Preclinical models for oral squamous cell carcinoma investigations

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Abstract

Background: Coronavirus Disease 2019 (COVID-19) first identified in China in December 2019, rapidly spread and reached pandemic level by March 2020. Achieving population level immunity against Severe Acute Respiratory Syndrome Coronavirus 2 (SAR-CoV-2) has been considered the main direction to ending the pandemic. Better characterization of immune response to SARS-CoV-2 is essential for effectively tackling the virus through informing prevention, treatment and control strategies.

Aim: The objectives of this study were to longitudinally evaluate IgG antibody response among recovered COVID-19 patients and vaccinated persons and second to assess protective efficacy by estimating risk of re-infection and breakthrough infection.

Methods: We evaluated two cohorts of adults: 1) recovered COVID-19 patients without vaccination (followed for the median 23 weeks) and 2) vaccinated persons without the history of COVID-19 infection (followed for the median 5 weeks). On each follow up visit blood was drawn for IgG antibody testing and participants were interviewed about the potential exposure to SARS-CoV-2 and diagnosis of infection.

Results: Study enrolled 250 persons, including 150 recovered from COVID-19 and 100 vaccinated against COVID-19. Among recovered 150 COVD-19 persons SARS-CoV-2 IgG antibody seropositivity decreased from 95.3% to 92.0% during the median 23 weeks of follow-up after diagnosis. Among vaccinated 100 persons 93 (93.0%) were IgG seropositive after the median 5 weeks of follow-up since the first dose of vaccine. Among vaccinated persons followed for at least 2 weeks, the rate of seropositivity was 96.8% (92/95). Kaplan-Meier analysis showed that among recovered persons the estimated probability of having SARS-CoV-2 IgG antibodies gradually declined over time dropping to 0.89 by week 36 after diagnosis. Among vaccinated persons, the probability of having SARS-CoV-2 IgG antibodies steeply increases over time reaching 0.99 by week 12 after vaccination. During the follow-up none of recovered patients had re-infection (incidence: 0.0 per 100 person-weeks, 95% CI: 0.0-0.1). No cases of breakthrough infections were reported among vaccinated persons (incidence: 0.0 per 100 person-weeks, 95% CI: 0.0-0.6).

Conclusions: Our study confirms that both natural infection and vaccination generate robust antibody response to SARS-CoV-2. There were no new infections diagnosed during the follow-up, corroborating previous findings of high protective efficacy of this response within the 6 months post-infection or vaccination(1). Data also indicates that SARS-CoV-2 specific IgG antibodies among recovered patients wane over time. Future studies to evaluate comprehensive immune response to the virus over extended periods of time, including dynamics of humoral and cellular immunity, are warranted to better inform prevention, treatment and control strategies.

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Keywords: COVID-19; Immunity; Natural infection; Vaccination; Immunoglobulin G.

Introduction

Coronavirus Disease 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SAR-CoV-2), identified in China in December 2019, rapidly spread around the world reaching pandemic level by March 2020 (2, 3). According to the World Health Organization (WHO) as of April 28, 2022 more than 508 million SARS-CoV-2 infections were reported globally resulting on more than 6 million deaths (4).

Achieving population level immunity against SARS-CoV-2, primarily through vaccination, has been considered the main direction to ending the pandemic. It has been shown that both natural SARS-CoV-2 infection and vaccination generate robust humoral response. SARS-CoV-2 specific IgA, IgM and IgG antibodies can be detected in the blood within 10-20 days after infection or vaccination (5, 6). IgG antibodies are retained for longer period compared to other classes, but titers also wane over time (7, 8). Because of waning immunity people remain under the risk of infection after recovery or vaccination as evidenced by reports of re-infections and breakthrough infections (9, 10).

The first case of COVID-19 in Georgia was diagnosed on February 26, 2020. Since then country experienced several serious outbreak waves with cumulative 1.65 million infections reported as of April 28, 2022 (11). Similar to the rest of the world, the number of new infections in Georgia has been declining since March 2022 with 7-day rate of new infections dropping to 42 per 1 million population compared to 5400 per 1 million registered during the last wave in February 2022 (11). Despite this favorable trend, available body of knowledge suggests that complete

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eradication of SARS-CoV-2 will not be possible and the risk of emergence of new, more contagious or virulent, strains remains substantial (12, 13).

Better characterization of immune response to SARS-CoV-2 is essential for effectively tackling the virus through informing prevention, treatment and control strategies. This is particularly important for countries with low COVID-19 vaccination uptake, such as Georgia with only 32% of population fully vaccinated as of end of April 2022 (11).

The primary objective of this study was to longitudinally evaluate IgG antibody response among recovered COVID-19 patients and vaccinated persons. The secondary objective was to assess protective efficacy by estimating risk of re-infection and breakthrough infection.

Methods

This was ambidirectional cohort study of two groups of adults (age ≥18 years): 1) recovered COVID-19 patients, who had not been vaccinated; 2) vaccinated persons with no previous history of COVID-19. For the recovered patients cohort baseline was defined as date of COVID-19 diagnosis, the cohort was prospectively followed during January-July, 2021. For the vaccinated cohort baseline was defined as date of vaccination, the cohort was prospectively followed during April-July, 2021. Retrospective component included information about the previous history of COVID-19, included date of diagnosis, type of care received (outpatient vs. inpatient). SARS-CoV-2 specific IgG antibodies were measured during the prospective component. At each follow-up visit blood specimen was drawn for IgG antibody testing and participants were interviewed about the potential exposure to SARS-CoV-2 and diagnosis of infection.

The main outcome of interest was proportion of participants positive for IgG antibodies measured by AdviseDx SARS-CoV-2 IgG II (Abbott, USA) assay. The assay was performed and interpreted in accordance with manufacturer's instructions. Assay result of ≥50 AU/ml was considered as positive (IgG is present in the blood). Bivariate comparisons were tested using Pearson's chi-square test for categorical data and Mann Whitney U test for continuous data. Factors associated with IgG positivity were assessed in multivariate regression analysis using Cox proportion hazards regression model to account for varying follow-up period.

The secondary outcome of interest was newly confirmed diagnosis of COVID-19 (defined as: re-infection among recovered patients cohort; breakthrough infection among vaccinated cohort). Time-to-event approach was used for deriving rates of re-infection and breakthrough infections, calculated as number of events (new diagnosis) divided by the number of total person-weeks of follow-up contributed to the observation period. All statistical analyses were performed using SAS v9.4 (SAS Inc, Cary, NC, USA). p value of <0.05 was considered statistically significant.

Results and discussion

Study enrolled 250 persons, including 150 recovered from COVID-19 and 100 vaccinated against COVID-19. Among 150 recovered patients, the median age was 46 (IQR: 33-56) years, 122 (81.3%) were women, vast majority of participants had immunological markers within the reference range (CD4, CD8, CD19 and CD16/56 cells). Overall 105 (70.0%) persons received outpatient care for their disease and 45 (30.0%) inpatient care. Participants were followed for the median 23 (IQR: 16-27) weeks.

Among 100 vaccinated persons the median age was 51 (IQR: 40-60) years, 77 (77.0%) were women, majority had immunological markers within the reference range, 86 (86.0%) received AstraZeneca vaccine versus 14 (14.0%) vaccinated with Pfizer BionTech, and were followed for the median 5 (IQR: 3-8) weeks. Comparison between the two study groups showed that vaccinated persons were older, had higher CD19 and CD16/56 cell counts, and shorter follow-up (Table 1).

Table 1. Characteristics of study population

	Recovered (n=150)	Vaccinated (n=100)	p value
Age, median (IQR)	46 (33-56)	51 (40-60)	0.007
Age categories, n (%)			
<45	71 (47.3)	34 (34.0)	0.04
45+	79 (52.67)	66 (66.0)	
Sex, n (%)			
Women	122 (81.3)	77 (77.0)	0.40
Men	28 (18.7)	23 (23.0)	
CD4 T cell count, median (IQR)	919 (737-1194)	1017 (761-1138)	0.25
CD8 T cell count, median (IQR)	546 (385-663)	560 (425-646)	0.74
CD4/CD8 ratio, median (IQR)	1.81 (1.35-2.40)	1.82 (1.40-2.44)	0.42
CD19 B cell count, median (IQR)	238 (181-337)	342 (227-404)	<0.0001
CD16/56 NK cell count, median (IQR)	238 (158-332)	290 (216-358)	0.004
Follow-up in weeks, median (IQR)	23 (16-27)	5 (3-8)	<0.001
COVID-19 care, n (%)			
Outpatient	105 (70.0)		
Inpatient	45 (30.0)		
Vaccine type, n (%)			
Vaxzevria (AstraZeneca)		86 (86.0)	
Comirnaty (Pfizer BionTech)		14 (14.0)	

Among recovered 150 COVD-19 persons 143 (95.3%) had detectable SARS-CoV-2 IgG antibodies at the first measurement performed after median 9 (IQR: 5-12) weeks since COVID-19 diagnosis. By the end of follow-up 5 initially IgG positive persons seroreversed, consequently the proportion of persons with IgG antibodies reduced to 92.0% (138/150) after the median 23 (IQR: 16-27) weeks of follow-up (Table 2). Seroversing persons did not significantly differ from non-seroreversing persons in terms age, gender, type of care received for COVID-19 and immune

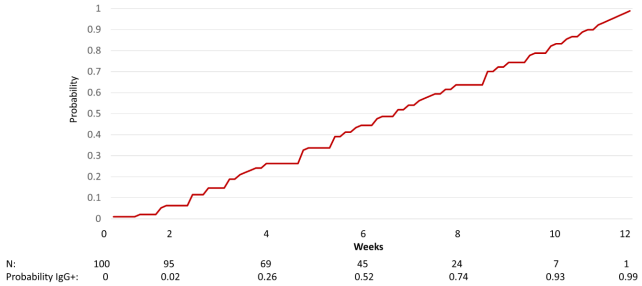
markers.

Among vaccinated 100 persons 89 (89.0%) had detectable SARS-CoV-2 IgG antibodies at the first measurement performed after median 3 (IQR: 2-4) weeks since the first dose of vaccine. By the end of follow-up 4 initially IgG negative persons seroconverted, consequently the proportion of persons with IgG antibodies increase to 93.0% (93/100) after the median 5 (IQR: 3 -8) weeks of follow-up. Among vaccinated persons followed for at least 2 weeks, the rate of seropositivity was 96.8% (92/95) (Table 2).

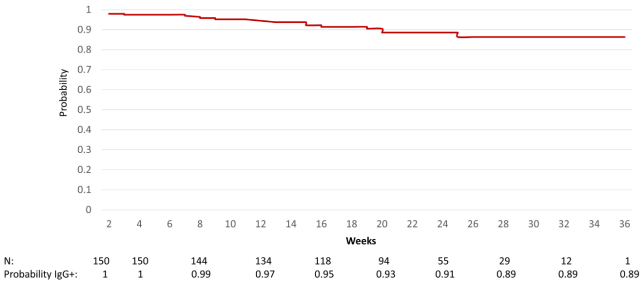
Table 2. SARS-CoV-2 IgG positivity

SARS-CoV-2 IgG positivity	Recovered	Vaccinated
IgG+ at first evaluation	95.3% (143/150)	89.0% (89/100)
IgG+ at the end of follow-up	92.0% (138/150)	93.0% (93/100)
IgG+ among those with ≥2-week follow-up		96.8% (92/95)

Kaplan-Meier analysis showed that among recovered persons the estimated probability of having SARS-CoV-2 IgG antibodies gradually declines over time dropping to 0.89 by week 36 after diagnosis (Figure 1).



Among vaccinated persons, the probability of having SARS-CoV-2 IgG antibodies steeply increases over time reaching 0.99 by week 12 after vaccination (Figure 2).



Factors associated with presence of SARS-CoV-2 IgG antibodies were evaluated in multivariate Cox proportional hazards model. Among recovered persons the only factor significantly associated with IgG positivity was increasing levels of CD19 B cells (HR 1.004, 95% CI: 1.000-1.008, p=0.04). No statistically significant differences were observed among patients receiving outpatient or inpatient care. Among vaccinated persons receiving Pfizer-BionTech vaccine (Comirnaty) was significantly associated with IgG positivity – HR 20.65 (2.04-209.25, p=0.01) (Table 3).

Table 3. Factors associated with SARS-CoV-2 IgG positivity

	Recovered		Vaccinated	
	HR (95% CI)	**p value**	**HR (95% CI)**	**p value**
Vaccinated vs. recovered				
Age (per year increase)	0.975 (0.931-1.021)	0.27	0.977 (0.909-1.049)	0.52
Men vs. women	0.226 (0.012-4.164)	0.32	0.745 (0.095-5.818)	0.78
CD4 T cell count (per unit increase)	0.997 (0.993-1.001)	0.14	1.001 (0.994-1.007)	0.84
CD8 T cell count (per unit increase)	1.003 (0.997-1.009)	0.34	0.996 (0.985-1.007)	0.49
CD4/CD8 ratio (per unit increase)	1.211 (0.143-10.279)	0.86	0.105 (0.003-4.083)	0.23
CD19 B cell count (per unit increase)	1.004 (1.000-1.008)	0.04	1.004 (0.998-1.010)	0.19
CD16/56 NK cell count (per unit increase)	1.002 (0.997-1.006)	0.49	0.997 (0.989-1.005)	0.48
Inpatient vs. outpatient	0.583 (0.106-3.203)	0.53		
Comirnaty vs. Vaxzevria vaccine			20.65 (2.04-209.25)	0.01

Overall participants contributed 3848 person-weeks of follow-up (3276 person-weeks among recovered patients cohort and 572 person-weeks among vaccinated cohort). During the follow-up none of recovered patients had re-infection (incidence: 0.0 per 100 person-weeks, 95% CI: 0.0-0.1). No cases of breakthrough infections were reported among vaccinated persons (incidence: 0.0 per 100 person-weeks, 95% CI: 0.0-0.6).

Our study confirms that both natural infection and vaccination generate robust antibody response to SARS-CoV-2. There were no new infections diagnosed during the follow-up, corroborating previous findings of high protective efficacy of this response within the 6 months post-infection or vaccination (1). Data also indicates that SARS-CoV-2 specific IgG antibodies among recovered patients wane over time. IgG positivity decreased from initial 95% to 92% over median 23 weeks of follow-up, while in Kaplan-Meier analysis the probability of having detectable IgG antibodies decreased to 89% by week 36 post infection. Similar results have been reported from other studies (14-16), however direct comparison of decline rate is not possible because of lack of standardization of antibody assays deriving varying results (17). In all cases,

trend towards waning immunity is same for all studies. Studies also suggest stronger antibody response is stronger among people with severe disease or those treated in inpatient settings (18, 19). We did not observe significant differences by type of care received – outpatient vs. inpatient, which is a definite marker of disease severity.

Antibody response among vaccinated persons indicate high seroconversion rates reaching 96% among persons with at least 2 weeks of follow-up after the vaccine dose. Our results are in line with previous reports demonstrating faster response to PfizerBiontech vaccine compared to AstraZeneca (20). Durability of immunity in our study could not be assessed because of short follow-up related to the fact that vaccination in Georgia was initiated only in March 2021. Similar to recovered patients, international experience indicates to waning immunity among vaccinated persons and this needs to be taken into account for planning response measures (21).

In this study we also evaluated association between host immune status and IgG antibody response. The only factor associated with antibody positivity was increasing levels of CD19 B cells among recovered patients. Our study was not powered to provide detailed insights into this relationship, but we can assume that this might be result of stimulation of B-cells by infection (22). It has been shown that low levels of T-cells, especially of CD4 cells, can play major negative role in antibody response against SARS-CoV-2 among HIV positive persons (23). In our study, all participants had T-cells within normal range and no significant associations were found.

Our study has several limitations. First of all, relatively small sample size likely affected statistical power. Secondly, the follow-up time was not sufficient to fully elicit data on long-term dynamics of antibody response and its protective efficacy, especially in the cohort of vaccinated persons. Finally, the study focused on antibodies only, while comprehensive immune response also incorporates neutralizing antibody response and cellular immunity (24).

Despite these limitations, our study provides important scientific and practical information for guiding effective response measures against the pandemic. Future studies to evaluate comprehensive immune response to the virus over extended periods of time, including dynamics of humoral and cellular immunity, are warranted to better inform prevention, treatment and control strategies.

Conclusions: Our study confirms that both natural infection and vaccination generate robust antibody response to SARS-CoV-2. There were no new infections diagnosed during the follow-up, corroborating previous findings of high protective efficacy of this response within the 6 months post-infection or vaccination(1). Data also indicates that SARS-CoV-2 specific IgG antibodies among recovered patients wane over time. Future studies to evaluate comprehensive immune response to the virus over extended periods of time, including dynamics of humoral and cellular immunity, are warranted to better inform prevention, treatment and control strategies.

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