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Time course and determinants of the antibody response to SARS-CoV-2 in Costa Rica: the RESPIRA study

Rolando Herrero[1*], Romain Fantin[1], Viviana Loría[1], Amada Aparicio[2], D. Rebecca Prevots[3], Michael Zúñiga[1], Roy Wong[2], Melvin Morera[2], Julia Butt[4], Marco Binder[5], Arturo Abdelnour[2], Alejandro Calderón[2], Roberto Castro[6], Bernal Cortes[1], Rebeca Ocampo[1], Juan Carlos Vanegas[1], Mitchell H. Gail[7], Ruth M. Pfeiffer[7], Julia Flock[8], Kim Remans[8], Lukas Eberhardt[5], Soheil Rastgou[5], Vladimir Magalhaes[5], Carolina Porras[1], Allan Hildesheim[1], Tim Waterboer[4] and for the RESPIRA study group

Abstract

Background Antibodies to SARS-CoV-2 are essential for protection or reduction in severity of subsequent disease. We studied antibody responses to spike protein receptor-binding domain (S1-RBD) and nucleocapsid (N) in a population-based sample of COVID-19 cases in Costa Rica.

Methods As part of the RESPIRA study, we selected an age-stratified random sample of PCR-confirmed COVID-19 cases diagnosed from March 2020 to July 2021. Antibodies were determined with multiplex serology in 794 unvaccinated subjects diagnosed 3 days to 17 months before recruitment to investigate immune response to natural infection. In addition, neutralizing antibodies were determined in 136 randomly selected participants. We estimated antibody positivity and GMTs by time since diagnosis and explored determinants using multivariate regression.

Results Most participants tested 15–29 days after PCR diagnosis were seropositive for N (90%) and S1-RBD antibodies (96%) and had the highest GMTs for both antibodies. Only 42% of subjects tested one year after infection were seropositive for N antibodies, compared to 97% for S1-RBD. GMTs for neutralizing antibodies peaked 15–89 days after infection and declined but remained positive for 95% of subjects thereafter. In multivariate models, antibodies were significantly higher among men and increased with age and severity of the clinical presentation. The correlation of multiplex and neutralizing antibodies was high (0.72 [95% CI = 0.63–0.79]) and stronger among women.

Conclusions A robust immune response against N and S1-RBD is elicited by COVID-19 a few days after infection. While S1-RBD antibodies are present after > 1 year, N antibodies decline significantly. Antibody levels are higher in men and increase with age and severity of disease. The different immune response patterns by sex warrant further investigation.

Trial registration RESPIRA Study ClinicalTrials.gov ID: NCT04537338 (3 September 2020).

Keywords SARS-CoV-2, COVID-19, Antibody response, RESPIRA, Costa Rica

*Correspondence:
Rolando Herrero
rherrero@acibfunin.com
Full list of author information is available at the end of the article

Background

More than 800 million cases and more than 7 million deaths from COVID-19 have been documented by WHO [1] around the world since the start of the pandemic in 2020.The numbers of cases and deaths are now much lower and the pandemic emergency was declared officially over by the World Health Organization in May 2023 [1, 2]. However, many areas of uncertainty remain about the future evolution of this new infection, and multiple aspects of the disease are still under study.

COVID-19 is usually characterized by mild or moderate respiratory symptoms, but in some cases, it can progress to severe systemic disease and death, especially in older patients with comorbidities [3–5]. In addition, many symptoms occurring or persisting after the acute infection, known as post-COVID syndrome, or long COVID, are present in a variable fraction of individuals [6–8], with important impact on the productivity and quality of life of patients [9, 10].

The disease is caused by the betacoronavirus SARS-CoV-2, which mutates over time and generates new variants. Some variants have the potential for higher pathogenicity and ability to escape immunity developed after previous infection or vaccination. The main variants, named by the WHO with Greek letters, are Alpha, Beta, Gamma, Delta and Omicron [11]. The adaptive immune response to infection is the main mechanism for reducing the impact of infectious diseases, as they can protect against infection and reinfection or against the more severe forms of the disease and death. The study of antibody responses to COVID-19, to which humans had not been exposed before, and the magnitude and duration of their protective efficacy against reinfection and severity of disease are essential to assess the level of protection at the individual and community level. Furthermore, this knowledge can be important for controlling future public health emergencies caused by coronaviruses or other pathogens, including the definition of quarantine periods or other preventive measures.

Immunity after natural infection involves humoral and cellular responses, and the main antigenic component of the virus is the spike (S) protein, which contains the receptor binding domain (RBD). The S protein binds to host cells via the angiotensin-converting enzyme 2 (ACE-2) receptor [12, 13] and is the main target of neutralizing antibodies [14]. Nucleoprotein plays an important role in transcription enhancement and viral assembly and is also an important target of the serologic response [14].

After infection, most infected subjects seroconvert, with a median of 12 days since symptom onset. Antibodies persist for variable periods, depending on the study, usually several months [15, 16]. Antibody levels have been shown to be higher comparing symptomatic with asymptomatic patients [17–19] or when the clinical presentation of the disease is more severe [20].

In a meta-analysis of studies investigating the protective efficacy of past infection against re-infection, symptomatic disease and disease severity among unvaccinated subjects, a strong protective effect against reinfection was demonstrated for most variants (average 85%) with the exception of Omicron (~55%), and a strong protection against severity of disease and death for all variants, including Omicron for a duration of at least one year [21].

Costa Rica is a country with 5 million inhabitants, a universal health system and adequate information for monitoring the evolution of the pandemic. Most health services and statistics for the entire country are centralized within the social security system (Caja Costarricense de Seguro Social or CCSS) and the Ministry of Health. In this context, we are conducting the RESPIRA study (Immune response to SARS-CoV-2 in Costa Rica), which allows the investigation of the time course and factors associated with the antibody response to SARS-CoV-2 in a population-based study of COVID-19 cases.

The main objective of this analysis is to describe the magnitude of the antibody response to the spike RBD (S1-RBD)) and the nucleocapsid (N) proteins of the SARS-CoV-2 virus in a group of cases diagnosed in Costa Rica during the first two waves of the pandemic. The first wave was associated with the ancestral variant and the second with the alpha and gamma variants between March 2020 and July 2021 [22]. In this manuscript, we present an analysis of antibody levels after natural infection with SARS-CoV-2 among both symptomatic and asymptomatic unvaccinated individuals and their socio demographic and clinical determinants.

Methods

Study population and specimens

RESPIRA includes a cohort of 999 COVID-19 cases diagnosed between March 2020 and July 2021, during the first and second waves of the pandemic in Costa Rica (Figure S1), The first wave was caused by the ancestral SARS-CoV-2 variant and the second by variants alpha and gamma [22]. The cohort of cases and a group of controls were followed for 2 years to investigate protective efficacy of antibodies generated after natural infection and vaccination to prevent re-infection and severe COVID-19 disease. In this report we present data on the levels of antibodies at the recruitment visit in unvaccinated cases of COVID-19.

Selection and recruitment of cases was from November 2, 2020 to September 3, 2021, with 70% of participants being mainly from the metropolitan area around the capital city and 30% from Guanacaste, a more rural province. Together, these regions represent 70% of the population

of Costa Rica. Recruitment of participants was stratified to obtain similar numbers in four age groups: 0–19, 20–39, 40–59 and 60 or more. Detailed methods of the study have been published [23].

A series of age- and region-stratified random samples of cases diagnosed by PCR from nasopharyngeal swabs (NPS) was selected from the nationwide surveillance lists of the CCSS and the Ministry of Health. To initially assess the time course of the antibody response, we selected a minimum of 50 cases diagnosed in each month since the beginning of the pandemic in Costa Rica. When the original case was ineligible or refused participation in the study, the participant was replaced with another case with similar characteristics. Most of the cases in this early phase of the pandemic were likely first infections and none of them reported having been infected in the past.

Eligibility criteria for cases included diagnosis by PCR, willingness to donate a blood specimen, plans to remain in the country for the next 12 months, ability to communicate and absence of incapacitating medical conditions. By design, 30% of the cases were recruited within 14 days after PCR diagnosis and 70% had longer periods after diagnosis, up to 17 months.

Enrollment visits were conducted at home or at one of the study clinics. An in-person interview of participants obtained information on sociodemographic characteristics, medical history (including symptoms during their COVID-19 episode), lifestyle (smoking, alcohol, physical activity), preventive practices during the pandemic (distancing, mask use), and SARS-CoV-2 vaccination status.

Blood was collected for detection of antibodies. The blood samples were collected at home or study clinics and initially stored in cold boxes at 2–10 °C. Samples were centrifuged, aliquoted and frozen in liquid nitrogen at local laboratories within 12 h of collection, and subsequently transferred to our central repository for storage at -80 °C.

Antibody testing

Analysis of antibody responses to SARS-CoV-2, common cold coronaviruses HKU-1, OC43, 229E, and NL63, as well as human polyomavirus BK, Varicella zoster virus (VZV), and human Cytomegalovirus (CMV) was performed at the German Cancer Research Center (DKFZ) as described [24]. Briefly, recombinantly expressed nucleocapsid protein (protein N) and the receptor-binding-domain (RBDs) of the spike protein (S1-RBD) of coronaviruses, as well as protein VP1 of polyomavirus BK, protein gE and gl of VZV, and protein pp65 of CMV, were linked to the bead surface of fluorescently labelled polystyrene beads (Luminex Corp., Austin, TX, USA). Antigen-loaded beads were combined to achieve a suspension array that was incubated with serum sample (1:100 serum dilution). A Luminex 200 Analyzer (Luminex Corp., Austin, TX, USA) was used to distinguish the bead sets and their respective antigens, and to quantify the amount of serum antibody bound to the antigen by a reporter fluorescence as median fluorescence intensities (MFI) of at least 100 beads per set measured. A comparison of 1:100 versus 1:1,000 serum dilutions is presented in the supplementary material.

The thresholds we defined to consider a blood sample as seropositive (SP) were based on samples collected during two pre-pandemic studies in Costa Rica: the Microbiome Project [25] and the ESCUDDO Feasibility study [26]. Ninety-one independent pre-pandemic samples were used, and the thresholds were defined as the estimation of the 99.9 percentile of a normal law (the mean plus 3 standard deviations). The 99.9th percentile was chosen to ensure specificity. Thus, a blood sample was considered as positive if antibodies were above 3,614 MFI for protein N antibodies, and if antibodies were above 482 MFI for S1-RBD antibodies. For all other viruses, previously established and standardized cutoffs were used to determine seropositivity [27, 28].

For a subset of sera ($n=136$), their functional capacity in terms of virus neutralization was tested. For this purpose, a lentivirus-based pseudovirus neutralization assay was employed using a codon-optimized SARS-CoV-2 (Wuhan Hu-1) spike protein for pseudovirus development. The pseudovirus encoded the firefly luciferase gene used for quantitative measurement of virus entry into ACE2-transduced A549 lung epithelial cells. Briefly, pseudovirions were incubated with a 10-step twofold dilution series of test sera for 1 h at 37 °C before being added to ACE2-A549 cells. 24 h later, cells were lysed and luciferase activity was measured. Luciferase signals were standardized to 0% (uninfected cells) to 100% (cells infected with pseudovirus without serum) and plotted over serum dilution. The 50% inhibitory titer was determined by logistic regression. For quality control each batch included positive (recombinant human monoclonal Spike antibody) and negative (pre-pandemic human serum) controls. The assay was validated by comparing defined sera against an independent authentic virus (SARS-CoV-2) neutralization test, as summarized in the supplemental materials section, together with additional technical details of the assay.

The population of the districts included in RESPIRA was 2.86 million (Figure S2). 213,000 cases were reported to the Costa Rica national surveillance system during the case selection period. We selected 3181 PCR-confirmed cases and 1657 were contacted and deemed eligible, of which 999 were recruited, for a 60% participation rate. After exclusion of 23 cases without antibody results, 180

who reported COVID-19 vaccination before recruitment and 2 for whom we did not have certainty about the vaccination date, the group for this analysis consisted of 794 individuals, of which 136 were selected to test their recruitment sera for SARS-CoV-2 neutralization. Those with a history of vaccination were excluded because vaccination induces a strong immune response to the spike antigen. The present analysis presents results of antibody testing at the recruitment visit into the study, which occurred between 3 and 506 days after diagnosis. The analysis is based on the comparison of subjects with different times since diagnosis.

Statistical analysis

Bivariate analysis was implemented for both protein N and S1-RBD of SARS-CoV-2 and the other viruses, using the geometrical mean and the percentage of positivity as main indicators. The SARS-CoV-2 indicators were calculated by sex, age-group (0-19y, 20-39y, 40-59y, 60y+), severity (mild, moderate, hospitalized), comorbidities (hypertension, diabetes, obesity, asthma, high cholesterol), time since diagnosis and pandemic wave (ancestral *vs*. Alpha-Gamma).

A mild case was defined as a non-hospitalized case having two or fewer symptoms associated with hospitalization in our study (fever, shortness of breath, chest tightness, disorientation/confusion, fatigue). A moderate case was defined as a non-hospitalized case having three or more of these symptoms. There were very few completely asymptomatic cases ($n=25$).

In order to investigate the independent associations of the different variables with the antibody levels, multivariate analyses were implemented using a linear regression model and protein N, S1-RBD or neutralization as dependent variable (log-scale). Independent variables (noted X) were age, sex, severity, comorbidities, pandemic wave and time since diagnosis.

$$ln(antibodieslevel) = \alpha + \beta X$$

The multivariate analysis was based on a univariate model, and a fully-adjusted model (age, sex, severity, comorbidities, time since diagnosis and pandemic wave). The use of a log-scale dependent variable allowed us to interpret the exp(β) as a GMT-ratio: if $\beta = x$, the GMT of the category is *exp(x)* times the GMT of the reference category.

Results

The median age of the case group in this analysis was 38 years, and 37 years for those tested for pseudovirus neutralization. The study was designed to include similar numbers of subjects in each of the 4 age groups used for sampling (<20, 20–39, 40–59, 60+). However, due to lower participation rates among individuals under 20 and over 60 years, the final study population had a slight over-representation of young- and middle-aged adults. (Table 1). 53.5% of participants (47.1% of those with neutralization testing) were female. 63.7% of the cases (64.0% of those with neutralization testing) had mild symptoms and 4.7% (5.2% of those with neutralization testing)

Table 1 Selected characteristics of cases with multiplex results and pseudoneutralization results

	Cases with multiplex results		Cases tested for pseudo neutralization	
	(*n* = 794)	%	(*n* = 136)	%
Age				
0–19	178	22.4	36	26.5
20–39	242	30.5	40	29.4
40–59	231	29.1	33	24.3
60+	143	18.0	27	19.9
Sex				
Men	369	46.5	72	52.9
Women	425	53.5	64	47.1
Severity				
Mild	506	63.7	87	64.0
Moderate	251	31.6	42	30.9
Hospitalized	37	4.7	7	5.2
Comorbidities				
Without comorbidity	407	51.3	71	52.2
Any comorbidity[a]	387	48.7	65	47.8
Specific comorbidities				
Hypertension	174	21.9	32	23.5
Diabetes	85	10.7	14	10.3
Obesity	98	12.3	13	9.6
Asthma	130	16.4	19	14.0
High Cholesterol	170	21.4	30	22.1
Days since diagnosis				
0 to 6	32	4.0	3	2.2
7 to 14	215	27.1	41	30.2
15 to 29	51	6.4	10	7.4
30 to 59	139	17.5	28	20.6
60 to 89	49	6.2	10	7.4
90 to 119	54	6.8	8	5.9
120 to 179	76	9.6	9	6.6
180 to 269	60	7.6	14	10.3
270 to 364	85	10.7	9	6.6
365+	33	4.2	4	2.9
Wave				
Ancestral	572	72.0	97	71.3
Alpha/Gamma	222	28.0	39	28.7

[a] Comorbidities include hypertension, diabetes, obesity, asthma and high cholesterol

reported hospitalization. Comorbidities were common, with hypertension being the most commonly reported condition.

Thirty-eight percent of cases recruited between days 0 and 6 were positive for antibodies against protein N, increasing to 76% between days 7 and 14, reaching a positivity of 91% between 30 and 59 days and declining to a minimum of 42% after one year (Table 2). The GMTs increased from 1428 (95%CI = 806–2527) at 0–6 days to a maximum of 6730 between 15 and 29 days (95%CI = 5348–8468), declining thereafter to a minimum GMT of 2631 (95%CI = 1815–3738), a reduction of about 60% (adjusted p value < 0.01 for decline after 15 days).

For protein S1-RBD, seropositivity was 59% among subjects recruited 0–6 days after diagnosis and reached 96% between 15 and 29 days, subsequently declining to 87% but increasing again to 97% after 1 year. The GMTs for antibodies against S1-RBD followed a pattern of increase in the first month after infection similar to protein N antibodies, with a peak at 4791 (95%CI = 3715–6179) and a decline to about 3000 6–9 months after infection and a subsequent increase to 3894 (95%CI = 2861–5299) after one year, a reduction of about 20% compared to the peak levels (adjusted p value 0.02 for decline after 15 days). The GMTs by time since diagnosis are depicted in Figs. 1a and 1b.

Table 2 Protein N and S1-RBD antibody level (GMTs) and positivity according to the time between diagnosis and blood sample

Time since diagnosis (in days)	N	Protein N		S1-RBD	
		GMT [95%IC]	%pos	GMT [95%IC]	%pos
0 to 6	32	1428 [806—2527]	38%	850 [480- 1508]	59%
7 to 14	215	4163 [3597—4819]	76%	2566 [2186—3011]	89%
15 to 29	51	6730 [5348—8468]	90%	4791 [3715—6179]	96%
30 to 59	139	6006 [4998—7217]	91%	4202 [3505—5039]	93%
60 to 89	49	5469 [4070—7349]	84%	3975 [2899—5452]	92%
90 to 119	54	5801 [4363—7714]	87%	4655 [3551—6102]	94%
120 to 179	76	4470 [3474—5752]	75%	3448 [2596—4579]	89%
180 to 269	60	3549 [2644—4763]	63%	2998 [2126—4228]	87%
270 to 364	85	3466 [2849—4218]	58%	3276 [2622—4092]	93%
365 +	33	2631 [1815 -3738]	42%	3894 [2861—5299]	97%

GMT Geometric mean title, *%pos* Percentage of samples positive to Protein N (or S1-RBD), Thresholds for positivity, *Protein N* 3614MFI, S1-RBD: 482MFI

Table 3 shows GMTs and positivity for antibodies against N and S1-RBD proteins by relevant variables. GMTs for both protein N and S1-RBD, were higher in men than in women ($p < 0.01$) and showed a clear pattern of increase with age (p for trend < 0.01 for both antibodies) and severity of the disease (p for trend = 0.02 for N and < 0.01 for S1 RBD antibodies). The difference in antibody positivity by sex was statistically significant for N antibodies but not significant for S1-RBD. Subjects with comorbidities had comparable GMTs and positivity than those without any, but cases with hypertension had higher levels of antibodies than people without hypertension, mainly against protein N ($p = 0.03$, not shown in table).

With respect to age, the GMTs for antibodies against protein N were 3344 among subjects under 20 years and 5569 among those over 60 (p for trend < 0.01). Corresponding positivities were 64% and 83% (p for trend < 0.01). A similar but less pronounced pattern was observed for antibodies against the S1-RBD protein. Antibodies against both proteins increased with increasing severity of the COVID-19 infection reported by the cases (p for trend = 0.02 for N and < 0.01 for S1 RBD), and this was observed for both sexes (Table S1), with the highest levels observed for men with the most severe disease (hospitalized), although the difference was less striking for S1 antibodies between hospitalized men and women. The levels of N antibodies during the first pandemic wave, associated with the ancestral variant of the virus were comparable to those of the second wave ($p = 0.29$). Antibodies and positivity against S1-RBD were significantly higher during the first wave ($p < 0.01$) (see multivariate model below). The associations with time since diagnosis in the multivariate model demonstrate the significant decline for N antibodies over time that is less pronounced for S1-RBD.

In order to explore the independent contribution of each of the variables to the intensity of the antibody response, we created a multivariate model adjusting for age, sex, severity of the COVID-19-episode, presence of comorbidities, time since diagnosis and pandemic wave. We observed that age, sex, severity and time since diagnosis were independent determinants of the antibody response, with little modification of the ORs comparing the unadjusted with the adjusted models (Table 4). Considering individual comorbidities, only hypertension was associated with higher antibody levels, but this association was not present in the adjusted model. GMTs for S1-RBD antibodies were 27% higher in the adjusted model among subjects diagnosed during wave 1 compared to wave 2 (95%CI = 1.03–1.56).

To further explore the gender difference in antibody levels, we analyzed the trajectory of the GMTs over time

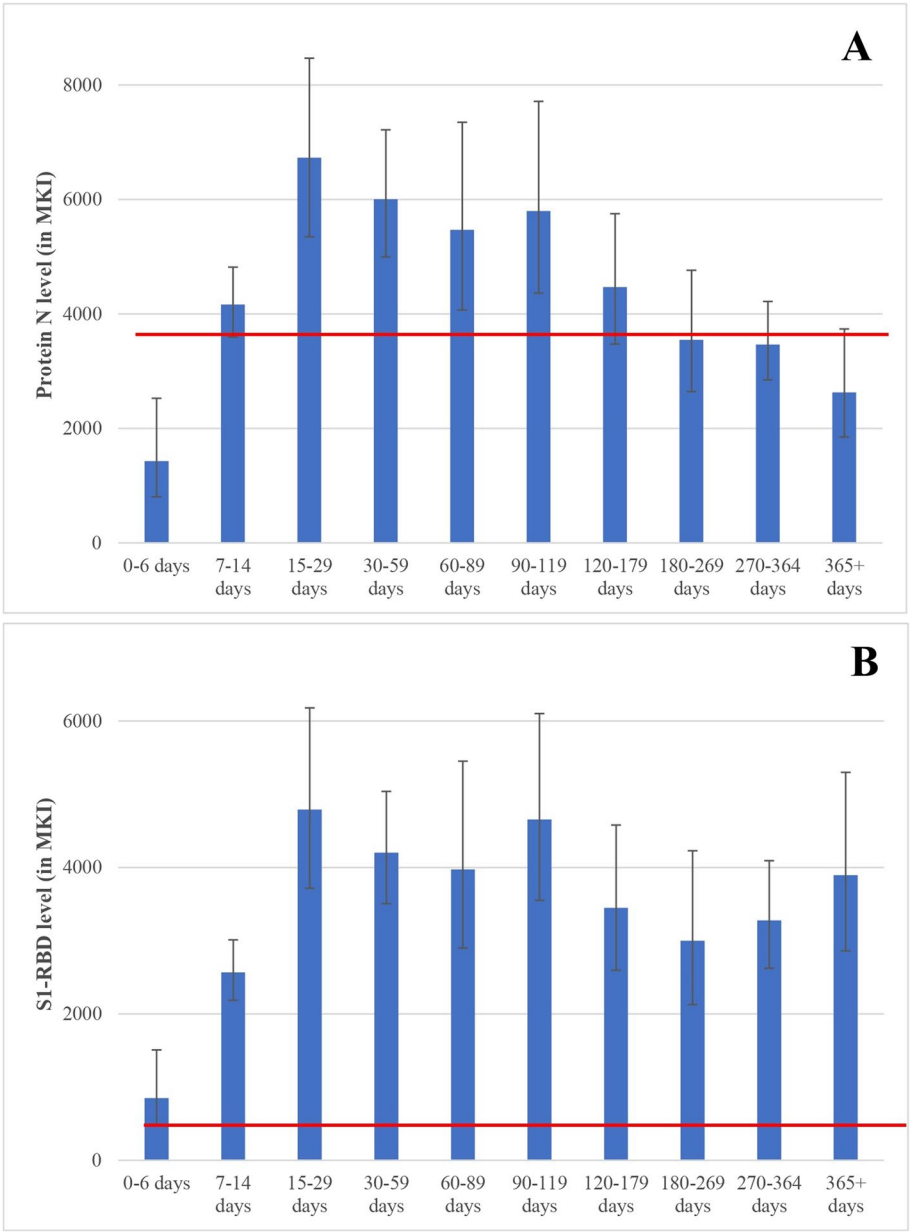

Fig. 1 Protein N (**a**) and S1-RBD (**b**) antibody level (GMTs) as determined with Luminex method according to the time between diagnosis and blood collection in a sample of 794 PCR-confirmed COVID-19 cases from Costa Rica. Legend: Red line corresponds to the seropositivity threshold used in the analysis

for both sexes separately. For both antibodies, men had higher levels than women beginning in the first few days after diagnosis and during the first 9 months, thereafter declining to a level comparable to women (Figs. 2a and b). The declines after age 15 were significant (p values < 0.01) for protein N in men and for S1-RBD they were significant in men ($p < 0.01$) but not in women ($p = 0.40$).

Neutralizing antibodies were already positive in the first two weeks after diagnosis in 86% of cases (Table 5), reaching 95% after 90 days despite declining levels. The factors associated with pseudovirus neutralization were comparable to those observed for the antibodies determined with the Luminex assay, with independent associations of neutralizing antibodies with time since diagnosis, male sex, age and severity of the clinical presentation. Positivity and antibody levels were higher for subjects diagnosed during wave 1 but the difference was not statistically significant (Table 5).

Table 3 Protein N and S1-RBD antibody level (GMTs) and positivity by age, sex, severity and comorbidities

	Protein N			S1-RBD	
	N	GMT [95%IC]	%pos	GMT [95%IC]	%pos
Sex		$p < 0.01$	$p < 0.01$	$p < 0.01$	$p = 0.07$
Men	369	5079 [4586—5624]	79%	3693 [3299—4133]	92%
Women	425	3827 [3413—4292]	71%	2891 [2565—3258]	88%
Age		p-trend < 0.01	p-trend < 0.01	p-trend < 0.01	p-trend = 0.09
0–19	178	3344 [2777—4027]	64%	2625 [2137 -3225]	86%
20–39	242	3815 [3309—4398]	72%	2896 [2514—3336]	91%
40–59	231	5308 [4671—6032]	81%	3759 [3265—4329]	92%
60 +	143	5569 [4694—6608]	83%	3998 [3307—4835]	91%
Severity		p-trend = 0.02	p-trend = 0.11	p-trend < 0.01	p-trend = 0.09
Mild	506	4144 [3748—4582]	73%	2972 [2667—3311]	89%
Moderate	251	4534 [3957 -5194]	76%	3630 [3178—4147]	93%
Hospitalized	37	6861 [5454—8631]	84%	4854 [3244—7264]	92%
Comorbidities		$p = 0.17$	$p = 0.19$	$p = 0.10$	$p = 0.29$
None	407	4139 [3716—4610]	73%	3026 [2700—3391]	91%
One or more[a]	387	4616 [4123 -5168]	77%	3479 [3082—3927]	89%
Hypertension	174	5127 [4362—6026]	80%	3643 [3018—4397]	87%
Diabetes	85	4752 [3694—6112]	74%	3669 [2771—4858]	87%
Obesity	98	4689 [3662—6004]	80%	3581 [2762—4641]	88%
Asthma	130	4025 [3303—4903]	72%	3114 [2515—3857]	88%
High Cholesterol	170	5022 [4271—5904]	81%	3642 [3079—4308]	91%
Wave		$p = 0.29$	$p = 0.56$	$p < 0.01$	$p < 0.01$
Ancestral	572	4482 [4119—4877]	74%	3528 [3221—3864]	92%
Alpha/Gamma	222	4077 [3421—4858]	76%	2600 [2172—3113]	85%

GMT Geometric mean title, *%pos* Percentage of samples positive to Protein N (or S1-RBD), Thresholds for positivity, *Protein N* 3614MFI, *S1-RBD* 482MFI

[a] Comorbidities include hypertension, diabetes, obesity, asthma and high cholesterol

The Pearson correlation between Luminex antibodies against protein N and neutralizing antibodies (both in log-scale) was 0.72 [95% CI = 0.63–0.79], and was stronger among women (0.81 [95% CI = 0.70–0.88]) than among men (0.58 [95% CI = 0.41–0.72]) ($p = 0.05$). The correlation of S-RBD antibodies as tested by Luminex with the pseudoneutralization (both in log-scale) was 0.82 [95% CI = 0.76–0.87], and was 0.88 [95% CI = 0.82–0.93] in women and 0.76 [95% CI = 0.64–0.84] in men ($p = 0.07$).

In order to determine if previous exposure to other coronavirus and other common viral infections affected the immune response to COVID-19, we also assessed the prevalence of antibodies against other selected coronaviruses (HKU, OC43, 229e and NL63), cytomegalovirus (CMV), BP1 BK virus and varicella-zoster virus (VZV) in the entire population and calculated the levels and positivity of the COVID-19 antibodies against proteins S and N within positive and negative strata of each of the other antibodies (Table S2). Antibodies against all these viruses were generally high in this population, and antibodies against N and S1 RBD proteins of SARS-CoV-2 were significantly higher among subjects positive for coronavirus 229e and VZV, but these associations were no longer observed in a multivariate model adjusting for age and sex, with the exception of coronavirus OC43, which was associated with a significant reduction in the antibody levels for both N and S1-RBD (Table S3 and S4).

Discussion

The COVID-19 pandemic resulted from dissemination of a new, highly transmissible coronavirus to which humans had not been exposed in the past and for which the immune system of most people had very limited ability to respond. Rapid characterization of the virus and successful development of protective interventions in record time were essential to accelerate the end of the pandemic, but there is a need for more investigation of all aspects of the disease in preparation for future events.

The RESPIRA study is being conducted to investigate the antibody response to SARS-CoV-2, its duration and determinants after natural infection and after vaccination, in addition to their protective efficacy against subsequent infection. In this manuscript, we

Table 4 Multivariate model of log-levels of antibodies against N and S1-RBD proteins by sex, age, severity, presence of comorbidities, pandemic wave and time since diagnosis

Model	Protein N		S1-RBD	
	Unadjusted ratio [IC95%]	Adjusted ratio [IC95%]	Unadjusted ratio [IC95%]	Adjusted ratio [IC95%]
Sex				
Men	1.33 [1.14—1.55]	1.26 [1.08—1.46]	1.28 [1.08—1.51]	1.23 [1.05—1.45]
Women	1	1	1	1
Age				
0–19	1	1	1	1
20–39	1.14 [0.92—1.41]	1.19 [0.97—1.47]	1.10 [0.88—1.39]	1.13 [0.91—1.42]
40–59	1.59 [1.28—1.97]	1.52 [1.22—1.89]	1.43 [1.14—1.80]	1.28 [1.01—1.63]
60 +	1.67 [1.31—2.12]	1.57 [1.22—2.03]	1.52 [1.17—1.98]	1.38 [1.05—1.81]
Severity				
Mild	1	1	1	1
Moderate	1.09 [0.92—1.30]	1.11 [0.94—1.31]	1.22 [1.02—1.46]	1.25 [1.05—1.49]
Hospitalized	1.66 [1.14—2.40]	1.47 [1.03—2.11]	1.63 [1.10—2.43]	1.40 [0.95—2.06]
Comorbidities				
None	1	1	1	1
At least one*	1.12 [0.95—1.30]	0.90 [0.77—1.06]	1.15 [0.97—1.36]	0.97 [0.81—1.16]
Pandemic Wave				
Ancestral	1.10 [0.92—1.31]	1.18 [0.97—1.44]	1.36 [1.13—1.63]	1.26 [1.02—1.57]
Alpha/Gamma	1	1	1	1
Time since diagnosis				
0 to 6	0.21 [0.13—0.34]	0.24 [0.15—0.39]	0.18 [0.11—0.29]	0.20 [0.12—0.34]
7 to 14	0.62 [0.45—0.86]	0.66 [0.48 – 0.91]	0.54 [0.38—0.76]	0.58 [0.41—0.82]
15 to 29	1	1	1	1
30 to 59	0.89 [0.63—1.26]	0.96 [0.68—1.36]	0.88 [0.61—1.27]	0.96 [0.66—1.40]
60 to 89	0.81 [0.53—1.24]	0.81 [0.54 – 1.23]	0.83 [0.53—1.30]	0.81 [0.52—1.26]
90 to 119	0.86 [0.57—1.30]	0.80 [0.53—1.20]	0.97 [0.63—1.51]	0.91 [0.59—1.40]
120 to 179	0.66 [0.45—0.97]	0.64 [0.44 – 0.94]	0.72 [0.48—1.08]	0.68 [0.45—1.02]
180 to 269	0.53 [0.35—0.79]	0.51 [0.35 – 0.77]	0.63 [0.41—0.96]	0.60 [0.39—0.92]
270 to 364	0.52 [0.35—0.75]	0.50 [0.35 – 0.73]	0.68 [0.46—1.02]	0.65 [0.44—0.97]
365 +	0.39 [0.24—0.63]	0.37 [0.23—0.59]	0.81 [0.49—1.34]	0.77 [0.46—1.26]

Ratio: exponential of the coefficient associated with the category. Adjusted ratios were calculated in a model adjusted for sex, age, severity and presence of comorbidity

* Comorbidities include hypertension, diabetes, obesity, asthma and high cholesterol

present seroconversion rates and GMT levels of antibodies against N and S1-RBD for a group of COVID-19 cases recruited into the study between a few days and more than a year after a PCR confirmed COVID-19 diagnosis. We also investigated the functional capacity of the antibodies by determining presence and levels of neutralizing antibodies in a subset of participants.

Seroconversion starts in the first few days and almost all individuals develop antibodies after infection. About two weeks after infection, 75% and 90% of individuals have developed N and S1-RBD antibodies, respectively and by one month this fraction is 90 and 96%. This is consistent with observations be several investigators [29–32]. Previous studies of time to seroconversion have shown an average of 10 days from PCR diagnosis to seroconversion [33].

In RESPIRA, where only about 5% of cases were severe (hospitalized), N antibodies peaked after one month, regardless of severity of the disease (data not shown) and declined after a few months to a lowest after one year, when only about 40% of infected individuals remained seropositive. The S1-RBD antibodies were more stable and declined more slowly, and after one year 97% of individuals were still positive.

The decline in N antibodies after one year coincided with a significant decline in the levels of neutralizing

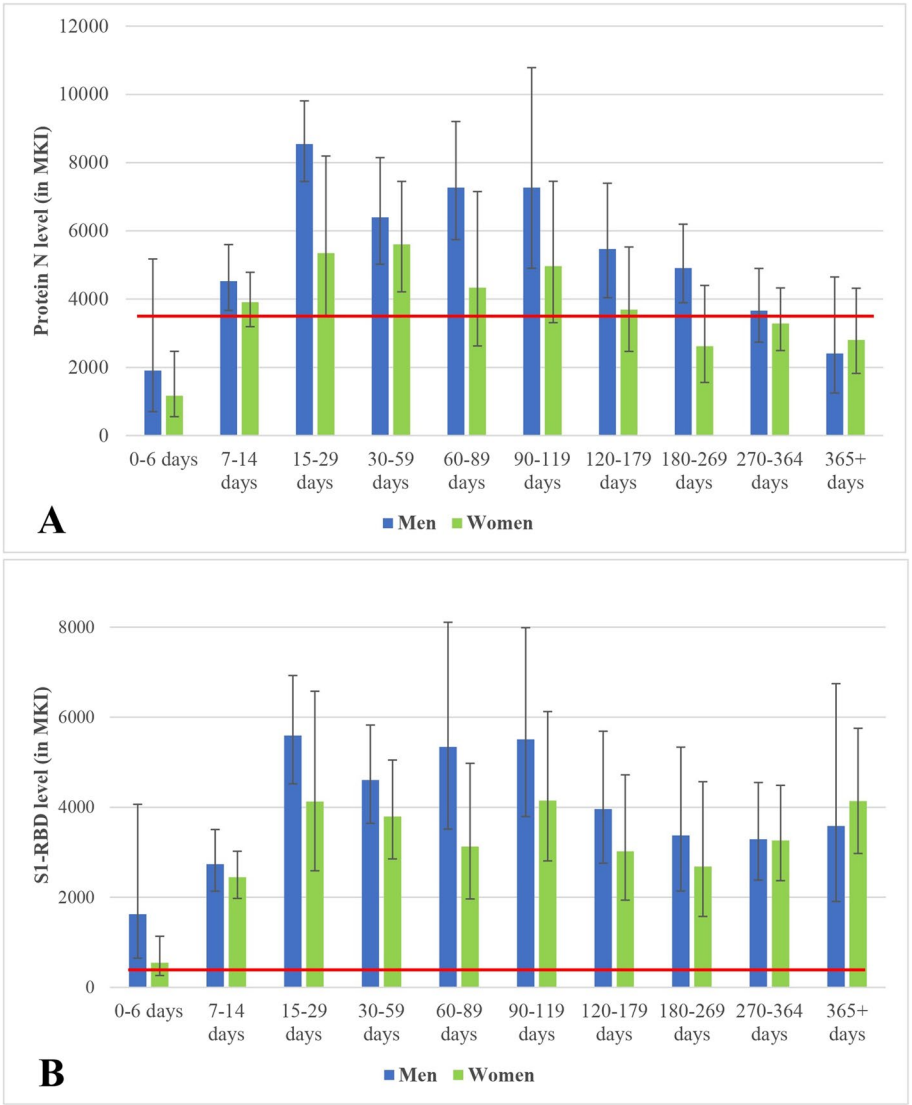

Fig. 2 Protein N (**a**) and S1-RBD (**b**) antibody level (GMTs) as determined with Luminex method according to the time between diagnosis and blood collection in a sample of 794 PCR-confirmed COVID-19 cases from Costa Rica, by sex. Legend: Red line corresponds to the seropositivity threshold used in the analysis

antibodies, while S1-RBD antibodies declined less than N antibodies and remained positive during the entire period, indicating that the latter can be markers of past exposure for prolonged periods but may not necessarily predict protection against subsequent infection. Indeed, the antibody response to the S glycoprotein, that contains the receptor-binding domain is considered the best indicator of past infection for individuals or populations [30]. However, the utility of the S1 RBD antibodies as markers of past infection is lost in vaccinated populations as the main antigen in vaccines is protein S.

Several studies have found seroconversion rates of more than 90% regardless of the severity of the infection [18, 19, 33, 34]. However, the magnitude of the antibody response is stronger for subjects with more severe disease, and some studies have observed a stronger response in male patients, although none of the studies have investigated the independent effect of severity, age and sex in a multivariate model [14, 35–37].

Women generally have stronger innate and adaptive immune responses than males, with faster clearance of pathogens and greater vaccine efficacy in females than in males. For example, antibody responses to influenza are twice as high in women than men. The stronger immune response also makes them more susceptible to autoimmune diseases [38, 39] Takahashi et al. reported that

Table 5 Multivariate model of log-level of pseudoneutralization levels by time since diagnosis, sex, age, severity and presence of comorbidities

	Pseudoneutralization in unvaccinated cases (one sample per person)				
	N = 136				
	N	%pos	Pseudoneutralization	Model	
			GMT [95%IC]	Unadjusted ratio [IC95%]	Adjusted ratio [IC95%]
Overall	136	92	595 [397—891]		
Days since diagnosis[a]					
0 to 14	44	86	458 [192—1092]	0.41 [0.16—1.03]	0.52 [0.20—1.32]
15 to 89	56	95	1128 [631- 2017]	1	1
90 +	36	94	303 [164—558]	0.27 [0.10—0.72]	0.19 [0.07—0.53]
Sex					
Men	72	94	745 [449—1236]	1.61 [0.72—3.62]	1.55 [0.70—3.43]
Women	64	89	462 [241—887]	1	1
Age					
0–19	36	89	343 [152—775]	1	1
20–39	40	93	411 [205—823]	1.20 [0.42—3.46]	0.90 [0.30 – 2.69]
40–59	33	91	703 [294—1683]	2.05 [0.67—6.24]	1.23 [0.37—4.05]
60 +	27	96	1748 [703—4351]	5.10 [1.57—16.52]	2.40 [0.60 – 9.63]
Severity					
Mild	87	90	447 [261—766]	1	1
Moderate	42	95	898 [477—1692]	2.01 [0.83—4.85]	2.14 [0.88 – 5.20]
Hospitalized	7	100	1770 [260—12021]	3.96 [0.63—24.92]	3.98 [0.62 – 25.52]
Comorbidities					
Without comorbidity	71	92	441 [255—760]	1	1
With at least one comorbidity[b]	65	92	826 [451—1514]	1.88 [0.84—4.20]	1.05 [0.42—2.60]
Wave					
Ancestral	97	94	693 [440 – 1090]	1.70 [0.70 – 4.15]	2.34 [0.88 – 6.20]
Alpha/Gamma	39	87	408 [171 – 972]	1	1

GMT Geometric mean title. *%pos* Percentage of samples positive to pseudoneutralization. Thresholds for positivity: Pseudoneutralization > 1. Ratio: exponential of the coefficient associated with the category. Adjusted ratios were calculated in a model adjusted for sex, age, severity, wave, time since diagnosis and presence of comorbidity

[a] The categories of days since diagnosis are different from those in Table 4 because the number of subjects tested for pseudoneutralization (*n* = 136) is much lower than for S1-RBD and N antibodies (*n* = 794). Thus, the categories were collapsed for Table 5

[b] Comorbidities include hypertension, diabetes, obesity, asthma and high cholesterol

the levels of several proinflammatory innate immune chemokines and cytokines were higher in male patients after COVID-19 infection while a more robust T cell response among female patients was observed compared to male patients. In particular, activated CD8 T cells were significantly elevated only in female patients but not in males compared to healthy volunteers [40].

In RESPIRA, men had higher antibody levels, including neutralizing antibodies, and the association was independent of age and severity of the disease, which were also independently associated with the antibody levels. There is a limited number of studies evaluating COVID-19 antibody responses by sex, and several studies have reported higher antibody levels in men, especially after vaccination [41–43]. Since previous reports did not adjust for the mutual effects of age and severity of the COVID-19 infection, which are associated with antibody levels, we elaborated a multivariate model and these associations appeared to be independent of each other. Furthermore, men have higher antibodies than women in each stratum of severity and in each age stratum. It is unclear if the explanation is the presence of more undetected comorbidities or higher severity by age and sex or a real biological difference in response to SARS-CoV-2 by sex.

Other investigators have described lower antibody levels elicited by SARS-CoV-2 in children [44–46] and higher levels of antibodies in relation to older age and

severity of the infection, likely as a result of longer exposure or increased viral replication in the more severe cases. Older patient may have higher antibody levels because of previous exposure to other coronaviruses [47, 48].

SARS is known to be a neutralization-sensitive virus because the RBD is exposed, and neutralizing antibodies have been used for therapeutic purposes [49]. The decline in neutralization we observed is important as it could predict loss of protection against subsequent infection or against severe disease. Interestingly, the correlation of neutralizing antibodies with multiplex antibodies was stronger among women despite lower levels of both anti N and anti S antibodies, and antibodies in men peaked earlier but after a few months were comparable to those of women, indicating differential immune responses that could explain differences in clinical presentation of the disease by sex.

We noted higher antibody levels in cases diagnosed during the first wave of the pandemic, where the predominant variant in Costa Rica was the ancestral. The increased antibody levels were statistically significant only for S1-RBD but not for N or neutralizing antibodies. We explored if the association with age, sex, severity and comorbidities in the multivariate models were different by pandemic wave (data not shown), but the associations were similar. The association of antibody levels with increasing age and comorbidities was significantly stronger during the second wave (p for interaction for S1-RBD antibodies < 0.01). Whether this represents a different immune response to the different variants warrants further investigation. The second wave had a lower representation of subjects with longer follow-up, but the general trajectory of the antibody levels over time was similar for both waves (data not shown).

Finally, to explore if pre-existing immunity to other coronaviruses modifies the antibody response to SARS-CoV-2 [50], we developed multivariate models to assess the association of coronaviruses HKU1, OC43, 229e and NL63, in addition to CMV, VZV and BK VP1 with antibody levels against N and S1-RBD of SARS-CoV-2. The only significant association Iin the adjusted model was a 25% lower level of N and S1-RBD antibodies among subjects with previous antibodies against betacoronavirus OC43, Reduced incidence of COVID-19 infection and less severe disease has been reported among subjects previously infected with OC43 [51, 52], suggesting the need to further explore this association.

This is the first study in Latin America having evaluated the immune response to COVID-19 in a series of well characterized unvaccinated cases selected from official surveillance listings with different times since infection, including the assessment of neutralizing antibodies. Among the limitations of the study is that the assessment of the antibody response over time was done in different subjects recruited with different times since diagnosis. In addition, since the study was based on a group of cases selected from the general population of cases, we had a limited number of severe cases to explore in more detail additional aspects of the immune response.

The follow-up in RESPIRA, where all subjects and their controls are being followed for 2 years with interviews and collection of specimens for immunologic studies and PCR testing is expected to allow prospective investigation of the associations we observed and assessment of protection by antibodies and vaccination.

Conclusions

A robust immune response against N and S1-RBD is elicited by COVID-19 shortly after infection. While S1-RBD antibodies are present after > 1 year, N antibodies decline significantly. Antibody levels are higher in men and increase with age and severity of disease. The different immune response patterns by sex warrant further investigation.

Abbreviations

S1-RBD	Spike protein receptor binding domain
N	Nucleocapsid
PCR	Polymerase chain reaction
GMT	Geometric mean titers
RESPIRA	Acronym from Spanish name of the study (Respuesta Inmune al SARS-Cov-2)
CCSS	Costa Rican Board of Social Security
NPS	Nasopharyngeal swab
VZV	Varicela-zoster virus
CMV	Cytomegalovirus
DKFZ	German Cancer Center
MFI	Median fluorescence intensity

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10742-8.

Supplementary Material 1. Figure S1 Evolution of incident cases in Costa Rica from March 2020 to February 2022 by date of diagnosis according to the surveillance system of the Ministry of Health and Caja Costarricense de Seguro Social. Legend: Dates of diagnosis of cases for this study and dates of accrual of cases and controls for this study are indicated by horizontal lines.

Supplementary Material 2. Figure S2. Flowchart of study subjects included in the RESPIRA study to evaluate the immune response to SARS-CoV-2 in Costa Rica.

Supplementary Material 3.

Acknowledgements

Not applicable.

Data sharing

Data are available upon written request after approval by the investigators and the participating institutions.

Authors' contributions
RH, AH, TW, AAparicio designed the study RH, AH, RP, CP obtained the funding RH, AAparicio, VL,CP, AH supervised the entire study TW, JB, MB superised the laboratory work RO, MM, RC,AC, RP, RW,AAbdelnour supervised the field woek MZ, BC, JF, KR,VM, SR LE conducted the laboratory work JCV,RF selected sample and did the ststistical analyses MHG, RP supported statistical analysis All authors reviewed and commented on the manuscript.

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Data availability
Data are available upon written request after approval by the investigators and the participating institutions.
(Agencia Costarricense de Investigaciones Biomédicas (ACIB)—Fundación INCIENSA.
(FUNIN)), and the Caja Costaricense de Seguridad Social (CCSS). Investigators. interested in more details about this study, including protocols, informed consent forms,
and data use agreement should contact the principal investigator and the corresponding.
author, Rolando Herrero (rherrero@acibfunin.com).

Declarations

Ethics approval and consent to participate
The RESPIRA study was approved by the Ethical Committee of the Costa Rican Social Security Administration (Caja Costarricense de Seguro Social), protocol #R020-SABI-00261. The committee is authorized and supervised by the National Research Council (CONIS) of the Ministry of Health of Costa Rica under Research Law number 9234 of April 2014. The research was conducted in compliance with all local regulations and the Declaration of Helsinki. All participants, or their parents in the case of minors, signed informed consent forms approved by the ethical committee. Children between 12 and 17 additionally signed informed assent forms. The signature was performed in the presence of a witness who was not part of the study staff, according to the Costa Rican research law.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Agencia Costarricense de Investigaciones Biomédicas-Fundación INCIENSA (ACIB-FUNIN), San José, Costa Rica. [2]Caja Costarricense del Seguro Social, San José, Costa Rica. [3]Epidemiology and Population Studies Unit, Laboratory of Clinical Immunology and Microbiology, Division of Intramural Research, NIAID, National Institutes of Health, Bethesda, MD, USA. [4]Infections and Cancer Epidemiology, German Cancer Research Center, Heidelberg, Germany. [5]Virus-Associated Carcinogenesis, German Cancer Research Center, Heidelberg, Germany. [6]Ministerio de Salud, San José, Costa Rica. [7]Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. [8]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany.

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