI, 82.5 to 95.2) and 96.5% (95% CI, 90.1 to 98.8), respectively. Although there was no difference in the proportion of detectable antibody between the immunosuppressed patients and HCWs using the Fortress assay (P=0.30), immunosuppressed patients were less likely to have a positive serologic test using the Abbott assay compared with HCWs (P=0.002).

To investigate potential missed cases of patients who were SARS-CoV-2 IgG positive in our overall cohort screened by the Abbott assay alone, we re-examined the 822 study patients without confirmed infection; 147 of 822 (17.9%) patients had an antibody index value of >0.25 by the Abbott assay, of which 100 had a value between 0.25 and 1.4 and 47 patients had a value ≥1.4. All but four patients were retested using the Fortress assay, and discordant results were seen in 18 of 143 (12.6%) patients. Twelve (12%) of 100 patients negative on the Abbott assay were positive on the Fortress assay, whereas six positive patients by the Abbott assay were negative on the Fortress assay. When these 18 samples were tested by the LFIA, agreement was seen with the Fortress assay in 14 of 18 (77.8%) patients (Supplemental Material). Analyzing the concordance of the assays, we found only a moderate agreement between the Abbott and Fortress assays (κ=0.73 [0.64–0.82]) and between the Abbott and LFIA assays (κ=0.60 [0.46–0.74]), whereas concordance between the Fortress and LFIA assays was strong (κ=0.86 [0.77–0.95]).

On amalgamating the results of the Fortress and Abbott assays, the overall seroprevalence in our transplant cohort increased to 10.4% (95% CI, 8.5 to 12.6).

The finding of a seroprevalence of 10.4% (95% CI, 8.5 to 12.6) in a cohort of shielded patients with kidney transplants was higher than expected, albeit in patients from a region with a community seroprevalence rate of 13% (Ward H, Atchison CJ, Whitaker M, Ainslie KCE, Elliott J, Okell LC, et al.: Antibody prevalence for SARS-CoV-2 in England following first peak of the pandemic: REACT2 study in 100,000 adults. medRxiv, 2020 10.1101/2020.08.12.20173690). Notably, our study demonstrates the influence of the assay utilized to detect SARS-CoV-2 antibodies and hence, estimate seroprevalence in an immunosuppressed cohort.

Our results indicate that the Fortress ELISA and LFIA are more sensitive than

Table 3. Patient characteristics by antibody status and assay in patients who were RT-PCR positive

Patient No.	Abbott	Fortress	LFIA	Timing of Test Postdiagnosis (d)	Age, yr	Sex	Ethnicity	First Year Post-Transplant	Induction Agent Used	Immunotherapy at Diagnosis
22	Negative	Negative	Negative	35	53	Man	BAME	Yes	Alemtuzumab	FK
29	Negative	Negative	Negative	28	28	Woman	BAME	Yes	Alemtuzumab	FK
36	Negative	Negative	Negative	27	72	Man	White	Yes	Alemtuzumab	FK, MMF
1	Negative	Positive	Positive	65	63	Man	BAME	No	Unknown	FK, MMF
6	Negative	Positive	Positive	70	48	Man	BAME	No	Alemtuzumab	FK
11	Negative	Positive	Positive	55	46	Woman	BAME	Yes	Alemtuzumab	FK, MMF
13	Negative	Positive	Positive	60	79	Woman	BAME	No	Alemtuzumab	FK
16	Negative	Positive	Positive	46	43	Man	BAME	Yes	Alemtuzumab	FK, MMF
18	Negative	Positive	Positive	37	67	Woman	BAME	No	Alemtuzumab	FK, MMF
31	Negative	Positive	Positive	29	76	Man	White	No	Alemtuzumab	FK, MMF
38	Negative	Positive	Positive	9	55	Man	BAME	No	Alemtuzumab	FK
33	Negative	Positive	Negative	21	66	Man	BAME	Yes	Basiliximab	FK, MMF
7	Positive	Positive	Negative	73	50	Man	White	No	Alemtuzumab	FK
26	Positive	Positive	Negative	28	49	Woman	BAME	Yes	Alemtuzumab	FK, Pred
24 patients were positive across all three assays				32 (22–41) (median)	52±13 (mean)	14 (58.3%) man	5 (20.8%) White	3 (12.5%) yes	19 (79.2%) alemtuzumab	9 (37.5%) (FK, MMF, Pred)

Paired historic sera from all patients were negative across the three assays. BAME, Black, Asian, and minority ethnic; FK, tacrolimus; MMF, mycophenolate mofetil; Pred, prednisolone.

the Abbott test at detecting SARS-CoV-2 antibodies in kidney transplant recipients. We also showed that there was better concordance between the Fortress and LFIA assays compared with the Fortress and Abbott assays, which also suggests the potential importance of the target antigen in the serologic assays. The LFIA and Fortress assays share the RBD as their target antigen, whereas the Abbott assay utilizes the NP. It may be proposed that the RBD is more immunogenic than the NP, making it a better stimulus for an immune response in patients who are immunosuppressed. Recently published evaluation studies from Public Health England support this, suggesting that assays targeting NP are less sensitive in immunocompetent populations too.[6] Further, we have demonstrated that the Abbott assay was significantly less likely to detect antibody in the immunosuppressed population compared with HCWs. In addition to the greater sensitivity of RBD assays, there is evidence that RBD antibodies may provide information on functional immunity given reported correlations between RBD antibodies and neutralizing antibodies.[7,8] It, therefore, follows that assays utilizing the RBD, rather than the NP, may be clinically more relevant for immunosuppressed patients.

Our study would have been strengthened by analyzing larger numbers of patients who were RT-PCR positive, incorporating serial sampling, and including demographic data on our HCWs, and we acknowledge that we have not been able to exclude discordance related to the detection of IgM or IgA by the Fortress assay. However, to our knowledge, this is the first SARS-CoV-2 seroprevalence study in patients with transplant**s**, and we have shown that immunoassays that incorporate the RBD as their antigenic target may be superior in testing for SARS-CoV-2 antibodies, without compromising specificity (Table 2). This finding may be seen in immunocompetent people but seems to have a greater effect in an immunosuppressed transplant population.[6]

DISCLOSURES

P. Kelleher reports scientific advisor or membership as an editorial board member for HIV medicine. S. McAdoo reports consultancy agreements with GSK; and honoraria from Rigel Pharmaceuticals, ThermoFisher Scientific, and Celltrion. L. Moore reports research funding from the Chelsea and Westminster Hospital Charity and National Institute of Health Research; honoraria from Eumedica and Profile Pharma; scientific advisor or membership with bioMerieux, Pfizer, and Umovis Lab; and speakers bureau for bioMerieux, Pfizer, and Umovis Lab. N. Mughal reports honoraria from Baxter, Eudmedica, and Pfizer. M. Willicombe reports research funding from Chiesi Pharmaceuticals. All remaining authors have nothing to disclose.

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C. Clarke, S. Mcadoo, M. Prendecki, and M. Willicombe conceived the project; S. Gleeson, E. Santos, and M. Willicombe obtained samples; C. Clarke, L. Greathead, M. Guckian, S. Mcadoo, L. Moore, N. Mughal, M. Prendecki, and E. Santos processed the samples; S. Gleeson, L. Greathead, and M. Willicombe obtained data; P. Kelleher and P. Randell supervised research; M. Prendecki and M. Willicombe analyzed the

data; C. Clarke, S. Mcadoo, M. Prendecki, and M. Willicombe wrote the first draft of paper; and all authors reviewed and approved the final manuscript.

DATA SHARING STATEMENT

Data are available from the corresponding author upon reasonable request.

SUPPLEMENTAL MATERIAL

This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2020081152/-/DCSupplemental.

Supplemental Table 1. SARS CoV-2 antibody detection in PCR-positive healthcare workers and historic control.

Supplemental Table 2.Comparison of SARS CoV-2 antibody detection by the Fortress and Abbott Assays.

Supplemental Figure 1. ROC curve analysis of the Abbott and Fortress serology tests for diagnosis of previous SARS-CoV-2 infection.

Supplemental Table 3. Comparison of assay results.

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Supplemental Data

Fortress Diagnostics COVID-19 Total Antibody Validation Results

The manufacturer's validation study included 1000 plasma/serum samples from 500 subjects (with PCR-proven infection) and 500 healthy controls, giving a test performance of:

- Sensitivity: 94.60% (95% CI: 92.26, 96.26)
- Specificity: 100% (95% CI: 99.24, 100.00)

Samples tested

- 259 serum samples were used for the sensitivity analysis and stratified into different groups.
    - Samples from 237 different patients
    - 22 patients had 2 samples
- 74 samples from archived sera (pre-outbreak) from patients with no history of COVID-19 (expected result non-reactive)
- 185 samples from PCR-positive cases with known time from onset of symptoms (both patients and staff).
- All samples were tested in parallel with a SARS CoV-2 NP IgG assay (Abbott Architect).

Table S1.

		Equivocal	Negative	Positive	Total
Negative controls		1 (1.35%)	73 (98.7%)	0	74
Positive controls	<7 days	0	12 (46.2%)	14 (53.9%)	26
	7-14 days	1 (2.17%)	4 (8.70%)	41 (89.1%)	46
	>14 days	0	4 (5.41%)	70 (94.6%)	74
	>21 days	0	3 (7.7%)	36 (92.3%)	39
Total		2	96	161	259

Giving a sensitivity of 93.8% (87.7-97.5) at >14 days post diagnosis of infection and specificity 100.0% (95.1-100.0%).

A comparison against the Abbot assay was performed using the same samples. Abbott assay gave a sensitivity of 87.6% (80.1-93.1) at >14 days post diagnosis of infection.

Table S2.

			FORTRESS			
			Equivocal	Negative	Positive	Total
ABBOTT	Negative control		1	73	0	74
	<7 days	Fortress	0	12	14	26
		Negative		11	7	18
		Positive		1	7	8
	7-14 days	Fortress	1	4	41	46
		Negative	1	4	11	16
		Positive		0	30	30
	>14 days	Fortress		4	70	74
		Negative		3	5	8
		Positive		1	65	66
	>21 days	Fortress		3	36	39
		Negative		3	3	6
		Positive		0	33	33
			2	96	161	259

Figure S1. ROC curve analysis of the Abbott and Fortress serology tests for diagnosis of previous SARS-CoV-2 infection

ROC curves of Abbott serology testing including samples from patients with confirmed SARS-CoV-2 infection by RT-PCR and historic control samples with varying cut-off values and corresponding sensitivity and specificity indicated. (A) Manufacturers specified cut off of 1.4 and (B) Optimal cut-off of 0.24.

ROC curves of Fortress serology testing including samples from patients with confirmed SARS-CoV-2 infection by RT-PCR and historic control samples with varying cut-off values and corresponding sensitivity and specificity indicated. (C) Manufacturers specified cut-off of 1 and (D) Optimal cut-off of 1.

Confidence intervals for the area under the curve (AUC) was calculated using the Wilson/Brown method and optimal cut-off calculated using the Youden index.

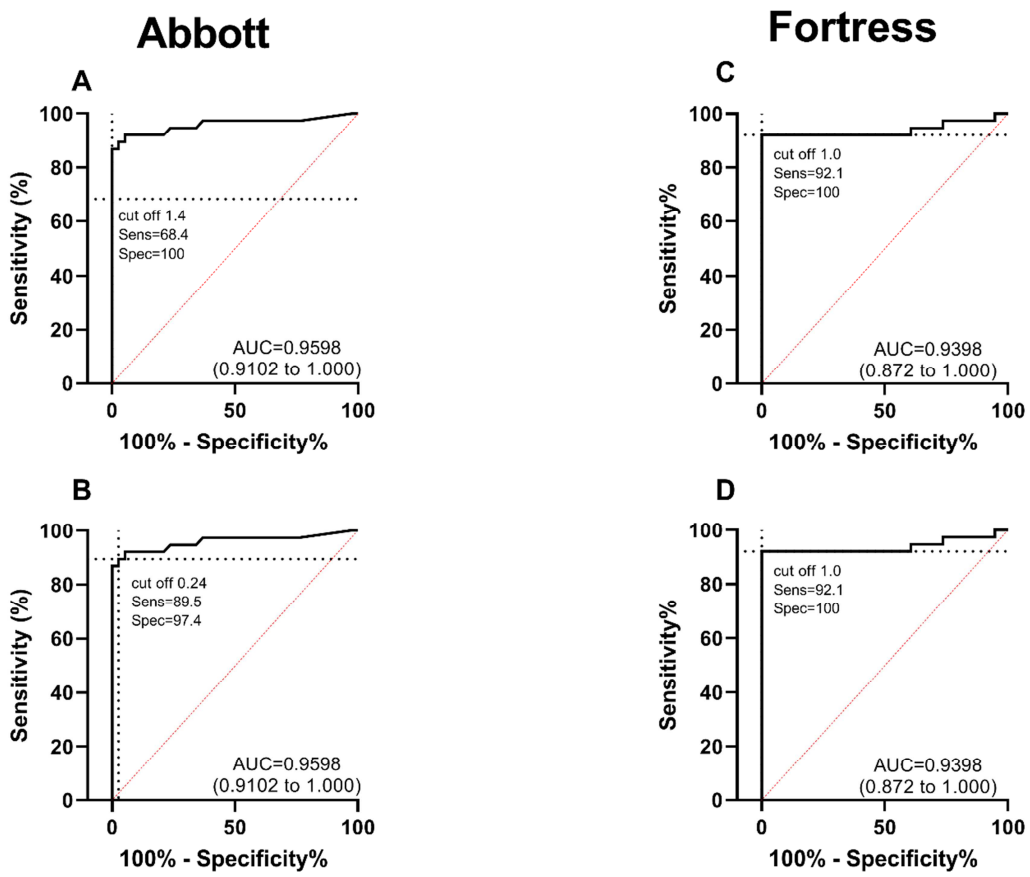
Abbott
Fortress
A
B
C
D
Sensitivity (%)
Sensitivity%
100% - Specificity%
cut off 1.4
Sens=68.4
Spec=100
AUC=0.9598
(0.9102 to 1.000)
cut off 0.24
Sens=89.5
Spec=97.4
AUC=0.9598
(0.9102 to 1.000)
cut off 1.0
Sens=92.1
Spec=100
AUC=0.9398
(0.872 to 1.000)
cut off 1.0
Sens=92.1
Spec=100
AUC=0.9398
(0.872 to 1.000)

Table S3. Comparison of assay results

	Abbott Assay		**Fortress Assay**		**Abbott Assay**		**LFIA**		**Fortress Assay**		**LFIA**
Negative	150	**Positive**	21		62	**Positive**	16		47	**Positive**	0
		Negative	129			**Negative**	46			**Negative**	47
Positive	69	**Positive**	63		69	**Positive**	59		84	**Positive**	75
		Negative	6			**Negative**	10			**Negative**	9
Total	**219**		**219**		**131**		**131**		**131**		**131**