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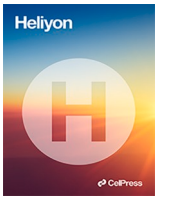

Research article

Comparison of adverse events following the second/third dose of BNT162b2 in a medical institute in Japan

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ABSTRACT

Background: Vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are crucial for ending the pandemic of coronavirus disease 2019 (COVID-19). Currently, the cumulative effect of booster shots of mRNA vaccines on adverse events is not sufficiently characterized.
Methods: A survey-based study on vaccine adverse events was conducted in a Japanese medical institute after the third dose of Pfizer BNT162b2. Adverse events were grouped using network analysis, and a heteroscedastic probit model was built to analyse adverse events.
Results: There were two main clusters of adverse events, systemic and local injection site-associated events. Subject background and the experience of previous vaccine-related adverse events were variably associated with the occurrence and intensity of adverse events following the third dose. Among adverse events, only lymphadenopathy increased prominently following the third dose, while the largest increase in other systemic adverse events occurred generally following the second dose.
Conclusions: The effect of repeated booster vaccines on the frequency and intensity of adverse events differs depending on the kind of adverse event.

1. Introduction

Vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are crucial for ending the pandemic of coronavirus disease 2019 (COVID-19), but there is still lingering apprehension over adverse events. Previous reports noted an increase in adverse events following booster shots of these vaccines, and this raises the risk of avoidance of boosters in the population. Understanding the characteristics of adverse events following vaccination allows public health authorities and clinicians to explain possible adverse events to populations or patients confidently based on real data, which may mitigate the sense of uncertainty and abject fear. The accumulation of scientific data is also a means to fight against the disinformation that is so prevalent regarding COVID-19 and its vaccines. However, few studies in Japan have investigated adverse events following the third dose of the COVID-19 vaccine and how their frequency or intensity changes from previous doses [1]. There is a concern that reactogenicity to mRNA vaccines might differ between different parts of the world due to various factors, including the difference in the frequency of human leukocyte antigen types [2]. Thus, it is necessary to collect data on adverse events among Japanese.

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2. Materials and methods

2.1. Study design

To investigate the adverse events following three doses of the BNT162b2 vaccine, we conducted a survey using Google Forms for faculty members of Nihon University School of Medicine, employees of the Nihon University Itabashi Hospital, medical students of the Nihon University School of Medicine, and nursing students of Nihon University Nursing School from December 27, 2021, to March 5, 2022. The online survey questionnaires included the characteristics of the participants, such as age, sex, occupation, medical histories, ABO blood type, smoking, and antipyretics use. Regarding adverse reactions to the COVID-19 vaccine, participants were asked about the following symptoms, pain, and swelling at the inoculation site, fever, fatigue or malaise, headache, being uncomfortable and/or vomiting, diarrhea, abdominal pain, arthralgia, rash, sore throat, and anaphylactic reaction. This study was approved by the Ethics Committee of Nihon University School of Medicine (approval number: P21-06-1). All procedures were performed under the guidelines of our institutional ethics committee and adhered to the tenets of the Declaration of Helsinki.

2.2. Statistical analyses

2.2.1. Network analysis

Networks were used to visualize the connections of adverse events. Networks were built with the IsingFit method in the IsingFit package, igraph and qgraph of R (ver. 4.1.3) [3,4] to assess the connection of adverse events. The model employed in the IsingFit package is a binary equivalent of Gaussian approximation methods, which is applicable only to two-state data; interactions are considered pairwise, and the data need to be cross-sectional. This package builds a figure of a network. In this figure, each symptom is

Table 1
Demographic characteristics of responders in this study (N = 793). Values within parentheses represent percentages.

Vaccination status			
	Completed third dose		787 (99.2)
	Up to the second dose		5 (0.6)
	Never vaccinated		1 (0.1)
Age	Age group	median age (lower quartile, upper quartile) in years	
	(Total)	34 (18, 49)	
	<25 years	21 (20, 23)	235 (29.6)
	25–40 years	29 (26, 39)	290 (36.6)
	≥40 years	58 (45, 75)	262 (33.0)
Sex	Female		415 (52.3)
	Male		376 (47.4)
Occupation	Medical doctors		165 (20.8)
	Nurses		49 (6.2)
	Other licenced medical professionals		46 (5.8)
	Medical students		238 (30.0)
	Nursing students		72 (9.1)
	Others		221 (27.9)
Medical histories	Allergic reaction to vaccines in the past		24 (3.0)
	Food or medication allergy		151 (19.0)
	Fat or glucose metabolism disorders		34 (4.3)
	Malignancy		5 (0.6)
	Obstetrical or gynaecological conditions other than malignancies		9 (1.1)
	Asthma		14 (1.8)
	Hypertension		31 (3.9)
Current use of medication			188 (23.7)
ABO blood type	A		273 (34.4)
	AB		82 (10.3)
	B		192 (24.2)
	O		225 (28.4)
Smoking	Naive		663 (83.6)
	Past smokers		107 (13.5)
	Current smokers		61 (7.7)
Antipyretics use	Prior to vaccination		
	First dose		123 (15.5)
	Second dose		181 (22.8)
	Third dose		176 (22.2)
After vaccination with BNT162b2 but without/before the onset of any event	First dose		163 (20.6)
	Second dose		224 (28.2)
	Third dose		545 (68.7)
After the onset of any event	First dose		122 (15.4)
	Second dose		263 (33.2)
	Third dose		342 (43.1)

represented with a circle or node, and a pair of nodes are connected with a line (edge). A green edge between two nodes represents a positive connection between two symptoms, and a red edge shows a negative connection. The thickness of the edge indicates the strength of the connection between two symptoms [5,6]. Clusters of nodes, or communities, were identified with the walktrap algorithm [7].

2.2.2. *Heteroscedastic probit model*

After delineation of the structures of communities in networks, some adverse events that consistently formed communities in all three networks were grouped together and were used for further analysis. The homogeneity of error variance over a range of observations, or homoscedasticity, was checked with the Breusch–Pagan (BP) test with Koenker's correction [8,9]. For the analysis of the adverse events following the second and third booster shots, the heteroscedastic probit model (HPM) [10] was created to assess the effect of various responder factors on a given adverse event group. Adverse events following the second dose were classified as follows: no report as 0 and reported as 1. Adverse events following the third dose were classified into three ordered factorial values: no report of adverse event as 0; worst adverse event ever (worser adverse events following the third dose compared to both the first and the second doses, or the first experience of the event following the third dose) as 2; and non-worst event (having experience of the event following the third dose but no report of worse reactions compared to the first and second doses and unable to be classified as 2) as 1. Unlike ordered probit and ordered logit models that assume homoscedasticity, the HPM is less prone to produce biased parameter estimates and misspecification of errors in predicting a latent variable with heteroscedastic latent variables [10,11]. While the magnitude of a regression coefficient of a homoscedastic model often lacks relevance to the actual value of a latent variable, a heteroscedastic model is useful in predicting the real value of the latent variable. In a heteroscedastic model, this is accomplished with the use of marginal effects. A marginal effect shows the influence of the change in an explanatory variable from a particular value to another particular value on the probability of a specific value of the latent variable. The HPM estimates threshold values for a latent variable, at which value the marginal effect of an explanatory variable differs. The goodness of fit for the HPMs was evaluated with McFadden's test. We

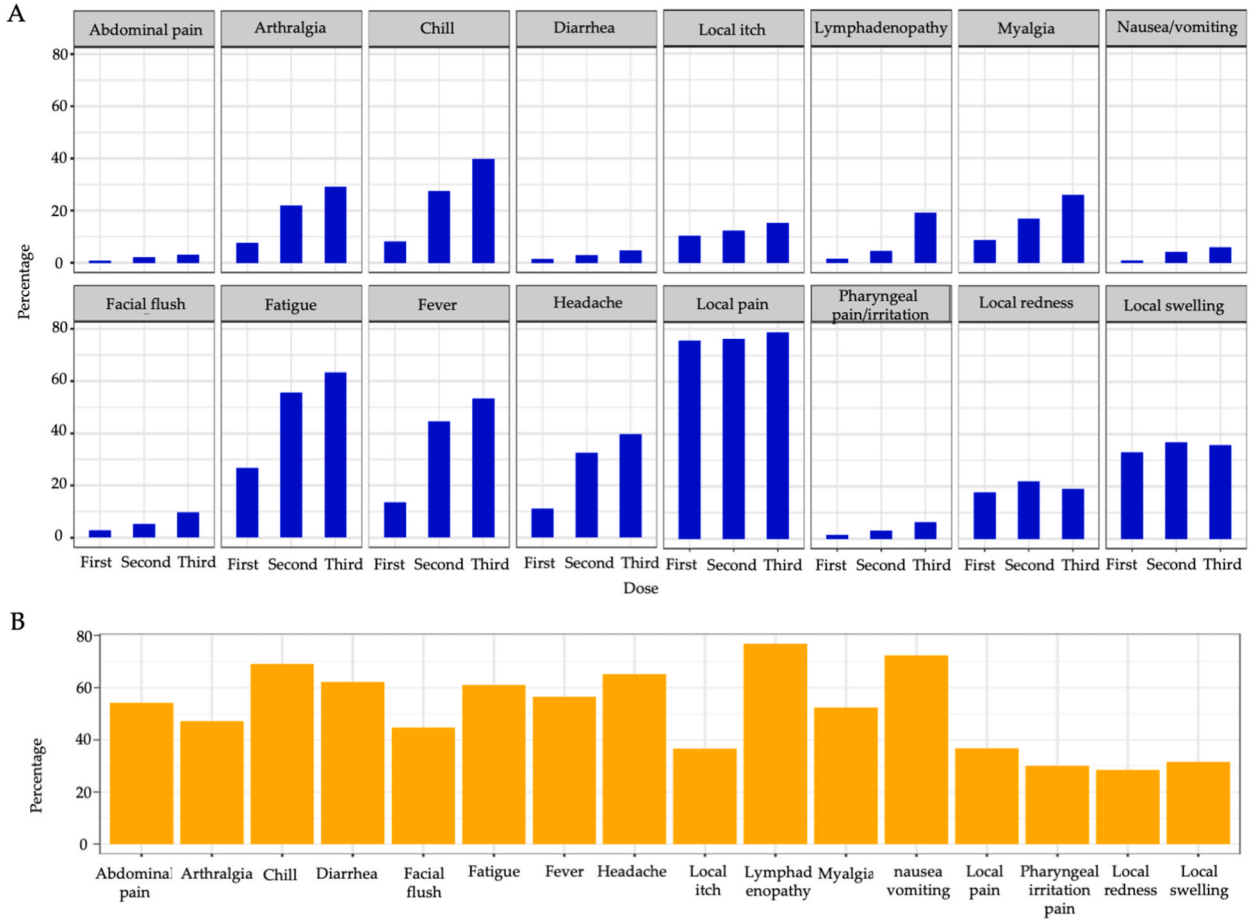

Fig. 1. Reported adverse events following each dose of BNT162b2. (A) Most adverse events increased sequentially following booster doses. Lymphadenopathy increased prominently only after the third dose. (B) The percentage of those who experienced the worst ever symptoms for each of the main adverse events among those with adverse events following the third dose. Systemic adverse events following the third dose tended to be more intense than those following the third dose compared to the second dose, but local adverse events were less likely to worsen.

also built an additional model to analyse lymphadenopathy following the third dose. These models were built and analysed with R, and the lmtest and oglmx packages were used for the BP test and HPM, respectively [12,13]. The correlation of variables was checked visually and then with variance inflation factors or the chi-squared test. A p value < 0.05 was considered statistically significant.

3. Results

3.1. Demographic characteristics of the study population

After the exclusion of people who did not consent or had inconsistent vaccination status reports, we obtained 793 responses. A total of 99.2 % of subjects completed the third dose of the SARS-CoV-2 vaccine. A total of 0.6 % of subjects did not receive the booster after the second dose of the vaccine, and 0.1 % had never been vaccinated (Table 1). Only thirteen participants had a history of COVID-19. Other characteristics are also summarized in Table 1.

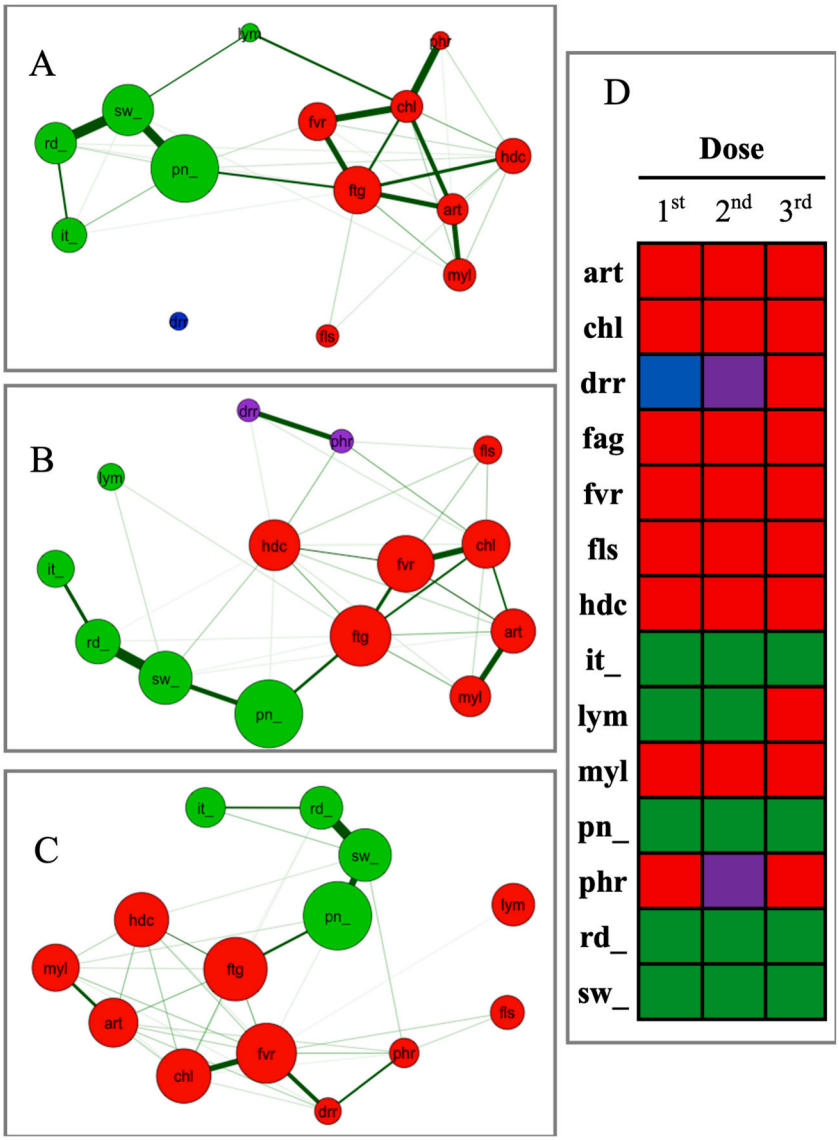

Fig. 2. Networks following the first (A), second (B) and third (C) doses of the BNT162b2 vaccine. Four injection-associated local events (i.e., pain, swelling, redness and itching) were consistently clustered together. Seven systemic adverse events (i.e., arthralgia, chills, facial flush, fatigue, fever, headache and myalgia) formed another cluster in all three networks. The colour of each node represents which cluster a given node belongs to in a network, and clusters seen across all three networks are represented with the same node colours (D). Abbreviations [art; arthralgia, chl; chills, drr; diarrhea, fag; fa-tigue, fvr; fever, fls; facial flush, hdc; headache, it; itching at the local injection site, lym; lymphadenopathy, myl; myalgia, pn; pain at the local injection site, phr; pharyn-geal irritation/pain, rd; redness at the local injection site, sw; swelling of the local injection site].

3.2. Reactogenicity of the BNT162b2 mRNA COVID-19 vaccine

Fig. 1 (and Table S1) summarizes the distribution of self-reported adverse events. Anaphylactic reactions were reported in 0.4 %, 0.4 %, and 0.5 % of the subjects following the first, second, and third doses, respectively. Neither myocarditis nor pericarditis was reported. Most adverse events gradually increased from the first dose to the third dose or prominently increased following the second dose. Notably, only lymphadenopathy increased prominently following the third dose (Fig. 1A). The percentage of those who experienced the worst ever symptoms following the third dose for each of the main adverse events is shown in Fig. 1B. Systemic adverse events tended to be more intense than previous doses, but local adverse events were less likely to worsen.

Table 2
Heteroscedastic probit model for the grouped systemic adverse events following the second dose of BNT162b2.[a]

Mean estimate								
Parameter estimate		SE	95 % CI			t value	p value	
Sex (comparison to female) rowhead								
Male	−0.11	0.06	−0.22	–	−0.54	−1.82	0.0689	
Age (comparison to[b] <25 years) rowhead								
25–40 years	0.00	0.02	−0.04	–	−0.07	0.22	0.8256	
≥40 years	−0.10	0.05	−0.20	–	−0.49	−1.74	0.0820	
Blood type (comparison to type A) rowhead								
AB	0.19	0.37	−0.53	–	−0.85	0.52	0.6004	
B	−0.16	0.08	−0.33	–	−0.80	−1.96	0.0499	*
O	−0.08	0.05	−0.19	–	−0.45	−1.55	0.1211	
Medical history rowhead								
Asthma	−0.10	0.06	−0.21	–	−0.52	−1.56	0.1188	
Metabolism disorders	−0.01	0.06	−0.12	–	−0.24	−0.11	0.9104	
Allergic reaction to vaccine	0.10	0.11	−0.12	–	−0.13	0.91	0.3648	
Smoking (comparison to naive) rowhead								
Past	0.06	0.07	−0.08	–	−0.10	0.81	0.4198	
Current	−0.07	0.05	−0.16	–	−0.38	−1.34	0.1814	
Adverse events following the first dose of BNT162b2 rowhead								
Systemic events	0.06	0.05	−0.04	–	−0.02	1.17	0.2423	
Local events	0.15	0.09	−0.03	–	0.10	1.68	0.0928	
Use of antipyretics (comparison to those who did not take any)[c] rowhead								
Timing 1	−0.05	0.04	−0.13	–	−0.29	−1.10	0.2731	
Timing 2	−0.07	0.04	−0.16	–	−0.37	−1.49	0.1354	
Timing 3	−0.07	0.05	−0.16	–	−0.38	−1.39	0.1633	

Marginal effects on the occurrence of adverse events

	Marginal effect	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	−0.04	0.03	−0.09	–	0.01	−1.43	0.1527	
Age (comparison to <25 years)								
25–40 years	−0.05	0.02	−0.10	–	0.00	−1.94	0.0530	
≥40 years	−0.13	0.03	−0.18	–	−0.08	−4.99	6.10×10^{-7}	***
Blood type (comparison to type A)								
AB	−0.02	0.05	−0.12	–	0.08	−0.43	0.6663	
B	−0.10	0.05	−0.19	–	−0.01	−2.25	0.0248	*
O	−0.05	0.04	−0.13	–	0.03	−1.29	0.1979	
Medical history								
Asthma	−0.06	0.06	−0.18	–	0.05	−1.08	0.2792	
Metabolism disorders	−0.01	0.05	−0.11	–	0.09	−0.23	0.8220	
Allergic reaction to vaccine	0.03	0.07	−0.11	–	0.17	0.44	0.6604	
Smoking (comparison to naive)								
Past	0.06	0.03	−0.01	–	0.12	1.63	0.1041	
Current	−0.05	0.04	−0.13	–	0.02	−1.37	0.1717	
Adverse events following the first dose of BNT162b2								
Systemic events	0.23	0.03	0.17	–	0.30	7.08	1.46×10^{-12}	***
Local events	0.18	0.04	0.09	–	0.26	4.07	0.0001	***
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.00	0.04	−0.07	–	0.07	0.12	0.9032	
Timing 2	0.02	0.03	−0.05	–	0.09	0.60	0.5480	
Timing 3	−0.01	0.03	−0.08	–	0.06	−0.33	0.7424	

SE, standard error.

[a] General properties of the model, Breusch–Pagan test, Chi-square 73.38, $p = 2.53 \times 10^{-9}$,McFadden's pseudo R-square test 0.20, Log-likelihood −384.84.

[b] Statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001.

[c] Timing 1, After the onset of adverse events following the first dose of BNT162b2, Timing 2, Prior to the second dose of BNT162b2, Timing 3, After the second dose of BNT162b2 but without or before the onset of adverse events.

3.3. Network analyses

A network was built for each vaccine dose (Fig. 2A–B and C for the first, second and third, respectively). Four adverse events, pain, swelling, itching and redness of the local injection site, were clustered in the same group in all 3 networks and formed a 'local event group'. The other 7 adverse events, arthralgia, chill, facial flush, fatigue, fever, headache, and myalgia were consistently clustered in the same group in all networks and formed a 'systemic event group (Fig. 2D).

Table 3
Heteroscedastic probit model for the grouped local adverse events following the second dose of BNT162b2.[a]

Mean estimate							
	Parameter estimate	SE	95 % CI			t value	p value
Sex (comparison to female)							
Male	−0.10	0.18	−0.45	–	−0.97	−0.54	0.5912
Age (comparison to <25 years)							
25–40 years	0.03	0.07	−0.10	–	−0.16	0.46	0.6476
≥40 years	0.02	0.05	−0.08	–	−0.13	0.42	0.6726
Blood type (comparison to type A)							
AB	0.00	0.02	−0.04	–	−0.07	0.15	0.8779
B	0.00	0.01	−0.02	–	−0.05	0.20	0.8440
O	0.00	0.01	−0.02	–	−0.04	0.07	0.9420
Medical history							
Asthma	0.00	195.49	−383	–	−751	0.00	1.0000
Metabolism disorders	−0.01	0.03	−0.08	–	−0.16	−0.40	0.6865
Allergic reaction to vaccine	0.02	0.05	−0.08	–	−0.13	0.35	0.7260
Smoking (comparison to naive)							
Past	−0.03	0.08	−0.19	–	−0.41	−0.39	0.6995
Current	−0.04	0.09	−0.22	–	−0.46	−0.39	0.6976
Adverse events following the first dose of BNT162b2							
Systemic events	0.04	0.11	−0.18	–	−0.31	0.38	0.7040
Local events	0.33	0.26	−0.18	–	−0.03	1.26	0.2060
Use of antipyretics (comparison to those who did not take any)[b]							
Timing 1	−0.02	0.06	−0.15	–	−0.31	−0.38	0.7008
Timing 2	0.00	0.02	−0.03	–	−0.06	0.15	0.8812
Timing 3	−0.01	0.02	−0.04	–	−0.08	−0.31	0.7603
Marginal effects on the occurrence of local adverse events (outcome 1)							
	Marginal effect	SE	95 % CI			t value	p value
Sex (comparison to female)							
Male	−0.34	63.05	−124	–	123	−0.01	0.9957
Age (comparison to <25 years)							
25–40 years	0.04	11.16	−21.8	–	21.9	0.00	0.9972
≥40 years	0.03	8.02	−15.7	–	15.8	0.00	0.9968
Blood type (comparison to type A)							
AB	−0.01	2.62	−5.13	–	5.12	0.00	0.9974
B	0.05	8.05	−15.7	–	15.8	0.01	0.9953
O	0.03	4.53	−8.85	–	8.90	0.01	0.9956
Medical history							
Asthma	0.37	0.50	−0.61	–	1.35	0.74	0.4605
Metabolism disorders	−0.05	8.66	−17.0	–	16.9	−0.01	0.9954
Allergic reaction to vaccine	0.06	9.54	−18.6	–	18.8	0.01	0.9948
Smoking (comparison to naive)							
Past	−0.11	25.00	−49.1	–	48.9	0.00	0.9966
Current	−0.15	27.66	−54.4	–	54.1	−0.01	0.9956
Adverse events following the first dose of BNT162b2							
Systemic events	0.12	20.06	−39.2	–	39.4	0.01	0.9954
Local events	0.63	42.63	−82.9	–	84.2	0.01	0.9882
Use of antipyretics (comparison to those who did not take any)[b]							
Timing 1	−0.09	16.77	−33.0	–	32.8	−0.01	0.9955
Timing 2	0.04	7.51	−14.7	–	14.8	0.01	0.9953
Timing 3	0.01	4.74	−9.28	–	9.31	0.00	0.9976

SE, standard error.

[a] General properties of the model, Breusch−Pagan test, Chi-square 71.33, $p = 5.83 \times 10^{-9}$,McFadden's pseudo R-square test 0.45, Log-likelihood −220.44.

[b] Timing 1, After the onset of adverse events following the first dose of BNT162b2, Timing 2, Prior to the second dose of BNT162b2, Timing 3, After the second dose of BNT162b2 but without or before the onset of adverse events.

Table 4
Heteroscedastic probit model of systemic adverse events following the third dose of BNT162b2.[a]

Mean estimate								
	Parameter estimate[b]	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	0.02	0.02	−0.01	–	0.06	1.34	0.1797	
Age (comparison to <25 years)								
25–40 years	0.02	0.02	−0.02	–	0.06	0.97	0.3315	
≥40 years	0.03	0.02	−0.02	–	0.07	1.10	0.2711	
Blood type (comparison to type A)								
AB	−0.01	0.03	−0.06	–	0.04	−0.30	0.7641	
B	0.00	0.02	−0.05	–	0.04	−0.08	0.9388	
O	0.01	0.02	−0.03	–	0.06	0.61	0.5444	
Medical history								
Asthma	0.11	0.09	−0.08	–	0.29	1.16	0.2471	
Metabolism disorders	0.01	0.04	−0.08	–	0.10	0.24	0.8069	
Allergic reaction to vaccine	−0.07	0.03	−0.12	–	−0.02	−2.56	0.0105	**
Smoking (comparison to naive)								
Past	−0.01	0.02	−0.06	–	0.03	−0.53	0.5975	
Current	0.00	0.03	−0.07	–	0.06	−0.06	0.9520	
Systemic event								
First dose of BNT162b2	−0.03	0.02	−0.07	–	0.00	−1.74	0.0814	
Second dose of BNT162b2	−0.32	0.05	−0.42	–	−0.22	−6.32	2.58×10^{-10}	***
Local event								
First dose of BNT162b2	0.01	0.03	−0.05	–	0.07	0.23	0.8157	
Second dose of BNT162b2	−0.14	0.06	−0.26	–	−0.02	−2.33	0.0197	**
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.02	0.02	−0.02	–	0.05	0.78	0.4376	
Timing 2	−0.11	0.03	−0.18	–	0.05	−3.42	0.0006	***
Timing 3	0.00	0.02	−0.03	–	0.03	0.07	0.9403	
Marginal effects on the absence of systemic events (outcome 0)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	0.04	0.03	−0.02	–	0.09	1.33	0.1847	
Age (comparison to <25 years)								
25–40 years	0.03	0.03	−0.03	–	0.09	0.96	0.3391	
≥40 years	0.04	0.04	−0.03	–	0.11	1.09	0.2748	
Blood type (comparison to type A)								
AB	−0.01	0.05	−0.10	–	0.08	−0.28	0.7822	
B	0.00	0.04	−0.07	–	0.07	−0.06	0.9552	
O	0.02	0.03	−0.05	–	0.09	0.63	0.5286	
Medical history								
Asthma	0.11	0.08	−0.06	–	0.28	1.27	0.2027	
Metabolism disorders	0.02	0.06	−0.11	–	0.14	0.27	0.7899	
Allergic reaction to vaccine	−0.15	0.09	−0.32	–	0.03	−1.67	0.0945	
Smoking (comparison to naive)								
Past	−0.02	0.04	−0.09	–	0.06	−0.48	0.6279	
Current	−0.01	0.06	−0.12	–	0.11	−0.09	0.9306	
Systemic event								
First dose of BNT162b2	−0.06	0.03	−0.12	–	0.00	−1.94	0.0520	
Second dose of BNT162b2	−0.35	0.05	−0.44	–	−0.25	−7.28	3.25×10^{-13}	***
Local event								
First dose of BNT162b2	0.01	0.05	−0.09	–	0.12	0.23	0.8164	
second dose of BNT162b2	−0.17	0.06	−0.29	–	−0.05	−2.87	0.0041	***
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.02	0.03	−0.04	–	0.08	0.73	0.4629	
Timing 2	−0.15	0.04	−0.22	–	−0.08	−4.29	1.81×10^{-5}	***
Timing 3	0.04	0.03	−0.01	–	0.10	1.58	0.1151	
Marginal effects on the non-worst systemic events (outcome 1)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	0.04	0.03	−0.01	–	0.10	1.54	0.1239	
Age (comparison to <25 years)								
25–40 years	0.03	0.04	−0.04	–	0.11	0.91	0.3649	
≥40 years	0.06	0.04	−0.03	–	0.14	1.27	0.2052	
Blood type (comparison to type A)								
AB	−0.02	0.04	−0.09	–	0.05	−0.54	0.5926	
B	−0.02	0.03	−0.09	–	0.04	−0.61	0.5388	

(*continued on next page*)

Table 4 (*continued*)

Marginal effects on the non-worst systemic events (outcome 1)								
	Marginal effects	SE	95 % CI			t value	p value	
O	−0.01	0.03	−0.07	–	0.05	−0.24	0.8091	
Medical history								
Asthma	−0.14	0.07	−0.28	–	−0.01	−2.10	0.0359	**
Metabolism disorders	−0.04	0.05	−0.15	–	0.06	−0.83	0.4078	
Allergic reaction to vaccine	−0.07	0.05	−0.17	–	0.03	−1.42	0.1564	
Smoking (comparison to naive)								
Past	−0.06	0.03	−0.13	–	0.00	−1.89	0.0585	
Current	0.03	0.05	−0.07	–	0.13	0.57	0.5656	
Systemic event								
First dose of BNT162b2	0.06	0.03	0.00	–	0.12	2.10	0.0359	**
Second dose of BNT162b2	0.16	0.04	0.09	–	0.23	4.32	2.0×10^{-5}	***
Local event								
First dose of BNT162b2	0.00	0.04	−0.08	–	0.07	−0.12	0.9044	
Second dose of BNT162b2	−0.02	0.05	−0.11	–	0.08	−0.34	0.7307	
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.04	0.03	−0.03	–	0.10	1.06	0.2900	
Timing 2	−0.11	0.04	−0.19	–	−0.04	−2.94	0.0033	***
Timing 3	0.05	0.04	−0.02	–	0.13	1.32	0.1853	
Marginal effects on the worst systemic event (outcome 2)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	−0.06	0.03	−0.13	–	0.00	−2.01	0.0445	*
Age (comparison to <25 years)								
25–40 years	−0.05	0.04	−0.14	–	0.03	−1.21	0.2267	
≥40 years	−0.08	0.05	−0.18	–	0.02	−1.59	0.1113	
Blood type (comparison to type A)								
AB	0.03	0.05	−0.06	–	0.12	0.60	0.5477	
B	0.02	0.04	−0.06	–	0.10	0.54	0.5873	
O	−0.01	0.04	−0.08	–	0.07	−0.15	0.8821	
Blood type (comparison to type A)								
AB	0.03	0.05	−0.06	–	0.12	0.60	0.5477	
B	0.02	0.04	−0.06	–	0.10	0.54	0.5873	
O	−0.01	0.04	−0.08	–	0.07	−0.15	0.8821	
Medical history								
Asthma	0.03	0.10	−0.17	–	0.24	0.33	0.7418	
Metabolism disorders	0.03	0.07	−0.11	–	0.18	0.46	0.6473	
Allergic reaction to vaccine	0.14	0.06	0.02	–	0.26	2.20	0.0280	**
Smoking (comparison to naive)								
Past	0.07	0.04	−0.01	–	0.15	1.79	0.0736	
Current	−0.03	0.06	−0.15	–	0.10	−0.43	0.6641	
Systemic event								
First dose of BNT162b2	−0.03	0.04	−0.10	–	0.04	−0.78	0.4345	
Second dose of BNT162b2	0.16	0.05	0.07	–	0.26	3.43	0.0006	***
Local event								
First dose of BNT162b2	0.00	0.06	−0.11	–	0.11	−0.04	0.9663	
Second dose of BNT162b2	0.16	0.06	0.04	–	0.28	2.67	0.0077	***
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	−0.05	0.04	−0.13	–	0.03	−1.26	0.2082	
Timing 2	0.23	0.05	0.13	–	0.32	4.83	1.37×10^{-6}	***
Timing 3	−0.05	0.04	−0.12	–	0.02	−1.39	0.1633	

SE, standard error.

[a] General properties of the model, Breusch−Pagan test, Chi-square 85.30, $p = 9.96 \times 10^{-11}$,McFadden's pseudo R-square test 0.17, Log-likelihood −613.1.

[b] Statistical significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[c] Timing 1, After the onset of adverse events following the first dose of BNT162b2, Timing 2, Prior to the second dose of BNT162b2, Timing 3, After the second dose of BNT162b2 but without or before the onset of adverse events.

3.4. Heteroscedastic probit model

Based on the results of network analyses, we defined the following two groups: the 'local event group', which consisted of itching, pain, redness and swelling of the injection site, and the 'systemic event group', which consisted of arthralgia, chills, facial flush, fatigue, fever, headache, and myalgia.

BP tests for all four formulas modelling the systemic/local adverse events following the second/third doses rejected the null hypothesis of homoscedasticity. The main results of the analysis are shown in Tables 2–6.

Table 5
Heteroscedastic probit model for local adverse events following the third dose of BNT162b2.[a]

Mean estimate								
	Parameter estimate[b]	SE	95 % CI			t value	p value	
Sex								
Male	0.00	0.00	−0.01	–	0.00	−0.98	0.3282	
Age (comparison to <25 years)								
25–40 years	−0.01	0.01	−0.02	–	0.00	−1.75	0.0798	
≥40 years	−0.01	0.01	−0.03	–	0.00	−1.93	0.0536	
Blood type (comparison to type A)								
AB	−0.01	0.01	−0.02	–	0.01	−1.08	0.2821	
B	−0.01	0.00	−0.02	–	0.00	−1.41	0.1579	
O	−0.01	0.00	−0.02	–	0.00	−1.55	0.1218	
Medical history								
Asthma	0.01	0.01	−0.01	–	0.02	1.05	0.2953	
Metabolism disorders	0.00	0.01	−0.01	–	0.01	0.62	0.5336	
Allergic reaction to vaccine	−0.01	0.01	−0.03	–	0.01	−1.00	0.3160	
Smoking (comparison to naive)								
Past	0.01	0.01	0.00	–	0.02	1.23	0.2195	
Current	0.01	0.01	−0.01	–	0.02	0.92	0.3587	
Systemic event								
First dose of BNT162b2	0.00	0.00	−0.01	–	0.00	−0.57	0.5716	
Second dose of BNT162b2	0.00	0.00	−0.01	–	0.01	0.27	0.7847	
Local event								
First dose of BNT162b2	0.00	0.01	−0.01	–	0.02	0.53	0.5954	
Second dose of BNT162b2	0.23	0.08	0.07	–	0.38	2.90	0.0038	***
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.00	0.00	−0.01	–	0.01	−0.41	0.6795	
Timing 2	0.01	0.01	0.00	–	0.02	1.78	0.0754	
Timing 3	0.00	0.00	−0.01	–	0.01	−0.19	0.8512	
Marginal effects on the absence of local events (outcome 0)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	−0.01	0.01	−0.03	–	0.00	−1.64	0.1001	
Age (comparison to <25 years)								
25–40 years	0.00	0.01	−0.01	–	0.02	0.43	0.6667	
≥40 years	0.03	0.01	0.00	–	0.06	2.12	0.0344	*
Blood type (comparison to type A)								
AB	0.00	0.01	−0.02	–	0.01	−0.05	0.9591	
B	0.00	0.01	−0.02	–	0.01	−0.16	0.8734	
O	0.00	0.01	−0.02	–	0.02	0.19	0.8529	
Medical history								
Asthma	0.00	0.02	−0.05	–	0.05	−0.03	0.9800	
Metabolism disorders	0.02	0.02	−0.02	–	0.07	1.10	0.2713	
Allergic reaction to vaccine	0.00	0.02	−0.03	–	0.04	0.17	0.8613	
Smoking (comparison to naive)								
Past	−0.01	0.01	−0.02	–	0.00	−2.70	0.0068	***
Current	0.00	0.01	−0.03	–	0.02	−0.20	0.8441	
Systemic event								
First dose of BNT162b2	0.00	0.01	−0.01	–	0.02	0.58	0.5630	
Second dose of BNT162b2	−0.01	0.01	−0.04	–	0.01	−1.07	0.2824	
Local event								
First dose of BNT162b2	−0.05	0.03	−0.11	–	0.01	−1.55	0.1211	
Second dose of BNT162b2	−0.64	0.05	−0.73	–	−0.55	−13.85	$<2.20 \times 10^{-16}$	***
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.00	0.01	−0.02	–	0.01	−0.31	0.7594	
Timing 2	−0.02	0.01	−0.04	–	0.00	−1.64	0.1020	
Timing 3	0.02	0.01	0.00	–	0.04	1.97	0.0485	*
Marginal effects on the non-worst local events (outcome 1)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	0.10	0.03	0.04	–	0.16	3.49	0.0005	***
Age (comparison to <25 years)								
25–40 years	0.13	0.04	0.06	–	0.20	3.68	0.0002	***
≥40 years	0.07	0.04	−0.01	–	0.15	1.62	0.1044	
Blood type (comparison to type A)								
AB	0.18	0.04	0.10	–	0.26	4.22	2.0×10^{-5}	***
B	0.11	0.04	0.03	–	0.18	2.84	0.0045	***

(*continued on next page*)

Table 5 (*continued*)

Marginal effects on the non-worst local events (outcome 1)								
	Marginal effects	SE	95 % CI			t value	p value	
O	0.10	0.03	0.04	–	0.17	2.99	0.0028	***
Medical history								
Asthma	0.00	0.10	−0.18	–	0.19	0.05	0.9605	
Metabolism disorders	0.10	0.09	−0.07	–	0.27	1.12	0.2638	
Allergic reaction to vaccine	0.13	0.07	−0.01	–	0.26	1.81	0.0702	
Smoking (comparison to naive)								
Past	−0.02	0.05	−0.12	–	0.07	−0.44	0.6625	
Current	−0.07	0.06	−0.18	–	0.05	−1.11	0.2663	
Systemic event								
First dose of BNT162b2	0.01	0.03	−0.05	–	0.07	0.21	0.8322	
Second dose of BNT162b2	0.03	0.04	−0.04	–	0.10	0.89	0.3720	
Local event								
First dose of BNT162b2	0.08	0.05	−0.03	–	0.19	1.47	0.1428	
Second dose of BNT162b2	0.54	0.03	0.47	–	0.60	16.63	$<2.20 \times 10^{-16}$	*
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.03	0.03	−0.04	–	0.10	0.86	0.3922	
Timing 2	−0.07	0.03	−0.13	–	0.00	−1.95	0.0512	
Timing 3	−0.06	0.03	−0.13	–	0.00	−1.87	0.0610	
Marginal effects on the worst local event (outcome 2)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	−0.09	0.03	−0.15	–	−0.03	−2.96	0.0031	***
Age (comparison to <25 years)								
25–40 years	−0.14	0.04	−0.21	–	−0.06	−3.68	0.0002	***
≥40 years	−0.10	0.04	−0.18	–	−0.02	−2.36	0.0184	**
Blood type (comparison to type A)								
AB	−0.18	0.05	−0.27	–	−0.09	−3.90	0.0001	***
B	−0.10	0.04	−0.18	–	−0.03	−2.74	0.0061	***
O	−0.10	0.03	−0.17	–	−0.04	−2.96	0.0031	***
Medical history								
Asthma	0.00	0.10	−0.21	–	0.20	−0.04	0.9681	
Metabolism disorders	−0.12	0.08	−0.28	–	0.03	−1.53	0.1260	
Allergic reaction to vaccine	−0.13	0.07	−0.27	–	0.01	−1.88	0.0607	
Smoking (comparison to naive)								
Past	0.04	0.05	−0.06	–	0.13	0.70	0.4846	
Current	0.07	0.06	−0.05	–	0.19	1.11	0.2683	
Systemic event								
First dose of BNT162b2	−0.01	0.03	−0.07	–	0.05	−0.36	0.7177	
Second dose of BNT162b2	−0.02	0.04	−0.09	–	0.05	−0.52	0.6051	
Local event								
First dose of BNT162b2	−0.03	0.06	−0.15	–	0.09	−0.49	0.6256	
Second dose of BNT162b2	0.11	0.04	0.02	–	0.19	2.40	0.0163	**
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	−0.03	0.04	−0.10	–	0.04	−0.78	0.4371	
Timing 2	0.08	0.03	0.02	–	0.15	2.52	0.0117	**
Timing 3	0.04	0.03	−0.02	–	0.10	1.26	0.2062	

SE, standard error.

[a] General properties of the model, Breusch−Pagan test, Chi-square 114.23, $p = 5.10 \times 10^{-16}$,McFadden's pseudo R-square test 0.30, Log-likelihood −539.40.

[b] Statistical significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[c] Timing 1, Prior to the third dose of BNT162b2, Timing 2, After the third dose of BNT162b2 but without or before the onset of adverse events, Timing 3, After the onset of adverse events following the second dose of BNT162b2.

3.4.1. The effect of age on adverse events

Those aged 25–40 years were associated with a reduced incidence of systemic events following the second dose, but the difference was not statistically significant ($p = 0.0530$). Following the third dose, those aged 25–40 years experienced more non-worst local events ($p = 0.0002$) but were less likely to experience the worst local events ($p = 0.0002$) than those aged <25 years. Those aged 25–40 years also had less non-worst lymphadenopathy following the third dose ($p = 2.00 \times 10^{-5}$). Age ≥40 years was associated with a reduced likelihood of systemic events following the second dose ($p = 6.10 \times 10^{-7}$), was likely to result in fewer local events following the third dose and was less likely to result in the worst local events compared to age <25 years ($p = 0.0344$ and $p = 0.018$, respectively). Lymphadenopathy tended to be absent ($p = 0.0358$) and less likely to be the non-worst event ($p = 0.0006$) in this older age group. Although age ≥40 years was associated with reduced worst lymphadenopathy, this was not statistically significant ($p = 0.0723$).

Table 6
Heteroscedastic model of lymphadenopathy following the third dose of BNT162b2.[a]

Mean estimate								
	Parameter estimate[b]	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	0.07	0.05	−0.03	–	0.18	1.39	0.1651	
Age (comparison to <25 years)								
25–40 years	0.05	0.06	−0.06	–	0.16	0.85	0.3968	
≥40 years	0.07	0.05	−0.03	–	0.18	1.39	0.1651	
Blood type (comparison to type A)								
AB	−0.06	0.10	−0.25	–	0.13	−0.60	0.5501	
B	0.12	0.09	−0.06	–	0.30	1.34	0.1810	
O	0.04	0.06	−0.08	–	0.17	0.67	0.5010	
Medical history								
Asthma	−0.51	2.51	−5.42	–	4.41	−0.20	0.8397	
Metabolism disorders	−93.24	426.97	−930	–	744	−0.22	0.8271	
Allergic reaction to vaccine	−0.23	1.49	−3.14	–	2.69	−0.15	0.8776	
Smoking (comparison to naive)								
Past	0.08	0.09	−0.10	–	0.27	0.88	0.3769	
Current	0.81	0.54	−0.25	–	1.87	1.50	0.1333	
Systemic event								
First dose of BNT162b2	0.07	0.06	−0.04	–	0.18	1.21	0.2282	
Second dose of BNT162b2	−0.27	0.13	−0.53	–	−0.02	−2.11	0.0347	*
Local event								
First dose of BNT162b2	−0.17	0.11	−0.38	–	0.05	−1.53	0.1259	
Second dose of BNT162b2	0.07	0.09	−0.12	–	0.25	0.72	0.4705	
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	0.04	0.08	−0.13	–	0.20	0.46	0.6477	
Timing 2	0.08	0.08	−0.08	–	0.23	0.95	0.3436	
Timing 3	−0.05	0.07	−0.18	–	0.08	−0.71	0.4775	
Lymphadenopathy								
First dose of BNT162b2	0.03	0.06	−0.08	–	0.15	0.55	0.5814	
Second dose of BNT162b2	1.75	0.43	0.92	–	2.59	4.11	4.0×10^{-5}	***
Marginal effects on the absence of lymphadenopathy (outcome 0)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	0.07	0.03	0.02	–	0.12	2.74	0.0061	***
Age (comparison to <25 years)								
25–40 years	0.02	0.03	−0.03	–	0.08	0.85	0.3943	
≥40 years	0.06	0.03	0.00	–	0.12	2.10	0.0358	**
Blood type (comparison to type A)								
AB	−0.02	0.04	−0.11	–	0.06	−0.55	0.5833	
B	−0.01	0.03	−0.07	–	0.05	−0.39	0.6990	
O	−0.01	0.03	−0.06	–	0.05	−0.22	0.8270	
Medical history								
Asthma	0.06	0.08	−0.10	–	0.21	0.74	0.4602	
Metabolism disorders	0.03	0.06	−0.08	–	0.15	0.59	0.5562	
Allergic reaction to vaccine	0.08	0.15	−0.22	–	0.38	0.52	0.6061	
Smoking (comparison to naive)								
Past	−0.03	0.03	−0.10	–	0.04	−0.89	0.3720	
Current	−0.05	0.05	−0.14	–	0.05	−1.01	0.3120	
Systemic event								
First dose of BNT162b2	0.02	0.03	−0.03	–	0.07	0.83	0.4080	
Second dose of BNT162b2	0.00	0.03	−0.06	–	0.06	−0.07	0.9420	
Local event								
First dose of BNT162b2	−0.10	0.03	−0.17	–	−0.04	−3.17	0.0015	***
Second dose of BNT162b2	0.02	0.04	−0.06	–	0.10	0.44	0.6583	
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	−0.04	0.04	−0.11	–	0.03	−1.06	0.2894	
Timing 2	−0.09	0.03	−0.14	–	−0.03	−3.20	0.0014	***
Timing 3	−0.02	0.03	−0.08	–	0.04	−0.76	0.4502	
Lymphadenopathy								
First dose of BNT162b2	0.14	0.02	0.11	–	0.17	8.84	$<2.20 \times 10^{-16}$	***
Second dose of BNT162b2	−0.58	0.09	−0.76	–	−0.41	−6.58	4.80×10^{-11}	*
Marginal effects on non-worst lymphadenopathy (outcome 1)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female)								
Male	−0.01	0.00	−0.01	–	0.00	−3.29	0.0010	***

(*continued on next page*)

Table 6 (*continued*)

Marginal effects on non-worst lymphadenopathy (outcome 1)								
	Marginal effects	SE	95 % CI			t value	p value	
Age (comparison to <25 years)								
25–40 years	−0.01	0.00	−0.01	–	0.00	−4.29	2.0×10^{-5}	***
≥40 years	−0.01	0.00	−0.02	–	0.00	−3.46	0.0006	***
Blood type (comparison to type A)								
AB	0.00	0.00	−0.01	–	0.00	−1.65	0.0991	
B	−0.01	0.00	−0.02	–	0.00	−3.17	0.0015	***
O	0.00	0.00	−0.01	–	0.00	−1.33	0.1850	
Medical history								
Asthma	−0.03	0.02	−0.08	–	0.02	−1.33	0.1846	
Metabolism disorders	−0.05	0.01	−0.06	–	−0.03	−7.62	2.63×10^{-14}	***
Allergic reaction to vaccine	−0.02	0.11	−0.24	–	0.20	−0.20	0.8415	
Smoking (comparison to naive)								
Past	−0.01	0.00	−0.01	–	0.00	−2.90	0.0038	***
Current	−0.01	0.02	−0.05	–	0.04	−0.34	0.7334	
Systemic event								
First dose of BNT162b2	0.00	0.00	0.00	–	0.00	0.02	0.9876	
Second dose of BNT162b2	0.02	0.00	0.01	–	0.03	3.84	0.0001	***
Local event								
First dose of BNT162b2	0.02	0.01	0.01	–	0.03	4.36	1.0×10^{-5}	***
Second dose of BNT162b2	0.00	0.00	0.00	–	0.01	2.72	0.0066	***
Use of antipyretics (comparison to those who did not take any)[c]								
Timing 1	−0.01	0.00	−0.01	–	0.00	−4.65	3.39×10^{-6}	***
Timing 2	0.00	0.00	−0.01	–	0.01	0.54	0.5885	
Timing 3	0.00	0.00	0.00	–	0.00	−1.03	0.3007	
Lymphadenopathy								
First dose of BNT162b2	−0.01	0.01	−0.03	–	0.01	−0.76	0.4487	
Second dose of BNT162b2	0.23	0.07	0.10	–	0.36	3.46	0.0005	***

Marginal effects on the worst lymphadenopathy (outcome 2)								
	Marginal effects	SE	95 % CI			t value	p value	
Sex (comparison to female) rowhead								
Male	−0.06	0.03	−0.11	–	−0.01	−2.46	0.0140	**
Age (comparison to <25 years) rowhead								
25–40 years	−0.02	0.03	−0.08	–	0.04	−0.64	0.5244	
≥40 years	−0.05	0.03	−0.11	–	0.00	−1.80	0.0723	
Blood type (comparison to type A) rowhead								
AB	0.03	0.05	−0.06	–	0.12	0.60	0.5510	
B	0.02	0.03	−0.04	–	0.08	0.74	0.4595	
O	0.01	0.03	−0.05	–	0.07	0.29	0.7700	
Medical history rowhead								
Asthma	−0.03	0.08	−0.18	–	0.13	−0.32	0.7462	
Metabolism disorders	0.01	0.06	−0.10	–	0.12	0.22	0.8234	
Allergic reaction to vaccine	−0.06	0.07	−0.19	–	0.08	−0.83	0.4055	
Smoking (comparison to naive) rowhead								
Past	0.04	0.03	−0.03	–	0.11	1.15	0.2506	
Current	0.06	0.06	−0.05	–	0.17	1.00	0.3185	
Systemic event rowhead								
First dose of BNT162b2	−0.02	0.03	−0.07	–	0.03	−0.83	0.4068	
Second dose of BNT162b2	−0.02	0.03	−0.07	–	0.04	−0.54	0.5868	
Local event rowhead								
First dose of BNT162b2	0.08	0.03	0.02	–	0.14	2.66	0.0078	***
Second dose of BNT162b2	−0.02	0.04	−0.10	–	0.06	−0.53	0.5940	
Use of antipyretics (comparison to those who did not take any)[c] rowhead								
Timing 1	0.04	0.04	−0.03	–	0.11	1.22	0.22167	
Timing 2	0.09	0.03	0.03	–	0.14	3.27	0.0011	***
Timing 3	0.02	0.03	−0.04	–	0.08	0.78	0.4332	
Lymphadenopathy rowhead								
First dose of BNT162b2	−0.13	0.01	−0.16	–	−0.11	−10.39	$<2.20 \times 10^{-16}$	***
Second dose of BNT162b2	0.35	0.07	0.21	–	0.49	5.02	5.19×10^{-7}	***

[a] General properties of the model, Breusch–Pagan test, R-square 51.86, p = 0.00012, McFadden's pseudo R-square test 0.22, Log-likelihood −346.57.

[b] Statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001.

[c] Timing 1, Prior to the third dose of BNT162b2, Timing 2, After the third dose of BNT162b2 but without or before the onset of adverse events, Timing 3, After the onset of adverse events following the second dose of BNT162b2.

3.4.2. The effect of sex on adverse events

Males were associated with a reduced risk of the worst systemic events following the third dose compared to females ($p = 0.0445$). Although the mean estimate showed that men were less likely to have systemic events following the second dose than females, this difference was not statistically significant ($p = 0.15267$). Although male sex was associated with an increased likelihood of non-worst local events following the third dose compared with female sex ($p = 0.0005$), males were less likely to have the worst local event following the third dose ($p = 0.0031$). Following the third dose, male sex was also associated with the absence of lymphadenopathy ($p = 0.0061$) and a reduced likelihood of non-worst and worst lymphadenopathy ($p = 0.0010$ and 0.0140, respectively) compared with female sex.

3.4.3. The effect of history of allergic reaction to previous vaccines on adverse events

A history of allergic reactions to other vaccines was negatively associated with the absence of systemic events and positively associated with the worst systemic event following the third dose ($p = 0.0015$ and 0.0280, respectively).

3.4.4. The effect of blood type on adverse events

Compared to blood type A, blood type B was associated with a reduced risk of systemic events following the second dose in the mean estimate and was associated with their absence ($p = 0.0499$ and 0.0248, respectively). Blood types AB, B and O were associated with an increased likelihood of a non-worst local event ($p = 2.00 \times 10^{-5}$, 0.0045 and $p = 0.0028$, respectively) and a reduced likelihood of the worst local event ($p = 0.0001$, 0.0061, and 0.0031, respectively). Blood type B was also associated with a reduced likelihood of non-worst lymphadenopathy following the third dose ($p = 0.0015$).

3.4.5. The effect of asthma on adverse events

Asthma was negatively associated with a non-worst third systemic event ($p = 0.0359$). The presence of conditions related to fat or glucose metabolism, including type 1 and 2 diabetes mellitus, dyslipidaemia and metabolic syndrome, was not associated with any adverse events.

3.4.6. The effect of smoking on adverse events

Past smokers were associated with a reduced likelihood of local event absence and a reduced likelihood of non-worst lymphadenopathy following the third dose ($p = 0.0068$ and 0.0038, respectively).

3.4.7. The effect of systemic events following previous doses on adverse events following later doses

The experience of the systemic event group following the first dose was associated with an increased likelihood of systemic events following the second dose ($p = 1.46 \times 10^{-12}$) and the non-worst systemic event following the third dose ($p = 0.0359$).

Those who experienced the second systemic event were less likely to be free of the third systemic event ($p = 2.58 \times 10^{-10}$) and more likely to have non-worst and worst systemic events and non-worst lymphadenopathy following the third dose ($p = 2.00 \times 10^{-5}$, 0.0006, and 0.0001, respectively).

3.4.8. The effect of local events following previous doses on adverse events following later doses

The experience of the local event group following the first shot was associated with an increased risk of systemic events following the second dose ($p = 5.00 \times 10^{-5}$). Following the third dose, the experience of the local event group was associated with less absence and an increased likelihood of both non-worst and worst lymphadenopathy ($p = 0.0015$, 1.00×10^{-5}, and 0.0078, respectively).

Those who experienced the second local event were less likely to be free of the third systemic event ($p = 0.0197$) and more likely to have the worst systemic event ($p = 0.0077$). They were less likely to be local event free ($p < 2.20 \times 10^{-16}$), more likely to have non-worst and worst local events ($p < 2.00 \times 10^{-16}$ and 0.0163, respectively) and tended to have non-worst lymphadenopathy ($p = 0.0066$).

3.4.9. The effect of antipyretics on adverse events

The use of antipyretics prior to the third dose was associated with a reduced likelihood of non-worst lymphadenopathy ($p = 3.39 \times 10^{-6}$). The use of antipyretics following the third dose of BNT162b2 but without any event or before the onset of an event was positively associated with the systemic event group following the third shot ($p = 0.0486$), with a reduction in absence and an increase in the non-worst and worst systemic event group following the third dose ($p = 0.0006$, 0.0033, and 1.37×10^{-6}, respectively). The use of antipyretics was also associated with an increase in local events following the third dose ($p = 0.0117$), a reduced likelihood of the absence of lymphadenopathy and increased worst lymphadenopathy ($p = 0.0014$ and 0.0011, respectively).

Those who had experienced lymphadenopathy following the first dose tended to lack lymphadenopathy and were less likely to experience the worst lymphadenopathy (p values were $<2.00 \times 10^{-16}$ for both). Those who experienced lymphadenopathy following the second dose were less likely to lack lymphadenopathy ($p = 4.80 \times 10^{-11}$) and more likely to experience non-worst and worst lymphadenopathy ($p = 0.0005$ and 5.19×10^{-7}, respectively).

4. Discussion

Our previous survey-based study on adverse events following the second dose of BNT162b2 showed that systemic adverse events were associated with young age and female sex [14]. This study showed that some adverse events associated with BNT162b2 formed

two distinct groups, and subject background (e.g., age, sex, blood type), experiences of adverse events following previous vaccination and some other factors variably affected the adverse events following the third dose of BNT162b2.

The immune response to the SARS-CoV-2 vaccine is age-related [15–17]. In this study, people aged ≥40 years experienced fewer systemic and local events following the second dose than those aged <25 years, which is consistent with another study that reported that systemic reactions such as fatigue, headache, and fever were less likely to occur in an age-dependent manner after the third dose in Japan [1]. This study also showed that those aged ≥25 years were less likely to experience worsening of injection site-related local adverse events and lymphadenopathy following the third booster shots compared to the second dose, but this was not true for other systemic adverse events.

Sex affected adverse events following the third dose; females experienced local and systemic adverse reactions more often than males [1]. We found that females tended to experience intensification of systemic adverse events compared to the second dose. Therefore, it is reasonable to explain to a female vaccine recipient a possible intense reaction following later doses of the vaccine. Sex also affected lymphadenopathy, which showed a similar trend to other systemic adverse events, and females were more likely to experience intensification of lymphadenopathy following the third booster shot compared to the second booster than males.

We found that a history of allergic reaction to other vaccines was positively associated with the worst systemic event and negatively associated with the absence of a systemic event after the third dose. Hence, it is reasonable to provide complete information about possible adverse events to a vaccine recipient with a previous vaccine-related allergic reaction.

In the present study, current smoking did not reduce the risk of adverse events, and only past smoking habits were associated with non-worst lymphadenopathy following the third dose. Other studies showed that current smokers had substantially lower antibody titres than past smokers among SARS-CoV-2-vaccinated people [18,19]. As higher IgG levels are associated with a greater risk of adverse events, current smokers were expected to experience fewer adverse events. These discrepancies warrant further study on the effect of smoking on immune responses.

The effect of blood type on reactogenicity to COVID-19 vaccines is a controversial issue [20,21]. Our results showed that blood type B was associated with a reduced risk of systemic events following the second dose compared with blood type A. We also found that blood types AB, B and O were associated with the non-worst local events but were negatively associated with the worst local event. Blood type B was also associated with a reduced risk of non-worst lymphadenopathy after the third dose. To address this inconsistency, future large-scale studies are needed.

In the present study, the use of antipyretics was associated with systemic and local events, especially in male lymphadenopathy. One explanation is that people who had experienced adverse events were more likely to use antipyretics. However, in the present study, experiences of systemic/local events after the first or second dose were associated with the experiences of adverse reactions after the third dose (especially in lymphadenopathy). Therefore, if the effect of antipyretics is not sufficient, the use of antipyretics can be a confounding factor of adverse events.

Our previous report showed that those who experienced systemic and local adverse events following the first dose were more likely to develop similar adverse events following the second dose [1]. In this study, we found that those who experienced systemic adverse events following the second dose experienced not only more frequent but also worse symptoms than with previous doses. Injection site-related local adverse events have a similar tendency to become more intense but to a lesser extent than systemic adverse events, and it may be appropriate to tell a person with a previous local event that these reactions do not necessarily intensify with successive boosters.

We also found that lymphadenopathy following the first dose was unlikely to occur again following the third dose, while experience of lymphadenopathy following the second dose was associated with the experience of lymphadenopathy after the third dose. Interestingly, only the occurrence rate of lymphadenopathy increases prominently following the third dose. Compared to other adverse events queried in this study, lymphadenopathy could be more objective and may represent the true extent of the immune response, less affected by psychological state, to the vaccine. However, it is also possible that delayed B- and T-cell memory responses cause a particular immune response in lymph nodes. Those with prominent lymph node enlargement following the first dose may have a large number of memory B cells or plasma cells whose IgG can reduce the non-neutralized naked antigen following boosters and therefore result in less naive lymphocyte activation and less lymphadenopathy following boosters. However, the exact mechanism of how the vaccine may cause lymphadenopathy is still not clear [22]. Given the dramatic intensification of lymphadenopathy following the third dose, those who receive three or more doses of boosters should be informed of this reaction before receiving the vaccine even when they have not developed lymphadenopathy following previous doses, especially those who are young or female.

Our study has some limitations. A previous study showed a positive association between immunoglobulin G (IgG) levels and adverse events [23]. Therefore, investigation of the relationship among age, IgG level, and adverse events is important. However, in the present study, IgG titres were not evaluated. During the study period, only thirteen participants reported having had a previous diagnosis of COVID-19, which made us unable to analyse the COVID-19 history as a factor influencing adverse events. Additionally, adverse events were queried based on the internet study form, and there was no way to know what adverse events occurred in those who did not answer, which may have biased this study.

5. Conclusions

In conclusion, to the best of our knowledge, this is the first study to clarify the association among different adverse events using networks following the first, second, and third doses of BNT162b2. Some adverse events following BNT162b2 formed two main groups: systemic and injection site-related local event groups. While previous experiences of systemic and local events were associated with worse adverse events following the third dose, local adverse events following the third dose were often not worse than those

previously experienced. Unlike other adverse events, lymphadenopathy increased sharply following the third booster. It may be appropriate to tell a vaccine candidate who is going to receive three or more boosters that lymphadenopathy may occur even without previous lymphadenopathy following the earlier doses, but local events do not necessarily intensify.

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Institutional review board statement

This study was approved by the Ethics Committee of Nihon University School of Medicine (approval number: P21-06-1). All procedures were performed under the guidelines of our institutional ethics committee and adhered to the tenets of the Declaration of Helsinki.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

Data are contained within the article or supplementary material.

CRediT authorship contribution statement

Takahiro Namiki: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kazuhide Takada:** Writing – review & editing, Investigation. **Satoshi Hayakawa:** Writing – review & editing, Supervision, Funding acquisition. **Shihoko Komine-Aizawa:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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