

RESEARCH ARTICLE

Analysis of accumulated SARS-CoV-2 seroconversion in North Carolina: The COVID-19 Community Research Partnership

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Abstract

Introduction

The COVID-19 Community Research Partnership is a population-based longitudinal syndromic and sero-surveillance study. The study includes over 17,000 participants from six healthcare systems in North Carolina who submitted over 49,000 serology results. The purpose of this study is to use these serology data to estimate the cumulative proportion of the North Carolina population that has either been infected with SARS-CoV-2 or developed a measurable humoral response to vaccination.

Methods

Adult community residents were invited to participate in the study between April 2020 and February 2021. Demographic information was collected and daily symptom screen was

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completed using a secure, HIPAA-compliant, online portal. A portion of participants were mailed kits containing a lateral flow assay to be used in-home to test for presence of anti-SARS-CoV-2 IgM or IgG antibodies. The cumulative proportion of participants who tested positive at least once during the study was estimated. A standard Cox proportional hazards model was constructed to illustrate the probability of seroconversion over time up to December 20, 2020 (before vaccines available). A separate analysis was performed to describe the influence of vaccines through February 15, 2021.

Results

17,688 participants contributed at least one serology result. 68.7% of the population were female, and 72.2% were between 18 and 59 years of age. The average number of serology results submitted per participant was 3.0 (± 1.9). By December 20, 2020, the overall probability of seropositivity in the CCRP population was 32.6%. By February 15, 2021 the probability among healthcare workers and non-healthcare workers was 83% and 49%, respectively. An inflection upward in the probability of seropositivity was demonstrated around the end of December, suggesting an influence of vaccinations, especially for healthcare workers. Among healthcare workers, those in the oldest age category (60+ years) were 38% less likely to have seroconverted by February 15, 2021.

Conclusions

Results of this study suggest more North Carolina residents may have been infected with SARS-CoV-2 than the number of documented cases as determined by positive RNA or antigen tests. The influence of vaccinations on seropositivity among North Carolina residents is also demonstrated. Additional research is needed to fully characterize the impact of seropositivity on immunity and the ultimate course of the pandemic.

Introduction

Estimating the proportion of the population previously infected with SARS-CoV-2, the agent of COVID-19, or who have been successfully vaccinated is imperative to optimally characterize the epidemiology of the pandemic and to make informed public health decisions about when and how to resume normal activities. Using case definitions based on clinically motivated testing for SARS-CoV-2 RNA or antigens is not reliable for multiple reasons. SARS-CoV-2 infections may not be recognized among asymptomatic or mildly symptomatic individuals [1–3]. In some communities, the lack of available testing for COVID-19 limited the ability to detect or diagnose cases, especially in the first few months of the pandemic. Often in rural areas access to care and testing is limited by external resources such as transportation. Our research group has also demonstrated that large-scale population-based cross-sectional sero-surveillance is similarly problematic because of rapid sero-reversion, especially among people with mild or asymptomatic disease [4].

To overcome these limitations, we established the COVID-19 Community Research Partnership (CCRP), a population-based longitudinal syndromic and sero-surveillance study. The CCRP includes >17,000 participants who submitted at least one serology result since April 16, 2020. These participants were recruited from six healthcare systems in North Carolina between

mid-April 2020 and February 2021. Over 49,000 longitudinal serology tests from CCRP participants were recorded, including some participants who completed up to eight sequential serology tests. The purpose of this study is to use these serology data to estimate the cumulative proportion of the population enrolled in our study that has either been infected with SARS-CoV-2 or developed a measurable humoral response to vaccination.

Materials and methods

Only the sero-surveillance portion of the CCRP in North Carolina is described in this paper. Community residents age 18 years or older within six North Carolina health systems were invited to participate in the study using multiple methods of communication, including email, websites, health system communications, and social and mass media (radio and television). Potential participants in two of the systems, Wake Forest Baptist Health and Atrium Health, were initially invited on April 16th, 2020. Potential participants in the other four health systems, WakeMed, New Hanover Regional Medical Center, medical associates of Campbell University School of Osteopathic Medicine, and Vidant Health were invited in November 2020. All participants provided informed consent for study procedures, including those required to secure a blood sample for serology testing. In the consent process, interested persons were provided a secure link to online informed consent. Demographic information was collected and daily symptom screen was completed using a secure, HIPAA-compliant, online portal. Participants were queried in the portal to determine healthcare worker status. The CCRP study was approved by the IRB of Wake Forest University Health Sciences.

A portion of participants were selected for serological testing. These were chosen to demographically represent the populations living in the region served by the health system. Participants were mailed kits for in-home collection of capillary blood via finger prick. The kits contained a lateral flow assay (LFA) to be used in-home to test for presence of anti-SARS-CoV-2 IgM or IgG antibodies. LFA results were recorded and interpreted using a smartphone application with central review (Scanwell Health, Inc. © 2020). In the first three months of the study, participants received a LFA by Syntron Bioresearch Inc., which detects IgM and IgG antibodies to the SARS-CoV-2 nucleocapsid antigens. However, this assay became unavailable during the study period. Beginning in July 2020, participants received the Scanwell SARS-CoV-2 IgM IgG Test from Teco Diagnostics, which detects IgM and IgG antibodies to the spike protein and nucleocapsid antigens. A subset of participants received two 20 μ L volumetric absorptive microsamplers (Mitra[®], Neoteryx) for sample collection, and these were analyzed centrally using the Syntron LFA. Both LFAs were validated at the Frederick National Laboratory for Cancer Research (FNLRCR) by the National Cancer Institute (NCI) using a panel of antibody-positive samples from patients with PCR confirmed SARS-CoV-2 infection or pre-pandemic controls [5, 6].

Participants were mailed test kits at various times throughout the study period depending on test kit availability, supply chain disruptions, and shipping delays, all of which were generally influenced by the pandemic itself. Participant enrollment occurred in an ongoing (rolling) fashion over time so that participants who enrolled earlier in the study period had more opportunities to be tested. Likewise, the decision by some to stop participating in the study limited the number of tests that could have been performed for these individuals. Lastly, the number of tests performed for each participant was influenced by the participant's willingness to complete each test or return samples for central testing.

Accumulated SARS-CoV-2 seroconversion, testing positive for IgM and/or IgG at least once during the study period, was estimated. This is presented as the probability of prior infection from the beginning of the study up to December 20, 2020 (end of observation period), the

time when vaccines were made available to certain members in the study population. A standard Cox proportional hazards model was constructed to illustrate the probability of seroconversion over time, taking into consideration covariates of age, sex, and healthcare worker status. The proportional hazards assumption was tested and not rejected using the Schoenfeld residuals [7]. A separate analysis was performed to describe the influence of vaccines. In this analysis, the period of observation was extended to February 15, 2021, and given the high likelihood of vaccination among healthcare workers, the Cox model was stratified by healthcare worker status. Results for this period represent the probability of prior infection or vaccination. Because of the dynamic nature of the CCRP population, with some dropping out after a period of participation, the data were censored on the day after the last negative serology. Participants who reported a serology result after the last day of observation for each analysis were considered censored on the last day if all prior serologies were negative. In the analysis, the hazards of healthcare worker status and biological sex were non-proportional, violating the proportionality hazard assumption of standard Cox model. We therefore estimated average hazard ratios (AHR) by using a weighted Cox regression [8, 9] to evaluate the effect of age and sex on time to seroconversion. Similar to the standard hazard ratio, an AHR of 1 indicates no difference in survival rates across all time points. An AHR greater than 1 means an increased risk, while an AHR lower than 1 means a reduction in risk over time [10]. Separate models were fit for healthcare workers and non-healthcare workers. All analyses were performed using R version 4.0.2 [11].

Results

Within the CCRP population of North Carolina, 17,688 participants contributed at least one serology result. Characteristics of these participants are listed in Table 1. 68.7% of the population were female and 72.2% of participants were between 18 and 59 years of age. Approximately 11% reported being a member of a minority race/ethnic group. The average number of serology test results submitted per participant was 3.0 (± 1.9). Healthcare worker profession was reported for 35.2% of study participants and 79.3% were female. The average number of serology test results submitted per healthcare worker was 3.5 (± 2.1), which was higher than non-healthcare workers (2.7 ± 1.7).

By December 20, 2020, the overall probability of seropositivity in the CCRP population since the beginning of the study was 32.6% (95% CI 28.4, 35.0). This probability can be considered the probability of prior infection since vaccines were not available for most people before this date. Fig 1 illustrates the accumulating probability over time. Many participants in the CCRP study identified as healthcare workers and were in the initial target group to receive a COVID-19 vaccine. Table 2 lists estimates of the probability of seropositivity before and after the availability of COVID-19 vaccines and according to healthcare worker status. By February 15, 2021 the probability among healthcare workers and non-healthcare workers was 83% (82, 85) and 49% (95% CI 47, 52), respectively. The analysis at this date represents the probability of either prior infection or vaccination. Fig 2 demonstrates a clear inflection upward in the curve around the end of December, which suggests a significant impact of vaccinations on serology results, especially for healthcare workers. Prior to the inflection, the probabilities were relatively close, suggesting that healthcare workers were not becoming infected at an appreciably higher rate than non-healthcare workers. A life table (Table 3) provides cumulative probabilities of seroconverting over time which correspond to the model illustrated in Fig 2.

Table 4 lists hazard ratios for risk of seroconverting among subgroups. Prior to December 20, 2020 neither sex nor age posed a significant risk of seropositivity among non-healthcare workers. By February 15, 2021, males were 19% less likely to have seroconverted during the

Table 1. Characteristics of North Carolina CCRP participants in the serology analysis (n = 17,688).

	Number (%)
Age (years)	
18–39	5,049 (28.5)
40–59	7,719 (43.6)
60+	4,920 (27.8)
Sex	
Female	12,160 (68.7)
Male	5,528 (31.3)
Race/Ethnicity	
Black or African American	542 (3.1)
Hispanic or Latinx	432 (2.4)
Other	1,042 (5.9)
White	15,672 (88.6)
Healthcare Worker Status	
No	11,461 (64.8)
Yes	6,227 (35.2)
Healthcare System Location	
Atrium Health	2,732 (15.4)
Campbell University	325 (1.8)
New Hanover Regional	506 (2.9)
Vidant Health	649 (3.7)
Wake Forest Baptist Health	11,558 (65.3)
WakeMed	1,918 (10.8)
Vaccination Reported (after Dec 20, 2020)	
Yes	8,041 (45.5)
No	9,647 (54.5)

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observation period (AHR 0.81, 95% CI 0.73, 0.90). Age did not have a significant impact on the risk of seroconverting among non-healthcare workers. As for the healthcare workers, again prior to December 20, 2020 the risk of seroconverting was not different for any of the sub-groups. However, by February 15, 2021 males were 10% more likely to have seroconverted, and older age groups were less likely to have seroconverted. The oldest age group of 60+ years was 38% less likely to have seroconverted (AHR 0.62, 95% CI 0.54, 0.72).

Discussion

Results of the COVID-19 Community Research Partnership (CCRP) suggest there may be more infections occurring in North Carolina than is documented based on reporting of positive SARS-CoV-2 RNA or antigen tests. Using US Census estimates of the total population in NC in 2019 and the number of reported positive tests according to the NC Department of Health and Human Services (DHHS) as of March 3, 2021 [12], the cumulative incidence of COVID-19 in NC is calculated to be approximately 8.3%, a number that is significantly less than the probability of prior infection on December 20, 2020 reported here (32.6%).

There are many aspects of the CCRP that are uniquely capable of determining the likelihood of prior infection in North Carolina. Unlike other serology studies that relied on cross-sectional analysis [13–17], the CCRP assessed serology status among participants over time with multiple possible measurements per participant. This is especially important as emerging evidence has documented short-term duration of seropositivity associated with SARS-CoV-2

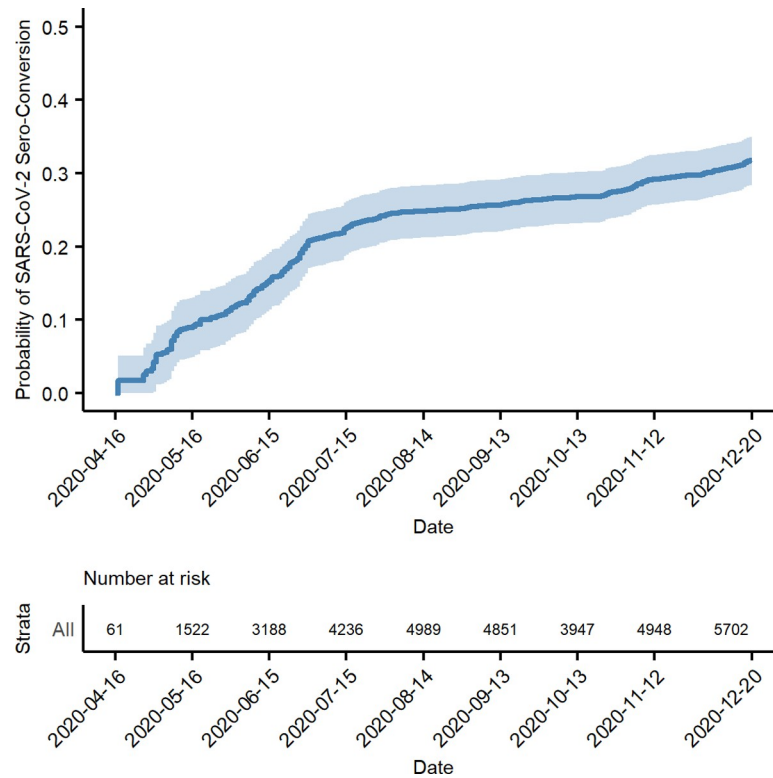


Fig 1. Probability of prior SARS-CoV-2 infection before the availability of vaccines.

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infection, as short as 2 months duration [4]. Traditional methods of performing cross-sectional studies to identify seroprevalence would fall short and underestimate progress toward population immunity [18, 19]. Even with the advantage of multiple measurements over time, there is still some risk of underestimation within the CCRP population. Individuals who joined the CCRP study relatively late in the pandemic may have already been infected and subsequently sero-reverted before their first test.

In order to fully characterize rates of SARS-CoV-2 infection during a pandemic, it is critically important to begin the process of identifying infections as early as possible. Efforts to identify infections during the CCRP study began in April 2020, an early stage of the pandemic and well ahead of peak infections that would occur in the coming winter months. In addition, the long time span (April 2020 to February 2021) of the study allowed for more thorough capture of seroconversions in the population and therefore the ability to determine accumulation of seropositivity, including among members of the population who would not have sought

Table 2. Probability of seropositivity according to healthcare worker status.

Period of Observation	Estimate (95% confidence interval)	
	Non-healthcare worker	Healthcare worker
April 16, 2020 to December 20, 2020 ¹	0.35 (0.31, 0.38)	0.27 (0.23, 0.31)
April 16, 2020 to February 15, 2021 ²	0.49 (0.47, 0.52)	0.83 (0.82, 0.85)

1. Probability of prior infection (before vaccines).

2. Probability of prior infection or vaccination.

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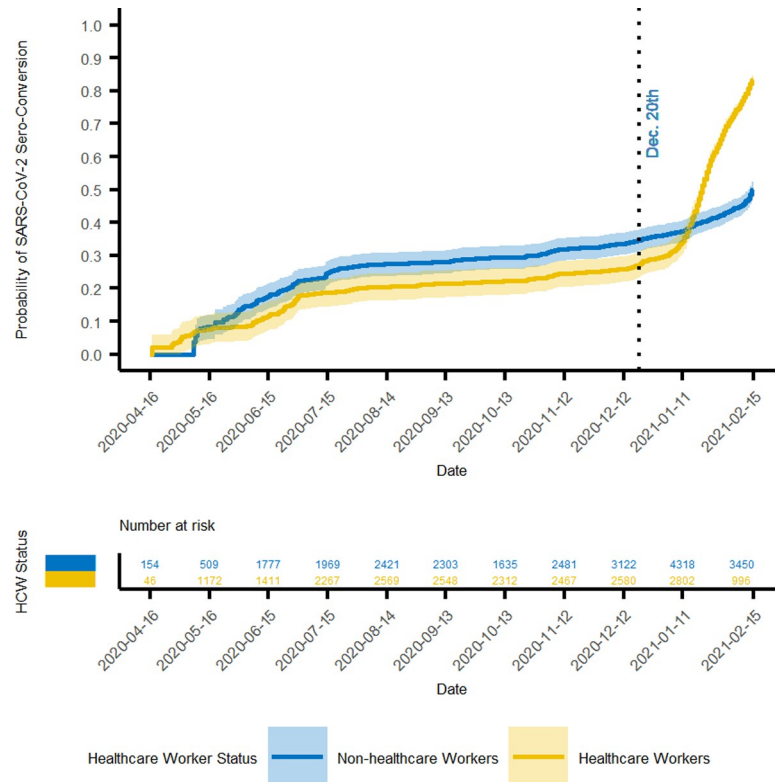


Fig 2. Probability of prior SARS-CoV-2 infection or vaccination.

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testing, e.g. asymptomatic infections or symptomatic individuals who have a known positive contact.

A COVID-19 vaccination campaign began in December 2020 in North Carolina and initially targeted healthcare workers and people age 65 or older. Owing to the nature of the study, which solicited participation within health system networks, the proportion of CCRP

Table 3. Cumulative probability of seroconverting over time.

Date	At risk		Events		Censored		Probability (95% CI)	
	Non-HCW	HCW	Non-HCW	HCW	Non-HCW	HCW	Non-HCW	HCW
4/16/2020	154	46	0	0	0	0	0.00 (0.00,0.00)	0.00 (0.00,0.00)
5/16/2020	509	1172	19	31	20	37	0.08 (0.04,0.12)	0.07 (0.03,0.12)
6/15/2020	1777	1411	115	61	266	63	0.17 (0.14,0.21)	0.11 (0.07,0.16)
7/15/2020	1969	2267	162	170	960	274	0.24 (0.21,0.28)	0.19 (0.14,0.23)
8/14/2020	2421	2569	89	54	207	105	0.27 (0.24,0.31)	0.2 (0.16,0.24)
9/13/2020	2303	2548	22	34	211	94	0.28 (0.24,0.31)	0.22 (0.17,0.25)
10/13/2020	1635	2312	40	22	630	236	0.29 (0.26,0.33)	0.22 (0.18,0.26)
11/12/2020	2481	2467	66	69	363	397	0.32 (0.28,0.35)	0.24 (0.2,0.28)
12/12/2020	3122	2580	69	52	247	210	0.34 (0.3,0.37)	0.26 (0.22,0.3)
1/11/2021	4318	2802	212	339	722	365	0.37 (0.34,0.4)	0.34 (0.3,0.38)
2/10/2021	3450	996	612	2023	3007	618	0.45 (0.42,0.48)	0.77 (0.75,0.79)

HCW = healthcare worker.

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Table 4. Risk of seroconversion within subgroups.

Characteristic	December 20, 2020			February 15, 2021		
	AHR	95% CI	p value	AHR	95% CI	p value
<u>Non HCW</u>						
Sex						
Female	–	–		–	–	
Male	1.15	0.97, 1.36	0.1	0.81	0.73, 0.90	<0.0001
Age Group						
18–39	–	–		–	–	
40–59	1.02	0.81, 1.28	0.9	0.99	0.87, 1.48	0.9
60+	1.05	0.83, 1.31	0.7	1.15	0.99, 1.32	0.055
<u>HCW</u>						
Sex						
Female	–	–		–	–	
Male	1.04	0.85, 1.29	0.7	1.10	1.01, 1.20	0.037
Age Group						
18–39	–	–		–	–	
40–59	1.02	0.84, 1.23	0.9	0.82	0.75, 0.88	<0.0001
60+	1.10	0.83, 1.47	0.5	0.62	0.54, 0.72	<0.0001

HCW = healthcare worker, AHR = average hazard ratio, CI = confidence interval.

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participants who are healthcare workers was generally high and may not represent the general population. As illustrated in Table 2 and Fig 2, the proportion of healthcare workers testing seropositive prior to December 20, 2020 is quite similar to that of non-healthcare workers. This suggests that an over-representation of healthcare workers in the CCRP is not contributing to a relatively high probability of prior infection as of December 20, 2020.

It is very clear though, the availability of COVID-19 vaccinations among healthcare workers had a strong influence on serology results. There was an inflection upward in the curves after December 20, 2020, which was more prominent among healthcare workers. The increase in probability among non-healthcare workers during this interval likely reflects vaccinations received by those age 65 years or older. The subgroup analysis identified age as a factor associated with lower probability of seroconversion by February 15, 2021. This finding may be due to a longer duration of time between first vaccine dose and detectable humoral response among older vaccine recipients. The pattern of increasing probability late in the study period suggests that vaccination efforts in North Carolina are contributing significantly to the proportion of the population that have developed a humoral response to one or more SARS-CoV-2 specific antigens. Enrollment in a COVID-19 vaccine clinical trial by individuals in the CCRP study is possible prior to December 20, 2020. However, a material impact on the results of this study is not expected from the very low number of such participants.

There are other limitations to this study that need to be acknowledged. The demographics of the CCRP study population do not match that of the general population in North Carolina. There was an imbalance in sex and race, with over-representation of females and Whites. There was under-representation of young adults less than age 30. Not to mention, adolescent and pediatric residents (age <18 years) were not included in this analysis of the CCRP. For these reasons, it may not be appropriate to generalize these results to all populations in North Carolina.

The performance characteristics of LFAs in the detection of anti-SARS-CoV-2 antibodies should be considered in the interpretation of these data. The sensitivity/specificity of the Syntrotron LFA are: IgM 93.3%/97.5%, IgG 73.3%/100%, IgM or IgG 96.7%/97.5%. The sensitivity/specificity of the Scanwell SARS-CoV-2 IgM IgG Test are: IgM 90%/100%, IgG 86.7%/100%, IgM or IgG 96.7%/100% [5, 6]. While these performance characteristics evoke some concern about the accuracy of test results, particularly negative results, the pattern of increasing probability of seropositivity over time along with the apparent influence of vaccinations provide some measure of internal validity. Not to mention, LFAs were possibly the only practical method of determining serology status for a study population of this magnitude.

Population immunity (“herd immunity”) is the point at which the incidence of infection decreases once a certain amount of the population has acquired immunity. Public health experts are particularly interested in sero-surveillance data as this helps in determining the number of infections in the population, which can be used as a surrogate of immunity. Results of the CCRP study may be particularly useful for this purpose because serology status was assessed in a longitudinal way, which for SARS-CoV-2 infections has its advantages over cross-sectional serology studies for reasons already mentioned. What is not yet known, though, is whether the cumulative proportion of the population that tested seropositive accurately represents the proportion that has acquired immunity. Indeed, it is possible that immunity may wane over time in conjunction with sero-reversion and/or declining antibody titers [1, 4, 20]. This could produce a condition in which some of the previously seropositive population has relative immunity or no immunity at all. Lastly, there are uncertainties concerning the degree to which immunity from vaccines or natural infection will extend to infections caused by newer variants of SARS-CoV-2 [21–24].

Randolph and Barreiro have calculated a population immunity threshold of 67% for SARS-CoV-2 [25]. Because the assumption that seropositivity equals acquired immunity is not yet proven for SARS-CoV-2 and because of the limitations in generalizing these results broadly, it may be premature to compare cumulative probability of seropositivity in the CCRP study to a given threshold. More research is needed to determine if the decline in cases and hospitalizations in North Carolina (February and March 2021) could be attributed to population immunity that is approaching such a threshold.

Results of the CCRP study provide valuable insights about the proportion of North Carolina residents who have been infected with SARS-CoV-2. These data suggest more North Carolina residents may have been infected than the number of documented cases as determined by positive RNA or antigen tests for SARS-CoV-2. This is consistent with the understanding that mildly symptomatic or asymptomatic individuals may not seek testing. The influence of vaccinations on seropositivity among North Carolina residents is also demonstrated. Additional research is needed to fully characterize the impact of seropositivity on immunity and the ultimate course of the pandemic.

Supporting information

S1 Dataset.

(XLSX)

Acknowledgments

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