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Journal of Infection and Chemotherapy

journal homepage: www.elsevier.com/locate/jic



Original Article

Antibody responses and SARS-CoV-2 infection after BNT162b2 mRNA booster vaccination among healthcare workers in Japan



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Keywords: Anti-spike antibody SARS-CoV-2 mRNA vaccine Booster vaccination Healthcare worker

ABSTRACT

Introduction: Vaccine effectiveness against SARS-CoV-2 infections decreases due to waning immunity, and booster vaccination was therefore introduced. We estimated the anti-spike antibody (AS-ab) recovery by booster vaccination and analyzed the risk factors for SARS-CoV-2 infections.

Methods: The subjects were health care workers (HCWs) in a Chiba University Hospital vaccination cohort. They had received two doses of vaccine (BNT162b2) and a booster vaccine (BNT162b2). We retrospectively analyzed AS-ab titers and watched out for SARS-CoV-2 infection for 90 days following booster vaccination.

Results: AS-ab titer eight months after two-dose vaccinations had decreased to as low as 587 U/mL (median, IQR (interquartile range) 360–896). AS-ab titer had then increased to 22471 U/mL (15761–32622) three weeks after booster vaccination. There were no significant differences among age groups.

A total of 1708 HCWs were analyzed for SARS-CoV-2 infection, and 48 of them proved positive. SARS-CoV-2 infections in the booster-vaccinated and non-booster groups were 1.8% and 4.0%, respectively, and were not significant. However, when restricted to those 20–29 years old, SARS-CoV-2 infections in the booster-vaccinated and non-booster groups were 2.9% and 13.6%, respectively (p = 0.04). After multivariate logistic regression, COVID-19 wards (adjusted odds ratio (aOR):2.9, 95% confidence interval (CI) 1.5–5.6) and those aged 20–49 years (aOR:9.7, 95%CI 1.3–71.2) were risk factors for SARS-CoV-2 infection.

Conclusions: Booster vaccination induced the recovery of AS-ab titers. Risk factors for SARS-CoV-2 infection were HCWs of COVID-19 wards and those aged 20–49 years. Increased vaccination coverage, together with implementing infection control, remains the primary means of preventing HCWs from SARS-CoV-2 infection.

Authorship statement

All authors meet the ICMJE authorship criteria. All authors have seen and approved the manuscript, and contributed significantly to the work.

All authors approved the publication of the manuscript, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

https://doi.org/10.1016/j.jiac.2022.07.010

Received 2 June 2022; Received in revised form 11 July 2022; Accepted 14 July 2022

Available online 20 July 2022

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HI, TK, KI, IY, KY, and HN conceptualized the study; HI, TK, MY, TT and HN designed the study; HA, SM, TY, KK and KM performed experiments and analyzed the data; HK, YO, MU and HC organized blood sampling, HI wrote the first draft of the manuscript.

1. Introduction

The SARS-CoV-2 Omicron variant (B.1.1.529) with as many as 36 mutations within the spike protein was first detected in Botswana in November 2021 [1], and it raised concerns about its escape from naturally acquired or vaccine-elicited immunity. Spike proteins are the mediator of host cell entry and the main targets of neutralizing antibodies. Previous studies associated with SARS-CoV-2 variants have demonstrated that mutations within the receptor binding domain (RBD) mediate the escape from vaccine-induced neutralizing antibodies [2–4].

Three vaccines (BNT162b2 manufactured by Pfizer-BioNTech, mRNA-1273 by Moderna, and ChAdOx1-S by Oxford/AstraZeneca) have been approved by MHLW (Japanese Ministry of Health, Labour and Welfare). These three vaccines use the original wild-type SARS-CoV-2 spike protein first identified in Wuhan, China, as the sole immunogen. These SARS-CoV-2 vaccines induced neutralizing humoral and cellular immunity and resulted in reductions of SARS-CoV-2 infections, hospitalizations, and deaths [5,6].

Vaccine effectiveness against SARS-CoV-2 infections decreases during the first six months primarily due to waning immunity rather than the variant escaping vaccine protection [7,8]. The neutralizing antibody responses and vaccine effectiveness progressively decrease post vaccination and are attenuated even more for emerging variants [9–13]. Then, a third dose of mRNA vaccine (''booster'') was approved for individuals vaccinated >6 months earlier, and it has been shown to be very effective, inducing high neutralizing antibody titers [10].

We previously revealed that two-dose vaccinations introduced humoral immunity in health care workers (HCWs) [14]. This present work is a sequential study using the same cohort. There have been several studies regarding the analysis of anti-Spike antibody (AS-ab) titer decreases during 6–8 months after two-dose vaccinations [3,15]. In the present study, we also measured AS-ab titers eight months after two-dose vaccinations, just before booster vaccination of HCWs at Chiba University Hospital (CUH), and also AS-ab titers three weeks after booster vaccination.

We used BNT162b2 for vaccination at CUH, initiating vaccination at this location on Dec. 21, 2021. The Omicron variant gradually became predominant from December 2021, with the number of thusly infected COVID-19 patients beginning to increase, then peaking in early February 2022 in Japan.

Wild-type spike glycoprotein, encoded by BNT162b2 vaccine, elicited neutralizing antibody against newly emerging variants other than the original Wuhan virus [16]. This booster vaccine was also proven to introduce neutralizing antibody against Omicron in Japan [15], indicating that booster vaccination would reduce infection and hospitalization. In this study, we observed the AS-ab titers change during eight months after two-dose vaccinations, and then just after three weeks following booster vaccination. Furthermore, we also noted the vaccination effects on HCWs by the 90-day infection and hospitalization rates.



Fig. 1. The subjects and flowchart of cohort in this study

The subjects of this study consisted of health care workers (HCWs) of Chiba University Hospital, and they were all registered in the former study.

A total of 1774 HCWs were analyzed to determine the effects of booster vaccination. They completed the 2nd anti-Spike antibody (AS-ab) examination three weeks after two-dose vaccinations.

As for AS-ab analysis, we analyzed all HCWs who participated in blood sampling. As for vaccination effectiveness analysis, a total of 1708 HCWs, except for 66 who retired, were included. There were 357 HCWs without AS-ab titers. The numbers of booster vaccinations and non-booster vaccinations were 1633 and 75, respectively.

2. Subjects and methods

The subjects were HCWs at CUH, and they were also the vaccination cohort of a former study [14]. They had received two doses of BNT162b2 mRNA COVID-19 vaccine, with a 21-day interval, in March and April 2021. The total number of subjects at registration was 2015. The number of participants was then restricted to 1774 HCWs, who received two-dose vaccinations and underwent blood sampling for AS-ab titer estimation (Fig. 1). The former study analyzed the AS-ab titer response [14]. The Japanese government published the booster vaccination program for HCWs for the control of SARS-CoV-2 infection, and CUH then initiated booster vaccination from the 1st of Dec. 2021.

For HCWs, CUH began booster vaccination from Dec. 21, 2021, and provided opportunities for AS-ab titer examinations both before and after booster vaccination. Background information had already been collected in the former study [14].

Blood samples were obtained 0–1 week before and three weeks after booster vaccination. AS-ab titers were analyzed using Elecsys® Anti-SARS-CoV-2S on Cobas 8000 e801 module (Roche Diagnostics, Rotkreuz, Switzerland). This system allows for the quantitative detection of antibodies, predominantly IgG, aiming at the SARS-CoV-2 spike protein RBD.

AS-ab titers were measured at CUH in this study, while they were measured at SRL (Tokyo, Japan), a commercial clinical laboratory, in the former study. For standardization of the two laboratories, 50 serum samples from the former study, stored at CUH, were randomly chosen and measured for AS-ab titers for quality control. We confirmed that they were well correlated (r = 0.994) with each other.

The AS-ab titers after booster vaccination were expected to increase to more than 250 U/mL, the upper range of the manufacturer's protocol. The serums were diluted 400-fold and the new measurement range was 160-100,000 U/mL, as the former range before dilution was 0.4-250 U/mL. If AS-ab titers were beyond this range, the serums were diluted 900-fold and remeasured. If the results were lower than 160 U/mL, the serums were remeasured using the undiluted solution.

The AS-ab titers were retrospectively analyzed. The total number of HCWs was 1351, as 66 had retired and 357 had dropped out from blood sampling. Then, at three weeks after booster vaccination, the number of HCWs was 1272, as three had dropped out from booster vaccination and 76 from blood sampling.

SARS-CoV-2 infection was also observed during 90 days after booster vaccination, and it was diagnosed by PCR using nasopharyngeal swab. Some participants were diagnosed by antigen test using nasopharyngeal swab or a salivary approach. HCWs who had been diagnosed with SARS-CoV-2 infection were required to report to the Department of Infection Control. In addition, those who had been determined to have had close contact with COVID-19 patients were also required to report, even if they were symptom-free.

All HCWs were required to report their physical condition to the staff of the health management department every day before work. In addition, we had provided HCWs with opportunities of SARS-CoV-2 PCR testing whenever they complained of poor physical condition. By such a daily health management system for the HCWs, we kept track of the HCWs infected with SARS-CoV-2.

The numbers of booster and non-booster vaccinations were 1633 and 75, respectively. As for the non-booster HCWs, the duration of observation was 90 days from Dec. 21, 2021, when we initiated booster vaccination.

Univariate analysis was performed to identify factors associated with SARS-CoV-2 infection after booster vaccination. Then, multivariate linear regression analysis was performed using factors showing a p-value of <0.1 by univariate analysis. Even if the p-value was \geq 0.1 by univariate analysis, the following three factors — booster vaccination, sex, age groups — were used for multivariate linear regression analysis.

Statistical analyses were performed using JMP Pro 15 (SAS Institute, Cary, NC, USA). A two-sided p-value <0.05 was considered statistically

significant.

The former study was approved by the Chiba University Ethics Committee on February 24th, 2021 (No. HS202101-03) and on April 21st, 2021 (No. HS202104-01), respectively. All study subjects gave written informed consent before undergoing any study procedures. The present study was approved by the Chiba University Hospital Ethics Committee on March 10th, 2022 (No. M10227). We prepared home page information associated with this study procedure, and provided participants with the opportunity to opt out.

3. Results

The changes of AS-ab titers are shown in Table 1. The AS-ab titer three weeks after two-dose vaccinations was 2060 U/mL (median, IQR (interquartile range) 1250–2650) [14]. The AS-ab titer then decreased to as low as 587 U/mL (median, IQR 360–896) eight months after the two-dose vaccinations. Then, three weeks following the booster vaccination, the AS-ab titer had increased to 22471 U/mL (median, IQR 15761–32622).

The differences of AS-ab titers among age groups are also shown in Table 1. The AS-ab titers three weeks after two-dose vaccinations differed significantly among age groups (P < 0.01), and they were lower in the older groups. The AS-ab titers at eight months after two-dose vaccinations, just before booster vaccination, also differed among age groups (P < 0.01), being lower in the older groups. However, there were no significant differences in AS-ab titers among age groups three weeks after booster vaccinations.

The risk factors for SARS-CoV-2 infection during 90 days after booster vaccination were analyzed (Table 2). A total of 1708 subjects were listed for analysis, and 48 HCWs had become infected with SARS-CoV-2. SARS-CoV-2 infections according to age groups differed significantly (P < 0.05), with the incidence rate between 20 and 49 years being around 3.4%, while that among those aged 50 years or more was less than 1%. Other significant factors were COVID-19 wards and nurses. Booster vaccination was not a factor.

SARS-CoV-2 infection and vaccine effectiveness (VE) were analyzed according to age classification (Table 3). In total, SARS-CoV-2 infections in booster vaccination and non-booster groups were 1.8% and 4.0%, respectively, the difference not being significant. VE was as low as 31.1% (95%CI (confidence interval) -116%-78%). However, when restricting the analysis to the 20-29-year group, SARS-CoV-2 infections in the booster vaccination group and non-booster group were 2.9% and 13.6%, respectively (p = 0.04), and VE was 78.7% (95%CI 28.1%-93.4%) (see Table 4).

Kaplan-Meier curve analysis for SARS-CoV-2 infection is shown in Fig. 2. There were significant differences in the possibility of clinical contact with COVID-19, nurse and age, but not with booster vaccination.

There were no hospitalizations among the booster nor the nonbooster subjects.

After multivariate logistic regression analysis for SARS-CoV-2 infection, the adjusted odds ratio (aOR) of booster vaccination was 0.6 (95%CI 0.2–2.4). On the other hand, working at COVI-19 wards was a risk factor for SARS-CoV-2 infection, and its aOR was 2.9 (95%CI 1.5–5.6). Age lower than 50 years was another risk factor, and its aOR was 9.7 (95%CI 1.3–71.2). Male and nurse were not factors for SARS-CoV-2 infection.

Based on this result, we attempted to set an appropriate cut-off level of AS-ab titer required to prevent SARS-CoV-2 infection. ROC (Receiver Operating Characteristic) curve analysis of the relationship between ASab titer and SARS-CoV-2 infection revealed an AUC (area under the curve) of 0.58. From this result, it was not possible to set a cut-off level of antibody titer required to prevent SARS-CoV-2 infection.

4. Discussion

The effects of booster vaccination were estimated by the change of

Table 1

Change of anti-Spike antibody titers and COVID-19 vaccinations.

	3 weeks after 2-dose vaccinations			8 months after 2-dose vaccinations				3 weeks	3 weeks after booster vaccinations			
Age	n	Median	(IQR) (U/mL)	P-value	n	n Median (IQR) (U/mL) P-value		n	Median (IQR) (U/mL)		P-value	
Total	1774	2060	(1250–2650)		1351	587	(360–896)		1272	22716	(15761-32622)	
20–29 yr.	412	2315	(1530-2935)	< 0.01	298	722	(466–1057)	< 0.01	280	22573	(15943-33497)	0.53
30-39 yr.	559	2080	(1310-2660)		406	635	(380–905)		377	22857	(15577-32905)	
40-49 yr.	440	2035	(1235-2560)		348	534	(342-818)		324	22279	(16040-33083)	
50–59 yr.	288	1875	(1045-2420)		241	510	(318-801)		234	23413	(14519-32253)	
≥60 yr.	75	1360	(740–2120)		58	347	(224–616)		57	18843	(14159–30610)	

2021.

QR: interquartile range.

Table 2
Univariate analysis of SARS-CoV-2 infection during 90 days from Dec. 21,

		n	SARS-CoV-2	(%)	Р
			infection		value
Total		1708	48	(2.8)	
Age	20-29	395	14	(3.5)	0.04
Ū	yr.				
	30–39	539	18	(3.3)	
	yr.				
	40-49	427	15	(3.5)	
	yr.				
	50–59	278	1	(0.4)	
	yr.				
	\geq 60 yr.	69	0	(0.0)	
Male	yes	575	13	(2.3)	0.36
	no	1133	35	(3.1)	
Booster vaccination	yes	1633	45	(2.8)	0.47
	no	75	3	(4.0)	
History of SARS-CoV-2	yes	17	0	(0.0)	1.00
infection	no	1691	48	(2.8)	
Working at COVID-19	yes	172	13	(7.6)	< 0.01
wards	no	1536	35	(2.3)	
Occupation	Doctor	474	10	(2.1)	< 0.05
	Nurse	552	24	(4.4)	
	Other	682	14	(2.1)	
Body Mass Index (kg/m ²)	$<\!\!25$	1229	28	(2.3)	0.25
	≥ 25	230	8	(3.5)	
Smoking	yes	360	12	(3.3)	0.22
	no	1099	24	(2.2)	
Diabetes Mellitus	yes	24	1	(4.2)	0.45
	no	1435	35	(2.4)	
Steroid therapy	yes	40	1	(2.5)	1.00
	no	1419	35	(2.5)	
Immunosuppressive	Yes	9	0	(0.0)	1.00
therapy	no	1450	36	(2.5)	

Table 3

SARS-CoV-2 infection and hospitalization according to age and booster vaccination during 90 days after booster vaccination from Dec. 21, 2021.

	Booster Vaccination	n	SARS-CoV-2 infection	(%)	P value
Total	yes	1633	45	(2.8)	0.47
	no	75	3	(4.0)	
20-29	yes	373	11	(2.9)	0.04
yr.	no	22	3	(13.6)	
30-39	yes	508	18	(3.5)	0.62
yr.	no	31	0	(0.0)	
40-49	yes	414	15	(3.6)	1.0
yr.	no	13	0	(0.0)	
50-59	yes	272	1	(0.4)	1.0
yr.	no	6	0	(0.0)	
\geq 60 yr.	yes	66	0	(0.0)	
	no	3	0	(0.0)	

AS-ab titers between before and after vaccination in this study. The ASab titer of 2060 U/mL, acquired three weeks after the two-dose vaccinations, had declined to as low as 592 U/mL during eight months. However, booster vaccination induced strong humoral immune

Table 4

Multivariate logistic regression for SARS-CoV-2 infection during 90 days after booster vaccination.

	aOR	95% CI	P value
20–49 yr	9.7	1.3–71.2	< 0.05
Working for COVID-19 wards	2.9	1.5-5.6	< 0.01
Booster vaccination	0.7	0.2-2.4	n.s
Male	0.95	0.5-1.9	ns
Nurse	1.8	0.97–3.4	ns

aOR: adjusted odds ratio, CI: confidence interval.

response and increased the AS-ab titer up to 22471 U/mL. Vaccine efficacy or effectiveness against COVID-19 decreased by six months, owing in part to waning immunity [7,8]. The decrease of AS-ab titer, as shown in this study, also reflects the waning of immunity. Several countries have introduced booster vaccination for the recovery of immunity, as it had waned after two-dose vaccinations. Seki et al. reported an AS-ab titer response by booster vaccination from 456 U/mL to 28158 U/mL, which was measured with the same kit as used in our study [15]. They also concurrently measured the neutralization antibody titers against Omicron variant, and the neutralization antibody response was in parallel with the AS-ab response. The AS-ab titer could be an index for estimating the neutralization antibody. Neutralization antibody was shown to be highly predictive of immune protection from SARS-CoV-2 infection [17]. We tried, but ultimately failed to set an appropriate antibody titer cut-off level that can prevent SARS-CoV-2 infection. Although AS-ab titers varied, it is speculated that even those with lower levels may have reached a level that could prevent SARS-CoV-2 infection.

BNT162b2 had been approved prior to emergence of the Omicron variant, and therefore primary vaccine series resulted in a low-to-absent neutralization of the SARS-CoV-2 Omicron variant. However, booster vaccination with BNT162b2 elicited neutralizing titers against wild-type virus higher than those after two vaccination doses, as well as broader neutralization against the Delta and Beta variants [18]. BNT162b2 booster vaccine recipients exhibited potent neutralization against Omicron variant [15,19]. Although neutralization antibody titer against Omicron was lower than against wild-type and Delta, our booster vaccination was shown to provide protection against Omicron.

We analyzed the AS-ab titer according to age classification. The ASab titer at three weeks after two-dose vaccinations differed among age classes, with the older HCWs' response being attenuated in comparison with younger HCWs. The same trend was also observed in the present study, as eight months after two-dose vaccinations the AS-ab titer still decreased to a lower level. Older HCWs' responses were significantly lower. However, the AS-ab titer after booster vaccination did not show any difference among the various age groups. Although the number of HCWs aged 65 years or older was small in the present study, repeated vaccinations would induce immunity and be beneficial for older persons. In consideration of the AS-ab titer being correlated with neutralization antibody [15], elderly people would benefit greatly from booster vaccination.

Three doses of mRNA vaccine provide protection against both the



Fig. 2. Kaplan-Meier curve analysis of COVID-19 after booster vaccination COVID-19 incidence was analyzed by Kaplan-Meyer curve. Four factors were analyzed. There were significant differences in working at COVID-19 wards, nurse and age, but there was no significant difference in booster vaccinations.

Omicron and Delta variants, although protection against Omicron variants would be less compared with Delta variants [20]. VE is not so durable, decreasing to less than 40% in both mRNA vaccines after 10 weeks [21]. Another study also revealed the decline of VE against Omicron, stating that booster vaccination would be effective for the recovery of VE [22]. Therefore, our concern is regarding VE in HCWs. Contrary to expectations, there was no significant difference in COVID-19 between booster and non-booster vaccinations. When limiting to HCWs aged 20–29 years, the COVID-19 incidence was significantly lower in HCWs having received booster vaccination. However, the vaccine efficacy among HCWs aged 30 years or more was not sufficiently analyzed in this study. The booster vaccination rate was 95.6% in this cohort, and the number of non-vaccinated HCWs was 75. As for the 53 HCWs aged 30 years or older, there was no SARS-CoV-2 infection.

Working in COVID-19 wards was shown to be a risk factor of SARS-CoV-2 infection, as HCWs with the obvious possibility of clinical contact with COVID-19 present an increased risk of SARS-CoV-2 infection. CUH had specialized in the medical care of severe COVID-19, such as those requiring high-dose oxygen inhalation, needing ECMO (Extracorporeal Membrane Oxygenation) treatment, hemodialysis, and requiring mechanical ventilation. Many patients had underlying disorders such as cardiovascular disease, chronic respiratory disease, chronic kidney disease, diabetes, steroid treatment, and pregnancy, which were all risk factors for severe diseases. Because HCWs must frequently carry out their medical treatment responsibilities at close range to patients, those working in COVID-19 wards might had been at increased risk of SARS-CoV-2 infection even if infection control measures had been taken. Thus, from the results of this study, we were able to clarify the necessity to strengthen infection control measures in COVID-19 wards. In other studies, the effectiveness of two doses of mRNA vaccine against severe COVID-19 had been confirmed [16,23]. Booster vaccination also provides protection against COVID-19-associated hospitalization [24]. In consideration of the waning vaccine effectiveness against SARS-CoV-2 infection, HCWs should receive booster vaccinations for the prevention of SARS-CoV-2 infection and hospitalization, as well as adhere to the infection control especially in the COVID-19 ward.

The age range of 20–49 years was also found to be another risk factor of SARS-CoV-2 infection, as it was mainly observed in this age range, except for the younger generation aged less than 20 years, in the period of Omicron dominance in Japan in this study period. We speculated that social factors, in addition to occupational factors, were also reflected in these results.

The occupation of nursing was also at higher risk of SARS-CoV-2 infection by univariate analysis. First, nurses were the main members working at COVID-19 wards. Second, the present cohort consisted mainly of nurses, totaling 32%. Furthermore, 87.1% of the nurses were aged 20–49 years, younger than the other HCWs in this cohort. The factor of nurse was confounded by other factors, COVID-19 wards and the age range of 20–49 years, and thus nurse was not selected as an independent factor for SARS-CoV-2 infection.

This study has several limitations. First, this study was retrospectively conducted with a cohort from a previous study. Second, this study was conducted at a single facility. Third, samples decreased owing to several factors. Some HCWs retired before booster vaccination. Others did not wish AS-ab measurement. Fourth, our study measured the AS-ab titers. The neutralization antibody titers for some variants were speculative. Although there was a relatively strong correlation between anti-S protein antibody and neutralization antibody, they did depend on the reliance on other studies [15]. Fifth, the variants of each of the HCWs who developed COVID-19 were not analyzed. According to the information regarding prevailing variants in Japan, the rate of the Omicron variant gradually increased from the year-end of 2021, just at the timing of booster vaccination at CUH. Sixth, information associated with health-and-risk of SARS-CoV-2 infection was missing. Seventh, as for analysis of the infection rate after booster vaccination, the control groups without booster vaccination consisted of only 75 HCWs. Fortunately, the booster vaccination rate rose up to 96% at CUH, much higher than the Japanese booster vaccination rate of around 30% at the end of February (Japanese Cabinet Office).

Booster vaccination induced the recovery of AS-ab titers and reduced the risk of SARS-CoV-2 infection. However, contrary to expectations, vaccination effectiveness was confirmed only among the HCWs aged 20–29 years. HCWs working at COVID-19 wards and HCWs aged 20–49 years were presented with risk factors for SARS-CoV-2 infection. Increasing the vaccination coverage, together with implementing infection control, remains the primary means of protecting HCWs against SARS-CoV-2 infection.

Funding

This study was funded by a donation to Chiba University Hospital. The content is solely the responsibility of the authors and does not necessarily reflect the official views of CUH.

Declaration of competing interest

The authors whose names are listed in the manuscript have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

Acknowledgement

We are grateful to the HCWs who participated in this study at CUH.

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