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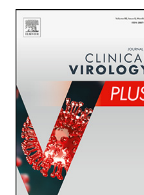

High SARS-CoV-2 seroprevalence in Lagos, Nigeria with robust antibody and cellular immune responses

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ABSTRACT

Background: Early evidence suggested that the impact of the COVID-19 pandemic was less severe in Africa compared to other parts of the world. However, more recent studies indicate higher SARS-CoV-2 infection and COVID-19 mortality rates on the continent than previously documented. Research is needed to better understand SARS-CoV-2 infection and immunity in Africa.

Methods: In early 2021, we studied the immune responses in healthcare workers (HCWs) at Lagos University Teaching Hospital (n = 134) and Oxford-AstraZeneca COVID-19 vaccine recipients from the general population (n = 116) across five local government areas (LGAs) in Lagos State, Nigeria. Western blots were used to simultaneously detect SARS-CoV-2 spike and nucleocapsid (N) antibodies (n = 250), and stimulation of peripheral blood mononuclear cells with N followed by an IFN-γ ELISA was used to examine T cell responses (n = 114).

Results: Antibody data demonstrated high SARS-CoV-2 seroprevalence of 72·4% (97/134) in HCWs and 60·3% (70/116) in the general population. Antibodies directed to only SARS-CoV-2 N, suggesting pre-existing coronavirus immunity, were seen in 9·7% (13/134) of HCWs and 15·5% (18/116) of the general population. T cell responses against SARS-CoV-2 N (n = 114) were robust in detecting exposure to the virus, demonstrating 87·5% sensitivity and 92·9% specificity in a subset of control samples tested. T cell responses against SARS-CoV-2 N were also observed in 83.3% of individuals with N-only antibodies, further suggesting that prior non-SARS-CoV-2 coronavirus infection may provide cellular immunity to SARS-CoV-2.

Conclusions: These results have important implications for understanding the paradoxically high SARS-CoV-2 infection with low mortality rate in Africa and supports the need to better understand the implications of SARS-CoV-2 cellular immunity.

1. Introduction

Early projections of SARS-CoV-2 spread in Africa fueled fears that the healthcare infrastructure on the continent would be ill prepared to cope with the anticipated COVID-19 hospitalizations and deaths [1]. However, as of October 2022, 12·1 million COVID-19 cases and 256,000 deaths have been reported in Africa, representing ~2% and ~4% of global statistics, respectively. Early studies indicated a less severe epidemiological picture of COVID-19 in Africa, with a higher proportion of cases resulting in asymptomatic infections and lower mortality compared to other parts of the world [2]. Several factors may help explain these phenomena including the younger age, lower age-related co-morbidities, climate and environmental factors, and weak health systems for disease surveillance [3–5].

In Africa, SARS-CoV-2 prevalence studies conducted in blood donors, healthcare workers (HCWs), pregnant women, and others have de-

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scribed varying rates throughout the pandemic, ranging from 0·4% in Cape Verde (June-July 2020) to more than 49% in Kenya (August 2020-October 2021) and 79% across 12 states in Nigeria (June-August 2021) [6–8]. In most cases, the reported prevalence of antibodies against SARS-CoV-2 was several orders of magnitude higher than would be expected from PCR confirmed cases in the same time period. However, more recent evidence indicates an underestimation of COVID-19′s impact in Africa. In Zambia, postmortem surveillance conducted between June 15 and October 1, 2020, detected SARS-CoV-2 RNA in ~20% of subjects sampled within 48h of death, and only two of the 70 had been diagnosed with SARS-CoV-2 prior to death [9]. A follow-up study showed that during peak transmission periods, approximately 90% of all deceased individuals tested positive for COVID-19 [10]. While these studies demonstrate under-reporting of SARS-CoV-2 infection, there remains a lack of evidence for major increases in mortality, at least suggestive of lower pathogenic impact.

The humoral response to SARS-CoV-2 has been the primary focus of most studies conducted in Africa. A study of pre-pandemic samples in Gabon and Senegal, demonstrated significant pre-existing immunity based on antibodies directed to the SARS-CoV-2 spike (S) and nucleocapsid (N) proteins, in contrast to samples from Canada, Brazil and Denmark [11]. However, Gabonese sera with or without antibodies to SARS-CoV-2 N were unable to neutralize the virus in vitro or in mouse infection studies. These data, along with other published studies confirm pre-existing humoral responses cross-reactive to SARS-CoV-2 that may be qualitatively and quantitatively distinct in Africa.

The study of T cell responses has also been used in research and clinical settings during the COVID-19 pandemic to provide further insights into the immune response to infection and/or vaccination. In contrast to humoral responses, T cell responses to human coronaviruses may be long-lasting even many years after infection [[12],[13]]. SARS-CoV-2-specific T cells were maintained at six to nine months following primary infection, indicating that T cell immunity may persist beyond antibody responses [12]. Preclinical development of SARS-CoV-2 vaccines have demonstrated T cell responses accompanying antibody development and clinical studies are now demonstrating similar timelines following vaccination [14]. These findings, together with studies that have demonstrated a role for T cells in viral clearance, suggest that cell-mediated immune responses may be an important component of protection against SARS-CoV-2 [15]. Here we report the results of antibody and T cell analyses using Nigerian samples from HCWs and a group of vaccine recipients from the general population prospectively followed post-vaccination. To our knowledge, this is the first study to examine both the antibody and T cell response to SARS-CoV-2 in West Africa.

2. Methods

2.1. Study population and ethics statement

Our original study in early 2021 proposed to only recruit HCWs, a population with high-risk occupational exposure to COVID-19. However, the roll-out of COVID-19 vaccines in Nigeria at the end of March 2021 enabled collaboration with the Lagos State COVID-19 Taskforce to study of vaccine recipients from communities in Lagos state. The Oxford-AstraZeneca vaccine, with its low cost and simple refrigeration requirements enabled equitable access for low- and middle-income countries and was the first non-profit vaccine to report efficacy. The recommended two doses were administered intramuscularly with an interval of 8–12 weeks between doses.

The HCW cohort was recruited from the Lagos University Teaching Hospital (LUTH). Study participation included a brief clinical examination, clinical questionnaire including demographics and exposure, and venous blood sample. HCWs included some with documented prior COVID-19 infection by PCR ($n = 40$). HCW samples were collected between March and October 2021.

The Lagos State Vaccine study population included individuals from the general population from Agbowa, Amuwo, Ikorodu, Iwaya, and Oshodi, Lagos State, Nigeria, all of whom, at the time of enrollment (baseline), had no documented history of SARS-CoV-2 infection and were administered the first dose of the Oxford-AstraZeneca COVID-19 vaccine between March to April 2021 and the second dose 8 weeks later. A brief questionnaire on demographics and exposure was administered at baseline, and venous blood samples were collected at baseline (immediately prior to administration of the first dose), 7-, 14-, and 84-days after vaccination for immune response testing.

All individuals from each of the two cohorts provided written informed consent for the collection of samples and data. The HCW study received ethical clearance from the Harvard T.H. Chan School of Public Health Institutional Review Board (IRB, Protocol #: IRB-21–0329) and the LUTH Health Research Ethics Committee (HREC, Protocol #: ADM/DCST/HREC/APP/4192). The original Lagos State COVID-19 Vaccine study was reviewed and approved by the LUTH HREC (Protocol #: ADM/DCST/HREC/APP/4207), and the secondary analysis of these samples was determined not human subjects research by the Harvard IRB (Protocol #: IRB21–1350).

2.2. Virion lysate

Briefly, Vero E6 cells infected with SARS-CoV-2 (Isolate USA-WA1/2020, BEI Resources NR-52,281) and propogated for five days. Supernatant was then clarified at 10,000 x g for 20 mins at 4 °C, precipated with PEG-8000 and NaCl, and then resolved by sucrose gradient ultracentrifugation at 170,000 x g for 90 mins at 4 °C. Viral pellets were lysed with complete NP40 buffer containing protease inhibitors.

2.3. Western blot

Aliquots of virion lysates were added to nonreducing buffer (final concentrations of 2% SDS, 0·5 M Tris pH 6·8, 20% glycerol, 0·001% bromophenol blue) and subjected to 12% PAGE and Western blot analysis using patient serum (1:250) as primary antibody and anti-human IgG horseradish peroxidase (HRP) (1:2000; ThermoFisher Scientific, Waltham, MA) as secondary antibody. Visualization was performed using Metal Enhanced DAB Substrate Kit (ThermoFisher Scientific, Waltham, MA) per the manufacturer's instructions. Reference antisera to recombinant based S and N antigens verified the SARS CoV-2 antigens. In addition, control negative (pre-pandemic) and PCR-confirmed SARS-CoV-2 positive samples were used to verify SARS-CoV-2 S and N reactivity with each immunoblot assay.

2.4. Image analysis

Western blots were analyzed using image processing software, ImageJ (NIH), to machine-read and quantify SARS-CoV-2 S antibody signals. The average pixel intensity was quantified at the Western blot SARS-CoV-2 S band, background areas, and control band. The control bands were visualized with an anti-SARS-CoV-2 S (41,050-D005, SinoBiological, China) antibody. The background-adjusted SARS-CoV-2 S band signal was then normalized to the background-subtracted control band and expressed as % of the control.

2.5. T cell stimulation

From each individual, 3 mL whole blood was collected in a vacutainer tube treated with lithium or sodium heparin (BD, Franklin Lakes, NJ), and tubes were inverted ten times to ensure that the blood mixed thoroughly with the anticoagulant. One (1) mL whole blood was then pipetted into three MASI Stimulator Tubes (Mir Biosciences, New Jersey) either coated with SARS-CoV-2 N, positive control (phytohemagglutinin, PHA), or negative control (PBS). All tubes were then inverted 25 times to ensure that the whole blood mixed thoroughly along the inner

walls of the MASI Stimulator Tubes. The MASI Stimulator Tubes were incubated in a humidified incubator at 37 °C with 5% CO_2 for 18–22 h. After the incubation step, the MASI Stimulator Tubes were centrifuged at 2500 x g for five minutes and the supernatant was aliquoted into 1 mL cryotubes and immediately processed by the IFN-γ ELISA.

The assay used to study T cell responses is based on a commercial assay (Mir Biosciences, New Jersey), which uses a bacterial toxin to deliver proteins of interest into the cytosol of antigen presenting cells for peptide processing and presentation to T cells. Specifically, the assay uses the *Bacillus anthracis* lethal toxin with deletion of its N-terminal catalytic domain (LFn) [16]. LFn has previously been used study T cell responses to HIV, Ebola, Zika and dengue viruses [17–21].

For the LFn-SARS-CoV-2 N construct, the N-terminal domain of lethal factor and SARS-CoV-2 N separated by a $(GGS)_2$ linker was codon optimized and cloned into the pET28b(+) expression vector with a C-terminal His-tag. This was expressed in *E. coli* BL21 (DE3) and purified by immobilized metal affinity chromatography. The final product was desalted into PBS, pH 7.2 and used to coat the inner wall of the MASI Stimulator Tube. The other MASI Stimulator Tubes were coated with PHA as a positive control and PBS as a negative control. Whole blood from each individual were exposed to either LFn-SARS-CoV-2 N, PHA or PBS and supernatants were processed via a commercial IFN-γ ELISA (Mir Biosciences, Inc., New Jersey).

2.6. IFN-γ ELISA

Supernatants (100 μl) collected from the MASI Stimulator Tubes were screened for the presence of human IFN-γ by the MASI-COVID enzyme-linked immunosorbent assay (ELISA) (Mir Biosciences, Boston, MA), according to the manufacturer's instructions. The presence of IFN-γ was captured using a microplate reader (optical density 450 nm). Assay performances were monitored using internal controls and cutoffs were determined as specified by the manufacturer for the kit. A result was considered positive if the IFN-γ response measured >5.4 IU/mL, according to the manufacturer's instructions.

2.7. Statistics

For both the HCW and general population vaccine recipient cohorts, we calculated baseline SARS-CoV-2 S and N antibody seroprevalence determined by Western blot as percentage of total. Using these seroprevalence categories, we evaluated T cell responses after stimulation with SARS-CoV-2 N by plotting IFN-γ ELISA signal in a subset of HCW baseline samples and 7-day post-vaccination general population samples. Additionally, for the vaccine recipient cohort, we used the Western blot and image analysis to evaluate development of SARS-CoV-2 S antibodies in sequential samples post-vaccination by calculating mean S signal and change over time. The mean S antibody signal between groups was compared by T-test. Finally, we calculated sensitivity and specificity of IFN-γ responses against SARS-CoV-2 N. All statistics and plots were generated using Prism (version 9·0·0).

2.8. Role of the funding source

Authors declare that the funder did not have any role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

3. Results

Our study included a cohort of 134 HCWs working at LUTH. Forty HCWs previously tested positive for COVID-19 by RT-PCR and 94 did not have history of documented SARS-CoV-2 infection (Table 1). The second cohort included 116 individuals from the general population across five local government areas of Lagos State: 24 from Agbowa, 23 from Amuwo, 25 from Ikorodu, 22 from Iwaya, and 22 from Oshodi (Table 1). For these individuals, baseline and follow-up samples were collected between March and July 2021.

We analyzed the SARS-CoV-2 seroprevalence among HCWs based on antibodies directed against SARS-CoV-2 $S + N$ or S-only. The overall seroprevalence of SARS-CoV-2 in HCWs was 72·4% (97/134) (Table 1). Of HCWs with previous RT-PCR confirmation of COVID-19 (convalescent), 100% (40/40) had SARS-CoV-2 $S + N$ or S-only antibodies (Table 1). Of HCWs without any prior history, 59·6% (56/94) had SARS-CoV-2 $S + N$ antibodies, whereas 1·1% (1/94) had S-only antibodies (Table 1).

We next examined HCWs with antibodies directed against SARS-CoV-2 N-only, suggestive of pre-existing coronavirus immunity. There was reactivity to N-only in 9·7% (13/134) of HCWs, all with no history of PCR-confirmed SARS-CoV-2 infection (Table 1). Additionally, 17·9% (24/134) of HCWs were seronegative to SARS-CoV-2.

We similarly analyzed the SARS-CoV-2 antibody profiles in the general population vaccine recipients. At baseline, the overall SARS-CoV-2 seroprevalence, based on antibodies against $S + N$ or S-only, was 60·3% (70/116), with reactivity to SARS-CoV-2 N-only in 15·5% (18/116), suggestive of pre-existing coronavirus immunity (Table 1). 24·1% of the general population vaccine recipients were seronegative (28/116). Across the five local government areas, SARS-CoV-2 seroprevalence ranged from 34·8% (8/23) in Amuwo to 72% (18/25) in Ikorodu. The reactivity to SARS-CoV-2 N-only ranged from 4.5% (1/22) in Iwaya to 22·7% (5/22) in Oshodi (Table 1).

Given that the Oxford-AstraZeneca COVID-19 vaccine is designed to generate immunity against SARS-CoV-2 S, we next examined the evolution of SARS-CoV-2 S antibodies post-vaccination. Most individuals, regardless of their SARS-CoV-2 antibody status at baseline, had detectable S antibodies post-vaccination (Fig. 1A-E). Interestingly, three of 116 individuals (2·6%), all of whom were seronegative at baseline, failed to develop SARS-CoV-2 S antibodies, even four weeks after the second vaccine dose (Fig. 1D-E).

At baseline, some individuals already had SARS-CoV-2 S antibodies, suggesting prior SARS CoV-2 infection, we also examined the percentage of S development that occurred in each timepoint post-vaccination. In individuals with SARS-CoV-2 $S + N$ antibodies at baseline, 74·4% of S antibody development occurred within 7-days post-vaccination, followed by 24·5% and 1·2% within 14- and 84-days post-vaccination, respectively (Fig. 2). For individuals with S-only antibodies or were seronegative at baseline, ~36·6% of S antibody development occurred within 7-days post-vaccination, followed by 56·4% and ~7% within 14- and 84-days post-vaccination, respectively (Fig. 2). *De novo* S antibody production in individuals with SARS-CoV-2 N-only antibodies at baseline differed compared to other groups, with relatively consistent levels between time periods. For these individuals, 22·9%, 39·9%, and 37·2% of S antibody development occurred within 7-, 14-, and 84-days post-vaccination, respectively (Fig. 2).

Lastly, we examined the T cell response against SARS-CoV-2 N in HCWs and individuals in the general population 7-days post-vaccination. In HCWs with SARS-CoV-2 $S + N$ antibodies, 82·6% (19/23) also had a T cell response against N, whereas a single HCW with N-only antibodies had a positive T cell response (1/1;100%) (Fig. 3A, Table 2). T cell testing was performed in 8 HCWs who were seronegative; 75·0% (6/8) did not have a T cell response against SARS-CoV-2 N (Fig. 3A, Table 2).

In individuals of the general population with SARS-CoV-2 $S + N$ antibodies, 51% (26/51) had a T cell response against SARS-CoV-2 N (Fig. 3B, Table 2). In individuals with N-only antibodies at baseline, 81.8% (9/11) had T cell responses against N (Fig. 3B, Table 2). In individuals who were seronegative, none (0/20) demonstrated T cell responses against SARS-CoV-2 N (Fig. 3B, Table 2).

Finally, we calculated the sensitivity and specificity to determine the efficacy of using T cell IFN-γ against SARS-CoV-2 N as a diagnostic biomarker for exposure. The positive control group consisted of 14 HCWs with previous RT-PCR confirmation of COVID-19 and the neg-

Table 1
SARS-CoV-2 antibody seroprevalence among healthcare workers and the vaccine recipients at baseline.

	Number	SARS-CoV-2: $S + N$, Number Positive (%)	SARS-CoV-2: S-only, Number Positive (%)	SARS-CoV-2: N-only, Number Positive (%)	Seronegative, Number (%)
HCW - COVID-19 convalescent	40	38 (95·0)	2 (5·0)	0 (0)	0 (0)
HCW - No history of COVID-19	94	56 (59·6)	1 (1·1)	13 (13·8)	24 (25·5)
Overall - HCWs	134	94 (70·15)	3 (2·24)	13 (9·70)	24 (17·91)
General Population – Vaccine Recipients (baseline)					
Agbowa	24	18 (75·0)	0 (0)	3 (12·5)	3 (12·5)
Amuwo	23	8 (34·8)	1 (4·4)	5 (21·7)	9 (39·1)
Ikorodu	25	18 (72·0)	0 (0)	4 (16·0)	3 (12·0)
Iwaya	22	12 (54·5)	1 (4·6)	1 (4·5)	8 (36·4)
Oshodi	22	12 (54·6)	0 (0)	5 (22·7)	5 (22·7)
Overall – Vaccine Recipients – All Sites	116	68 (58·62)	2 (1·72)	18 (15·52)	28 (24·14)

Abbreviations: S, Spike; N, Nucleocapsid.

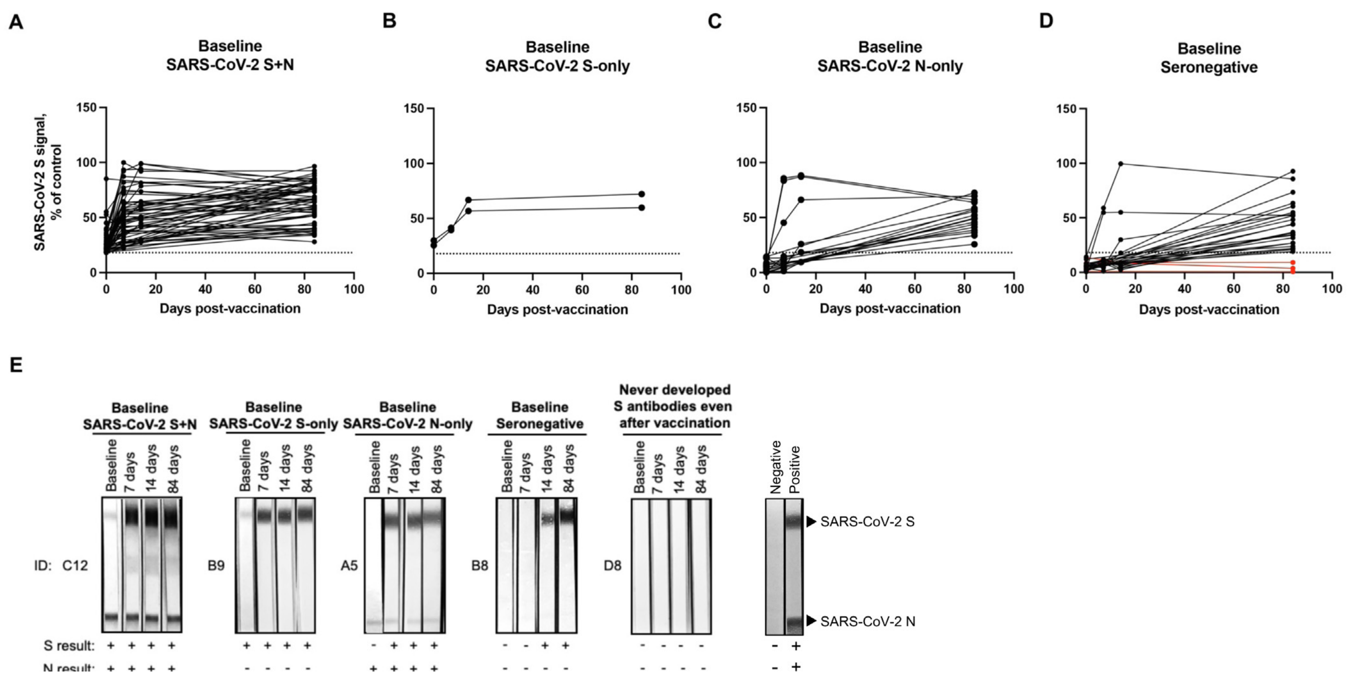

Fig. 1. Evolution of SARS-CoV-2 spike (S) in individuals in the general population post-vaccination. Sera sequentially collected from vaccine recipients in the general population were subjected to Western blot analysis. The post-vaccination evolution of SARS-CoV-2 S antibodies for each individual with baseline antibodies to A) SARS-CoV-2 $S + N$, B) S-only, C) N-only, or D) who were seronegative (red lines represent individuals who never developed S antibodies even after two vaccine doses) determined by image analysis where the S antibody signal was calculated and plotted as a % of control. The x-axis corresponds to number of days post-vaccination. The y-axis corresponds to background subtracted SARS-CoV-2 S signal normalized to the control line for each Western blot. Dashed line, cutoff. E) Representative image of Western blots for groups of individuals, including loading controls using COVID-19 positive and negative serum.

Table 2
T cell IFN-γ to SARS-CoV-2 N among HCWs and the vaccine recipients.

	Number Tested	SARS-CoV-2 Serostatus: $S + N$ Number Positive, T cell IFN-γ SARS-CoV-2 (% Positive)	SARS-CoV-2 Serostatus: S-only Number Positive, T cell IFN-γ SARS-CoV-2 (% Positive)	SARS-CoV-2 Serostatus: N-only Number Positive, T cell IFN-γ SARS-CoV-2 (% Positive)	Seronegative Number Positive, T cell IFN-γ SARS-CoV-2 (% Positive)
HCW- COVID-19 convalescent	14	12/14 (85.7)	–	–	–
HCW-No history of COVID-19	18	7/9 (77.8)	–	1/1 (100)	2/8 (25)
General Population – Vaccine Recipients	82	26/51 (51.0)	–	9/11 (81.8)	0/20 (0)
Total Tested[a]	114	50/74 (67.57)	-	10/12 (83.33)	2/28 (7.14)

[a] Total Tested is the sum of T cell tests among healthcare workers and vaccine recipients in the general population.

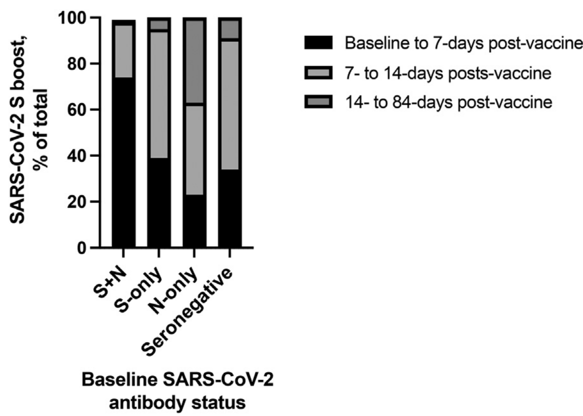

Fig. 2. Production of SARS-CoV-2 spike (S) in individuals in the general population post-vaccination. Sera sequentially collected from individuals in the general population were subjected to Western blot analysis. The level of antibody production was determined by subtracting the mean SARS-CoV-2 S antibody signal between baseline and 7-days post-vaccination, 7- and 14-days post-vaccination, and 14- and 84-days post-vaccination. The S signals from each time period were then summed and plotted as a% of total in a stacked format.

Table 3
Statistics of SARS-CoV-2 N T cell IFN-γ assay.

Statistic	Value	95% Confidence Interval
Sensitivity	87.5%	57.2% to 98.2%
Specificity	92.9%	76.5% to 99.1%
Positive Predictive Value	87.5%	57.2% to 98.2%
Negative Predictive Value	92.9%	76.5% to 99.1%
Accuracy	90.5%	77.4% to 97.3%

ative control group consisted of 28 HCWs and individuals in the general population who were seronegative. The resulting sensitivity was 87·5% (95% Confidence Interval (CI) = 57.2%–98.2%) and the specificity was 92·9% (95% CI= 76.5%–99.1%); the positive predictive value was 87.5% (95% CI = 57.2%–98.2%) and negative predictive value of 92.9% (95% CI = 76.5%–99.1%) (Table 3).

4. Discussion

In Africa, the immunological response to SARS-CoV-2 and non-SARS human coronaviruses represents a critical research gap that may shed light on the paradoxically high SARS-CoV-2 infection with low mortality rate compared to other parts of the world. The goal of the present study was to profile the antibody and T cell responses in natural SARS-CoV-2 infection and COVID-19 vaccination in two Nigerian cohorts. There are four findings worthy of mention from this study. First, antibodies directed against SARS-CoV-2 $S + N$, suggestive of previous exposure to the virus, were observed in a majority of individuals prior to vaccination. These results agree with other SARS-CoV-2 seroprevalence studies, suggesting a very high infection rate by 2021. Second, having antibodies to SARS CoV-2 N-only is suggestive of pre-existing coronavirus immunity, as represented by HCWs and the general population with N-only antibodies prior to vaccination. Third, most vaccine recipients with prior COVID-19 infection developed SARS-CoV-2 S antibody within 7 days of vaccination. Finally, T cell IFN-γ against SARS-CoV-2 N is a robust method to detect exposure to the virus or related coronaviruses as demonstrated by the high detection rate among the HCWs with prior PCR-confirmed COVID-19 and in those with N-only antibody responses.

Studies have shown that the antibody response to natural infection by SARS-CoV-2 is highly variable [22]. In some cases, individuals never produce a detectable antibody response, or low antibody titers have been observed in individuals with mild or asymptomatic infections [[22],[23]]. The vast majority of antibody studies have utilized ELISA assays to recombinant SARS CoV-2 antigens. Multiple studies now indicate reduced specificity of these assays when analyzing samples originating from sub-Saharan Africa [[11],[24]]. Of note, our study employed virion-based immunoblots which enabled simultaneous visualization of antibody responses to multiple SARS-CoV-2 antigens with multiple positive and negative controls to ensure specificity.

Our antibody data showed a high SARS-CoV-2 seroprevalence in 2021 in Nigeria with strong antibody reactivity. A majority of individuals, including 72.4% of HCWs and 60·3% of the general population vaccine recipients, had antibodies directed against SARS-CoV-2 $S + N$ or S-only prior to vaccination (Table 1). These data are in comparison to studies conducted in Nigeria between June and December 2020 showing lower seroprevalence rates ranging between 17%–25% [[25],[26]]. Possible explanations for the higher seroprevalence observed in 2021 may be due to the emergence of the highly transmissible Delta variant and the high risk of exposure for the HCWs in our study.

While COVID-19 vaccines have also been shown to induce robust antibody responses, there is strong evidence that these responses decline in the months following the second or booster doses [[27],[28]]. Our data demonstrate that a majority of general population vaccine recipients, regardless of baseline antibody status, developed SARS-CoV-2 S antibodies post-vaccination (Fig. 1A-E). However, three individuals (2·6% across the vaccine population) failed to develop an antibody response after two vaccine doses (Fig. 1D-E). It is possible that the Western blot had inadequate sensitivity to detect low titer S antibody responses.

Moreover, previous SARS-CoV-2 immunity appears to impact the level of S antibody production. Vaccination with the Oxford-AstraZeneca COVID-19 vaccine produced 74% of S antibodies within seven days in individuals who already had SARS-CoV-2 $S + N$ antibodies at baseline (Fig. 2). In contrast, only 39%, 23%, or 34% of S antibody production occurred within seven days in individuals who demonstrated S-only or N-only antibodies, or were seronegative at baseline, respectively (Fig. 2). Importantly, analysis was not performed on samples beyond 84-days post-vaccination; therefore, we are unable to character-

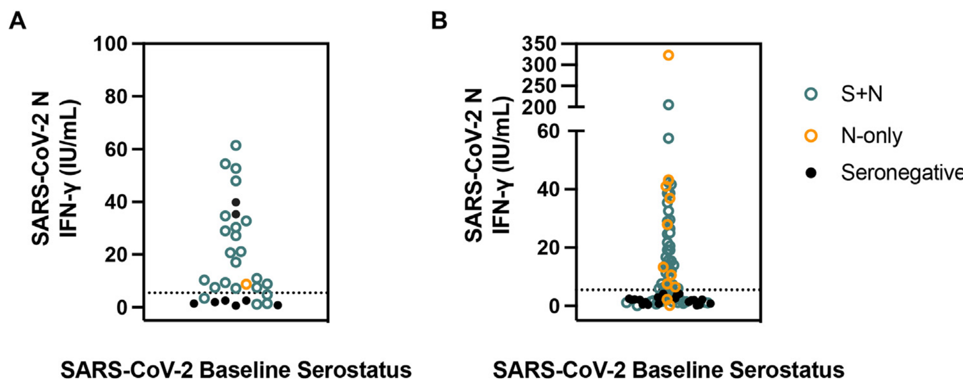

Fig. 3. T cell responses to SARS-CoV-2 nucleocapsid (N) in healthcare workers (HCWs) and individuals in the general populations. Whole blood samples were stimulated with SARS-CoV-2 N, then supernatants were processed via an IFN-γ enzyme-linked immunosorbent assay for A) healthcare workers and B) individuals in the general population who had antibodies to SARS-CoV-2 $S + N$, N-only, or were seronegative. Responses are expressed as an IU/mL. Dashed line, assay kit cutoff.

ize antibody waning. However, several studies have demonstrated substantial antibody waning after vaccination with the Oxford-AstraZeneca COVID-19 vaccine [29–31].

During infection with SARS-CoV-2, S and N proteins are major targets of both antibodies and T cells [32]. While SARS-CoV-2 S protein is more genetically diverse among coronavirus-infected humans and animals, the N protein is highly conserved [33]. Our antibody data suggests pre-existing coronavirus immunity with 9·7% (13/134) of HCWs and 15·5% (18/116) of individuals in the general population who only had SARS-CoV-2 N antibodies prior to vaccination. These results recapitulate other studies highlighting a similar phenomenon [[11],[24],[34]]. SARS-CoV-2 N seropositivity could suggest undocumented infections with other as yet unrecognized coronaviruses, including animal coronaviruses. There is 93%–100% homology between SARS-CoV-2 and several bat and pangolin N proteins at the amino acid level, and both alpha and betacoronaviruses have been isolated from animals in Central and West Africa [[11],[35],[36]]. The dynamics of antibody waning after natural infection is not well studied. It is possible that waning of S antibodies may result in the SARS-CoV-2 N antibody only profile. Prospective serologic studies are needed in these populations to further elucidate this finding.

We also report, for the first time, T cell responses against SARS-CoV-2 N in West Africans. The T cell assay used in this study does not require peripheral blood mononuclear cell (PBMC) isolation, offering ease-of-use and a more time-sensitive procedure from sampling to result. Instead, an individual's whole blood is collected in lithium heparin tubes, followed by T cell stimulation in the kit's specialized tubes coated with SARS-CoV-2 N protein or positive/negative controls. After stimulation, supernatants are processed via a one-step ELISA to measure IFN-γ secreted by T cells that responded to the SARS-CoV-2 N protein.

Evidence suggests that most individuals infected with SARS-CoV-2 generate IFN-γ-producing T cells that can be detected in peripheral blood as early as 2–4 days from the onset of symptoms or between 7 and 14 days after vaccination [[37],[38]]. A recent study of cellular responses in residents of rural Kenya who had not experienced any respiratory symptoms nor had contact with COVID-19 cases or received COVID-19 vaccines demonstrated that 78% of individuals possessed broadly reactive T cells specific to multiple SARS-CoV-2 antigens. Interestingly, 60% of these asymptomatic individuals lacked SARS-CoV-2 spike antibodies. Our results demonstrate robust T cell responses against SARS-CoV-2 N (Table 2). A high sensitivity and specificity in detecting exposure to SARS-CoV-2 based on whole blood IFN-γ was also observed (Table 3). In HCWs with previous RT-PCR confirmation of COVID-19, the sensitivity of T cell IFN-γ against SARS-CoV-2 N was 87·5%. The specificity of the assay in individuals who were seronegative was 92·9%.

A majority of individuals with N-only antibodies demonstrated T cell responses to SARS-CoV-2 N, suggesting that prior non-SARS-CoV-2 coronavirus infection may provide cellular immunity to SARS-CoV-2. In individuals from the general population who were seronegative, none had detectable T cell IFN-γ responses (Fig. 3B). However, two HCWs who were seronegative had a positive T cell response. These may have been false positives or the T cell response to SARS-CoV-2 N was skewed by pre-existing immunity to other pathogens including globally endemic human non-SARS coronaviruses [33].

This study has limitations. Our study populations of HCWs and the general population of vaccine recipients were relatively small. Second, while our results are suggestive of pre-existing coronavirus immunity, it was not within the scope of the study to confirm the specific coronavirus responsible for the response. Our study utilized virion-based immunoblots to simultaneously detect antibody responses against SARS-CoV-2 S and/or N. The detection of antibodies against multiple SARS-CoV-2 antigens is a unique feature of the assay. In contrast, ELISAs, generally allow for antibody analysis against a single SARS-CoV-2 antigen. Moreover, as cellular responses have been shown to be cross-reactive, the T cell assay used in this study is not able to distinguish between SARS-CoV-2 and other human coronaviruses. Studies will be required to determine whether pre-existing coronavirus immunity may alter susceptibility to SARS-CoV-2 infection and/or COVID-19 severity. Third, the focus of the T cell response was on SARS-CoV-2 N. While we observed robust responses to N, there are other proteins that have been shown to be targeted by T cells, which may correlate with immunity and disease severity. Moreover, in individuals with N-only antibodies, we were only able to examine T cell responses in a total of twelve individuals. Further analysis is needed to confirm whether individuals with SARS-CoV-2 N-only antibodies were previously infected with non-SARS coronaviruses and whether this pre-existing immunity elicits a T cell response against SARS-CoV-2 in African populations. Finally, due to the limited amount of blood collected for each patient, we were unable to define the CD4- and CD8-specific T cell responses.

5. Conclusion

Our study demonstrates high SARS-CoV-2 seroprevalence as well as possible preexisting coronavirus immunity in the Nigerian population in 2021, and also provides new data on T cell responses as well as S antibody production after vaccination based on prior infection/exposure. Our findings highlight the need for further investigations to better understand the immune mechanisms and consequences related to pre-existing coronavirus immunity in West Africa. Pre-existing B cell and/or T cell memory may have important implications for natural infection and disease outcomes. Identification of conserved antibody and/or T cell epitopes may hold promise for improved vaccines protecting against current and future coronaviruses.

Data availability statement

All data produced in the present study are available upon reasonable request to the corresponding author.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

BBH is a co-founder of Mir Biosciences, Inc., a company that develops T cell-based diagnostics/vaccines for infections, cancer, and autoimmunity.

No other competing financial interests.

CRediT authorship contribution statement

Sulaimon Akanmu: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – review & editing, Supervision. **Bobby Brooke Herrera:** Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Beth Chaplin:** Methodology, Validation, Formal analysis, Data curation, Writing – review & editing, Visualization. **Sade Ogunsola:** Methodology, Validation, Data curation, Writing – review & editing. **Akin Osibogun:** Methodology, Validation, Formal analysis, Data curation, Writing – review & editing, Supervision. **Fatima Onawoga:** Methodology, Validation, Data curation, Writing – review & editing. **Sarah John-Olabode:** Methodology, Validation, Data curation, Writing – review & editing. **Iorhen E. Akase:** Methodology, Validation, Data curation, Writing – review & editing. **Augustina Nwosu:** Methodology, Validation, Data curation, Writing – review & editing. **Donald J. Hamel:** Methodology, Validation, Formal analysis, Data curation, Writing – review & editing. **Charlotte A. Chang:** Methodology, Validation, Formal analysis, Data curation, Writing – review & editing,

Visualization. **Phyllis J. Kanki:** Conceptualization, Methodology, Validation, Formal analysis, Data curation, Writing – review & editing, Visualization, Supervision, Funding acquisition.

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