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SARS-CoV-2 vaccination at a High-Complexity Reference Hospital: Implementation and results of an active pharmacovigilance program

Joaquín Sáez Peñataro

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SARS-CoV-2 vaccination at a High-Complexity Reference Hospital: Implementation and results of an active pharmacovigilance program

Doctoral thesis dissertation presented by Joaquín Sáez Peñataro to apply for the degree of doctor at the University of Barcelona

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Presentation: February 2025

Declarations and signatures

Acknowledgements

A las personas más importantes de mi vida: mi Madre, y mis Abuelitos, Felipe y Lola. Por enseñarme a soñar, a nunca rendirme, a caminar hacia adelante a pesar de las dificultades y a aprender de los errores. Por apoyarme y por quererme incondicionalmente. A vosotros, os lo debo todo.

A mi gata Lila. Por las horas que hemos pasado juntos escribiendo esta Tesis, uno al lado del otro.

*Hello darkness, my old friend
I've come to talk with you again
Because a vision softly creeping
Left its seeds while I was sleeping
And the vision that was planted in my brain
Still remains
Within the sound of silence
In restless dreams I walked alone
Narrow streets of cobblestone
'Neath the halo of a street lamp
I turned my collar to the cold and damp
When my eyes were stabbed by the flash of a neon light
That split the night
And touched the sound of silence
And in the naked light I saw
Ten thousand people, maybe more
People talking without speaking
People hearing without listening
People writing songs that voices never share
No one dared
Disturb the sound of silence
"Fools" said I, "You do not know
Silence like a cancer grows
Hear my words that I might teach you*

Take my arms that I might reach you"

But my words like silent raindrops fell

And echoed in the wells of silence

And the people bowed and prayed

To the neon god they made

And the sign flashed out its warning

In the words that it was forming

And the sign said, "The words of the prophets

Are written on the subway walls

And tenement halls

And whispered in the sounds of silence"

Simon and Garfunkel, "The Sound of Silence", álbum "Wednesday Morning, 3 A.M.", 1964

Ésta es una de mis canciones preferidas, porque es la canción que solía escuchar con uno de mis mejores amigos, en esas tardes interminables de verano, intentando traducirla al castellano, y entender su mensaje. En esa época tan tierna e inocente, cuando nuestra única preocupación era traducir una canción, o si quedaríamos después con los gemelos y Jona para ir al cine, o si conseguiríamos reproducir la fórmula de la fusión nuclear fría, quién me iba decir que unas decenas de años después, la recordaría, y que ahora entendería ese mensaje profético que nos dejaron Simon y Garfunkel en un lejano 1964.

Ha sido un camino muy largo. Ha pasado mucho tiempo desde aquel momento en el que la Hermana Eulalia nos explicó la estructura de la célula. Me parecía increíble que una persona, un cuerpo humano, estuviera formado por trillones (casi infinitos) seres vivos mucho más pequeños. Me emocionó tanto que se lo expliqué a mi abuelo, aquella tarde, en la rotonda de las Palmeritas en Elda, mientras me comía el helado de mora que me solía comprar. Y allí fue cuando le dije: "Abuelito, hoy la Hermana Eulalia nos ha explicado que la célula está formada por membrana, núcleo y citoplasma. Y yo quiero aprender y saber más. Quiero ser médico". Pues hoy, Abuelito, te dedico esta Tesis. Es una Tesis humilde y sencilla, aunque

bien hecha (que para eso me la han dirigido dos grandes Maestros). También esta Tesis va dirigida a ti, Abuelita, y a ti, Mamá. Hemos recorrido juntos un largo camino. En este viaje, desde aquella tarde en la rotonda de Las Palmeritas, hasta ahora, he contado con las personas más importantes de mi vida, mis dos abuelos Felipe y Lola, y mi madre, Pepita. No ha sido fácil en absoluto. Pero durante los primeros años de vida tuve el cariño de mi abuela, mi segunda madre, así como los consejos de mi abuelo, mi ejemplo de vida. Y desde entonces hasta ahora he tenido la inmensa suerte de contar, indefectiblemente, con mi Madre, a quien le dirijo la dedicatoria más especial de esta Tesis. Mamá, no sé cómo has tenido tanta paciencia conmigo, tanto amor, tanta capacidad para encontrar sabiduría y las palabras justas en cada momento. Tú, Mama, la “chiquilla” que fue azafata y modelo en la Feria Internacional del Calzado; que salía con Pedrito Rico; que conoció a Dalí, a Montserrat Caballé y a Massiel; que vivió tantas aventuras en Galicia con su seiscientos; has sido también una luchadora incansable. Cuando no teníamos a nadie, luchaste como una campeona para sacarme adelante, y no sólo eso, también para que pudiera estudiar. Así que, aunque no haga falta decirlo, lo diré. Sin ti, nunca jamás habría llegado a este punto de la Tesis. Por tanto, esta Tesis es tanto tuya, como mía. Gracias, mi valiente y grandísima Madre, mamá.

Dicen que lo importante es recorrer el camino, como bien expresa Jack Kerouak en su obra *“On the road”*, o *“En el camino”*. Y de alguna manera, puedo confirmarlo. He vivido buenos y malos momentos en Elda, pero siempre recordaré con cariño a mis Hermanas del Colegio de las Carmelitas. También al Instituto Monastil y los buenos profesores que tuve. Ahora me viene a la mente el bueno de Boro, mi excelso profesor de Educación Física. Tan bueno, que me aprobó a pesar del nulo interés que mostré en sus clases. También me acuerdo mucho de Paco y sus cigotos, de Elena y su estricta revisión de la ortografía, y de mi gran amigo Paco, el profesor de Inglés, QEPD. Grandes como nadie. Y cómo no, aquellos momentos tan interesantes en la Universidad, donde conocí a mi amor platónico. En la Universidad tuve también el inmenso honor de asistir a las clases de grandes profesores, como el Dr Sánchez del Campo, el Dr Pedro Zapater, el Dr Horga, el Dr Roberto Gallego, la Dra Francisca Colom, el Dr Correa (QEPD), el Dr Segura, el Dr Terol, o el Dr Salvador Martínez. Y allí además conocí a grandísimos amigos, como David Paco, William, Tenza, Pedro, Roger, y otros muchos con quienes viví noches interminables, y amaneceres inolvidables en el Puerto de Alicante.

Y el camino continuó. Que la fiesta no pare. Después de la Facultad acabé (aún no sé bien cómo), en Neurocirugía. Con todo mi cariño, recuerdo y agradezco la ayuda del Dr Poblete, el Dr Sánchez, los Dres Carales, y el Dr García-Amorena. Pero no se me daba bien eso de operar, y decidí que quería ser farmacólogo clínico. Y acabé en el despacho de Joan Albert (por aquel momento tutor de residentes de farmacología clínica), explicándole que quería cambiar de especialidad. Para el recuerdo ha quedado su cara, expresión del más estimado poema de Bécquer. Ya sé que fue un “triple salto mortal”. Pero puedo confirmar, sin miedo a equivocarme, que, desde aquel momento, hasta ahora, nunca, ni un sólo día, me he arrepentido de esa decisión que tomé. Soy farmacólogo clínico, y repetiría. Es verdad que, a esa satisfacción, hay que agradecer el hecho de haber podido hacer la residencia en un Servicio como el del Clínic, con mucha gente buena y muy sabia, de la que he aprendido muchas cosas estos años (y sigo aprendiendo). Fueron años felices en los que compartí momentos inolvidables con mi querido hermano y co-R Nil. Aquel viaje a Nijmegen hacia 2014, con Nil y con Gonzalo, ha quedado impreso en mi corazón con tinta indeleble. Años después, tuve el inmenso honor de convertirme en adjunto del Servicio, y después en tutor de residentes. He aprendido mucho de mis resis, y les guardo un cariño inmarcesible e infinito: Pau, Maria, Carlos (Chelas), Vane, Vicky, Alejandro ¡Gracias por todo, chicos!

Estos años también he tenido el inmenso honor de ser profe en la Universidad. Doy gracias a mis estudiantes por haberme enseñado tanto, sobre todo, la parte más importante: que hacer docencia en realidad, significa aprender con tus discentes. Un abrazo enorme al Dr Sergi Mas y a los compañeros del Departamento, y muy especialmente, a la Dra Amàlia Lafuente, allá donde esté (QEPD).

Y todavía recuerdo, con cariño, al Dr Xavier Carné y sus reflexiones sobre metodología, la Declaración de Helsinki, y ECRIN, mucho ECRIN. En ECRIN de hecho, pasé unos años bien bonitos, donde aprendí aspectos muy interesantes sobre la gestión de ensayos clínicos, viajé, conocí personas amables, y tuve la suerte, después de algunos años, de mantener esa relación con muchos de ellos (*To my ECRIN colleagues, for all that I have learned with you, for your kindness, for letting me be part of the ECRIN family!*).

Y en el Servicio como digo, he tenido la suerte de compartir profesión durante años con gente maravillosa, como Sara, Ana, Judit, Joan Albert, José, Ali, Cris (x2), Albert, y tantos compañeros que han ido y viniendo a lo largo de los años, de los cuales también he podido

aprender muchas cosas, y guardar un excelente recuerdo. De hecho, en los últimos años hemos ido creciendo y nuestra “pequeña familia” se ha enriquecido y convertido en un gran equipo multidisciplinar. Estoy muy agradecido de aprender cada día tanto, y tanto, de vosotros.

No puedo hablar del Servicio sin hablar de mi querido Juan Bascuas, un gran compañero con el que tengo la suerte de trabajar en mil cosas desde hace años. Juan es una persona a la que tengo un gran respeto y admiración tanto profesional, como personal. Una persona imprescindible a la que agradezco, tanto su dedicación en todo lo que hace, como su paciencia conmigo, como su forma de alegrarnos el día con sus “*kind reminders*” y sus memes. Juan, eres grande, y tienes un lugar especial en esta tesis, como en mi vida. Por muchos lunes más juntos haciendo el troll.

Después de unos cuantos saltos mortales adicionales, vino el COVID, y después las vacunas. Aunque ahora parece una eternidad, casi casi recuerdo, como si fuera ayer, el día en el que se administró la primera vacuna en Hospital Clínic. Para mí fue emocionante poder verlo junto con el Dr Joan Bartra, alergólogo, y el Dr Gonzalo Calvo, regulador experimentado. En ese punto supe que quería hacer mi tesis sobre la seguridad de las vacunas COVID ¡Y lo más bonito fue que una nueva parte del camino empezaba en ese punto! Me encantaron aquellos días en los que íbamos al centro de vacunación a vigilar efectos adversos, diseñábamos circuitos de seguimiento, e improvisábamos, de la mejor manera que podíamos y sabíamos, las guías de actuación con todas las incertezas que había en aquel momento. La Dra Anna Vilella ha sido una figura inspiradora para mí. Aprendí mucho de ella, y abrió una puerta muy importante para crear la Unidad Funcional de Vacunas, y establecer la colaboración con farmacovigilancia. Me encanta el mundo de las vacunas, me encanta colaborar con todos los compañeros de la Unidad, y siempre tengo muy presente a la Dra Vilella: Anna, moltes gràcies per tot. He après molt de tu, i ha estat un plaer col·laborar tan bé!

Y cómo no, he de destacar que tuve el inmenso honor de coincidir en varias ocasiones con el Dr Trilla, un enorme referente de quién pude aprender la importancia de vacunación, y de quién me he inspirado también en la redacción de esta tesis.

Por supuesto, también conocí a otras personas muy sabias, de las que pude aprender mucho, como Eli Gómez, o Sebas. Personas realmente maravillosas que aportan paz, conocimiento,

alegría, y muchas cosas bonitas al equipo. Personas por las que volvería a “montar guardia” al centro de vacunación.

No todo fue un camino de rosas. Hubo momentos buenos y otros no tanto. Pero siempre he tenido la suerte de contar con personas maravillosas, como Lucía, Pau y Sara, quienes fueron un gran apoyo para mí, especialmente en 2021, un año particularmente difícil. También he contado con personas increíbles, como la increíble Eulàlia y la admirable Mireia, que, con mucha paciencia, me han dado valiosos consejos para enfocar la tesis. ¡Gracias infinitas!

Y ciertamente, me puse a implementar mi proyecto de tesis ¡Pero qué proyecto! Llamar a casi 7.000 personas, dos veces (tras cada dosis de vacuna), con una base de datos estructurada que permitiera su análisis, y un protocolo sistemático que permitiese ser fiable. No sería justo si no reconociese, y agradeciese con todo mi cariño y respeto profesional y personal, el enorme equipo que trabajó incansablemente para llevar a cabo el proyecto. Además de mi Juan Bascuas que nos coordinaba a todos para llevar a buen puerto el proyecto, he de destacar la labor enorme del equipo de “llamadores”: Luis Aparicio, Susanna Bañuelos, Pilar Cano, Vanessa de Dios, Anna Massó, Celia Puente, Carlos Sánchez, Martina Villarreal, así como a Sandra Serrano, data manager, por su expertise y amabilidad. Quiero destacar que hay muy pocos data managers que realmente manejen como maneja Sandra, ¡crack donde las haya! Ha sido un verdadero placer contar con todos. Y, de hecho, creo que hasta lo pasamos bien, entre llamada y llamada, con mil anécdotas divertidas (que dejaré para recordar en la celebración post-tesis). Gracias de verdad. Sin vosotros, este proyecto no habría podido culminarse. Por supuesto, también he de dar las gracias al Àrea del Medicament, y en especial, a la Dra Concha Camacho, por apoyar el proyecto desde el principio, y en especial, al Programa de Farmacovigilancia.

Así pues, fue una época “convulsa” de muchas incertezas, algún miedo, mucho trabajo, pero también bonita en cuanto cabe. Además, siempre he contado con el apoyo infinito del Programa de Farmacovigilancia, tanto del Órgano de Coordinación (pinyol), como del Comité de Farmacovigilancia con nuestros “embajadores”. En el Comité he tenido el placer de colaborar con embajadores y embajadoras tan eminentes e increíbles como la Dra Saurí de Oncología, el Dr Hernández de Autoinmunes, o el Dr Sanmartí de Reumatología, entre otros imprescindibles colegas. Quiero que sepáis que el mejor momento de la semana es el

miércoles a las 14:00, cuando nos reunimos el pinyol. Tener este equipo es de las mejores cosas que me ha ofrecido este Hospital (de tantas muy buenas). Y os agradezco vuestro apoyo infinito, que me deis la oportunidad de aprender cada día de vosotros, y que hagáis que cada día me guste más lo que hago. Gracias enormes: Rosa, Sònia, Joan, Mariona, Cris, Vicky, Maria Jesus, Ester, Maria, Carla, Elena, Andrea, Juan ¡Creo que ningún eldense estuvo nunca tan bien acompañado en un equipo!

Debo agradecer, profundamente, el apoyo recibido desde Direcció General y especialmente de Direcció Mèdica, con el Dr Castells en la creación del Programa Clínic de Farmacovigilancia, y ahora con la Dra Sánchez que sigue dándonos apoyo. Muchas gracias por haber creído en este proyecto, por habernos ayudado, y por habernos dado el soporte institucional fundamental para consolidar un proyecto hermoso.

Y dentro de este equipo hay personitas muy bonitas que hacen que mi día a día sea más fácil. Mi súper team de farmacovigilancia en el Servicio, con Maria, Elena, Andrea, y ahora desde hace poco ¡súper Carla! Teneros cerca es un gran apoyo para mí cada día, y me encanta aprender con vosotras estableciendo deadlines, hitos por completar, y siempre, mucha ilusión. Gracias enormes por tanta paciencia, tanta buena profesionalidad, tanta amabilidad, y hacer que me lo pase genial con cada proyecto que emprendemos ¡Por muchos más!

Por supuesto, no puedo dejar de agradecer a mi querido amigo Joan Bartra. Joan es mi amigo, pero también un referente profesional y personal para mí. Joan, muchas gracias por los buenos consejos, por tu paciencia, por enseñarme siempre con amabilidad y una sonrisa, por crear buen ambiente, por haber consolidado el Programa con tu “tarannà” imprescindible, por estar siempre a mi lado, por haber sido el “pal de paller” que ha enganchado a tanta gente a este mundo de la farmacovigilancia. Tú me has enseñado que la rigurosidad científica no está reñida con el buen humor, el buen ambiente, y una buena predisposición. Quiero que sepas que eres una persona muy importante, y que, para mí, eres mi “tercer Director de tesis”. Gracias por todo, *my dear friend*.

Y me dejo lo mejor para el final. Quiero dar las gracias a mis dos Directores de Tesis. Los dos muy diferentes entre sí, pero dos grandes Maestros, de los que he aprendido mucho en todos los años que los conozco. Hemos vivido muchas cosas juntos. Y sólo puedo deciros que sois muy grandes para mí. Creo que el idioma español, en toda su riqueza, se me queda corto para expresar tanto mi agradecimiento, como el enorme cariño y respeto profesional

que tengo hacia vosotros, mis queridos Gonzalo y Ferran, Ferran y Gonzalo. Grandes donde los haya, y mitos de la farmacología clínica, esta tesis tiene vuestras huellas bien impresas, y estoy muy orgulloso de que así sea. Os quiero.

Pues bien, ahora acaba una parte del camino, y sólo cogeré un poco de aire para empezar con ilusión la siguiente etapa, como siempre, adelante, como siempre, “gota malaya”. Como bien decía Nach en su canción “El camino del guerrero” de 2005: *“... El guerrero de la rima nunca se desanima. Se apoya en fuerzas divinas pero no las subestima. Su alma es como un ave y él vuela. Desvela claves y sabe que ahí fuera. Fieras espera que mueran... Aunque duelan los motivos su objetivo, cumple sin incertidumbre. No sucumbe hasta que vislumbre la cumbre. Y tumbe al mal brutal en un ritual combativo. Permaneciendo en guardia aunque parezca dormido... Se siente vivo y da gracias. Y no hay lugar que le pueda intimidar bajo ninguna circunstancia. Entrena y la pena no frena su emblema; Paciencia. Para el todo es posible con práctica y experiencia... Perseverancia, confianza, poder Preciado. Armado con dictados, alados aliados al lado. Legado de vivencia, experiencias. De un pasado que marcó su espíritu... No le pierde el ímpetu, tampoco el tedio. Se muestra serio. Toma cada decisión con tensión pero sin nervios. Pone remedio al desánimo y no culpa al prójimo. Viendo grandes verdades en los detalles anónimos... Recorre ciudades, se enfrenta a sus rivales. Conoce sus defectos y también sus cualidades. Ante las dificultades siempre se esfuerza y se crece. Si se desvanece y perece se lo merece... Cuando su don establece nadie puede superarlo. Permanece en silencio si intentan impresionarlo. Aprende de sí mismo...”*

Joaquín S. Peñataro

En Barcelona, a 07 de febrero de 2025

Funding

This thesis did not receive external funding. The research supporting this thesis was conducted using the resources of the Clinical Pharmacology Department and the Medicines Area of Hospital Clínic.

Table of contents

Declarations and signatures	2
Acknowledgements	6
Funding	15
Table of contents	16
Index of tables	18
Index of figures	19
Abbreviations and acronyms	20
List of articles in the Thesis	22
Thesis summary	24
1 Introduction.....	32
1.1 The race for a vaccine against SARS-CoV-2: Overview of the general context.	34
1.2 Development of the first SARS-CoV-2 vaccines.....	42
1.2.1 Vaccine platforms under development.....	45
1.3 Rollout of the first authorised SARS-COV-2 vaccines	60
1.3.1 Accelerated development of vaccine candidates	60
1.3.2 Development and release of the first mRNA vaccines	65
1.3.3 Comparison of BNT162b2 and mRNA-1273	74
1.3.4 1.2.4 The need for continuous safety monitoring after the release of BNT162b2 and mRNA-1273	82
2 Hypothesis.....	92
3 Objectives	98
4 Material, methods, and results.....	104
5 Discussion	296
5.1 Implementation and performance of a prospective and comparative pharmacovigilance system	299
5.1.1 Strengths and limitations.....	303
5.1.2 Future perspectives	306
5.2 Assessment of tolerability and reactogenicity in the VigilVacCOVID study	310
5.2.1 Comparison between BNT162b2 and mRNA-1273	310
5.3 Comparison between HCPs and SOTRs	329
5.3.1 Risk factors of reactogenicity	338
5.3.2 Strengths and limitations.....	342
5.4 Future perspectives.....	349
5.5 Reactogenicity and Immunogenicity.....	358

5.5.1	Reactogenicity and elicited humoral and cellular immunogenicity	358
5.5.2	Strengths and limitations.....	367
5.6	Future perspectives.....	372
5.7	General discussion.....	377
6	Conclusions	382
7	References	386

Index of tables

Table 1. Multistakeholder interactions and key factors involved in expediting SARS-CoV-2 vaccine development and approval	40
Table 2. Summary of main vaccine platforms, advantages, limitations, and illustrative examples	57
Table 3. BNT162b2 and mRNA-1273 differences in formulation	76
Table 4. Storage and administration conditions	77
Table 5. Main clinical outcomes of Phase III clinical trials with BNT162b2 and mRNA-1273	80
Table 6. Tolerability and reactogenicity profile of BNT162b2 and mRNA-1273 in Phase III clinical trials after the second vaccine dose	82
Table 7. Comparison of reactogenicity rates between BNT162b2 and mRNA-1273	313
Table 8. Tolerability and systemic reactions reported in pivotal clinical trials	320
Table 9. Tolerability and systemic reactions reported in real-world studies	321
Table 10. Tolerability and systemic reactions reported in spontaneous reporting pharmacovigilance databases	322
Table 11. mRNA-1273 results in the COVE clinical trial compared with real-world data from the VigilVacCOVID study	332
Table 12. Overview of adverse reactions associated with COVID-19 vaccines as reported in SSRs/PSURs during 2021–2022	347

Index of figures

Figure 1. Interaction between stakeholders	42
Figure 2. Structure of SARS-CoV-2	43
Figure 3. Clinical development of candidate vaccines	44
Figure 4. Structure and functions of SARS-CoV-2 components	46
Figure 5. Structure of mRNA vaccines	53
Figure 6. Types of RNA vaccines: Traditional and saRNA constructs.....	54
Figure 7. Expedited clinical development of SARS-CoV-2 vaccines	63
Figure 8. mRNA vaccine development and approval pathways	73
Figure 9. Spontaneous reports received in Eudravigilance for mRNA-1273 (Elasomeran)	318
Figure 10. Spontaneous reports received in Eudravigilance for BNT162b2 (Tozinameran)	319

Abbreviations and acronyms

ACE2	Angiotensin-converting enzyme 2
ACT	Access to COVID-19 Tools Accelerator
AdV	Adenovirus
AEFI	Adverse event following immunisation
AESI	Adverse event of special interest
AMC	Advance Market Commitment
APC	Antigen-presenting cell
AR	Adverse reaction
BARDA	Biomedical Advanced Research and Development Authority
CEPI	Coalition for Epidemic Preparedness Innovations
cDNA	cloned DNA
CHMP	Committee for Medicinal Products for Human Use
CMA	Conditional marketing authorisation
COVID-19	Coronavirus disease 2019
CoVPN	COVID-19 Prevention Network
DC	Dendritic cell
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
EMA	European Medicines Agency
ETF	Emergency Task Force
EU	European Union
EUA	Emergency Use Authorization
EUL	Emergency use listing
FDA	Food and Drug Administration
Gavi	The Global Alliance for Vaccines and Immunisation
ICMRA	International Coalition of Medicines Regulatory Authorities
ICP	Immune correlate of protection
IDMC	Independent Data Monitoring Committee
LNP	Lipid nanoparticles
MA	Marketing Authorization
MAA	Marketing authorisation application

MERS-CoV	Middle East respiratory syndrome coronavirus
MHC	Major histocompatibility complex
MHRA	Medicines and Healthcare Products Regulatory Agency
MRCT	Multi-regional clinical trial
MVA	Modified vaccinia Ankara
mRNA	Messenger ribonucleic acid
NDV	Newcastle disease virus
NIAID	National Institute of Allergy and Infectious Diseases
ORFs	Open reading frames
OWS	Operation Warp Speed
PIP	Paediatric Investigation Plan
PRIME	Priority Medicines
R&D	Research and Development
RBD	Receptor binding domain
RG	Reverse genetics
RMP	Risk Management Plan
SAP	Statistical analysis plan
SARS-CoV-2	Severe Acute Respiratory Syndrome coronavirus-2
STV	Solidarity Clinical trials Vaccines
UK	United Kingdom
US	United States
VAC4EU	Vaccine monitoring Collaboration for Europe
VE	Vaccine efficacy
VSV	Vesicular stomatitis virus
WHO	World Health Organization

List of articles in the Thesis

Thesis in compendium of publications format. The thesis consists of **4 objectives and 2 articles**.

Article 1:

Authors: **Joaquín Saez-Peñataro**, Ferran Torres, Joan Bartra, Juan Bascuas, Anna Vilella, Marta Tortajada, Sebastiana Quesada, Elisenda González, Ester Lopez-Suñé, Antoni Castells, Sandra Serrano, Concepción Camacho, Antoni Trilla, Gonzalo Calvo.

Title: Tolerability and Reactogenicity Profile of mRNA SARS-Cov-2 Vaccines from a Mass Vaccination Campaign in a Tertiary Hospital: Between-Vaccine and Between-Population Prospective Observational Study (VigilVacCOVID Study)

Journal: BioDrugs (IF 2022:6.8, Q1 Pharmacology and Pharmacy, according to Journal Citation Report (JCR))

Year of publication: 2022

Volume: 36

Pages: 509-520

Reference: Sáez-Peñataro J, Torres F, Bartra J, et al. Tolerability and Reactogenicity Profile of mRNA SARS-Cov-2 Vaccines from a Mass Vaccination Campaign in a Tertiary Hospital: Between-Vaccine and Between-Population Prospective Observational Study (VigilVacCOVID Study). BioDrugs. 2022;36(4):509-520. doi:10.1007/s40259-022-00543-9

Article 2:

Authors: Joaquín Sáez-Peñataro, Gonzalo Calvo, Juan Bascuas, Maria Mar Mosquera, Maria Ángeles Marcos, Natalia Egri, Ferran Torres

Title: Association between Reactogenicity and Immunogenicity in a Vaccinated Cohort with Two mRNA SARS-CoV-2 Vaccines at a High-Complexity Reference Hospital: A Post Hoc Analysis on Immunology Aspects of a Prospective Cohort Study

Journal: BioDrugs (IF 2023:5.2, Q1 Immunology, according to Journal Citation Report (JCR))

Year of publication: 2024

Volume: 12

Pages: 665

Reference: Sáez-Peñataro J, Calvo G, Bascuas J, et al. Association between Reactogenicity and Immunogenicity in a Vaccinated Cohort with Two mRNA SARS-CoV-2 Vaccines at a High-Complexity Reference Hospital: A Post Hoc Analysis on Immunology Aspects of a Prospective Cohort Study. *Vaccines (Basel)*. 2024;12(6):665. Published 2024 Jun 17. doi:10.3390/vaccines12060665

Thesis summary

RESUMEN EN CASTELLANO

TÍTULO DE LA TESIS

Vacunación contra SARS-CoV-2 en un Hospital de Referencia de Alta Complejidad: Implementación y resultados de un programa de farmacovigilancia activa.

INTRODUCCIÓN

El virus coronavirus-2 del síndrome respiratorio agudo severo (SARS-CoV-2) es el agente causante de la pandemia COVID-19, que ha infectado a cientos de millones de personas y provocado millones de muertes en todo el mundo. Esta emergencia desencadenó una intensa y rápida respuesta a gran escala, con un inmenso esfuerzo internacional sin precedentes que se tradujo en un rápido desarrollo de vacunas, un logro nunca visto en la historia de la medicina. Las primeras vacunas aprobadas en diciembre de 2020 fueron BNT162b2 y mRNA-1273, dos vacunas de ARNm. Aunque la plataforma de ARNm no se había utilizado anteriormente en una vacuna autorizada, era una tecnología prometedora que ofrecía muchas ventajas, como la adaptabilidad de las vacunas y la rápida escalabilidad de su fabricación. Además, tanto BNT162b2 como mRNA-1273 habían mostrado buenos resultados de eficacia y seguridad en ensayos clínicos previos de fase III. Sin embargo, era necesario un seguimiento de seguridad post-autorización más prolongado para caracterizar mejor el perfil de seguridad de estas vacunas, especialmente las reacciones adversas raras y a largo plazo. Este seguimiento de vigilancia posterior a la autorización era de suma importancia teniendo en cuenta que, por primera vez en la historia, millones de personas se vacunarían en un breve periodo de tiempo.

HIPÓTESIS

1. **Hipótesis principal:** El estudio VigilVacCOVID permitirá evaluar diferencias entre las vacunas BNT162b2 y mRNA-1273, y entre profesionales sanitarios y pacientes trasplantados, en términos de reactogenicidad e inmunogenicidad a corto plazo.
2. **Hipótesis específicas:**

- La reactogenicidad será principalmente leve y comparable con ensayos clínicos previos.
- La vacuna mRNA-1273 mostrará mayor reactogenicidad que BNT162b2.
- Los profesionales sanitarios tendrán mayor reactogenicidad que los pacientes trasplantados.
- Factores como edad, sexo y comorbilidades influirán en la reactogenicidad, especialmente tras la segunda dosis.
- Habrá una asociación entre reactogenicidad e inmunogenicidad.
- Los trasplantados vacunados con mRNA-1273 tendrán menor inmunogenicidad y reactogenicidad que los profesionales sanitarios vacunados con la misma vacuna.

OBJETIVOS

1. Evaluar comparativamente las vacunas BNT162b2 y mRNA-1273 en profesionales sanitarios y pacientes trasplantados, durante la campaña de vacunación en un hospital terciario, respecto a reactogenicidad y tolerabilidad.
 - Comparar el perfil de seguridad observado en un entorno real con los ensayos clínicos.
 - Evaluar la incidencia y el tipo de reacciones adversas según la dosis y factores demográficos.
2. Analizar la relación entre reactogenicidad e inmunogenicidad.
3. Comparar respuestas de inmunogenicidad y reactogenicidad entre las vacunas y poblaciones estudiadas.

MÉTODOS

El proyecto VigilVacCOVID, en el que se basa esta tesis, fue un estudio observacional, post-autorización, de evaluación y monitorización de la seguridad de dos vacunas de RNAm (BNT162b2 y mRNA-1273). El estudio fue aprobado por el CEIm del Hospital Clínic de Barcelona (códigos HCB/2021/0684 y HCB/2021/0685). Este estudio constó de dos subestudios:

- 1) Un seguimiento prospectivo tras cada dosis de vacuna, de dos poblaciones vacunadas con ambas vacunas de ARNm durante la campaña de vacunación en el Hospital Clínic: profesionales sanitarios y pacientes trasplantados. El seguimiento se realizó mediante entrevista estructurada a través de llamada telefónica. Se recogieron las reacciones adversas reportadas por las personas vacunadas, así como características basales, como factores demográficos, comorbilidades e infección previa por SARS-CoV-2 (objetivos 1-4).
- 2) Posteriormente, se realizó una revisión retrospectiva de la inmunogenicidad humoral y celular de las personas vacunadas que habían sido incluidas en la base de datos de seguridad del primer subestudio, y se compararon los resultados de seguridad e inmunogenicidad (objetivo 5).

RESULTADOS PRINCIPALES

Subestudio 1:

El estudio proporcionó datos de una comparación sistemática y prospectiva de dos vacunas de ARNm, mostrando una mayor reactogenicidad de mRNA-1273. Ambas vacunas presentaron un adecuado perfil de seguridad y tolerabilidad en vida real, consistente con los resultados de seguridad procedentes de los ensayos clínicos.

Se observó una menor reactogenicidad en pacientes trasplantados, en comparación con profesionales sanitarios. Además, la edad, el sexo, el antecedente de alergia a medicamentos, y la infección previa por SARS-CoV-2 se asociaron con un mayor riesgo de reactogenicidad.

Subestudio 2:

Se observó una asociación significativa entre reactogenicidad e inmunogenicidad. Específicamente, se observó una asociación entre la incidencia de fiebre, malestar y mialgias con el desarrollo y los títulos de anticuerpos frente a SARS-CoV-2. No se observaron resultados estadísticamente significativos en relación con la inmunidad celular; sin embargo, se observó una tendencia hacia una correlación numérica entre la inmunidad celular y una mayor frecuencia de algunas reacciones adversas.

CONCLUSIONES

1. **El estudio VigilVacCOVID** proporcionó evidencia adicional en un entorno de vida real sobre la seguridad e inmunogenicidad de BNT162b2 y mRNA-1273:
 - Las reacciones adversas locales (dolor en el brazo) y sistémicas (fatiga, fiebre, cefalea) fueron las más frecuentes, generalmente leves.
 - El perfil de seguridad fue comparable con los ensayos clínicos.
 - La vacunación con mRNA-1273 mostró mayor reactogenicidad que BNT162b2.
 - Los pacientes trasplantados vacunados con mRNA-1273 presentaron menor reactogenicidad que los profesionales sanitarios.
 - Factores como edad, sexo, infecciones previas por SARS-CoV-2 y alergias aumentaron el riesgo de reactogenicidad.
 - Las reacciones adversas graves fueron más comunes tras la segunda dosis.
2. **Relación entre reactogenicidad e inmunogenicidad:**
 - Existe una asociación significativa entre reacciones sistémicas (fiebre, mialgia) y una mayor inmunogenicidad humoral.
 - Aunque no concluyente, podría haber una asociación con la inmunogenicidad celular.
 - Ambas vacunas tuvieron tasas similares de inmunogenicidad en profesionales sanitarios, pero mRNA-1273 mostró mayor reactogenicidad. Los pacientes trasplantados vacunados con mRNA-1273 presentaron menor inmunogenicidad humoral y reactogenicidad que los profesionales sanitarios vacunados con la misma vacuna.
 - No se encontró asociación entre inmunogenicidad y la intensidad de las reacciones adversas o el número de dosis recibidas.

Palabras clave: Farmacología, mecanismos de acción de los medicamentos, evaluación de medicamentos, acción de los medicamentos

RESUM DE LA TESI EN CATALÀ

TÍTOL DE LA TESI

Vacunació contra SARS-CoV-2 en un hospital de referència d'alta complexitat: implementació i resultats d'un programa de farmacovigilància activa.

INTRODUCCIÓ

El virus coronavirus-2 del síndrome respiratori agut sever (SARS-CoV-2) és l'agent causant de la pandèmia de la COVID-19, que ha infectat centenars de milions de persones i ha provocat milions de morts arreu del món. Aquesta emergència va desencadenar una resposta intensa i ràpida a gran escala, amb un immens esforç internacional sense precedents que es va traduir en un desenvolupament accelerat de vacunes, un èxit mai vist en la història de la medicina.

Les primeres vacunes aprovades el desembre del 2020 van ser BNT162b2 i mRNA-1273, dues vacunes d'ARNm. Tot i que la plataforma d'ARNm no s'havia utilitzat anteriorment en una vacuna autoritzada, es considerava una tecnologia prometedora que oferia molts avantatges, com l'adaptabilitat de les vacunes i l'escalabilitat ràpida de la seva fabricació. A més, tant BNT162b2 com mRNA-1273 havien mostrat bons resultats d'eficàcia i seguretat en assajos clínics previs de fase III.

Tanmateix, calia un seguiment més prolongat de la seguretat postautorització per caracteritzar millor el perfil de seguretat d'aquestes vacunes, especialment pel que fa a reaccions adverses rares i a llarg termini. Aquest seguiment era de gran importància, ja que, per primera vegada a la història, milions de persones es vacunarien en un període de temps molt curt.

HIPÒTESI

1. **Hipòtesi principal:** L'estudi VigilVacCOVID permetrà avaluar diferències entre les vacunes BNT162b2 i mRNA-1273, així com entre professionals sanitaris i pacients trasplantats, en termes de reactogenicitat i immunogenicitat a curt termini.
2. **Hipòtesis específiques:**

- La reactogenicitat serà principalment lleu i comparable amb la descrita en assajos clínics previs.
- La vacuna mRNA-1273 mostrarà una reactogenicitat més elevada que BNT162b2.
- Els professionals sanitaris tindran una reactogenicitat més gran que els pacients trasplantats.
- Factors com l'edat, el sexe i les comorbiditats influiran en la reactogenicitat, especialment després de la segona dosi.
- Hi haurà una associació entre reactogenicitat i immunogenicitat.
- Els pacients trasplantats vacunats amb mRNA-1273 presentaran menor immunogenicitat i reactogenicitat que els professionals sanitaris vacunats amb la mateixa vacuna.

OBJECTIUS

1. Avaluar comparativament les vacunes BNT162b2 i mRNA-1273 en professionals sanitaris i pacients trasplantats durant la campanya de vacunació en un hospital terciari, pel que fa a reactogenicitat i tolerabilitat.
 - Comparar el perfil de seguretat observat en un entorn real amb els assajos clínics.
 - Avaluar la incidència i el tipus de reaccions adverses segons la dosi i els factors demogràfics.
2. Analitzar la relació entre reactogenicitat i immunogenicitat.
3. Comparar les respostes d'immunogenicitat i reactogenicitat entre les vacunes i les poblacions estudiades.

MÈTODES

El projecte VigilVacCOVID, en què es basa aquesta tesi, va ser un estudi observacional postautorització per avaluar i monitoritzar la seguretat de dues vacunes d'ARNm

(BNT162b2 i mRNA-1273). L'estudi va ser aprovat pel CEIm de l'Hospital Clínic de Barcelona (codis HCB/2021/0684 i HCB/2021/0685).

Aquest estudi va constar de dos subestudis:

1. **Seguiment prospectiu després de cada dosi de la vacuna**, en dues poblacions vacunades amb les vacunes d'ARNm durant la campanya de vacunació a l'Hospital Clínic: professionals sanitaris i pacients trasplantats. El seguiment es va realitzar mitjançant una entrevista estructurada per trucada telefònica. Es van recollir les reaccions adverses reportades pels vacunats, així com característiques basals com factors demogràfics, comorbiditats i infecció prèvia per SARS-CoV-2 (objectius 1-4).
2. **Revisió retrospectiva de la immunogenicitat humoral i cel·lular** en les persones vacunades incloses en la base de dades de seguretat del primer subestudi, per comparar els resultats de seguretat i immunogenicitat (objectiu 5).

RESULTATS PRINCIPALS

Subestudi 1:

L'estudi va proporcionar dades d'una comparació sistemàtica i prospectiva de dues vacunes d'ARNm, mostrant una reactogenicitat més gran de mRNA-1273. Totes dues vacunes van presentar un perfil de seguretat i tolerabilitat adequat en condicions reals, consistent amb els resultats de seguretat dels assajos clínics. Es va observar una menor reactogenicitat en pacients trasplantats en comparació amb els professionals sanitaris. A més, l'edat, el sexe, l'antecedent d'al·lèrgia a medicaments i la infecció prèvia per SARS-CoV-2 es van associar amb un major risc de reactogenicitat.

Subestudi 2:

Es va observar una associació significativa entre reactogenicitat i immunogenicitat. Concretament, es va identificar una relació entre la incidència de febre, malestar i mialgies amb el desenvolupament i els títols d'anticossos contra SARS-CoV-2. No es van trobar resultats estadísticament significatius en relació amb la immunitat cel·lular; no obstant això, es va observar una tendència cap a una correlació numèrica entre la immunitat cel·lular i una major freqüència d'algunes reaccions adverses.

CONCLUSIONS

1. L'estudi VigilVacCOVID va proporcionar evidència addicional en un entorn real sobre la seguretat i immunogenicitat de BNT162b2 i mRNA-1273:
 - Les reaccions adverses locals (dolor al braç) i sistèmiques (fatiga, febre, cefalea) van ser les més freqüents, generalment lleus.
 - El perfil de seguretat va ser comparable als assajos clínics.
 - La vacunació amb mRNA-1273 va mostrar una major reactogenicitat que BNT162b2.
 - Els pacients trasplantats vacunats amb mRNA-1273 van presentar menor reactogenicitat que els professionals sanitaris.
 - Factors com l'edat, el sexe, infeccions prèvies per SARS-CoV-2 i al·lèrgies van augmentar el risc de reactogenicitat.
 - Les reaccions adverses greus van ser més freqüents després de la segona dosi.
2. **Relació entre reactogenicitat i immunogenicitat:**
 - Hi ha una associació significativa entre reaccions sistèmiques (febre, mialgia) i una major immunogenicitat humoral.
 - Encara que no concloent, podria haver-hi una associació amb la immunogenicitat cel·lular.
 - Ambdues vacunes van mostrar taxes similars d'immunogenicitat en professionals sanitaris, però mRNA-1273 va presentar més reactogenicitat. Els pacients trasplantats vacunats amb mRNA-1273 van mostrar menor immunogenicitat humoral i reactogenicitat que els professionals sanitaris vacunats amb la mateixa vacuna.
 - No es va trobar cap associació entre immunogenicitat i la intensitat de les reaccions adverses o el nombre de dosis rebudes.

Paraules clau: Farmacologia, mecanismes d'acció dels medicaments, avaluació de medicaments, acció dels medicaments

I Introduction

1.1 The race for a vaccine against SARS-CoV-2: Overview of the general context.

The Severe Acute Respiratory Syndrome coronavirus-2 (SARS-CoV-2) virus is the causative agent of the COVID-19 pandemic, which has infected hundreds of millions of individuals and resulted in millions of deaths globally (1). In March 2020, the World Health Organization (WHO) declared the novel coronavirus outbreak (COVID-19) a global pandemic (2,3). By that date, nearly 120,000 cases had been reported, with transmission observed in more than 114 countries. This emergency triggered an intense and rapid large-scale response. By April 8, 2020, just a few weeks after the pandemic was declared, 115 vaccine candidates had entered pre-clinical development, and five were being investigated in Phase I clinical trials (4)

This enormous, unprecedented international effort resulted in an unparalleled rapid vaccine development, an achievement never seen before in the history of medicine (5). The reasons for this rapid turnaround were multifactorial, and they implied **multilateral global collaboration** and **work sharing** between different stakeholders, such as regulatory authorities, government, and the private sector, to expedite vaccine development (Table 1). On the one hand, the collaboration between multinational pharmaceutical companies and biotechnology firms accelerated the development process by bringing together specialised expertise, facilitating scaling activities, and utilising an extensive distribution network (6). It is also important to highlight the at-risk investment made by manufacturers, who began preparations for large-scale vaccine manufacturing prior to the availability of late-stage clinical trial results, thereby enabling vaccines to be strategically positioned and fully prepared for mass supply (7). These collaborations would not have been possible without the establishment of solid funding mechanisms through private-public initiatives (8), which played a crucial role in accelerating the development of SARS-COV-2 vaccines. For instance, **Operation Warp Speed (OWS)** is a public-private partnership in the United States that encompasses collaboration among key stakeholders, such as the Food and Drug Administration (FDA), private companies, and other federal agencies. The coalition was established under direct support from the White House and under the auspices of the **Biomedical Advanced Research and Development Authority (BARDA)** and represented a real breakthrough in public policy (9). By the first quarter of 2020, the

partnership had already made substantial investments in various vaccine candidates (8). Furthermore, the **Coalition for Epidemic Preparedness Innovations (CEPI)** was created as an innovative global partnership between public, private, philanthropic, and civil society organisations to accelerate the development of equitable access to vaccines. The coalition provides funding and support for the development of innovative vaccine platforms. During the pandemic, the partnership founded an international network of centralised laboratories to allow testing and the comparison of immunological vaccine responses and supported the development of 17 vaccines against SARS-CoV-2 (10). Likewise, in terms of supply chain and vaccine availability, several companies established partnerships with contract manufacturers to ensure an adequate supply of vaccines to meet the projected demand. These agreements contributed to supporting the at-risk investment made by manufacturers (11). Simultaneously, regulatory and regional authorities implemented risk mitigation measures to reduce the risks of vaccine shortages while ensuring high standards of quality, safety, and efficacy (12).

At the governmental level, several early efforts were also made to support the accelerated development of SARS-CoV-2 vaccines. On 16 March 2020, the G7 committed to supporting the initiation of collaborative research projects for treatments and vaccines against SARS-CoV-2. Accordingly, the United States (US), United Kingdom (UK), and European Union (EU) were among the earliest to invest in mRNA vaccine research (6). For its part, on 16 September 2020, the European Commission announced its intention to introduce a legislative proposal to establish a European Biomedical Research and Development (R&D) agency, modelled after the US's BARDA (13). Additionally, “**push and pull**” incentive mechanisms were designed and smoothly implemented to support innovative vaccine development. These mechanisms implied public-private partnerships in research and development, establishment of production facilities, conducting multiple clinical trials across various geographic regions, and implementation of tax incentives (examples of “push” funding), while in turn, governments guaranteed purchase commitments for new vaccines (“pull” funding) at the culmination of the product development phase (6).

On the other hand, regulatory authorities also reinforced their collaboration during the pandemic. The **International Coalition of Medicines Regulatory Authorities (ICMRA)** is an independent organisation that was established to foster global strategic collaboration

and information exchange among regulatory bodies. ICMRA acted as a forum to support strategic coordination and international cooperation among global medicine regulatory authorities, promoting a unified approach to the development and accessibility of SARS-CoV-2 vaccines (14).

In addition, regulatory authorities strengthened their commitment and cooperation to align on regulatory requirements during the COVID-19 pandemic. Both the European Medicines Agency (EMA) and FDA engaged in ongoing, real-time discussions with manufacturers throughout the pandemic. They also released additional questions and answers (Q&A) documents to provide guidance and support for early development initiatives, such as the EMA's **PRIority MEDicines (PRIME)** scheme (15). Furthermore, target product profiles were created by both agencies to provide a clear guide for developers regarding requirements for vaccines (16,17), as well as specific guidance on **risk management plan (RMP)** requirements for SARS-CoV-2 vaccines (18). They also advised regarding the monitoring activities to be implemented in the EU for SARS-CoV-2 vaccines, including the roles, responsibilities, and interactions of involved stakeholders (19). This guidance served to harmonise the preparation of RMPs by companies and their evaluation by regulators. Dedicated regulatory efforts made in terms of pharmacovigilance and vaccine surveillance are fully described in section 1.3.

In line with these efforts, the EMA established the **Emergency Task Force (ETF)** in accordance with the Regulation on EMA's Reinforced Role (Regulation (EU) 2022/123) (20). The Regulation established the group as a permanent entity and assigned it a crucial role in readiness for upcoming future crises. The ETF was responsible for: i) providing scientific advice to developers of medicines; ii) reviewing scientific data on these medicines, including data requested from their developers; iii) engaging in preliminary discussions with these developers; iv) providing scientific support to facilitate clinical trials in the EU; v) supporting EMA's scientific committees by evaluating applications for authorisation and monitoring their safety; vi) providing scientific recommendations on the use of medicines prior to their authorisation; and vii) collaborating closely with different stakeholders and with European and international organisations.

Furthermore, the **Access to COVID-19 Tools (ACT) Accelerator** was a collaborative initiative involving governments, scientists, businesses, and global health organisations such

as CEPI and the WHO, aimed at expediting the development and accessibility of SARS-CoV-2 vaccines within a forum where governments, research institutions, and healthcare systems can engage in structured and efficient information exchange (21).

The deployment of the SARS-CoV-2 vaccines has been unprecedented compared with previous vaccination efforts. The vast number of individuals, doses, and human resources involved necessitated an extensive global vaccination program, in which inequalities were a particular risk for low and middle-income countries (22). **The Global Alliance for Vaccines and Immunisation (Gavi)** was established in 2000 to facilitate vaccination initiatives for children in developing nations, with a special focus on the most impoverished regions. Gavi introduced the **Advance Market Commitment (AMC)**, an innovative program characterised by public-private health funding. The primary aim of this initiative is to ensure the availability of high-quality vaccines in low-income and low-middle-income countries at significantly reduced prices, supported financially by high-income countries (23). COVAX was one of three pillars of ACT Accelerator, the pillar dedicated to SARS-COV-2 vaccines, and it was launched in April 2020 in response to the pandemic (24). COVAX, coordinated by Gavi, CEPI, and the WHO, acted as a platform supporting research, development, and manufacturing of several SARS-CoV-2 vaccine candidates, and negotiated their pricing to enable rapid, fair, and equitable access to newly developed SARS-CoV-2 vaccines.

The EMA also facilitated the advancement of processes and scientific progress in the pharmacovigilance of SARS-CoV-2 vaccines by engaging in specific research projects, such as the **ADVANCE** project (25), which aims to improve the monitoring of the benefit-risk balance of vaccines. The project characteristics, as well as its continuation through the **VAC4EU** project, are described in section 1.3.

Moreover, regulatory approval processes were streamlined by accelerated pathways with fast-track assessments and rolling reviews that enabled companies to submit further data as and when it was generated. National authorities endorsed and applied these rolling review schemes during clinical trial authorisations and rapid scientific advice procedures (26,27). These optimised regulatory pathways led to more efficient regulatory timelines while assuring a robust and consistent efficacy and safety evaluation (28).

Additionally, innovative clinical trial designs were adopted and endorsed by regulatory authorities, such as “seamless clinical trials”, where certain phases of vaccine development

are consolidated into a single protocol, which is continually adapted on an as-needed basis, e.g., Phase I/II or Phase I/II/III. The implementation of these adaptive clinical trial methods greatly contributed to shortening development timelines (29). The development and authorisation of the first mRNA SARS-CoV-2 vaccines is explained in section I.II.

Finally, on the development level, pre-existing non-clinical data from research conducted during previous coronavirus outbreaks facilitated that early stages of vaccine design could be conducted more expeditely. In this sense, the complete spike glycoprotein of SARS-CoV-2 was selected as the vaccine antigen, primarily based on prior experience with SARS-CoV and MERS, which demonstrated that such vaccines confer viral protection (30). Besides, clinical research had already been conducted with the platforms that subsequently comprised the basis of the SARS-CoV-2 vaccines. For instance, over the past three decades, viral vector vaccines had been extensively researched and had previously demonstrated the ability to elicit robust and durable immune responses in the context of other infectious diseases and cancer (31). Similarly, messenger ribonucleic acid (mRNA) technologies had been the subject of extensive research and development for over two decades before gaining prominence during the SARS-CoV-2 pandemic (32–34). Overall, this previous experience facilitated initial consultations with regulatory authorities regarding the application of platform technology strategies for vaccine development. Consequently, these principles were embraced during the SARS-CoV-2 pandemic, permitting the extrapolation of safety data across various vaccine candidates. Additionally, this led to the implementation of a platform-based non-clinical toxicology program to support first-in-human clinical trials and led to a reduction in the duration of the exploratory and preclinical testing phase, which is typically several years (35), to a few months (36).

As a whole, the increasing public health derived from the rapid transmission and significant disease burden of the novel coronavirus motivated a paradigm shift in vaccine development. There were multiple and multidimensional key contributing factors that helped to expedite this new framework. A descriptive summary of these factors, their dimensions, and implications for vaccine development and accessibility can be found in Table 1 and Figure 1.

As the final output of this intricate system of collaborative public-private efforts, the first vaccines against SARS-CoV-2 were approved at the end of 2020. These vaccines were based

on an innovative technology that had never before been applied in an approved vaccine: mRNA technology. The approval was based on a careful assessment of their benefit-risk balance, supported by high efficacy rates and an adequate safety profile; nevertheless, some uncertainties remained regarding potential safety risks that might emerge, particularly given that millions of individuals had never been vaccinated in such large-scale campaigns conducted over short time periods. For this reason, it became essential to implement pharmacovigilance plans and programs to monitor the safety profile using real-world data. It is within this context that the VigilVacCOVID project was conceived, forming the conceptual foundation of the present thesis. The following section 1.2 will introduce the key regulatory and clinical development processes that preceded the approval of COVID-19 vaccines, concluding in section 1.3 with the context in which these pharmacovigilance programs were proposed and implemented.

Table 1. Multistakeholder interactions and key factors involved in expediting SARS-CoV-2 vaccine development and approval

Factor dimension	Description and examples	Implications for vaccine development and accessibility
PLATFORM DEVELOPMENT		
Use of pre-existing data	Pre-existing data were accessible due to prior coronavirus outbreaks. Platforms such as mRNA and adenovector were already used in ongoing clinical trials.	The use of pre-existing data paved the way for the adoption of these platforms, enabling the extrapolation of safety data across various vaccine candidates.
Vaccine clinical development	Use of innovative clinical trial designs, such as “seamless adaptive clinical trials” instead of sequential designs.	Timelines of clinical development of vaccine candidates were shortened.
MULTILATERAL GLOBAL COLLABORATION		
Public-private collaboration	Funding mechanisms to support financial risk. <u>Examples:</u> Operation Warp Speed (OWS)(8), CEPI(10) Governmental efforts, such as “push and pull” incentive mechanisms.	Provided frameworks for manufacturers and industries to assume the financial risk and advance the development and manufacturing of vaccine candidates.
REGULATORY EFFORTS		
Regulatory alignment	EMA/FDA strengthened their commitment and cooperation to align on regulatory requirements. <u>Examples:</u> harmonisation of regulatory documents and guidance documents. The FDA and EMA worked closely with the WHO and other key stakeholders. <u>Examples:</u> ICMRA, ACT, ETF	Harmonisation of regulatory processes and real-time guidance with industries and manufacturers contributed to clarifying and expediting the development phase of vaccines.

Factor dimension	Description and examples	Implications for vaccine development and accessibility
Regulatory framework and strategies	Regulatory authorities optimised regulatory processes through accelerated review timelines, rollout procedures and vaccine-specific guidances. Regulatory authorities endorsed the implementation of seamless designs for vaccine development.	Expedited authorisation of vaccines, while assuring a strong analysis of efficacy and safety data.
VACCINE ACCESS		
Up-scaled manufacturing	Vaccine developers started scale-up manufacturing processes well before to be prepared for mass supply.	Rapid access to SARS-CoV-2 vaccines after authorisation.
Shortages regulation	Regulatory and regional authorities implemented risk mitigation measures to reduce risks of vaccine shortages.	Assurance of vaccine access throughout the pandemic.
Equitable access	AMC scheme: COVAX, coordinated by Gavi, CEPI, and the WHO, worked to ensure equitable access to SARS-CoV-2 vaccines in low and middle-income countries.	Assurance of vaccine access in low and middle-income countries throughout the pandemic.

Table 1. Contributing factors to expedite SARS-CoV-2 vaccine development and public accessibility. Table created by the author.

Figure 1. Interaction between stakeholders

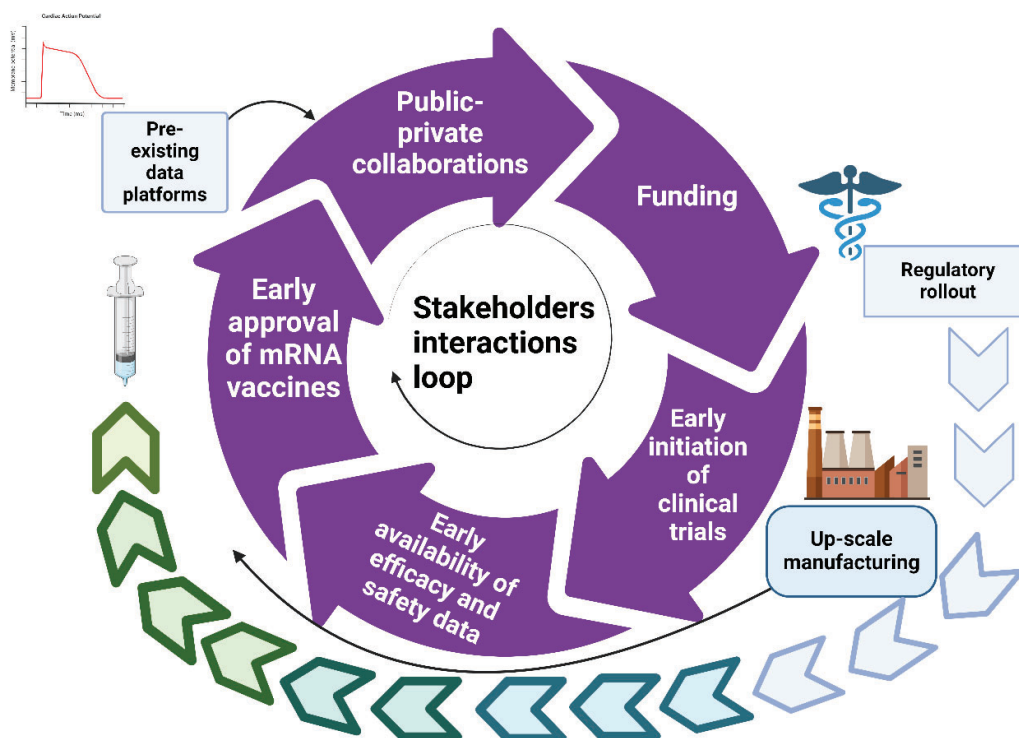


Figure 1. Interaction loop between the different stakeholders, and the final outcome of this interaction: the early approval of the first mRNA vaccines. Created in BioRender.com

1.2 Development of the first SARS-CoV-2 vaccines

The initial and pivotal step in the development of a vaccine against SARS-CoV-2 was the characterisation of the viral target. This milestone was achieved at a remarkably early stage of the SARS-CoV-2 outbreak, marked by the sequencing and subsequent publication of the viral genome in early January 2020 (37). This genomic data facilitated the prompt identification of the sequence of the Spike (S) protein, the antigen responsible for eliciting an immune response (38). Figure 2 shows the structure of the SARS-CoV-2 virus.

Figure 2. Structure of SARS-CoV-2

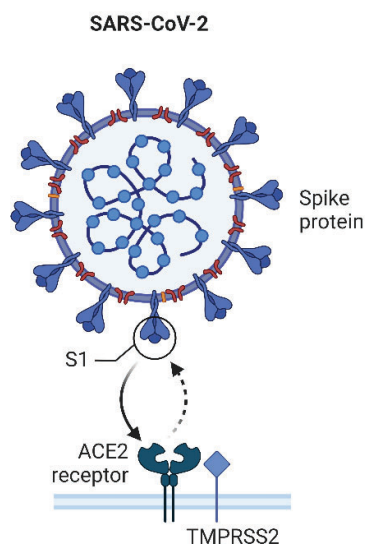


Figure 2. Structure of SARS-CoV-2 virus, with the Spike (S) protein, and its ACE2 receptor. Created in BioRender.com

The first administration of a SARS-CoV-2 vaccine dose to a participant in a human clinical trial took place on March 16, 2020 (39,40). This Phase I clinical trial (“Safety and Immunogenicity Study of 2019-nCoV Vaccine (subsequently known as mRNA-1273) for prophylaxis of SARS-CoV-2 Infection (COVID-19), was an open-label, dose-ranging clinical trial that explored the safety, reactogenicity, and immunogenicity of an mRNA vaccine candidate, mRNA-1273, produced by ModernaTX, Inc. (41). Clinical trial results showed immune responses in all participants. Likewise, no clinical trial-limiting safety concerns were identified, and the most frequently reported adverse reactions (ARs) included fatigue, chills, headache, myalgia, and pain at the injection site (40).

By April 2020, a total of 115 vaccine candidates had commenced pre-clinical development, and five were undergoing investigation in Phase I clinical trials. By September 2020, the number had rapidly increased to 321 vaccine candidates. Of these, 33 vaccine candidates were in clinical trials, and six candidates were in Phase IIb/III or Phase III, two of which

used inactivated viruses, two non-replicant viral vectors, and two the mRNA platform. Figure 3 shows time trends in vaccine candidate development.

Figure 3. Clinical development of candidate vaccines

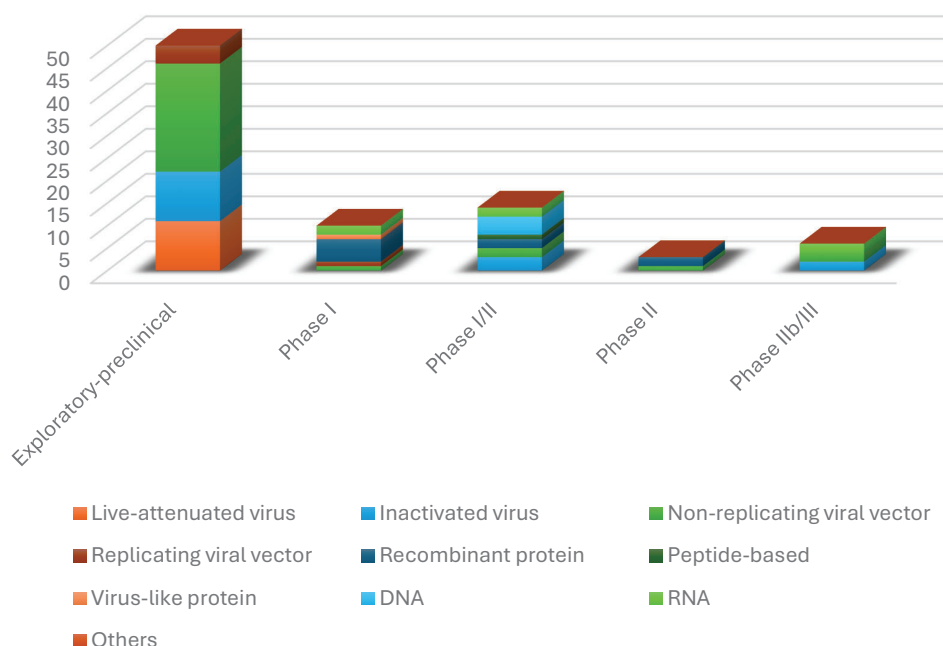


Figure 3. Status of clinical development of the different vaccine candidates, as of September 2020. Modified from Le TT et al (4).

Nine months after the start of the first clinical trial, the first vaccine was authorised by the FDA, on December 11, 2020 (42), and by the EMA on December 21, 2020 (43), showing an accelerated and unprecedented development process. In subsections 1.2.1, 1.2.2, and 1.2.3 below, we will address the different platforms of SARS-CoV-2 vaccine candidates, their streamlined clinical development, and the regulatory framework with fast-track and rollout reviews that enabled their early authorisation.

1.2.1 Vaccine platforms under development

SARS-CoV-2 is closely related to SARS-CoV, which circulated between 2002 and 2004. Both SARS-CoV and the Middle Eastern respiratory syndrome coronavirus (MERS-CoV) have caused epidemics with high case fatality rates in humans (44,45). Despite this, no licensed vaccines against coronaviruses were available for humans, likely due to the limited geographic distribution and the sporadic nature of human deaths caused by these viruses. Several SARS-CoV vaccines were developed preclinically, with two candidates reaching Phase I clinical trials, one inactivated vaccine (46) and a DNA vaccine (47). However, these clinical trials did not advance further as the epidemics subsided, and these viral infections have not re-emerged in humans since 2004. In contrast, vaccines against MERS-CoV have been actively pursued, and these preclinical studies have identified the spike protein as the key antigenic target for coronavirus vaccines (30,48). The identification of the **spike protein** was one of the key milestones that accelerated the development of several vaccine candidates during the pandemic.

Similarly, as mentioned earlier, another pivotal step in the development of a vaccine against SARS-CoV-2 was the characterisation of the viral target in early January 2020. SARS-CoV-2 is classified under the genus *Betacoronavirus* within the family *Coronaviridae*. It is an enveloped virus comprising a single-stranded, positive-sense RNA genome that is 29,903 nucleotides long and contains 11 open reading frames (ORFs) encoding 27 viral proteins. ORF1a/b spans 21,290 nucleotides and encodes 16 non-structural proteins (nsp1–nsp16). The remaining 8613 nucleotides of the genome encode four structural proteins and six accessory proteins. The structural proteins include the **spike (S)**, responsible for virus attachment and a major antigenic component; **envelope (E)**; **matrix (M)**; and **nucleocapsid (N)** proteins. The accessory proteins are ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10 (49,50). The structure and functions of the different components of SARS-CoV-2 are illustrated in Figure 4. The S protein binds to the human angiotensin-converting enzyme 2 (ACE2) receptor. The protein has two subunits: **S1** or **receptor binding domain (RBD)**, which is the binding domain to the host cell-surface receptor, and **S2**, which is the domain that mediates viral cell membrane fusion (51). SARS-CoV-2 binds to host ACE2 receptors with its S protein and enters the cell. The virus subsequently undergoes uncoating, followed by the synthesis of viral proteins and replication of its nucleic acid utilising the host cell's transcriptional and

translational machinery, ribosomes. Given that the genetic material of SARS-CoV-2 is positive-sense single-stranded RNA, transcription is not required. Subsequently, new virions are assembled from the viral components, re-encapsulated, and released from the host cell to infect additional cells (36).

Figure 4. Structure and functions of SARS-CoV-2 components

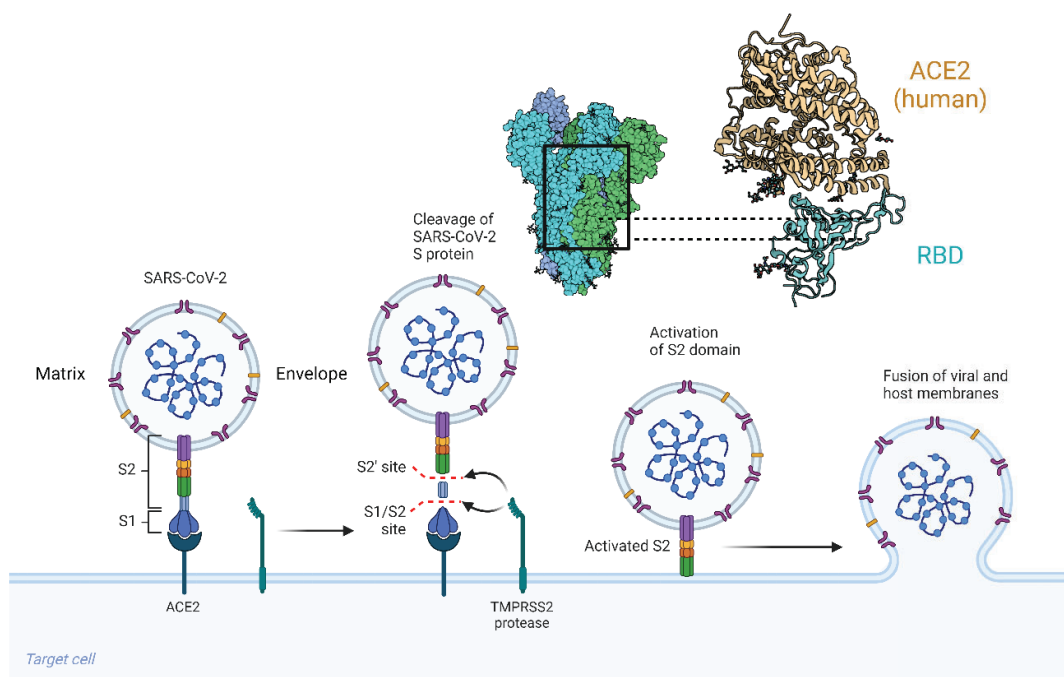


Figure 4. Structure of SARS-CoV-2. Protein S is shown with its two domains, S1 and S2. Created in BioRender.com

A vaccine is a biological agent that elicits an immune response to specific antigens from infectious pathogens, enabling the immune system to combat a disease that it has not previously encountered (52). According to the European Union vaccination glossary, “a vaccine typically contains an agent that resembles a disease-causing microorganism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. The agent stimulates the body's immune system to recognise the agent as foreign, destroy it, and "remember" it so that the immune system can more easily recognise and destroy any of these microorganisms that it later encounters” (53). **S protein-based vaccines** against SARS and MERS demonstrated potent immunogenicity and protective

effects in pre-clinical and Phase I clinical trials (54). Therefore, most vaccine platforms focused on eliciting an immune response against the **S protein**.

Traditional vaccine technologies were based on using either an attenuated version of the virus or a viral fragment. The SARS-CoV-2 pandemic has underscored that, in recent years, the field has shifted towards **reverse vaccinology** (55,56). This modern approach focuses on vaccine development driven by the discovery and understanding of the viral genome. Therefore, the genetic sequence of the S protein from the SARS-CoV-2 virus served as a blueprint for the design of several vaccine platforms, especially for the development of **nucleic acid vaccines**, which will be described later.

Similarly, **reverse genetics (RG)** is a potent technique extensively employed for the genetic manipulation of RNA viruses using their full-length cloned DNA (cDNA), facilitating the development of effective antiviral strategies (57). Obtaining infectious viral RNA from cDNA has historically posed significant challenges in coronaviruses due to their large genomes and the instability of certain regions within the viral genome. To address these challenges, a robust RG system for SARS-CoV-2 and other RNA viruses was rapidly developed during the initial stages of the SARS-CoV-2 pandemic (58). The fast development of these RG systems has been useful in fostering the development of different vaccine platforms such as **live-attenuated** and **vector-based vaccines**. These systems also enabled the understanding of SARS-CoV-2 biology and pathogenesis, and by allowing the study of different aspects of the viral life cycle, including replication and pathogenesis (59,60).

Finally, another important aspect in the design and development of vaccine platforms was the selection of adjuvants. Adjuvants encompass a range of molecules or larger microbial-derived products that broadly impact the immune system or target a specific immune response. These can include or be composed of immunostimulants or immunomodulators. Adjuvants are often incorporated into vaccines to potentiate the immune response. Various adjuvants can modulate different types of immune responses, so the selection or combination of adjuvants in a vaccine depends on the vaccine type and considerations related to efficacy and safety (61–63).

1.2.1.1 Description of mRNA and other vaccine platforms under development

The developed platforms can be divided into ‘traditional’ approaches (**inactivated or live-virus vaccines**), new platforms that had resulted in licensed vaccines before the SARS-CoV-2 pandemic (**recombinant protein vaccines and vectored vaccines**), and breakthrough platforms without a previously licensed vaccine (**RNA and DNA vaccines**).

1.2.1.1.1 Inactivated virus vaccines:

Inactivated vaccines are produced by growing a working virus seed of SARS-CoV-2 in a susceptible cell line, such as Vero E6, followed by inactivation by heat, irradiation, or chemical insult (64,65). As the entire virus is presented to the immune system, the immune responses are expected to target not only the S protein of SARS-CoV-2 but also the matrix, envelope, and nucleoprotein. Production processes can be complex as large amounts of viruses are needed and antigen integrity needs to be confirmed in the laboratory. However, inactivated vaccines are more stable and safer than live attenuated vaccines. Therefore, this vaccine platform is suitable for immunocompromised patients (36). An example of this kind of SARS-CoV-2-developed vaccine is **CoronaVac** (initially known as PiCoVacc), which was developed by Sinovac Biotech Ltd, China. These vaccines are usually administered intramuscularly and can contain aluminium hydroxide or other adjuvants.

1.2.1.1.2 Live-attenuated vaccines:

Live-attenuated virus vaccines are typically developed by employing a non-virulent strain of the virus and/or engineering a genetically attenuated variant. This modified virus undergoes limited replication cycles that are inadequate to induce disease but are capable of eliciting immune responses similar to those prompted by natural infection (66–68). Virus attenuation can be accomplished by adapting the virus to adverse conditions, such as exposure and growth at low temperatures in a non-susceptible host or cells. Additionally, genetic modification can be employed through the RG system to achieve codon de-optimisation or deletion of genes essential for triggering innate immune recognition, thereby diminishing innate immune responses and potential reactogenicity events. However, disadvantages to these vaccines include safety concerns because of the excretion of the virus in faeces, thereby leading to a potential risk of transmission to unvaccinated individuals, especially

immunocompromised ones. Likewise, the development of this platform is time-consuming if it is carried out by traditional methods and technically challenging when RG is used (7,68). The first live-attenuated vaccine candidate, called COVI-VAC, was developed by Codagenix© in cooperation with the Serum Institute of India. The clinical trial for this candidate began on December 11, 2020 (NCT04619628). So far, no live-attenuated vaccine candidate has been tested in Phase-III clinical development.

1.2.1.1.3 Protein subunit vaccines:

Subunit vaccines are based on synthetic peptide(s) or recombinant protein(s) of the target pathogen. Unlike inactivated viruses or live-attenuated viruses, subunit vaccines only contain specific viral antigenic fragments and do not include any additional components of the pathogenic viruses, such as the S protein or a region of it, such as the RBD (69,70). Consequently, this method mitigates the risks associated with incomplete viral inactivation, reversion to virulence, and pre-existing anti-vector immunity (68), being typically regarded as very safe. Besides, subunit vaccines can precisely target well-characterised neutralising antigenic epitopes and, when combined with adjuvants, enhance immunogenicity and/or efficacy (68,71). The first candidate was developed by **Novavax, Inc.** (Gaithersburg, Maryland). However, there are several limitations to this platform, particularly concerning SARS-CoV-2, where the expression of the S protein is challenging. This issue might be addressed by expressing only the RBD, however, this might be suboptimal since it does not encompass all the antigenic epitopes found in the full-length S protein (7). In addition, the formulation process is usually complex and necessitates the development of new production protocols and stability assays for each new antigen (70).

1.2.1.1.4 Vectored vaccines:

Replication-incompetent vectors are based on another virus that has been engineered to express the spike protein and has been disabled from replication *in vivo* by the deletion of parts of its genome by RG (7,68). The majority of these candidates are based on adenovirus (AdV) vectors, although modified vaccinia Ankara (MVA), human parainfluenza virus vectors, influenza virus, adeno-associated virus, and Sendai virus are also used (72–77). The majority of these vectors are administered intramuscularly, enter the cells of the vaccinated individual, and subsequently express the protein S, eliciting an immune response. This

strategy offers numerous advantages: i) handling live SARS-CoV-2 during production is not required; ii) there is substantial experience in manufacturing large quantities of certain vectors (7). For instance, an Ad26–MVA-based prime-boost vaccine against the Ebola virus had previously been authorised in the European Union (78); and iii) these vectors effectively stimulate both B-cell and T-cell responses without the use of adjuvants. However, this platform has some disadvantages: i) some vectors can be partially neutralised by pre-existing host vector immunity (74); ii) the viral vector can induce an immune response that subsequently interferes with future vaccines based on the same vector (79); iii) some vectors may undergo recombination events with other viruses during the production process (70); and iv) there is a theoretic risk of mutagenic events as viral vectors require viral integration into the host genome (80).

The first vaccine candidate was developed by CanSino Biological Inc./Beijing Institute of Biotechnology, using Adenovirus 5 (AdV5) (68). The second candidate was developed by Gamaleya Research Institute, Health Ministry of the Russian Federation, using the vectors AdV5/AdV26. The third vaccine candidate was developed by Janssen Pharmaceutical (Beerse, Belgium), using an AdV26-based vector (7). In addition, a chimpanzee adenovirus vector was developed by the University of Oxford and later used by AstraZeneca in their ChAdOx1 nCoV-19 vaccine (81).

Replication-competent vectors are often derived from attenuated or vaccine strains of viruses that have been genetically modified to express a transgene, such as the spike protein. This method can lead to a more vigorous induction of immunity, as the vector partially propagates in the vaccinated individual and frequently elicits a strong innate immune response. Moreover, certain vectors can be administered via mucosal surfaces, potentially eliciting mucosal immune responses. Some vaccine platforms have been developed based on replication-competent vectors, such as an engineered measles vaccine strain developed by Institut Pasteur and Themis (later acquired by Merck), a vector based on the influenza virus developed by Beijing Wantai Biological Pharmacy (7), and others based on vesicular stomatitis virus (VSV) (82), horsepox, and Newcastle disease virus (NDV) (83).

1.2.1.1.5 Nucleic acid-based vaccines (DNA and RNA vaccines):

These are new-generation vaccines that contain DNA or RNA sequences that encode the targets for pathogenic antigens. Overall, these vaccines offer several advantages, such as the production of an antigenic protein that is similar to that causative of the infection, or the stimulation of both cellular and humoral immunogenicity, without the need for complex protein folding or production processes. Consequently, this type of vaccine is easier to design and proceed into the clinical trial phase compared with other platforms. This is precisely the key characteristic that facilitated the expedited development and early availability of mRNA vaccines (84,85).

DNA vaccines are based on the insertion of the SARS-CoV-2 S gene into bacterial plasmids that have a potent mammalian promoter, such as CMV and/or SV40. Hence, these vaccines are also known as “plasmid-vectored DNA vaccines”. Plasmids are then extensively produced in competent bacterial cells (68). Plasmid DNA vaccines offer several benefits, including: i) the ability to target and stimulate both humoral and cellular immune responses; ii) straightforward and adaptable large-scale production and formulation processes that can be completed quickly, making them suitable for rapid deployment during the pandemic; iii) flexibility for incorporating multiple antigens; and iv) the possibility of room-temperature storage of the final vaccine product. However, these vaccines also have significant limitations (70), such as: i) low immunogenicity in humans, necessitating multiple doses to achieve optimal protection; ii) the theoretical risk of carcinogenesis due to potential integration into cellular chromosomes; and iii) the need for delivery devices to make them efficient, such as pyro-drive jet injectors.

The first DNA-based vaccine candidate for SARS-CoV-2 (INO-4800) was developed by Inovio Pharmaceuticals (Plymouth Meeting, PA) with the International Vaccine Institute and Advaccine (Suzhou, China) Biopharmaceutical Co., Ltd. Others have been developed, like AG0301-COVID19, developed by AnGes, Takara Bio (Kusatsu, Shiga, Japan) in collaboration with Osaka University, or nCov vaccine, developed by the Zydus Cadila (Ahmedabad, India) (7,68).

On the other hand, **RNA vaccines** represent a more recent advancement in vaccine development. Interest in messenger RNA (mRNA) vaccines began around 1990, after *in vitro*

and animal model studies showed that mRNA could be successfully introduced into cells (86).

The mechanism of action of mRNA vaccines works at a higher level than plasmid-vectored DNA vaccines. Instead of directly providing the gene sequence for an antigen, this type of vaccine directly delivers the transcribed mRNA, which is based on a single-stranded mRNA that encodes the spike protein of SARS-CoV-2. The mRNA molecule is made up of a 5' cap, untranslated region (UTR) sequences before and after the coding sequence and a polyadenosine (poly-A) tail at the 3' end of the single strand. These structures provide stability in the cytoplasm and promote the process of translation. This mRNA is synthesised *in vitro* and transported into cells using lipid nanoparticles (LNPs), which are specifically designed to enhance and support *in vivo* delivery of the mRNA. On the one hand, LNPs prevent mRNA from being degraded by RNases in the extracellular space, and on the other hand, they support the uptake of genetic material into the cells at the injection site (87). Similarly, mRNA in vaccines can be modified by incorporating 1-methylpseudouridine. Kariko and colleagues discovered that the incorporation of nucleoside base modifications such as N1-Methylpseudouridine significantly increases antigen expression and prevents excessive innate immune responses towards synthetic mRNA. This discovery laid the foundation for successful mRNA-based vaccine development (88). This breakthrough discovery earned Dr Karikó and Dr Andrew Weissman the Nobel Prize in 2023 (89). The structure of mRNA vaccines is represented in Figure 5.

Figure 5. Structure of mRNA vaccines

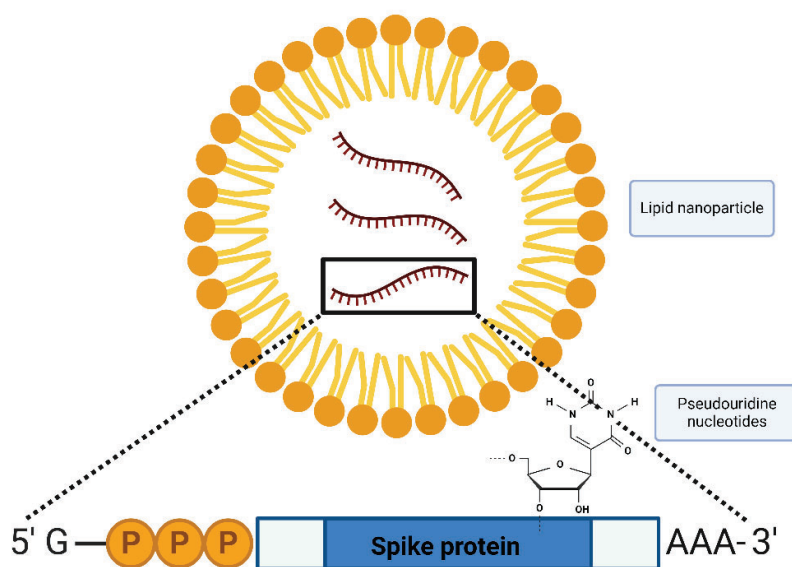


Figure 5. The structure of mRNA vaccines is depicted, with LNPs, 5' and 3' UTRs, and pseudouridine nucleotides. Created in BioRender.com

Once inside the cells, the mRNA is translated by the host cell machinery to the corresponding antigen, which is a viral functional protein that is presented to major histocompatibility complex (MHC) proteins, thereby eliciting a robust CD8+ T cell response, as well as CD4+ T-cell and B cell-mediated antibody responses. Given that mRNA is inherently unstable and susceptible to rapid degradation in the extracellular environment or cytoplasm, the LNP coating serves to protect the mRNA from enzymatic degradation outside the cell (7,68,90).

There are two main types of mRNA platforms: traditional or non-replicating and *in vivo* self-replicating vaccines. As described earlier, non-replicating mRNA platforms are based on a simple ORF that is flanked by the 5' UTR and 3' poly-A tail. The first traditional mRNA vaccines to be developed and approved were BNT162b2 (Pfizer-BioNtech) (91) and mRNA-1273 (Moderna) (40). For its part, self-amplifying mRNA (sa-mRNA) is primarily derived from the genomes of positive-stranded RNA viruses, such as alphaviruses and flaviviruses.

The sa-mRNA encodes the viral replication machinery that is essential for intracellular RNA amplification, with the objective of enhancing the expression levels of the gene of interest (GOI). Self-amplifying vaccines generate viral antigens on a continuous basis, thereby eliciting a stronger and longer-lasting immune response (7,68,87,92). The concept of nucleoside base modifications has also been successfully applied to saRNA, in which 5-methylcytidine-modified saRNA shows increased transfection and translation efficiency, as well as reduced innate immune responses (93). The first sa-mRNA vaccine to be developed and approved was ARCT-154 (Arcturus Therapeutics and CSL) (94). New innovative platforms, such as trans-amplifying mRNA (ta-mRNA) and circular RNA (circRNA), are currently under development (95). In Figure 6, saRNA is depicted, compared with traditional non-replicating RNA.

Figure 6. Types of RNA vaccines: Traditional and saRNA constructs

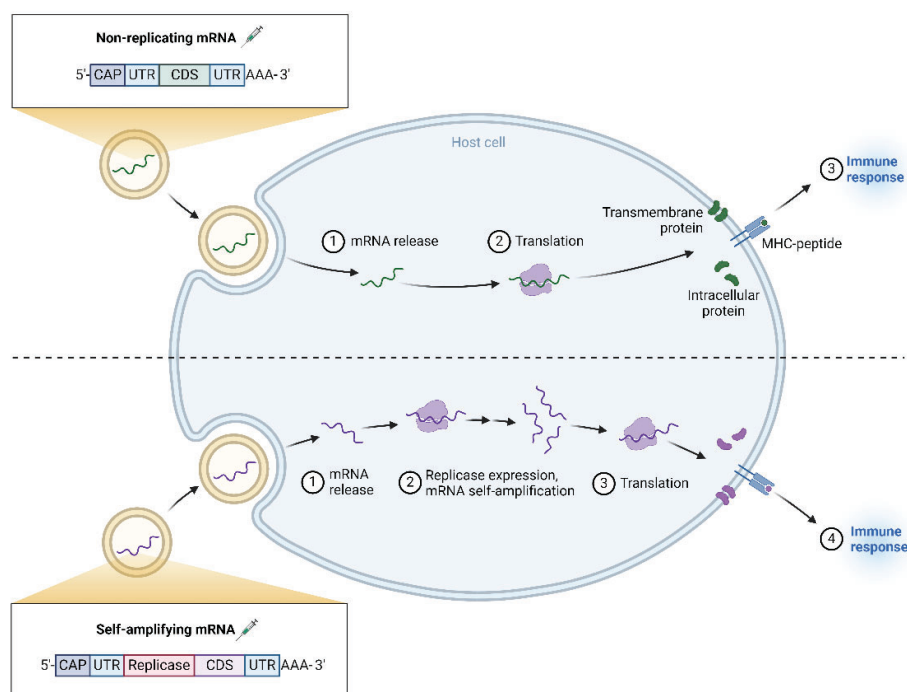


Figure 6. Traditional and saRNA constructs are represented. The replicase of saRNA vaccine constructs enables the intracellular amplification of RNA

Overall, RNA vaccines offer numerous advantages, including the ability to target both humoral and cellular immune responses, the absence of integration into the human genome (unlike plasmid-vectored DNA vaccines), no anti-vector immunity, and no risk of viral infection. Besides, and very importantly, the development of an mRNA vaccine is a relatively simple procedure. Initially, once the target antigen from the pathogen of interest is identified, its gene is sequenced and chemically synthesised with specific modifications, including the incorporation of signal peptides and transmembrane domains to direct the antigen to particular cellular locations. This modified gene is then cloned into plasmid DNA. Subsequently, the plasmid DNA undergoes *in vitro* transcription, producing the mRNA vaccine candidate, which is then prepared for evaluation in preclinical trials. The time required for the multiplication, growth, and inactivation or isolation of the desired part of the virus is saved. This conventional process usually consumes substantial material resources, such as the tissues used for virus cultivation (e.g., chicken or mammalian eggs). Conversely, once the genetic sequence of the antigen is determined, mRNA-based vaccines can be rapidly produced in biomedical laboratories globally. Therefore, the production of mRNA vaccines is not only less time-consuming but also more cost-effective, as laboratories typically possess the necessary materials. This manufacturing efficiency supported large-scale vaccine production during the pandemic.

Additionally, the mRNA platform is highly versatile and adaptive. Since mRNA consists of identical fundamental building blocks across all organisms, a novel vaccine can be readily developed by simply altering the nucleotide sequence to encode a pathogen-specific antigen. The sole requirement to initiate the process is knowledge of the specific sequence encoding the antigen. This aspect is highly valuable to adapt to subsequent viral variants of concern and to provide a quick vaccination response (68,70,87,96).

Finally, vaccination utilising RNA induces a strong innate immune response. The RNA facilitates the expression of the vaccine antigen within host cells and possesses inherent adjuvant properties (97). This platform had previously shown high immunogenicity and antigen-specific T-cell responses in cancer clinical trials (32), thereby manifesting high efficacy and potency at low doses. This makes it possible to lower the volume required and thus the time and cost of manufacture (11).

Nonetheless, this platform also has certain limitations, such as: i) it requires storage at extremely low temperatures to maintain vaccine stability; ii) the production process is costly, rendering this type of vaccine less feasible for low-income countries; and iii) the lack of systematic methods to identify the primary mechanisms of physicochemical degradation in the final formulated mRNA vaccine.

Despite its limitations, the quick up-scale potential, as well as the advantages related to the production process, and the information gained related to the mRNA platform before the pandemic rapidly fostered the development and success of this vaccine platform. Table 2 depicts a summary of the main vaccine platforms described.

Table 2. Summary of main vaccine platforms, advantages, limitations, and illustrative examples

Vaccine platform	Advantages	Limitations	Examples
Inactivated virus vaccines	• More stable and safer than live-attenuated vaccines. • Suitable for immunocompromised patients.	• Production processes can be complex as large amounts of viruses are needed and antigen integrity needs to be confirmed in the laboratory.	CoronaVac (Sinovac Biotech Ltd, China).
Live-attenuated vaccines	• Inadequate to induce disease but capable of eliciting immune responses like natural infection. • Genetic modification through reverse genomics can achieve codon de-optimisation or deletion of genes essential for triggering innate immune recognition, thereby diminishing reactogenicity.	• Potential risk of transmission to unvaccinated individuals, especially immunocompromised vaccinees. • Development is time-consuming and technically challenging when reverse genomics is used.	COVI-VAC (Codagenix© in cooperation with the Serum Institute of India).
Protein subunit vaccines	• Very safe: Only contain specific viral antigenic fragments and do not include any additional components of the pathogenic virus. Mitigation of potential risks such as incomplete viral inactivation, reversion to virulence, and pre-existing anti-vector immunity. • Subunit vaccines can precisely target well-characterised neutralising antigenic epitopes and, when combined with adjuvants, enhance immunogenicity and/or efficacy.	• Expression of S protein is technically challenging. • Expression of RBD alone is suboptimal since it does not encompass all the antigenic epitopes found in the full-length S protein. • The formulation process is usually complex and requires the development of new production protocols and stability assays for each new antigen.	Novavax, Inc. (Gaithersburg, Maryland).



Vaccine platform	Advantages	Limitations	Examples
Vectorized vaccines	• Handling live SARS-CoV-2 during production is not required. • There is substantial experience in manufacturing large quantities of certain vectors. • Stimulation of both B-cell and T-cell responses without the use of adjuvants.	• Some vectors can be partially neutralised by pre-existing host vector immunity. • Viral vectors can induce an immune response that subsequently interferes with future vaccines based on the same vector. • Theoretic risk of mutagenic events.	Replication-incompetent vectors: AdV5 (CanSino Biological Inc./Beijing Institute of Biotechnology). AdV5/AdV26 (Gamaleya Research Institute, Health Ministry of the Russian Federation) AdV26 (Janssen Pharmaceutical, Beerse, Belgium). Chimpanzee adenovirus, ChAdOx1 nCoV-19 (Oxford-AstraZeneca). Replication-competent vectors: Measles vaccine strain (Institute Pasteur and Themis). Influenza virus (Beijing Wantai Biological Pharmacy).
DNA vectored-based vaccines	• Ability to target and stimulate both humoral and cellular immune responses. • Straightforward and adaptable large-scale production and formulation processes. • Flexibility for incorporating multiple antigens. • Capability for room-temperature storage of the final vaccine product.	• Low immunogenicity in humans, requiring multiple doses to achieve optimal protection. • Theoretical risk of carcinogenesis due to potential integration into cellular chromosomes.	INO-4800 (Inovio Pharmaceuticals, Plymouth Meeting, PA).



Vaccine platform	Advantages	Limitations	Examples
RNA vaccines	• Ability to target both humoral and cellular immune responses. • Absence of integration into the human genome. • No anti-vector immunity. • No risk of viral infection. • Adaptability to different antigens and variants of concern. • High efficacy and potency at low doses. Lower volumes required and lower time and cost of manufacture. • Scalability and rapid mass production.	• Require storage at extremely low temperatures to maintain vaccine stability. • Production process is economically costly. • Lack of systematic methods to identify the primary mechanisms of physicochemical degradation in the final formulated mRNA vaccine.	Traditional mRNA technology: BNT162b2 (Pfizer-BioNtech). mRNA-1273 (Moderna). Self-amplifying mRNA: ARCT-154 (Arcturus Therapeutics and CSL).

Table 2. Advantages and disadvantages of the different vaccine platforms. Table created by the author.

1.3 Rollout of the first authorised SARS-COV-2 vaccines

1.3.1 Accelerated development of vaccine candidates

Vaccine development, on average, normally takes 10 to 15 years (7,98). However, in the case of the first mRNA SARS-CoV-2 vaccines, regulatory approvals were granted shortly after the first results from Phase III clinical trials became available, less than 10 months after the start of the respective Phase I clinical trials (11). Historically, the shortest development timeline was for the mumps vaccine in 1967, which was developed in four years (99,100). However, contrarily to SARS-CoV-2, the disease had been prevalent for several decades, and live-attenuated mumps vaccines were already in use (100) since 1950 (101).

Overall, these prolonged timelines (10-15 years) are partially due to the sequential development that is normally required in regulatory processes, in which exploratory and preclinical stages and the clinical development Phases (Phase I-III clinical trials) normally take place sequentially. While this traditional approach has been the rule for the development of many vaccines, the urgency of the SARS-CoV-2 pandemic, where rapid and smooth access to vaccines and treatments was of utmost importance, highlighted the need to adapt this traditional approach (11).

As mentioned in section 1.1, previous experience allowed shortening preclinical timelines from several years to a few months. This effort was substantiated by a strong public-private collaboration of different stakeholders that helped to streamline the development process of several vaccine candidates. Additionally, the implementation of innovative clinical trial designs was also important to accelerate the development of vaccine candidates. Of these innovative designs, the seamless design was the first choice to accelerate development at the clinical stage (11).

In contrast to conventional fixed-sample designs, where the primary elements of a clinical trial (such as sample size, randomisation scheme, inclusion and exclusion criteria, or vaccine doses) remain unchanged until the clinical trial's completion, adaptive designs offer the flexibility to modify clinical trial procedures and/or statistical aspects during the course of the study. These modifications may include early termination for efficacy or futility, re-estimation of sample size, or adjustments to the randomisation scheme, and are informed by

accumulated data from interim analyses. To maintain the scientific integrity of the clinical research, such modifications must be pre-specified in the study protocol (102,103).

Phase I/II seamless clinical trials integrate dose-escalation phases for initial safety assessment (Phase I) with dose and schedule optimisation (Phase II) within a single study. Participants from the Phase I stage are combined with those from Phase II to identify the optimal dose and schedule. In contrast to conventional clinical trials, which separate the Phase II exploratory stage from the Phase III confirmatory stage, a seamless Phase II/III design integrates these two stages into a single continuous clinical trial. This strategy usually offers several advantages, such as i) the conservation of resources and time by eliminating the interim period between Phase II and Phase III clinical trials and ii) the inclusion of relevant Phase II data in the final analysis (103). Some of the designs applied to test candidate vaccines combined Phases I-III into a single seamless clinical trial, like Pfizer's clinical trial of BNT162b (104).

The implementation of adaptive seamless designs was clearly advantageous over conventional fixed-sample designs within the urgency of the pandemic and the urgent need to rapidly develop vaccine candidates that provided protection at the population level. Firstly, the understanding of SARS-CoV-2 biology and the epidemiology of the pandemic remained limited, indicating that vaccine clinical trial designs must be flexible and responsive to the evolving landscape (e.g., duration of infectivity, immunogenicity, immunity, viral variants of concern, etc.) as new data became available. Moreover, with over 100 vaccine candidates under development, seamless Phase II/III clinical trials enabled the evaluation of multiple candidate vaccines (or doses) concurrently (39). This approach allowed for the progression of only the most promising vaccine candidates (or doses) to subsequent phases of development. Under this design, the treatment arm demonstrating lower efficacy in the interim analysis could be discontinued from the clinical trial (a strategy known as “drop-the-loser arm”), with only the chosen dose proceeding to the second stage of the study. Additionally, if interim results from the first stage indicated promising efficacy, the number of subjects randomised to the placebo arm could be reduced, thereby mitigating potential ethical concerns related to the use of placebo.

For instance, Pfizer's research initially started with four mRNA vaccine candidates, among which BNT162b1 and BNT162b2 progressed to the Phase I-III clinical trial. The BNT162b1

candidate contained a modified mRNA sequence encoding the RBD of the S protein, whereas the BNT162b2 candidate included a modified mRNA sequence encoding the full-length spike protein. Despite the fact that both candidates elicited comparable antibody titres in Phases I and II, only BNT162b2 advanced to the Phase III stage because it demonstrated a more robust T-cell response (91,105).

Finally, adaptive design methods were especially useful in the monitoring of safety and under a thorough Data Safety Monitoring Board (DSMB) monitoring, with particular emphasis on addressing serious safety concerns. This aspect was of particular significance given the uncertainties surrounding SARS-CoV-2 vaccines and the importance of developing vaccines that were not only effective but also safe for subsequent mass administration.

In sum, the seamless design enabled a smooth and quick transition from early phase to confirmatory clinical trials, once there were positive interim data on safety and immunogenicity data. A rolling review of these interim results was made by Regulatory Authorities. Efficacy results were then submitted for final regulatory approval once phase III clinical trials met all primary efficacy endpoints (11). Figure 7 shows the general clinical development of SARS-CoV-2 vaccines, showing this seamless transition of phases with respect to the conventional clinical development. The specific process of mRNA vaccine development and roll out will be described in detail in the next section.

Figure 7. Expedited clinical development of SARS-CoV-2 vaccines

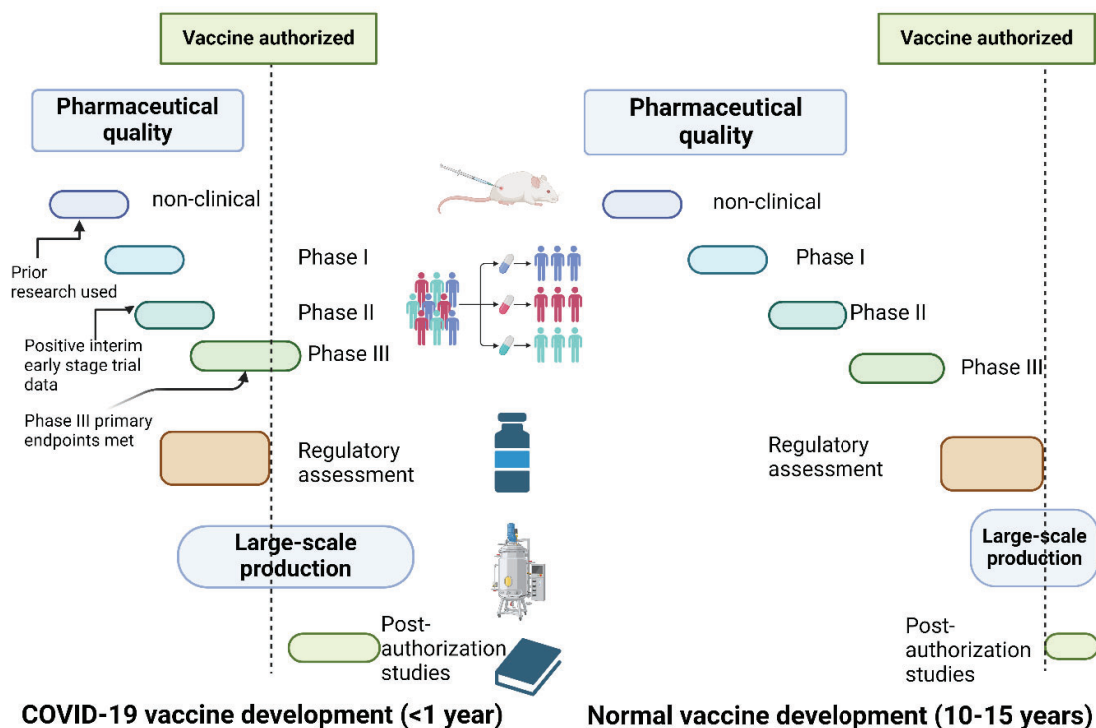


Figure 7. The expedited development of SARS-CoV-2 vaccines is represented, compared with conventional vaccine development. Created in BioRender.com

The mRNA-based vaccine BNT162b2, later named Comirnaty, was the first vaccine to get Emergency Use Authorization (EUA) from the FDA on December 11, 2020 (42), and a Conditional marketing authorisation (CMA) from the EMA on December 21, 2020 (43). The second vaccine to be authorised was mRNA-1273. The vaccine received EUA from the FDA on December 18, 2020 (106), and CMA from the EMA on January 06, 2020 (107). Later Ad26.COVS (Janssen) was granted EUA by the FDA on February 27 2021, and CMA by the EMA on March 11 2021 (11). The fourth vaccine was AZD1222 (Oxford/AstraZeneca), which received a CMA from the EMA on January 29 2021 (11).

Beyond the United States and the European Union, various other vaccines received national-level approvals. Sputnik V (Gam-COVID-Vac, developed by the Gamaleya Research Institute) was among the earliest SARS-CoV-2 vaccines to be registered for use in any country, with approval granted by the Russian Ministry of Health in August 2020. Similarly,

China's CoronaVac (developed by Sinovac), an inactivated whole-virus vaccine, received EUA in China in July 2020. In contrast to the vaccines approved by the EMA and FDA, these approvals utilised different expedited regulatory processes tailored to the specific needs of each country. However, significant concerns were raised within the scientific community and the public due to the limited data available prior to their approval (108).

Despite there being over 50 vaccines undergoing clinical trials and more than 240 in pre-clinical development (87,109), only two vaccines had received EUA and CMA by January 2021, and these were mRNA vaccines. No previous mRNA vaccines had been approved for human use, however, this platform had been previously tested in cancer clinical trials, showing promising results in terms of antigen-specific T-cell responses (32). This new approach to vaccination also offered other advantages, as described in section 1.2.1, such as their efficacy, immunogenicity, and safety (110,111). RNA vaccines offer adaptability in the design and expression of vaccine antigens, allowing them to closely replicate the structure and expression of the antigen as observed during natural infection. As a critical component of protein synthesis, RNA does not integrate into the host genome, is expressed transiently, and is subsequently metabolised and cleared by the body's natural mechanisms.

Therefore, RNA vaccines have been considered safe in previous studies (112,113). Additionally, mRNA-based vaccines can be rapidly produced in large quantities and with standardised methods, therefore enabling the targeting of any new antigen for which the mRNA is coded (33,87,114). Consequently, the RNA vaccine platform facilitated the swift development of vaccines in response to the pandemic. Specifically, these two vaccines became the primary focus in efforts to curb the rampant spread of the pandemic. Furthermore, mRNA has been considered since then a promising platform for future vaccine development (36,87). In the following subsections, we will describe the release of the two first mRNA vaccines, their main characteristics and differences, and the safety monitoring efforts implemented on a post-authorisation basis.

1.3.2 Development and release of the first mRNA vaccines

1.3.2.1 BNT162 (Pfizer-BioNTech)

On March 17, 2020, Pfizer and BioNTech announced plans to develop and test candidate vaccines. Under the collaboration terms, development costs would be shared equally between the two companies. BioNTech and its collaborators were responsible for supplying the vaccine candidates for clinical trials, utilising their European facilities certified for Good Manufacturing Practice (GMP) for the production of mRNA (115). In line with the development plans, BioNTech and Pfizer launched a coordinated program to compare different RNA-based vaccine candidates in umbrella-type clinical studies conducted in Germany and the United States, which was called ‘Project Lightspeed’. The program was designed to support the selection of a single vaccine candidate and dose level for a pivotal Phase III international safety and efficacy trial (5).

The project brought mRNA vaccine candidates from concept to clinical development in less than three months (115). Five initial vaccine candidates were assessed in preclinical repeat-dose toxicity studies using Wistar Han rats. Four of these candidates encoded different sequence variants of the RBD of the S protein (BNT162a1, BNT162b1, BNT162b3, and BNT162c1), while the fifth encoded the full-length S protein in its pre-fusion conformation (BNT162b2, with sequence variants V8 and V9; sequence V9 was the one included in the final approved vaccine) (116).

Shortly after the announcement, on April 23, the first Phase I/II clinical trial began in Germany (study BNT162-01, NCT04380701) (117). Originally, the study was planned to include two parts, i.e., Part A and Part B, however Part B was cancelled due to changes in the overall clinical development plan. Part A was a dose-finding part to investigate the optimal dose of four vaccine candidates (BNT162a1, BNT162b1, BNT162b2, and BNT162c2), allowing dose adjustments in younger participants. Doses tested in older participants and expansion cohorts were chosen based on the acceptability of dosing in younger participants.

The vaccines BNT162a1, BNT162b1, BNT162b2, and BNT162c2 were administered using a Prime/Boost (P/B) regimen with two doses given 21 days apart. The vaccine BNT162c2 was also administered using a single dose (SD) scheme. Four additional expansion cohorts

(cohorts 11, 12, 13, and 14) aged from 18 to 85 years received BNT162b2 using a P/B regimen only. In cohort 11, participants received BNT162b2 using one 3- μ g prime dose (Dose 1) and one 30- μ g boost dose (Dose 2) of BNT162b2. Cohorts 12, 13, and 14 received two 30 μ g doses of BNT162b2.

The first interim results were published by Mulligan MJ et al. (118), who reported the results of 45 participants, 18 to 55 years of age, who were randomised to three dose groups of BNT162b1, each with 15 participants. Group 1 was administered two doses of 10 μ g, separated by 21 days. Group 2 was given two doses of 30 μ g, also separated by 21 days. Group 3 was administered a single 100- μ g dose (118). RBD-binding IgG concentrations were measured 35 days after administration of the first dose, and they were compared to human convalescent sera (HCS) extracted from 38 patients 14 days after the PCR-confirmed diagnosis. RBD-binding IgG concentrations and SARS-CoV-2 neutralising titres in sera increased with the dose level and after a second dose. Local reactions and systemic events were dose-dependent, typically mild to moderate in severity, and transient in nature.

In the seven days after vaccination, injection site pain was the most frequent solicited local reaction, reported after the first dose by 58.3% (7 out of 12) in the 10- μ g BNT162b1 group, 100.0% (12 out of 12) in both the 30- μ g and 100- μ g BNT162b1 groups, and 22.2% (2 out of 9) in the placebo group. Conversely, the most common systemic events reported were mild to moderate fatigue and headache. Chills, muscle pain, and joint pain were reported by individuals who received BNT162b1 but not by individuals in the placebo arm.

A second vaccination at a dose of 100 μ g was not administered due to the observed increase in reactogenicity and the absence of a significant enhancement in immunogenic response when compared to a single 30- μ g dose. These results provided preliminary evidence that BNT162b1 was effective in producing antibodies against SARS-CoV-2, and that reactogenicity was tolerable. In an expanded report of the German trial, Sahin U et al. published the results of a second interim analysis of 60 participants who were vaccinated with BNT162b1 in Germany, in a second, non-randomised open-label phase. Twelve healthy participants (range 20–56 years) for each of the dose level groups (1 μ g, 10 μ g, 30 μ g, and 50 μ g) received the first dose on day 1 and a booster dose on day 22, and 12 participants received a 60- μ g prime dose on day 1 only. Reactogenicity was dose-dependent and was more pronounced after the booster dose. Solicited ARS, such as fever, chills, headache, muscle

pain, joint pain, injection site pain, and tenderness, were mostly mild or moderate and transient, with occasional severe (grade 3) manifestations. As regards immunogenicity results, there were substantial CD4+ and CD8+ T-cell responses, accompanied by potent antibody responses, with IgG concentrations binding to the RBD that were markedly higher than those observed in HCS (119).

Two of these candidates were included in the Phase I portion of the Phase I/II/III clinical trial in the US (study C4591001, NCT04368728) (104), which began shortly after the initiation of the trial in Germany on May 4 (5): **BNT162b1**, which encoded a secreted trimerized SARS-CoV-2 RBD, and **BNT162b2**, which encoded a prefusion stabilised membrane-anchored SARS-CoV-2 full-length spike protein. Walsh EE et al. randomly assigned healthy adults, aged 18 to 55 years and 65 to 85 years, to receive either placebo or one of two LNP-formulated, nucleoside-modified RNA vaccine candidates: BNT162b1 or BNT162b2. With BNT162b2 30 µg and BNT162b1 30 µg, the 50% neutralising geometric mean titres (GMTs) at 7 and 14 days after the second dose were 1.7–4.6 times the GMT of HCS in younger adults (aged 18–55 years), and 1.1–2.2 times the GMT of HCS in older adults (aged 65–85 years). BNT162b2 and BNT162b1 elicited comparable immune responses.

However, systemic ARs in response to BNT162b2 were milder than those in response to BNT162b1. Given the milder systemic reactogenicity profile observed with BNT162b2, with a comparable antibody response, the candidate selected for the Phase II/III portion of the trial was BNT162b2 (91). According to Walsh et al., LNP composition, formulation components, or the sequence selected for the vaccine RNA could have influenced the distinct safety profile since both vaccine constructs only differed in the nucleotide sequences that encoded the vaccine antigens and the overall size of the RNA constructs, which resulted in approximately five times as many BNT162b1 RNA molecules as BNT162b2 (91). Of note, the RNA nucleotide composition had previously been reported to affect its immune stimulatory activity and reactogenicity profile (120).

The authors also stated some uncertainties with respect to the trial's results. Firstly, the relative significance of humoral and cellular immunity in providing protection against COVID-19 had not been completely defined at the time of the publication of the results. Second, the cellular immune responses triggered by BNT162b2 were currently under

investigation. Third, the serum-neutralising responses induced by the vaccine candidates, compared to those induced by natural infection, appeared highly promising, however, the extent of protection against COVID-19 provided by this or any other standard remained uncertain. These were the typical uncertainties or challenges faced during the design and conduct of clinical development programs during the pandemic, as described in section 1.2.2.

Having proved immunogenicity in the Phase I/II clinical trial, in late July 2020, Pfizer announced that they would start Phase III testing with two 30 µg doses administered to over 30,000 subjects (5). In November 2020, Pfizer concluded Phase III testing and submitted preliminary results to regulatory Authorities (111). In the Phase II/III portion of the C4591001 clinical trial (NCT04368728) (104), Polack et al. evaluated the efficacy and safety of BNT162b2 in adults and adolescents ≥ 16 years of age who were healthy or had stable chronic medical conditions (including, but not limited to, hepatitis B virus infection, hepatitis C virus infection, or human immunodeficiency virus). Those with a medical history of COVID-19, treated with immunosuppressive therapy or diagnosed with an immunocompromising condition were excluded (111).

Participants were randomised to receive two 30 µg doses of either BNT162b2 or placebo, administered 21 days apart. BNT162b2 was 95.0% effective (95% confidence interval (CI) 90.3–97.6; > 0.9999 posterior probability of true vaccine efficacy (VE) being $> 30\%$) in preventing COVID-19 in participants who had no evidence of prior or existing SARS-CoV-2 infection (first primary efficacy endpoint). In an analysis including participants with and without evidence of prior SARS-CoV-2 infection, the efficacy of BNT162b2 was 94.6% (95% CI 89.9–97.3; > 0.9999 posterior probability of true VE being $> 30\%$) (second primary efficacy endpoint).

The cumulative incidence of COVID-19 cases began to diverge 12 days after the first dose between the BNT162b2 and placebo groups, suggesting an early onset of partial protection. Between doses one and two, BNT162b2 was 52.4% effective (95% CI 29.5–68.4). Between the administration of the second dose and seven days after, BNT162b2 was 90.5% effective (95% CI 61.0–98.9), suggesting the protective role of the booster dose. BNT162b2 was generally well tolerated; adverse reactions (ARs) were reported in 27% of BNT162b2 recipients. Few BNT162b2 or placebo recipients experienced severe ARs (1.1% vs 0.6%), serious ARs (0.6% vs 0.5%) or ARs that led to withdrawal from the trial (0.2% vs 0.1%). No

deaths were related to the administration of BNT162b2. Related serious ARs occurred in four BNT162b2 recipients (right axillary lymphadenopathy, right leg paraesthesia, shoulder injury related to vaccine administration, and paroxysmal ventricular arrhythmia).

Scale-up and manufacturing plans had been initiated before the conclusion of the clinical development of the vaccine. In July 2020, when the Phase II/III portion started, Pfizer and BioNTech disclosed that they had entered into an agreement with the United States government to supply 100 million doses of their vaccine candidate, BNT162, contingent upon receiving regulatory approval or an EUA. As of November 2020, BioNTech and Pfizer disclosed the establishment of commercial supply agreements for the years 2020 and 2021 with multiple nations, including Canada, Japan, the United Kingdom, the United States, and member countries of the European Union, contingent upon the clinical success and regulatory approval of their vaccine (115).

On 2 December 2020, BNT162b2 received a temporary EUA in the United Kingdom based on a rolling submission that included the interim Phase II/III data, with a median two-month follow-up per patient. This initial authorisation was soon followed by a series of EUAs or approvals for BNT162b2 in various countries, including Bahrain, Canada, Mexico, Saudi Arabia, and the United States, all of which occurred before December 14, 2020. Subsequently, on December 19, 2020, BNT162b2 received a CMA in Switzerland, and on December 21, 2020, the EMA granted a CMA for the EU. The initial authorisation included vaccinees aged 16 years or older (121–123). Meanwhile, study C4591001 would continue until completion of a planned follow-up period of two years after the second dose (111). On December 31, 2020, the WHO listed BNT162b2 for emergency use, making this vaccine the first to receive emergency validation from the WHO since the beginning of the pandemic (124).

1.3.2.2 mRNA-1273 (Moderna)

On January 13, 2020, The NIH and Moderna's Infectious Disease team finalised the mRNA-1273 sequence. The candidate vaccine, mRNA-1273, is an LNP-encapsulated, nucleoside-modified mRNA-based vaccine encoding the SARS-CoV-2 spike glycoprotein stabilised in its prefusion conformation (87). The vaccine had been co-developed by researchers at the National Institute of Allergy and Infectious Diseases (NIAID) and at Moderna (Cambridge,

MA). On February 7th, the initial batch of the Moderna vaccine was produced and prepared for analytical testing. On March 16th, the first patient in Phase I clinical trials was dosed with the mRNA-1273 vaccine; this was the first patient to be treated with an experimental SARS-CoV-2 vaccine under clinical trial (NCT04283461) (41).

In this first Phase I clinical trial, eligible participants were healthy adults, 18 to 55 years of age, who received two injections of trial vaccine 28 days apart at a dose of 25 µg, 100 µg, or 250 µg (40). The dose-escalation protocol outlined the enrolment of four initial sentinel participants in the 25-µg cohort, followed by the inclusion of four sentinel participants in the 100-µg cohort. If no halting rules were met after all participants in those two dose groups completed day 8, four sentinel participants in the 250-µg group were included, followed by the remainder of that dose group. No serious ARs were reported, and no prespecified trial halting rules were met. Solicited systemic ARs were more common after the second vaccination and occurred in 7 of 13 participants (54%) in the 25-µg group, all 15 in the 100-µg group, and all 14 in the 250-µg group. Local ARs were nearly all mild or moderate, and pain at the injection site was frequent. Solicited systemic and local ARs that occurred in more than half the participants included fatigue, chills, headache, myalgia, and pain at the injection site.

As regards immunogenicity, following the initial vaccination, antibody responses exhibited a dose-dependent increase. After administering the second vaccination, these titres significantly escalated. Serum-neutralising activity was observed in all participants assessed, using two different methods, with neutralisation levels generally comparable to those found in the upper range of a panel of HCS from control subjects. The 25-µg and 100-µg doses elicited CD4 T-cell responses that were strongly biased toward expression of Th1 cytokines, with minimal Th2 cytokine expression.

The trial was later expanded to include 40 older adults, who were stratified according to age (56 to 70 years or ≥71 years). All participants were assigned to receive two doses of either 25 µg or 100 µg of vaccine administered 28 days apart. Safety in this elderly population was similar to that reported in the first part of the trial. Solicited adverse events were predominantly mild or moderate in severity and most frequently included fatigue, chills, headache, myalgia, and pain at the injection site. Binding and neutralising-antibody responses appeared to be similar to those previously reported among vaccine recipients between the

ages of 18 and 55 years and were above the median of a panel of HCS. The vaccine elicited a strong CD4 cytokine response involving Th1 cells (125). In sum, these safety and immunogenicity findings supported the progression of the mRNA-1273 vaccine to later-stage clinical trials. Overall, the 100- μ g dose induced higher antibody titres than the 25- μ g dose, whereas the 250- μ g dose did not produce significant increases. These findings supported the evaluation of the 100 μ g dose in Phase II and III clinical trials.

One month after the initiation of the Phase I clinical trial, BARDA announced a grant of up to \$483 million to accelerate the development of the vaccine. Likewise, on May 1st, Moderna announced a strategic partnership with Lonza to produce approximately one billion doses of the mRNA-1273 vaccine annually (5). With this agreement, plans for scale-up manufacturing had already started during the early development phase of the vaccine.

Moderna's Phase II clinical trial began in May 2020 (NCT04405076) (126). In this clinical trial, 600 participants were stratified into two age cohorts (≥ 18 - <55 and ≥ 55) and were randomly assigned (1:1:1) to 50 or 100 μ g of mRNA-1273, or placebo, administered as two intramuscular injections, 28 days apart.

The most common solicited ARs were injection site pain, headache, and fatigue. Most ARs were mild or moderate in severity in both cohorts after the first and second vaccination. As regards immunogenicity, vaccination with the 100- μ g dose induced higher neutralising and binding antibodies than the dose of 50 μ g. This difference was especially high after the first dose. Antibodies increased substantially 14 days after the second dose to levels exceeding HCS. Therefore, the interim results of this clinical trial supported the safety and immunogenicity of mRNA-1273 and the progression to Phase III clinical testing.

The authors recognised some limitations in the publication, such as: i) the study population was not initially designed to be representative of individuals at risk for SARS-CoV-2 infection; ii) the study was not designed to statistically compare the superiority or equivalence between different doses of mRNA-1273; therefore, the conclusions were inherently qualitative; and iii) mRNA-1273 induced antibody responses that, on average, surpassed those observed in HCS; however, a serologic correlate of protection against SARS-CoV-2 had yet to be established at this time. This uncertainty was also present in the development of other SARS-CoV-2 vaccines (5).

Following the positive interim results from the Phase I and II clinical trials, BARDA maintained its support, leading to the initiation of Phase III trials for the mRNA-1273 vaccine on July 26, 2020. Given its safety and immunogenicity profile, the dose of 100 µg was selected for Phase III assessment.

In the Phase III clinical trial (COVE clinical trial), 30,420 participants 18 years of age or older, at high risk for SARS-CoV-2 infection or its complications, were randomly assigned in a 1:1 ratio to receive two intramuscular injections of mRNA-1273 (100 µg) or placebo 28 days apart (110). Between day 1 and November 25, 2020, a total of 269 COVID-19 cases were detected, resulting in an incidence rate of 79.7 cases per 1,000 person-years (95% CI, 70.5 to 89.9) among participants in the placebo group who showed no evidence of prior SARS-CoV-2 infection. In the primary analysis, 196 cases of COVID-19 were confirmed: 11 cases in the vaccine group (3.3 per 1,000 person-years; 95% CI, 1.7 to 6.0) and 185 cases in the placebo group (56.5 per 1,000 person-years; 95% CI, 48.7 to 65.3). These results demonstrated a 94.1% efficacy of the mRNA-1273 vaccine (95% CI, 89.3 to 96.8%; $P < 0.001$) in preventing symptomatic SARS-CoV-2 infection compared with placebo.

The study was initially planned based on an expected infection attack rate of 0.75%, which would have required a follow-up duration of six months after administering the two vaccine doses to accumulate 151 cases among the 30,000 participants. However, the pandemic trajectory accelerated across several regions in the United States during late summer and fall of 2020, leading to the rapid accrual of 196 cases after a median follow-up period of two months (110).

A significant secondary endpoint assessed the effectiveness of mRNA-1273 in preventing severe cases of COVID-19. Among the trial participants, 30 individuals developed severe COVID-19, all of whom were in the placebo group. This outcome suggested a VE of 100% for severe COVID-19.

As regards safety and reactogenicity, moderate and transient reactogenicity following vaccination was observed more frequently in the mRNA-1273 group. Serious adverse events were infrequent, with similar incidence rates in both groups.

These interim analyses were submitted for regulatory review, while trial participants continued with a two-year follow-up. Shortly after, the FDA and EMA granted Moderna's

vaccine access on the market on December 18, 2020, and January 06, 2020, respectively, for adults aged 18 and older (123,127) On 30 April 2021, the WHO listed mRNA-1273 under the Emergency use listing (EUL) procedure (128).

Approval timelines of BNT162b2 and mRNA-1273 are shown in Figure 8. Likewise, specific Phase III clinical outcomes of BNT162b2 and mRNA-1273 are described in greater depth in the next section.

Figure 8. mRNA vaccine development and approval pathways

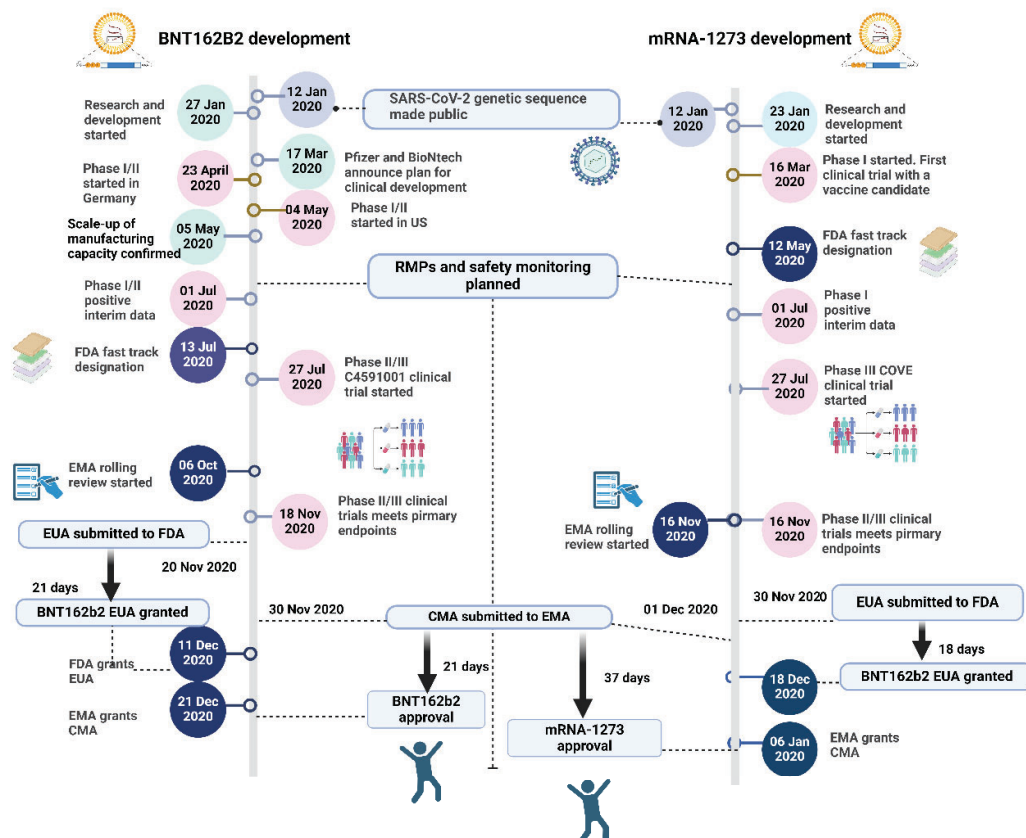


Figure 8. The development and approval timelines of BNT162b2 and mRNA-1273 are shown. Adapted from Kalinke U et al. (11) and Patel R et al. (5)

1.3.3 Comparison of BNT162b2 and mRNA-1273

1.3.3.1 Differences regarding structure and formulation

BNT162b2 was authorised as Comirnaty®. The active substance (single-stranded, 5'-capped mRNA produced using cell-free *in-vitro* transcription from the corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2) was named tozinameran (43), with Anatomical Therapeutic Chemical (ATC) code J07BN01. Conversely, mRNA-1273 was authorised as Spikevax®. The active substance (single-stranded, 5'-capped mRNA) produced using cell-free *in-vitro* transcription from the corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2) was named elasomeran, with ATC code J07BN01.

The vaccines are based on a novel mRNA technology as their active component, representing an innovative approach to vaccination. Each vaccine consists of a single-stranded mRNA molecule that encodes the spike protein of SARS-CoV-2, the S2-P antigen, consisting of the SARS-CoV-2 glycoprotein with a transmembrane anchor (5). As described in section 1.2.1, this type of protein can be found on the virus' surface and is known to allow SARS-CoV-2 to enter the host cells (129,130). BNT162b2 and mRNA-1273 share the same mRNA sequence. The mRNA consists of a 5' cap, untranslated regions (UTRs) preceding and following the coding sequence, and a poly(A) tail at the 3' end of the single strand. These elements confer stability to the molecule within the cytoplasm and enhance the translation process. Additionally, both vaccines incorporate nucleoside modifications, which involve substituting certain RNA nucleotides to create synonymous codons. These alternative codons are translated more efficiently due to the increased availability of their corresponding tRNA molecules within the cell (87).

For efficient translation, *in-vitro* transcribed mRNA should have essential structural elements, such as a 5' cap (especially the cap1 structure), 5' and 3' UTRs, and a poly(A)-tail surrounding. Numerous strategies have been investigated to accomplish a highly efficient 5' capping of *in-vitro* transcribed mRNA, such as post-transcriptional enzymatic reactions or co-transcriptional capping reactions (32,33). The 5' cap1 of mRNA-1273 was added post-transcriptionally using a vaccinia virus capping enzyme and vaccinia 2'-O-methyltransferase (131). In contrast, BNT162b2 used a co-transcriptional trinucleotide cap1 analogue (CleanCap), which provides nearly 100% capping efficiency (132).

Apart from the mRNA capping strategy, the UTRs of the mRNA play a crucial role in regulating gene expression. The 5' UTR, which serves as the ribosome entry site during translation, is capable of forming complex RNA secondary and tertiary structures that influence the initiation of translation (133). In contrast to the 5' UTR, the 3' UTR is crucial in governing the mRNA's destiny and functionality, encompassing processes such as degradation, translation, and localisation. The length of the 3' UTR significantly influences both the stability and translational efficiency of the mRNA. mRNAs with extended 3' UTRs tend to have a reduced half-life, while those with shorter 3' UTRs exhibit decreased translation efficiency (134–136). The UTRs at both ends of the mRNA vary between BNT162b2 and mRNA-1273, although the effect of these UTRs on mRNA translation is currently uncertain (137).

In addition to optimising the structural components of mRNA, modifications such as codon optimisation and the substitution of uridine with N1-methylpseudouridine (m1Ψ) can significantly improve gene expression. Modified nucleosides like pseudouridine (Ψ), commonly found in natural RNAs, help to mitigate TLR-mediated innate immune responses against mRNA and enhance both translational efficiency and biological stability. Given the advantageous impact of m1Ψ on mRNA immunogenicity and stability, both BNT162b2 and mRNA-1273 substituted all uridines with m1Ψ in their mRNA during *in-vitro* transcription. However, these vaccines differ in their m1Ψ content due to varying codon optimisation approaches (131,132). It remains uncertain whether the differences in m1Ψ content between these vaccines affect mRNA stability.

On the other hand, the RNA of BNT162b2 and mRNA-1273 are encapsulated within LNPs to enhance cellular uptake. LNPs protect mRNA from degradation by RNases. Furthermore, LNPs enable entry into host cells due to their phasic solubility with the cellular phospholipid bilayer. The LNP used by these two vaccines have some chemical differences; for instance, the type of lipid used for the LNPs (BNT162b2 uses ALC-0315 and mRNA-1273 uses SM-102) (87,137).

BNT126b2 and mRNA-1273 also differ in excipient composition. These excipients: i) balance the vaccine pH to resemble physiological pH, ii) maintain vaccine integrity and stability, and iii) help the molecules retain their structure during freezing (36). The composition of the mRNA vaccine formulations can be found in Table 3.

Table 3. BNT162b2 and mRNA-1273 differences in formulation

Ingredients	BNT162b2 (original formulation)	BNT162b2 (updated formulation)*	mRNA-1273
mRNA	Nucleoside-modified mRNA encoding the viral spike (S) glycoprotein of SARS-CoV-2	Nucleoside-modified mRNA encoding the viral spike (S) glycoprotein of SARS-CoV-2	Nucleoside-modified mRNA encoding the viral spike (S) glycoprotein of SARS-CoV-2
Lipids	2 [(polyethylene glycol)-2000]-N, N-ditetradecylacetamide (ALC-0159) 1,2-distearoyl-sn-glycero-3-Phosphocholine (DSPC) Cholesterol ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315)	2 [(polyethylene glycol)-2000]-N, N-ditetradecylacetamide (ALC-0159) 1,2-distearoyl-sn-glycero-3-Phosphocholine (DSPC) Cholesterol ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315)	Polyethylene glycol (PEG) 2000 dimyristoyl glycerol (DMG)): 1,2-dimyristoyl-rac-glycerol, methoxypolyethylene glycol 1,2-distearoyl-sn-glycero-3-Phosphocholine Cholesterol SM-102: heptadecane-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate
Excipients	Potassium chloride Monobasic potassium Phosphate Sodium chloride Dibasic sodium phosphate Dihydrate Sucrose Water for injections.	Tromethamine Tromethamine hydrochloride Sucrose Water for injections.	Tromethamine Tromethamine hydrochloride Acetic acid Sodium acetate Sucrose Water for injections.

*Table 3. Ingredients of the BNT162b2 and mRNA-1273 formulations; * Updated formulation: applies to all BNT162b2 vaccines for individuals aged 12 and older. It utilises different stabilising agents that help maintain the stability of the vaccine molecules during manufacturing, freezing, shipping, and storage processes. Modified from Muhar BK et al. (36) and the Summary of Product Characteristics (SmPC) of both vaccines (138,139).*

1.3.3.2 Differences regarding storage and administration

BNT162b2 and mRNA-1273 are administered as a series of two intramuscular (deltoid) injections, separated by 21 and 28 days, respectively. Both vaccines must be stored and transported at very low temperatures, which is a logistical caveat for their distribution (5), especially in rural areas or developing countries that lack ultra-cold freezers. Comparing mRNA-1273 and BNT162b2, the former has superior storage conditions and does not require previous dilution, contrarily to BNT162b2, which needs ultracold temperatures (-80°C to -60°C) for transportation and storage (36). Extended stability studies and the subsequent reformulation of the BNT162b2 vaccine (Table 5) have led to enhanced stability, which includes the ability to remain stable for up to 12 hours at room temperature after the vial is opened, as well as eliminating the need for dilution before administration. Table 4 shows the differences in dose, vial presentation, and storage conditions between BNT162b2 and mRNA-1273.

Table 4. Storage and administration conditions

Conditions	BNT162b2	mRNA-1273
Pharmaceutical presentation	Concentrate for dispersion for injection	Concentrate for dispersion for injection
Vaccination schedule	2 doses, 21 days apart	2 doses, 28 days apart
Dosage	30 micrograms of mRNA	100 micrograms of mRNA
Vials	2 mL clear multidose vial. Each vial contains 5 doses of 0.3mL. Pack size: 195 vials.	5 ml dispersion in a vial. Each vial contains 10 doses of 0.5mL. Pack size: 10 multidose vials.
Storage conditions	Unopened vial: 6 months at -90°C to -60°C, up to 5 days at 2 °C to 8°C, up to 2 hours up to 30°C, protected from light. After dilution: 6 hours at 2°C to 30°C.	Unopened vial: 7 months at -25°C to -15°C, 30 days at 2°C to 8°C, up to 12 hours at 8°C to 25°C, protected from light. Punctured Vial: 6 hours at 2°C to 25°C. Does not need dilution

Table 4. Storage and administration conditions of BNT162b2 and mRNA-1273. Modified from Fortner A et al. (87) and SmpCs of both vaccines (138,139).

1.3.3.3 Differences regarding safety and efficacy

Both BNT162b2 and mRNA-1273 demonstrated efficacy in the prevention of symptomatic COVID-19 and severe COVID-19. Table 7 describes the main Phase III clinical outcomes of BNT162b2 and mRNA-1273. The Phase III clinical trials that yielded these efficacy results have some methodological and statistical differences in their design; nevertheless, it can be stated with caution that both vaccines demonstrated comparable efficacy outcomes, independently of prior evidence of COVID-19.

Both studies included older patients (with a different cut-off for stratification). In the C4591001 clinical trial, the lower age limit included 16-year-old participants, whereas in the COVE clinical trial, the lower limit included 18-year-old participants. Efficacy outcomes were consistent, in both studies, in pre-defined subgroups of patients, for example, in age subgroups.

Both studies excluded immunosuppressed patients and pregnant women. The efficacy and safety results should be interpreted within the clinical and methodological context of the clinical trial that generated these results, as well as the specific population in which they were evaluated. In this sense, the results of both Phase III clinical trials were not extrapolatable to immunosuppressed patients and pregnant women, who were excluded as participants.

BNT162b2 and mRNA-1273 showed early VE after the first dose. However, the assessment was different in each trial. Polack FP et al., between the first and second dose, observed 39 cases of COVID-19 in the BNT162b2 group and 82 cases in the placebo group, resulting in a VE of 52% (95% CI, 29.5 to 68.4) (111); Baden LR et al. reported 30 severe cases in the vaccine arm and 0 in the placebo arm starting 14 days after the second injection, with a VE based on a Hazard Ratio of 1.00 (95% CI, NE-1.00) (110). Baden LR et al. also reported 225 symptomatic cases (independently of severity) in the vaccine arm and 11 in the placebo arm after the first dose (without specific dates), yielding a VE based on Hazard Ratio of 0.952 (95% CI, 0.912-0.974)

As regards reactogenicity, both BNT162b2 and mRNA-1273 showed higher reactogenicity after the second dose and in younger vaccinees. BNT162b2 was clearly less reactogenic than mRNA-1273. However, it should be taken into account that the differences in reactogenicity may reflect, at least partially, the differences in adverse event reporting between studies. In

the C4591001 clinical trial, the rate of any AR was calculated from the overall study population, whereas local and systemic ARs were collected from a “reactogenicity” subset of vaccinated persons, which was a smaller portion of the total sample size (n=8183 persons). Additionally, Baden LR et al. reported the proportion of any adverse event (not AR) after the first and second dose, whereas Polack FP et al. provided a broader overview by reporting the proportion of vaccinees with any AR after either dose.

The reactogenicity profile of both vaccines, with specific local and systemic ARs, is depicted in Table 6. As mentioned, the proportion of local and systemic ARs was higher with mRNA-1273, especially pain at the injection site, whereas the proportion of fever was higher with BNT162b2. Of note, solicited adverse events were less common in participants vaccinated with BNT162b2 who were positive for SARS-CoV-2 at baseline. This aspect was not reported with mRNA-1273 by Baden LR and colleagues.

Table 5. Main clinical outcomes of Phase III clinical trials with BNT162b2 and mRNA-1273

Clinical outcomes	Vaccine	
	BNT162b2 (C4591001 clinical trial)	mRNA-1273 (COVE clinical trial)
Target population stratification for clinical outcomes assessment	16 to <55 yr ≥55 yr	18 to <65 yr ≥65 yr
Participants excluded from the target population	Pregnant or breastfeeding women Immunosuppressed patients	Pregnant or breastfeeding women Immunosuppressed patients
Efficacy	Participants without prior evidence of COVID-19: 8 cases of COVID-19 among vaccine recipients and 162 among placebo recipients. VE*: 95.0% (95% CI, 90.3% to 97.6%) Participants with and without prior evidence of COVID-19: 9 cases of COVID-19 among vaccine recipients and 169 among placebo recipients VE: 94.6% (95% CI, 89.9% to 97.3%) Severe COVID-19: VE 75% ≥7 days after dose 2 (95% CI, -152.6% to 99.5%) VE starting 12 days after dose 1** VE consistent among study subgroups (age, sex, race, ethnicity, obesity, and presence of a coexisting condition)	Participants without prior evidence of COVID-19: 11 cases of COVID-19 among vaccine recipients and 185 among placebo recipients VE: 94.1% (95% CI, 89.3% to 96.8%) Participants with and without prior evidence of COVID-19: 12 with COVID-19 among vaccine recipients and 187 among placebo recipients VE: 93.6% (95% CI, 88.6% to 96.5%) Severe COVID-19: VE 100% (95% CI, could not be estimated to 1.0) VE starting 14 days after dose 1** VE consistent among study subgroups (age, presence of risk for severe COVID-19, sex, race, and ethnicity)

Clinical outcomes	Vaccine	
	BNT162b2 (C4591001 clinical trial)	mRNA-1273 (COVE clinical trial)
Overall reactogenicity	Proportion of participants experiencing at least one adverse event after dose 1 (related or unrelated): 26.7%	Proportion of participants experiencing at least one AR after vaccine dose 1: 87.8%
	Increased reactogenicity after vaccine dose 2 (exact proportion not specified for any AR)	Proportion of participants experiencing at least one AR*** after vaccine dose 2: 92.2%
	Solicited injection-site and systemic adverse events were more common among younger participants	Systemic events were reported more often by younger vaccine recipients than older vaccine recipients
	Solicited adverse events were less common in participants who were positive for SARS-CoV-2 at baseline	No clear analysis of the incidence of solicited AR*** according to SARS-CoV-2 status at baseline

Table 5. Comparison of efficacy and safety outcomes of BNT162b2 and mRNA-1273. Table created by the author.

*VE: vaccine efficacy; **early vaccine efficacy: Polack FP et al. and Baden LR et al. measured early VE differently ***AR: adverse reaction. References: Polack FP et al. (111) Baden LR et al. (110).

Table 6. Tolerability and reactogenicity profile of BNT162b2 and mRNA-1273 in Phase III clinical trials after the second vaccine dose

Tolerability and systemic solicited reactions*	BNT162b2				mRNA-1273			
	16-54 years		≥55 years		18-64 years		≥65 years	
	Mild to Mod.	Severe **	Mild to Mod.	Severe **	Mild to Mod.	Severe ***	Mild to Mod.	Severe ***
Tolerability (Local and immediate reactions)								
Injection site pain	7.6%	1.2%	65.7%	0.5%	90.1%	4.6%	83.4%	2.7%
Erythema	5.4%	0.5%	6.8%	0.5%	9.0%	2.0%	7.4%	2.1%
Swelling	5.9%	0.3%	7.3%	0.2%	12.6%	1.7%	10.8%	1.9%
Systemic Reactions								
Headache	48.5%	3.2%	38.4%	0.5%	62.8%	5.0%	46.4%	3.0%
Fatigue	54.8%	4.6%	47.7%	2.8%	67.6%	10.6%	58.4%	6.9%
Myalgia	37.3%	2.2%	27.8%	1.0%	61.3%	10.0%	46.9%	5.6%
Nausea/Vomiting	1.7%	0.2%	0.6%	0.1%	21.3%	<0.1%	11.8%	0.3%
Fever	30.2%	1.2%	21.5%	0.3%	17.4%	1.6%	10.2%	0.5%

Table 6. Comparison of the reactogenicity and tolerability profiles of BNT162b2 and mRNA-1273 according to Phase III clinical trials

Solicited reactions: Adverse reactions assessed per protocol in the clinical trial; **Severe: symptoms prevent daily activity; *Severe: resulting in debilitating symptoms). Mod.: Moderate*

1.3.4 1.2.4 The need for continuous safety monitoring after the release of BNT162b2 and mRNA-1273

Randomised clinical trials of mRNA-based vaccines reported efficacy rates for preventing SARS-CoV-2 of 94% to 95% (111). In these clinical trials, local and systemic reactions (reactogenicity) were recorded. Frequently reported reactions included injection site pain, fatigue, and headache, with greater reactogenicity being reported following the second dose, which was mainly mild to moderate and transient. However, clinical trials are usually developed in a highly controlled experimental setting, with pre-determined and restrictive inclusion criteria that hardly reflect the general population, which is the main target of mass vaccination campaigns. For instance, patients with chronic diseases were included only if the conditions were deemed stable by the investigators, and several subgroups of patients (i.e., transplanted, oncologic, or immunosuppressed patients) were largely underrepresented.

In this setting, post-authorisation studies can meet the urgent need to evaluate the safety of SARS-CoV-2 vaccines within a range of coexisting conditions, medical backgrounds, and in a real-world setting. Besides, mRNA vaccines were assessed and authorised under the same quality and safety standards as any other medicinal product; however, the recentness of their development raised the issue that a better understanding of their potential ARs was needed, especially rare or infrequent ones. For instance, one of the concerns was that the mRNA strand in the vaccine might elicit an unintended immune reaction (87).

Indeed, it was expected that, for the first time in history, millions of people would get a vaccination in a short time period, and therefore rare ARs could be detected more easily (140). The development of vaccines was implemented, as described earlier, in an expedited manner, thanks to huge international efforts and collaborations. This expedited development led to the early EUA and CMA of these vaccines. However, these early authorisations were granted with the condition that a longer safety follow-up was needed (141), with a careful monitoring of ARs as the vaccine distribution increased globally (5).

1.3.4.1 Continuous safety monitoring of SARS-CoV-2 vaccines from a regulatory point of view

The EU regulatory network played a leading role in monitoring the safety of COVID-19 vaccines throughout the pandemic. The unprecedented volume of case reports concerning suspected ARs following vaccination necessitated significant efforts to evaluate this safety data. The main objective was to identify and manage any potential risks while simultaneously excluding adverse health outcomes that were coincidental but appeared temporally related to vaccination.

Shortly before the authorisation of the first COVID-19 mRNA vaccines, the EMA published the Pharmacovigilance Plan of the EU regulatory network for COVID-19 vaccines (19), largely based on the experience acquired during the 2009 H1N1 influenza pandemic (142). In Europe, the good pharmacovigilance practices (GVP) included detailed requirements and guidance on the principles of risk management (GVP Module V), with a link to the format of the risk management plan (RMP template) as well as pharmacovigilance requirements for vaccines (GVP P.I) (19,143). Furthermore, the document “core RMP requirements for COVID-19 vaccines” (coreRMP19) was developed to facilitate and harmonise the

companies' preparation of RMPs and their evaluation by regulators. This document addressed the planning of the post-authorisation safety follow-up of COVID-19 vaccines by marketing authorisation holders (MAHs) and recommended ways to prepare for pharmacovigilance activities, considering the inherent uncertainties of the pandemic (18).

Additionally, MAHs of COVID-19 vaccines were expected to submit monthly summary safety reports to the EMA, in addition to regular Periodic Safety Update Reports (PSURs). These should include information on reported suspected ARs and adverse events of special interest (AESIs). The minimum elements to be addressed in these reports were listed in the coreRMP19 (19).

The Pharmacovigilance Risk Assessment Committee (PRAC) is the body within the EMA in charge of the evaluation of the safety of medicinal products intended for human use. It meets monthly and comprises representatives from all EU/EEA member states, as well as healthcare professionals, patient representatives, and European Commission delegates. The PRAC is responsible for making recommendations to the Committee for Medicinal Products for Human Use (CHMP) concerning all matters related to pharmacovigilance and risk management systems, including the detection, assessment, risk minimisation, and communication of information regarding adverse drug reactions (144). The PRAC led the assessment of all pharmacovigilance procedures such as PSURs, Post-authorisation Safety Studies (PASSs), and safety signals. In the context of mass vaccination, the PRAC conducted the assessment of additionally expedited Summary Safety Reports (SSRs) to swiftly identify any emerging safety concerns, initially on a monthly basis and subsequently every two months. These SSRs complemented the information provided by PSURs. Besides, to allow a smooth safety signal evaluation, the PRAC allowed flexible regulatory procedures. For instance, a signal procedure routinely follows a 60-day timetable (145); during the vaccine rollout, the timelines of many procedures were reduced to less than 30 days, and extraordinary meetings were held to allow rapid decision-making processes (142).

Moreover, safety concerns related to vaccines based on the same technology were frequently examined as a class effect. Based on the available data, concurrent evaluation of signals for multiple vaccines was also conducted. This approach ensured that PRAC recommendations were issued simultaneously for the respective vaccines, thereby facilitating comprehensive

communication with stakeholders. Last but not least, the scope of the “meeting Highlights from the PRAC” was extended to include information on other safety procedures involving COVID-19 treatments and vaccines, including signals, PSURs, PASS, and RMPs (146).

The EMA implemented exceptional measures to maximise the transparency of its regulatory activities on treatments and vaccines for COVID-19 that were approved or under evaluation (147). The EMA published regular pharmacovigilance updates on approved COVID-19 vaccines with the latest information. The format and contents were developed in collaboration with National Regulatory Authorities through PRAC. For instance, the Spanish National Agency published periodic reports on the safety of COVID-19 vaccines. These reports included aggregated safety reports and were publicly available (148). Additionally, after the COVID-19 vaccines were authorised, a specific webpage by the EMA offered the most recent information, including updates to product information, relevant research studies, and monthly safety reports of each vaccine (142). Likewise, to fight against vaccine hesitancy and uncertainties during mass vaccination, the EMA published “Frequently Asked Questions (FAQs)” or ‘Key Facts’ about COVID-19 vaccines (149).

Furthermore, the EMA commissioned studies from consortia specialising in observational research to enhance the safety evaluations conducted by the PRAC and to identify emerging safety concerns. These studies include pharmacoepidemiological investigations utilising extensive EU electronic healthcare databases, such as the ARS Toscana Database, PEDIANET (Italy); FISABIO, BIFAP, and SIDIAP (Spain); PHARMO (Netherlands); CPRD (UK); GePaRD (Germany); SNDS (France); Norwegian Health Registers; and Danish Registries (142). This point will be further described in the next subsection.

1.3.4.2 Continuous safety monitoring of SARS-CoV-2 vaccines: Surveillance monitoring systems

A systematic and regular review of aggregate safety data is essential, particularly early after vaccine introduction, as this is the timepoint when safety signals not identified during clinical development may emerge (150) and may impact mass vaccination. Indeed, there are certain adverse events that, due to rarity or pathogenesis, can only be detected during longer-term surveillance within a real-world setting after immunisation (151). Some of these rare adverse events following immunisation (AEFIs) can be deemed clinically relevant (i.e., AESIs).

AESIs, especially those with a low incidence rate, are the main target of post-authorisation surveillance monitoring.

More specifically, two potential safety concerns arose at the time of approval of the first COVID-19 vaccines because of the significant sequence homology between SARS-CoV-2 and SARS-CoV: the potential for antibody-dependent enhancement (ADE) and vaccine-associated enhanced respiratory disease (VAERD). ADE is an *in-vitro* phenomenon of enhanced viral entry or replication in the presence of vaccine-induced antibodies. VAERD corresponds to an ADE syndrome with respiratory affection. Both entities result in enhanced infection or an increase in 'severe' infections in vaccinated individuals infected by the homologous coronavirus. Neither ADE nor VAERD had been described in vaccine Phase III trials, however, much attention was paid when surveillance monitoring systems were implemented post-authorisation. Indeed, although these entities remained theoretical risks after vaccination, consensus recommendations were established regarding the importance of follow-up for monitoring potential disease enhancement. These recommendations were published and included in active surveillance programs (152).

Post-authorisation monitoring of vaccines is based on a combination of passive and active surveillance. Passive surveillance systems consist of databases into which spontaneous reports of AEFIs are entered, collected, and finally assessed by Regulatory Authorities. In Europe, the basis for passive reporting is Eudravigilance (EV), whereas in the FDA, adverse events are collected in the Vaccine Adverse Event Reporting System (VAERS) database. In addition, there are reporting databases at the National level, such as FEDRA in Spain, as well as National periodic reports of collected AEFIs, as previously described (148).

On the contrary, active surveillance systems aim to seek and collect all reports of pre-specified AEFIs from a representative sample, such as sentinel sites. At a hospital level, these types of active surveillance systems are implemented through hospital-based intensive monitoring systems for a clinically relevant target population of patients. The main advantage of this method is that the 'denominator' or size of the population where the AEFI originated is known in advance. Thus, a prospective collection of AEFIs through this system allows the calculation of real incidences (153).

The combination of safety data gathered from passive spontaneous reports and active surveillance systems allows the assessment of the safety profile of authorised vaccines within

a real-setting context and, in the long term, the re-evaluation of the risk-benefit relationship. In this sense, for a proper evaluation, the following conditions must be met:

- To define and understand what safety events (AEFIs) may be expected after vaccination.
- To perform an accurate prior analysis of background rates of potential AEFIs. This analysis allows correct signal evaluation of recorded AEFIs and assesses the relationship between vaccine exposure and the occurrence of a specific AEFI.
- To develop a comparative analysis with larger databases enriched with large international prospective cohorts.

In this regard, publicly funded initiatives were launched and directed towards the continuous monitoring of the safety of COVID-19 vaccines.

ACCESS (154), a project funded by the EMA to prepare a European infrastructure to monitor COVID-19 vaccines, began in May 2020. The main aims of the **ACCESS Project** were to: (i) develop a list of adverse events of AESIs, (ii) determine background rates of AESIs, and (iii) to develop template protocols for different types of studies to be adapted for full implementation to the local situation.

On the other hand, the Consortium **VAC4EU** (who was a key partner in the ACCESS Project) also started to work on the **Early-Covid-Vaccine-Monitor project** (155), which monitored COVID-19 vaccines in eight countries using two complementary methodologies:

- A prospective cohort event monitoring of vaccinated persons included and monitored through apps/internet. Incidence rates of suspected adverse drug reactions were calculated.
- A retrospective cohort study monitoring the incidence rates of ACCESS AESIs and COVID-19 prior to and post-vaccination.

Both ACCESS and Early-Covid-Vaccine-Monitor projects were funded by the EMA.

Conversely, the **Safety Platform for Emergency vACCines (SPEAC Project)**, supported by CEPI and the Brighton Collaboration, developed a list of AESIs that had been adapted and endorsed by the ACCESS project, the Global Vaccine Data Network (156), and public health Bodies in many countries (157).

Other prospective surveillance systems were implemented. For instance, the **V-safe Active Surveillance System** was established by the Centers for Disease Control and Prevention (CDC) in 2020 to collect collecting near-real-time data from COVID-19-vaccinated persons in the USA (158). The V-safe system was based on periodic Smartphone text messages that linked to web-based health surveys from the day of vaccination (day 0) through 12 months after the final dose of a COVID-19 vaccine. A total of 3,643,918 persons were enrolled in V-safe and completed at least one health survey within seven days following their first vaccine dose, whereas 1,920,872 participants completed at least one daily health survey within seven days following the second dose. Overall, the frequency of reported reactions was generally consistent with results observed in clinical trials.

Another hospital-based active surveillance system, implemented early after the approval of mRNA COVID-19 vaccines, was reported by Blumenthal KG et al. (159). This surveillance program specifically targeted allergic reactions detected post-vaccination in healthcare professionals through symptom surveys via email, text message, phone, and smartphone application links. Of 64,900 employees who received their first dose of a COVID-19 vaccine, 25,929 (40%) received the Pfizer-BioNTech vaccine, and 38,971 (60%) received the Moderna vaccine. At least one symptom survey was completed by 52,805 (81%). Acute allergic reactions were reported by 1,365 professionals (2.10%, 95% CI, 1.99%-2.22%), more frequently with the Moderna vaccine compared with Pfizer-BioNTech (2.20%, 95% CI, 2.06%-2.35% vs 1.95%, 95% CI, 1.79%-2.13%; $P=.03$). Anaphylaxis was confirmed in 16 employees (0.025%, 95% CI, 0.014%-0.040%): seven cases from the Pfizer-BioNTech vaccine (0.027%, 95% CI, 0.011%-0.056%) and nine from the Moderna vaccine (0.023%, 95% CI, 0.011%-0.044%) ($P=.76$). The study provided further evidence supporting the assumption that allergic reactions, especially severe and anaphylactic ones, are very rare with COVID-19 vaccines.

I.3.4.3 The Pharmacovigilance Program at the Hospital Clinic of Barcelona

The Pharmacovigilance Program at the Hospital Clinic was created to closely monitor patient safety and detect, report, treat, and prevent drug-related adverse events. The Pharmacovigilance Program constitutes a multidisciplinary group with representation of Preventive Medicine, Allergology, Immunology, Dermatology, Pharmacy, Clinical Pharmacology, Quality Management, and Nursery. Within the activity of safety monitoring of drug-related adverse events, the Pharmacovigilance Program implemented specific procedures for vaccine surveillance with integrated care processes for AEFI clinical management and reporting to Regulatory Authorities. These activities were specified in advance in a safety management plan and an active surveillance procedure that was in place before the initiation of mass vaccination of health professionals and patients at the Hospital Clínic.

Two main actions were foreseen in this safety management plan:

- To implement an intensive cohort event-monitoring study (the VigilVacCOVID study) to monitor the safety of mRNA vaccine administration at the Hospital.
- To establish a protocol for the early detection, assessment, and clinical management of detected ARs, as well as a consultation system to attend vaccinees that had doubts about potential ARs.

The current thesis will be focused on the description of the results of the VigilVacCOVID project. In line with the need to enrich post-authorisation safety data of vaccines in a “real-world” setting, the Pharmacovigilance Program proposed this study as a detailed analysis of the safety data gathered regarding two different mRNA vaccines (BNT162b2 and mRNA-1273) and a known denominator of the target vaccinated population. Besides, both pivotal clinical trials ((110,111)) suggested that mRNA-1273 had greater reactogenicity than BNT162b2. This project would then allow us to confirm, in a real-world setting after authorisation, whether mRNA-1273 indeed had a greater reactogenicity profile than its counterpart, BNT162b2.

On the other hand, given that both healthcare professionals and transplanted patients were vaccinated during the vaccination campaign, the analysis of collected data would also enable

the comparison of safety data and incidences in these two different populations. Furthermore, the analysis of other types of collected clinical data (i.e., such as COVID-19 infection and serologic status) would facilitate the exploration of alternative and innovative hypotheses, such as a possible relationship between these risk factors and the development of AEFIs.

Finally, a decreased immunogenicity was observed in elderly adults in previous clinical trials with BNT162n2 and mRNA-1273, along with a lower reactogenicity (91), which made us propose the hypothesis that there is an association between reactogenicity and elicited immunogenicity. The collection of humoral and cellular immunogenicity would allow us to compare reactogenicity and immunogenicity results, thereby substantiating these previous data, and assessing a potential association between these two variables.

2 Hypothesis

The main **underlying hypothesis** is that the implementation of the **VigilVacCOVID study**, an active pharmacovigilance program designed as an intensive cohort-event monitoring study, will enable the assessment of potential differences between two mRNA SARS-CoV-2 vaccines (BNT162b2 and mRNA-1273) and two different populations (healthcare professionals and transplanted patients) in terms of short-term reactogenicity and immunogenicity. Derived from the main hypothesis, the following specific hypotheses are proposed:

Hypothesis 1: The implementation of an active pharmacovigilance program will enable the assessment of potential differences related to the safety profiles of SARS-CoV-2 vaccines and two populations (healthcare professionals and transplanted patients) in a real-world setting.

Sub-hypothesis 1.1: The safety profile of vaccination will be characterised by local and systemic ARs of mostly mild severity.

Sub-hypothesis 1.2: The short-term reactogenicity and tolerability profile of SARS-CoV-2 vaccines, administered in a real-world context, will be comparable to the safety profile observed in pivotal clinical trials.

Sub-hypothesis 1.3: Vaccination with mRNA-1273 will exhibit greater reactogenicity than vaccination with BNT162b2.

Sub-hypothesis 1.4: Healthcare professionals will exhibit higher reactogenicity than transplanted patients after vaccination.

Sub-hypothesis 1.5: Variables such as age, sex, comorbidities, or previous infection with SARS-CoV-2 will determine an increased risk of reactogenicity.

Sub-hypothesis 1.6: There will be higher reactogenicity after the second vaccine dose.

Hypothesis 2: There will be an association between reactogenicity and immunogenicity induced by vaccination.

Hypothesis 3: Vaccination with mRNA-1273 in healthcare professionals will show greater immunogenicity and reactogenicity than BNT162b2. Solid-organ transplant recipients

vaccinated with mRNA-1273 will show lower immunogenicity and reactogenicity than healthcare professionals vaccinated with the same vaccine.

Hypothesis 4: There will be a stronger association between severe ARs and second vaccine doses and immunogenicity.

3 Objectives

The **general objectives** of this Thesis were:

1. To perform a comparative assessment of two SARS-CoV-2 vaccines (BNT162b2 and mRNA-1273) and two populations (healthcare professionals and transplanted patients) in a real-world setting during the mass vaccination campaign at a tertiary-level Hospital (Hospital Clínic), in terms of the reactogenicity and tolerability profile.
2. To assess the association between reactogenicity and immunogenicity after vaccination.

To achieve the **first general objective**, one specific objective and six specific sub-objectives were proposed:

Objective 1: To assess the safety profile of vaccination in a real-world setting, considering potential differences with previous pivotal clinical trials, and differences between vaccines and vaccinated populations.

Sub-objective 1.1: To characterise the safety profile of vaccination at a high-complexity reference hospital.

Sub-objective 1.2: To compare the safety profile of vaccination, measured at a high-complexity reference hospital, with data reported in pivotal clinical trials.

Sub-objective 1.3: To perform a comparative assessment of the reactogenicity and tolerability profile of BNT162b2 and mRNA-1273.

Sub-objective 1.4: To perform a comparative assessment of the reactogenicity and tolerability profile of healthcare professionals and transplanted patients vaccinated with mRNA vaccines.

Sub-objective 1.5: To assess potential associations between demographic factors (i.e., age and sex), SARS-CoV-2 seroprevalence status and comorbidities, and the development of ARs.

Sub-objective 1.6: To assess potential differences in the incidence and type of ARs between the first and the second dose.

Objective 1 (and its six related subobjectives) was addressed in the first article of this thesis.

To achieve the **second general objective**, three specific objectives were proposed:

Objective 2: To analyse the potential association between reactogenicity and elicited immunogenicity.

Objective 3: To compare immunogenicity and reactogenicity responses between mRNA-1273 and BNT162b2 in healthcare professionals, and between solid-organ transplant recipients and healthcare professionals vaccinated with mRNA-1273.

Objective 4: To analyse the severity and number of received doses as potential explanatory factors of the association between reactogenicity and immunogenicity.

Objectives 2, 3, and 4 were addressed in the second article of this thesis.



Tolerability and Reactogenicity Profile of mRNA SARS-Cov-2 Vaccines from a Mass Vaccination Campaign in a Tertiary Hospital: Between-Vaccine and Between-Population Prospective Observational Study (VigilVacCOVID Study)

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Accepted: 7 June 2022
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Abstract

Background The comparative safety profile of SARS-Cov2 vaccines requires further characterization in real-world settings.

Objectives The aim of the VigilVacCOVID study was to assess the short-term safety of BNT162b2 and mRNA-1273 during the vaccination campaign of healthcare professionals (HCPs) and solid-organ transplant recipients (SOTRs) at a hospital clinic.

Methods We conducted an observational, prospective, single-center, post-authorization study to characterize short-term adverse reactions (ARs) after vaccination. The primary endpoint was to assess between-vaccine differences (HCPs receiving BNT162b2 or mRNA-1273) and between-population differences (HCPs and SOTRs, both receiving mRNA-1273) in the risk of any ARs. Propensity score and covariate-adjusted multivariate models were used. The key secondary endpoint was to provide a descriptive assessment of the frequencies and intensity distribution of ARs.

Results We included 5088 HCPs and 1289 patients. mRNA-1273 showed greater reactogenicity than BNT162b2, with an odds ratio (OR) for any AR of 3.04 (95% confidence interval (CI) 2.48–3.73; *p* value: < 0.001) and a higher frequency and intensity of reported ARs. Compared with HCPs vaccinated with mRNA-1273, SOTRs showed a lower risk of ARs (OR = 0.36; 95% CI 0.25–0.50), with fewer and less severe ARs. Age, sex, and previous SARS-CoV-2 infection were statistically significant covariates for the risk of any AR. A history of drug allergy was significant in the comparison between vaccines (BNT162b2 vs. mRNA-1273), but not in that between SOTRs and HCPs.

Conclusions Our study shows that mRNA-1273 had greater reactogenicity than BNT162b2. Overall, both vaccines had an adequate tolerability profile. mRNA-1273 vaccination caused fewer ARs with milder severity in SOTRs.

1 Introduction

The short-term safety of SARS-Cov-2 mRNA vaccines is reasonably well characterized from clinical trial data [1, 2] and pharmacovigilance sources [3–6]. Real-world studies have been published beyond the controlled context of clinical trials [7–12]. It is important to continue to provide additional supporting evidence on the safety profile of SARS-CoV-2

vaccines, especially at a time characterized by severe distress and anxiety caused by the pandemic and the initial uncertainties surrounding vaccination. Millions of SARS-CoV-2 vaccine doses have been administered worldwide, with serious adverse reactions (ARs) being rare or very rare. However, it is important to continue collecting safety information on real-world scenarios to further elucidate aspects deserving evaluation. One aspect that needs to be addressed in real-world studies is the comparative tolerability and safety profile of the vaccines, such as BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), in clinical practice. Another aspect is the direct comparison of the reactogenicity and tolerability profile of vaccination in immunocompromised patients. It is known from experience with other vaccines that solid-organ transplant recipients (SOTRs)

The members of The VigilVacCOVID Group are listed in Acknowledgements.

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Key Points

Further vaccine safety data from large real-world cohorts are needed.

Our study provides data from a systematic, prospective comparison, showing higher reactogenicity of mRNA-1273 but an overall adequate tolerability profile of both SARS-CoV-2 vaccines, supporting existing evidence.

Our data show a lower reactogenicity in solid-organ transplant recipients (SOTRs), as expected. The impact of covariates such as age or previous SARS-CoV-2 infection suggest a potential role of immunogenicity in differences in the safety profile between SOTRs and healthcare professionals. These data provide additional supporting evidence for medical practice, given the proven efficiency of vaccination for public health and the increasing need for reassuring messages about the safety of SARS-CoV-2 vaccines.

present less robust immune responses than nontransplant patients [13]. Studies have found a reduced humoral immune response to BNT162b2 and mRNA-1273 mRNA vaccines in SOTRs after the first and second doses, measured by antispikes antibody responses [14, 15] in kidney [16], lung [17], heart [18, 19], liver [20], and in general in SOTRs [15], who are underrepresented in clinical trials. However, many immunosuppressed patients and patients with other comorbidities have already been vaccinated with up to four doses in many countries, and few prospective studies have been specifically designed to target these patients. So far, no specific safety signals have been detected in these patients. However, further prospective real-world data are required to strengthen this reassuring message on vaccination and provide additional evidence on how the reactogenicity profile is elicited, especially in immunocompromised populations compared with healthy vaccinated persons.

The main aim of the VigilVacCovid-HCP Project was to capture a complete short-term safety profile of the incidence of ARs, comparing two mRNA vaccines, BNT162b2 and mRNA-1273, in terms of the incidence and severity of ARs. In addition, the study aimed to compare two populations of participants vaccinated with mRNA-1273 (healthcare professionals (HCPs) and SOTRs), in terms of the incidence and severity of ARs.

2 Methods

An active surveillance program was developed by the Pharmacovigilance Technical Committee (PhVTC) of the Hospital Clinic of Barcelona. The PhVTC is a multidisciplinary

group with representatives from Clinical Pharmacology, Allergology, Pharmacy, Dermatology, Preventive Medicine, Immunology, Hospital Quality Management, and Nursing Professionals. Under the mandate of the PhVTC, the Department of Clinical Pharmacology, with the close collaboration of the Departments of Preventive Medicine and Epidemiology and the Department of Occupational Health Care, designed and implemented an active surveillance program with specific procedures for vaccine safety monitoring and integrated care processes for adverse event clinical management and reporting to the regulatory authorities. Vaccinated persons were informed of the program with a leaflet describing the objectives and methods before vaccination at the vaccination center. The information was explained again when calls were made after each dose. The survey was only delivered if the person agreed. The whole procedure was explained in the protocols assessed and approved by the Research Ethics Committee of the Hospital Clinic (references: HCB/2021/0684 and HCB/2021/0685).

The active surveillance program was designed as a prospective, observational, single-center post-authorization study aimed at characterizing short-term ARs after vaccination. The study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies [21] (see eTable 15 the Electronic Supplementary Material (ESM)).

2.1 Vaccination Campaign

The vaccination campaign began in the Hospital Clinic of Barcelona, a tertiary care Hospital, on 7 January 2021. The first group to be vaccinated were HCPs. SOTR patients started vaccination on 2 March 2021. The type of vaccine administered was determined by local authority guidance and availability depending on the national supply. Initially, BNT162b2 was the only vaccine available for HCPs. The vaccination plan for HCPs was amended on 22 January with the inclusion of mRNA-1273. Vaccination of SOTRs began on 1 March with mRNA-1273. Two doses were the usual vaccine dose at the time the study was conducted. As of May 2022, three doses in the general population and four doses in immunocompromised patients are recommended by the Spanish Ministry of Health [22].

2.2 Active Surveillance Program

The surveillance program was substantiated by a previously agreed pharmacovigilance plan that aimed to cover all vaccinated staff of the hospital and affiliated institutions (Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and Fundació Clinic per a la Recerca Biomèdica (FCRB)) and SOTR patients from 7 January until 30 April 2021. The primary objective was to make a comparative

descriptive assessment of the safety profile and reactogenicity of the two vaccines.

A structured questionnaire was designed containing demographic and medical data and ARs occurring after each vaccine dose.

A predefined list of ARs was agreed on based on the safety profile described in the Summary of Product Characteristics (SmPC) and the European Public Assessment Report (EPAR) of each vaccine [23–26] (see ESM eTable 13). These were considered solicited ARs. In addition, some ARs of special interest from the list of the ACCESS [3] and SPEAC Projects [5] and the Brighton Collaboration criteria were included in the predefined list of solicited ARs [27]. The following solicited ARs were included (as per Preferred Term (PT) level of the version 24.0 of the Medical Dictionary for Regulatory Activities (MedDRA): Injection site pain (PT: Injection site pain); arm pain (PT: pain in extremity); fatigue, asthenia (PT: fatigue); headache (PT: headache); fever (PT: pyrexia); muscle pain (PT: myalgia); malaise (PT: malaise); joint pain (PT: arthralgia); injection site swelling (PT: application site swelling); injection site redness (PT: application site redness); shoulder pain (PT: musculoskeletal pain); injection site pruritus (PT: vaccination site pruritus); headache (PT: headache); insomnia (PT: insomnia); nausea (PT: nausea); diarrhea (PT: diarrhea); and vomiting (PT: vomiting) (see ESM eTable 13).

A free-text option was provided to collect other adverse events not considered a priori in the predefined list. Reactions collected within the free-text option were considered unsolicited ARs.

Severe ARs were defined by intensity assessed on a Likert scale, with values ranging from 0 to 10, rated using instructions from the interviewer (see ESM eFig. 1).

2.3 Delivery of the Questionnaire

2.3.1 Preparatory Process

Nine professionals from the Medical Division were trained ad hoc and delivered the questionnaire through phone calls. A guiding document was drafted, which aimed to homogenize its administration. Simulations with ten dummy cases were performed to homogenize the delivery process amongst interviewers.

2.3.2 Implementation

Calls were made after each dose. At least three phone call attempts were made to each interviewee to maximize responsiveness. For HCPs, if we did not obtain a response on at least two separate calendar days, interviewees were then contacted at their institutional email addresses and asked whether: (i) They wanted to be further contacted by phone

at a preferred phone number and timeslot; (ii) they preferred to be contacted and answer the questionnaire by email; (iii) they did not want to be contacted further. For interviewees who chose to answer the questionnaire by email, an electronic version was enabled, constructed using Lyme Survey technology (enquesta.clinic.cat).

During the implementation process, there were regular team meetings for doubt resolution and consistency of data recording.

2.4 Variables Assessed

The structure of the questionnaire was based on two blocks, which were completed after the first and second doses for all participants.

The first block contained common background variables that were completed by all participants (HCPs and SOTRs) and specific background variables for each vaccinated group. The second block contained vaccine- and AR-related variables, which were completed by all participants.

2.4.1 First Block

Common background variables: Age, sex, medical background; SARS-CoV-2 infection history (positive PCR or antigen test, positive serology, and/or symptomatic infection with or without hospital admission).

Specific background variables: For HCPs, contact with SARS-CoV-2 patients in their daily activity (yes/no) was included in this first block. In case of SOTRs, type of transplant (liver, heart, kidney, kidney-pancreas) was considered.

2.4.2 Second Block

Vaccine and AR related variables: Date of vaccination; type of vaccine administered; AR start date, end date (where available), reported AR, and intensity of AR. Types of ARs were selected from a predefined list in the questionnaire. The interviewer asked a closed-question to assess whether the participant had had the AR (e.g., “Did you have fever?”). The interviewer recorded yes or no. These ARs were considered “solicited ARs.”

A free-text option called “other” was enabled to collect other ARs not considered a priori in the drop-down list. To explore “other ARs” the interviewer asked an open question (e.g., Have you had other types of AR?). Yes or no was recorded accordingly. If yes, the specific type of AR was recorded as a narrative description. These AR were considered “unsolicited ARs.”

2.5 Data Collection and Data Management for Clinical Follow-Up

A database was designed and implemented with MACROTM, which provided electronic data capture functionality (EDC) to support electronic data entry. Data completion and integrity of the database were assessed continuously to verify the information consistency and completeness. Hospital electronic records were reviewed to capture potential reactions not collected by the structured interview and to mitigate recall bias.

Solicited and unsolicited ARs were coded using the PT and System Organ Class (SOC) categories of version 24.0 of MedDRA. There was considerable heterogeneity in the way unsolicited ARs were reported and recorded. Therefore, unsolicited ARs were grouped within the closest related SOC and PT.

2.6 Statistical Analysis

2.6.1 Sample Size Calculation

There was no formal sample size calculation. We included all vaccinated HCPs and SOTRs who did not decline taking part in the survey.

2.6.2 Planned Analyses and Assessed Variables

Three groups were considered for analysis: HCPs vaccinated with BNT162b2, HCPs vaccinated with mRNA-1273, and SOTRs vaccinated with mRNA-1273. Two main comparisons were planned within these three groups: (i) A comparative analysis of the vaccine tolerability and reactogenicity profile between vaccines in HCPs, after the first and second doses; (ii) a comparative analysis of the tolerability and reactogenicity of vaccination between HCPs and SOTRs vaccinated with mRNA-1273, after the first and second vaccine doses. Tolerability was assessed by a Likert scale ranging from 0 to 10. Reactogenicity was assessed by the proportion of ARs in each group.

The key secondary endpoint was to describe solicited and unsolicited ARs recorded in each group, according to reported frequencies and intensity distribution. Comparison of the risk of any severe AR (Likert scale score 7–10) in the three study groups was also considered as an exploratory endpoint.

2.6.3 Statistical Analysis

Categorical variables were described as frequencies and percentages and continuous variables as mean $\pm$ standard deviation or median (25–75% interquartile range), as appropriate. Categorical data were compared using the

chi-square test and continuous variables using ANOVA with rank-transformed data. To assess baseline homogeneity, we used standardized differences (STDs, differences between groups divided by pooled standard deviation). To compare vaccination in HCPs, the inverse probability of the treatment weights (IPTWs) approach [28] was used to create a pseudo-population in which the two groups were balanced across baseline covariates. The stabilized weights were calculated using propensity scores (PS) [29] aimed to minimize the between-arm standardized differences [30]. Covariate balance was assessed using STDs to achieve absolute values < 0.20 ($< 20\%$), which are acceptable values of variability [31, 32]. Variables included in the IPTW construction were comorbidities; previous exposure to SARS-CoV-2; job position and SARS-CoV-2 occupational contact with patients; sex; age (per 1-year strata).

STDs were also calculated to compare HCPs and SOTRs and compare the three study groups. However, IPTW was not applied in the HCP and SOTR comparisons as they were very different populations and the key covariates inherent in each group were not modifiable, and the main outcome was to describe whether the same vaccine was tolerated differently in the two populations. The primary analysis of the comparison of the risk of ARs between vaccines in HCPs (BNT162b2 and mRNA-1273) after the first and second doses was based on IPTW methods. Sensitivity analyses were performed considering raw unadjusted data and covariate-adjusted data. Raw and adjusted regression models were used to estimate odds ratios (ORs) with 95% confidence intervals (CIs) for all model variables. The area under the curve of the receiver operating characteristics (AUC ROC) curve was calculated to assess model performance.

The primary analysis of the comparison of the risk of ARs between HCPs and SOTRs vaccinated with mRNA-1273 was based on covariate-adjusted data using only age, sex, previous SARS-CoV-2 infection, and a history of drug allergy. Adjusted regression models were used to estimate OR with 95% CI for all model variables. The AUC of the ROC curve was calculated to assess the performance of the model.

To analyze the key secondary endpoint, the rate of vaccinated persons with ≥ 1 AR was calculated for each vaccine and dose. The rate of each individual AR (solicited plus unsolicited) was calculated. Intensity was categorized, according to Likert scale values, as low grade or mild (grade 1; score 0–3), moderate grade (grade 2; score 4–6) and severe grade (grade 3; score 7–10). For each type of AR, the rate of vaccinated personnel with grade 3 ARs and median intensity was calculated.

In all analyses, we applied a two-sided type I error of 5%. SAS v9.4 (Cary, NC, USA) software was used throughout.

3 Results

3.1 Cohort Characteristics

Overall, 7907 HCPs were vaccinated with $\geq$ one vaccine dose. Of these, 6865 received two doses. Sixty four percent (5088) of HCPs participated in the survey and were included in the analysis. Of these, 3564 HCPs were vaccinated with BNT162b2 (of whom 2790 received the second dose), and 1524 were vaccinated with mRNA-1273 (of whom 1331 received the second dose). Likewise, 1806 SOTRs were vaccinated with two doses of mRNA-1273, of whom 77% (1289 patients) participated in the study. Of those, 1289 patients received one dose and 1132 received two doses.

Of the final population, a similar proportion of participants received the second dose in the three groups. There was a higher proportion of female participants amongst HCPs ($p \leq 0.001$, STD = 29), whereas the proportion was inverted in SOTRs, with a higher proportion of male vaccinated participants ($p \leq 0.001$, STD = 74). The mean age was comparable amongst HCPs (41.15 and 43.01 years; $p \leq 0.001$, STD = 14) but was higher in SOTRs (61.21 years; $p \leq 0.001$, STD = 137). Occupational SARS-CoV-2 contact was significantly higher in HCPs vaccinated with BNT162b2 compared with those vaccinated with mRNA-1273 ($p \leq 0.001$, STD = 29). Previous SARS-CoV-2 infection was higher in HCPs vaccinated with mRNA-1273 compared with those vaccinated with BNT162b2 and SOTRs ($p \leq 0.001$, STD = 21 and 35 for the comparisons between HCPs and SOTRs, respectively). SOTRs had a higher proportion of comorbidities, including drug allergies. Baseline characteristics of the population are shown in Table 1.

3.2 Adverse Reactions

3.2.1 Comparison Between Vaccines

In total 85.3% of the 5088 HCPs had $\geq$ one AR. The number of HCPs with any AR was 2878 (81.2%) after vaccination with BNT162b2 and 1463 (92.9%) after vaccination with mRNA-1273 (Table 2).

In the multivariate analysis, mRNA-1273 showed greater reactogenicity than BNT162b2, with an OR for any AR of 3.04 (95% CI 2.48–3.73; $p < 0.001$) in the main analysis adjusted by IPTW. Covariate-adjusted sensitivity analyses consistently showed significant results. Younger age, female sex, a history of drug allergies, and previous SARS-CoV-2 infection were significant in the covariate-adjusted model and increased the risk of ARs. Variables selected from the standard univariate model are shown in Table 3 with their respective ORs and 95% CIs. The ROC AUC curve had a value of 0.770 (95% CI 0.752–0.787). The exploratory

multivariate analysis based on severe ARs showed similar results to the model constructed for any AR (see ESM eTable 12).

Comparing BNT162b2 with mRNA-1273, local ARs such as injection site pain (1228 (34.7%) vs. 838 (49.4%)), arm pain (941 (26.6%) vs. 665 (39.2%)), and systemic ARs such as fatigue (915 (25.9%) vs. 533 (31.4%)), headache (850 (24.0%) vs. 547 (32.3%)), and fever (851 (24.0%) vs. 737 (43.5%)), were the most frequently reported solicited ARs for the two vaccines (see ESM eTable 2). Greater rates of injection site pain, fever, and arm pain were observed in the mRNA-1273 cohort than in the BNT162b2 cohort. Unsolicited AR rates were comparable between vaccines, except for hypersensitivity and lymphadenopathy, which were more frequent with mRNA-1273 (see ESM eTable 2, eFig. 2).

Mild (LS: 0-3) or moderate (LS: 3-6) ARs were reported in 66.3% of participants vaccinated with BNT162b2, and 43.6% of those vaccinated with mRNA-1273. Severe ARs (proportion of ARs with Likert scale score ≥ 7) were reported more frequently with mRNA-1273 than with BNT162b2 (56.4% vs. 33.7%, respectively). Severe systemic ARs were more frequent after the second dose: fatigue, headache, myalgia, malaise, arthralgia. No differences were observed after the second dose for severe local ARs. The mean and median Likert scale scores of ARs were higher with mRNA-1273. No significant differences were observed in the mean and median Likert scale scores between the different types of ARs nor between the first and second vaccine doses. Malaise was the AR with the highest mean and median Likert scale score in both groups (ESM eTables 3–6).

3.2.2 Comparison Between Healthcare Professionals (HCPs) and Solid-Organ Transplant Recipients (SOTRs)

SOTRs had fewer ARs than HCPs (1016 out of 1289 (78.8%) vs. 1463 out of 1524 (96%), respectively). A lower proportion of ARs was reported after the second dose in both groups (Table 4). In the covariate-adjusted multivariate analysis, the variable “population (SOTRs vs. HCPs)” was significant, with OR = 0.36 (95% CI 0.25–0.50). Younger age, previous SARS-CoV-2 infection, and female sex were significant and increased the risk of reactogenicity risk. A history of drug allergies was not a significant covariate in SOTRs in the covariate-adjusted multivariate analysis. Variables selected from the standard univariate model to build the multivariate model are shown in Table 5 with their respective ORs and 95% CIs. The ROC AUC curve had a value of 0.778 (0.752–0.805). The exploratory multivariate analysis considering severe ARs showed comparable results to the model constructed for any AR (see ESM eTable 12), except for drug allergies, which yielded significant results in SOTRs, unlike the main analysis with any AR.

Table 1 Baseline characteristics of participants: healthcare professionals (HCPs) and solid-organ transplant recipients (SOTRs)

	Population			Comparisons			
	HCP BNT162b2 (n = 3564) n (%)	HCP mRNA-1273 (n = 1524) n (%)	SOTR mRNA-1273 (n = 1289) n (%)	HCP BNT162b2 vs. HCP mRNA-1273		HCP mRNA-1273 vs. SOTR mRNA-1273	
				p value	STD (%)	p value	STD (%)
<i>Number of doses</i>							
1 dose	3564 (100)	1524 (100)	1289 (100)	–	0	–	0
2 doses	2790 (78.3)	1331 (87.3)	1132 (87.8)	< 0.001	24	0.698	1
<i>Gender</i>							
Male	1044 (29.3)	461 (30.2)	838 (65.0)				
Female	2520 (70.7)	1063 (69.8)	451 (35.0)	0.494	2	< 0.0001	74
<i>Age (years)</i>							
Mean (SD)	41.15 (12.60)	43.01 (14.20)	61.21 (12.29)				
Median (P25, P75)	41.00 (30.0, 52.0)	43.00 (30.0, 55.0)	62 (54.0, 70.0)	< 0.001	14	< 0.001	137
<i>Age strata (n, %)</i>							
18–30	974 (27.3)	395 (25.9)	23 (1.8)				
31–40	794 (22.3)	289 (19.0)	51 (4.0)				
41–50	806 (22.6)	316 (20.7)	164 (12.7)				
51–65	942 (26.4)	470 (30.8)	518 (40.2)				
> 65 years	48 (1.3)	54 (3.5)	533 (41.3)	< 0.0001	14	< 0.001	141
<i>Occupational SARS-CoV-2 contact</i>							
In contact with SARS-CoV-2 patients	1644 (46.1)	484 (31.8)	–	< 0.001	29	–	–
Without contact with SARS-CoV-2 patients	1915 (53.7)	1038 (68.1)	–	< 0.001	29	–	–
<i>Previous SARS-CoV-2 infection^a</i>							
Yes	354 (9.9)	262 (17.2)	78 (6.1)				
No	3210 (90.1)	1262 (82.8)	1211 (93.9)	< 0.001	21	< 0.001	35
<i>Comorbidities</i>							
Any comorbidity ^b	1267 (35.5)	512 (33.6)	705 (54.7)	0.404	4	< 0.001	43
Hypertension	122 (3.4)	61 (4.0)	310 (24.0)	0.309	3	< 0.001	60
Diabetes mellitus	38 (1.1)	18 (1.2)	223 (17.3)	0.719	1	< 0.001	58
Heart failure	10 (0.3)	2 (0.1)	37 (2.9)	0.314	3	< 0.001	23
Chronic bronchitis	7 (0.2)	6 (0.4)	7 (0.5)	0.202	4	0.561	2
Asthma	126 (3.5)	46 (3.0)	11 (0.9)	0.350	3	< 0.001	16
Rheumatic/immune-mediated disease	33 (0.9)	12 (0.8)	9 (0.7)	0.629	2	0.784	1
<i>Drug allergies</i>	294 (8.2)	113 (7.4)	145 (11.2)	0.315	3	0.000	13
<i>Food allergies</i>	154 (4.3)	54 (3.5)	19 (1.5)	0.199	4	0.001	13

^aDiagnosis by RCP, antigen test, and antibody positive serology^bNumber of vaccinees with at least one comorbidity

A smaller proportion of solicited and unsolicited ARs was reported in the group of SOTRs, compared with HCPs. There were fewer systemic ARs, such as fever (202 (15.7%) vs. 774 (50.8%)), malaise (113 (8.8%) vs. 400 (26.2%)), chills (109 (8.5%) vs. 341 (22.4%)), fatigue (251 (19.5%) vs.

532 (34.9%), myalgia (62 (4.8%) vs. 337 (22.1%), arthralgia (39 (3.0%) vs. 203 (13.3%)), as well as a smaller proportion of some local and reactogenic reactions, such as injection site redness (28 (2.2%) vs. 145 (9.5%)), injection site pruritus (21 (1.6%) vs. 87 (5.7%)) and arm pain (345 (26.8%) vs.

Table 2 Main results of the comparison of BNT162b2 vs. mRNA-1273 in HCPs. Raw and IPTW analyses on proportions of any adverse reaction

Patients with ≥ 1 adverse reaction	BNT162b2 (<i>n</i> = 3564) <i>n</i> (%)	mRNA-1273 (<i>n</i> = 1524) <i>n</i> (%)
Raw analysis		
Overall	2878 (80.8)	1463 (96.0)
Reactions after first dose	2054 (57.6)	1252 (82.2)
Reactions after second dose	2161 (60.6)	1209 (79.3)
IPTW analysis		
Overall	2873 (81.2)	1576 (92.9)
Reactions after first dose	2038 (57.6)	1344 (79.2)
Reactions after second dose	2190 (61.9)	1229 (72.5)

Frequency of adverse reactions in both vaccinated groups of HCPs (BNT162b2 and mRNA-1273) and after each dose of vaccine; *p* value < 0.001 for all comparisons. Main results are displayed in bold. HCPs healthcare professionals, IPTW inverse probability of the treatment weights

624 (40.9%)). There was also a lower proportion of immune-mediated ARs, such as hypersensitivity or lymphadenopathy (see ESM eTable 7, eFig. 3).

Mild (Likert 0–3) or moderate (Likert 3–6) ARs were reported in 75.6% of SOTRs and 41.9% of HCPs vaccinated with mRNA-1273. Severe AR (Likert score ≥ 7) were reported more frequently in HCPs after vaccination with mRNA-1273 (58.1% vs. 24.4%, respectively). Severe systemic ARs (fatigue, headache, myalgia, malaise, arthralgia) were more frequent after the second dose in HCPs, but not in SOTRs. No significant differences were observed after the second dose for severe local ARs in either group. Mean and median Likert scores were lower for SOTRs, for the whole set of ARs and for each specific AR (ESM eTables 8–11). Arthralgia was the AR with the highest mean and median Likert score in both groups.

4 Discussion

The relevance of our study lies in the provision of additional real-world evidence on the safety profile of vaccination, in a prospective comparative study where two vaccines (BNT162b2 and mRNA-1273) and two populations (HCPs and SOTRs) vaccinated with mRNA-1273 were compared using the same structured questionnaire.

Randomized clinical trials of mRNA-based vaccines reported an overall acceptable safety profile [1, 2]. The higher rate observed for mRNA-1273 in our study (increase of 11.7% in the rate of any observed AR compared with BNT162b2 (92.9% vs. 81.2%)) is consistent with that reported for the pivotal mRNA-1273 study, and the profile

of reported systemic and local ARs. However, while this higher rate for mRNA-1273 is consistent with reports from the respective clinical trials, the rate of any AR found for the two vaccines was substantially higher than that reported in clinical trials (81.2% vs. 27% and 92.9% vs. 87.8% for BNT162b2 and mRNA-1273, respectively). This may reflect differences in the adverse events reported between studies. For instance, data related to the pivotal clinical trial of BNT162b2 must be interpreted with caution, since the rate of any AR was calculated from the overall study population, whereas local and systemic ARs were collected from a “reactogenicity” subset of vaccinated persons. This is clearly a different way of reporting ARs in the pivotal Pfizer study. Despite these differences, our results are in line with safety information provided for BNT162b2 and mRNA-1273 in the respective Summaries of Product Characteristics [23, 26]

After the pivotal clinical trials, millions of doses of mRNA and other SARS-CoV-2 vaccines have been administered, including booster doses. Large-scale studies have been conducted, reporting that SARS-CoV-2 vaccines are safe [6, 33]. However, these studies are mainly based on electronic health records or adverse event reporting systems, and it is likely that mild or moderate symptoms are not systematically reported. Liu et al. analyzed 26 safety studies in a recent systematic review and meta-analysis of real-world studies of SARS-CoV-2 vaccines [34]. Sample sizes ranged from 77 to 2041 vaccinated persons. A large Japanese study of BNT162b2 assessed the safety of millions of vaccinated persons in Japan, although based only on spontaneous AR reporting [35]. Most of the 26 studies included were based on retrospective assessments of data or a single SARS-CoV-2 vaccine, and only a few small studies provided real-world comparative data. Most cases were based on retrospective assessment of data. Other studies with HCPs have been published, such as those by Riad et al. in HCPs in the Czech Republic and Slovakia [8, 9], with a similar sample size to the studies included in Liu’s revision (522 and 922 participants, respectively). A more recent Japanese study included 3254 HCPs vaccinated with BNT162b2 [11], and another recent Maltese study included 1480 HCPs vaccinated with BNT162b2 [36]. Another recent study, designed as a cohort event-monitoring study, included 22,184 participants vaccinated with BNT162b2, mRNA-1273, and other types of vaccines, such as Vaxzevria® [7]. Our results (in terms of proportions, severity, and types of reported AR) are in line with the results of these published studies.

Some prospective studies have specifically targeted safety in vaccinated SOTR. Cucchiari et al. found that patient-reported side effects on a semiquantitative scale (none/mild/moderate/severe) were consistent with the pivotal trial, and no donor-specific antibodies were detected [13]. Another prospective study in kidney transplant recipients (the RECOVAC-IR study) is ongoing [37]. Ou et al. studied

Table 3 Comparison 1: BNT162b2 vs. mRNA-1273 in HCPs. Risk of any adverse reaction (AR) estimated by inverse probability of the treatment weights (IPTW) and univariate and multivariate logistic regression models

Variables	Univariate model		Multivariate model ^a		IPTW model	
	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value	OR (95% CI)	<i>p</i> value
BNT162b2 (Ref: mRNA-1273)	5.72 (4.37–7.48)	< 0.001	5.40 (4.08–7.13)	< 0.001	3.04 (2.48–3.73)	< 0.001
Female sex	1.89 (1.61–2.22)	< 0.001	1.99 (1.68–2.37)	< 0.001	–	–
Age (risk change per 1 year younger)	1.02 (1.01–1.02)	< 0.001	1.02 (1.01–1.03)	< 0.001	–	–
<i>Age strata (year)</i>						
≤ 30	1 (Ref)	< 0.001	–	–	–	–
31–40	0.95 (0.74–1.21)		–	–	–	–
41–50	0.74 (0.59–0.94)		–	–	–	–
51–65	0.59 (0.48–0.73)		–	–	–	–
> 65	0.30 (0.19–0.47)		–	–	–	–
Previous SARS-CoV-2 occupational contact	1.05 (0.89–1.23)	0.573	–	–	–	–
Previous SARS-CoV-2 infection	1.95 (1.46–2.62)	< 0.001	1.77 (1.30–2.41)	< 0.001	–	–
Any previous comorbidity	1.31 (1.18–1.42)	< 0.001	–	–	–	–
Hypertension	0.87 (0.59–1.30)	0.505	–	–	–	–
Diabetes mellitus	1.03 (0.49–2.19)	0.934	–	–	–	–
Rheumatic/immune-mediated disease	0.93 (0.41–2.09)	0.865	–	–	–	–
Asthma	1.49 (0.91–2.44)	0.115	–	–	–	–
Heart failure	1.90 (0.24–14.70)	0.541	–	–	–	–
Chronic bronchitis	2.07 (0.27–15.93)	0.486	–	–	–	–
Previous drug allergies	1.46 (1.05–2.01)	0.022	1.57 (1.12–2.21)	0.010	–	–

Odds ratio (OR) (95% confidence interval (CI)) was used as the measure of the risk. Univariate and multivariate models were constructed as sensitivity analyses. The multivariate model was constructed using a forward stepwise selection approach of significant variables at univariate testing. Main results are displayed in bold

HCPs healthcare professionals

^aROC-AUC: 0.770 (0.752–0.787)

the safety and reactogenicity of BNT162b2 and mRNA-1273 in 741 SOTRs [38], and concluded that reactogenicity was similar to that reported in the original trials and severe reactions were rare. Overall, these previous safety data can help address vaccine hesitancy in SOTRs and other groups of immunocompromised patients. Nevertheless, further “real-world” data from prospective comparative studies in a “healthy population” are still needed to address in greater depth the comparative reactogenicity profile and thus consolidate this reassuring message. In a recent Israeli study, 80 liver transplant (LT) recipients and 25 controls were followed-up for 7–10 weeks after receiving the second dose of BNT162b2 [20]. Injection site reactions were reported at a similar frequency in both groups following the first and second doses. While the frequency of systemic events was similar in both groups after the first vaccination, they were significantly less frequent following the second dose among LT recipients. The most common systemic side effect was fatigue, followed by headache and myalgia. In our study, a

decrease in the frequency and severity of ARs was already seen after the first dose, including injection site reactions.

Our study helps provide additional prospective comparative data on solicited and unsolicited ARs in a large sample size, thus providing robust comparative data from two vaccines and two populations of vaccinated persons with the same vaccine. In line with previous clinical trials and real-world studies, our results show a predominance of local, mild AR, which can be seen as a reassuring message of the safety of vaccination.

In addition, our data provide some evidence on the impact of relevant covariates, such as age, sex, and previous SARS-CoV-2 infection, showing a consistent effect independently of the scenario studied. In HCPs, mRNA-1273 showed a higher rate of any observed AR compared with BNT162b2. The severity of AR (Likert score ≥ 3) was also higher for mRNA-1273. Lower age, female sex, previous SARS-CoV-2 infection, and a history of drug allergies were significant independent covariates associated with the risk

Table 4 Main results of the comparison of solid-organ transplant recipients (SOTRs) and healthcare professionals (HCPs). Proportions of any adverse reaction

Patients with ≥ 1 adverse reaction	SOTRs ($n = 1289$) n (%)	HCPs ($n = 1524$) n (%)
<i>Raw analysis^a</i>		
Overall	1016 (78.8)	1463 (96.0)
Reactions after first dose	875 (67.9)	1252 (82.2)
Reactions after second dose	699 (54.2)	1209 (79.3)

Frequency of ARs in SOTRs and HCPs (both vaccinated with mRNA-1273) and after each dose of vaccine. Main results are displayed in bold

^aNo IPTW or adjustment analyses were conducted since key covariates were inherent to any or other group and were not modifiable characteristics; p value < 0.001 for all comparisons

IPTW inverse probability of the treatment weights

of ARs, consistent with earlier reports [39–43]. The ROC AUC showed the acceptable performance of the multivariate model.

Comparison between HCPs and SOTRs (all vaccinated with mRNA-1273) showed a lower proportion and lower intensity of ARs in the latter group. This trend was

consistent in the covariate-adjusted multivariate analysis. Age, sex, previous exposure to SARS-CoV-2, and belonging to the group of SOTRs showed a significant impact in the development of ARs. Previous history of drug allergies, which was a significant covariate in the covariate-adjusted analysis of HCP, was only significant in SOTRs when severe ARs were considered. These findings strongly support the idea that immunosuppressive therapy—and reduced immunogenicity—can play a role in the short-term tolerability of mRNA SARS-CoV-2 vaccines.

Less clear is the influence of sex on the tolerability of vaccination in healthy persons and SOTRs. Pivotal clinical trials did not specifically address potential sex-related differences. We observed more ARs in vaccinated females. Indeed, sex-based differences do not seem to be circumscribed to vaccines, but rather seem a general pattern also shown by other types of medicinal products [44, 45]. Sex-based immunological differences have been hypothesized and our data could at least partially support this [46, 47]. However, other underlying mechanisms such as pharmacokinetics/pharmacodynamics, and social and behavioral causes still require further elucidation.

In sum, the results of this study show an increased reactivity of mRNA-1273 in terms of a higher proportion

Table 5 Comparison 2: solid-organ transplant recipients (SOTRs) vs. healthcare professionals (HCPs) (vaccinated with mRNA-1273). Risk of any adverse reaction (AR) estimated by univariate and multivariate logistic regression models

Variables	Univariate model		Multivariate model ^a	
	OR (95% CI)	p value	OR (95% CI)	p value
Population (SOTRs vs. HCPs)	0.16 (0.12–0.21)	< 0.001	0.36 (0.25–0.50)	< 0.001
Sex	2.83 (2.22–3.61)	< 0.001	1.55 (1.19–2.02)	0.001
Age (risk change per 1 year younger)	1.06 (1.05–1.08)	< 0.001	1.04 (1.03–1.05)	< 0.001
<i>Age strata (year)</i>				
≤ 30	1 (Ref)	< 0.001	–	–
31–40	1.09 (0.45–2.61)		–	–
41–50	0.40 (0.20–0.79)		–	–
51–65	0.25 (0.13–0.45)		–	–
> 65	0.07 (0.04–0.13)		–	–
Previous SARS-CoV-2 infection	2.99 (1.78–5.00)	< 0.001	2.09 (1.22–3.60)	0.008
Any previous comorbidity	1.05 (0.80–1.25)	0.667	–	–
Hypertension	0.69 (0.51–0.93)	0.017	–	–
Diabetes mellitus	0.75 (0.51–1.08)	0.125	–	–
Rheumatic/immune-mediated disease	2.71 (0.36–20.22)	0.332	–	–
Asthma	1.41 (0.56–3.55)	0.468	–	–
Heart failure	0.30 (0.15–0.59)	< 0.001	–	–
Chronic bronchitis	0.74 (0.16–3.35)	0.696	–	–
Previous drug allergies	3.20 (1.00–10.21)	0.049	1.42 (0.91–2.21)	0.120

The risk of ARs in the main comparison between vaccines was calculated. Odds ratio (OR) (95% confidence interval (CI)) was used as the measure of the risk. Univariate and multivariate models were constructed as sensitivity analyses. The multivariate model was constructed using a forward stepwise selection approach of significant variables at univariate testing

^aROC-AUC: 0.778 (0.752–0.805)

of ARs, but with an adequate tolerability profile of the two mRNA vaccines substantiated by the high number of mild and moderate ARs. A better reactogenicity and tolerability profile of mRNA-1273 was observed in SOTRs, in terms of a lower proportion of ARs and a lower proportion of severe ARs.

Our study had limitations linked to the observational and non-randomized nature, such as recall bias and potential confounders that may persist after the application of balancing and adjustment methods. In addition, vaccinated participants who agreed to participate may not be fully representative of the general population (e.g., participants who engage may be more prone to report ARs). The study was designed to capture short-term ARs and, due to its sample size, was not powered to detect rare ARs. In addition, severity (according to CIOMS (Council for International Organizations of Medical Sciences) criteria) was not assessed. Considering the results of clinical trials, which provided the core reference safety information when the study was designed, major complications or major reactions leading to hospital/emergency room admission or death within a short timeframe were in principle not expected. Therefore, severity was not collected in the questionnaire.

5 Conclusions

Our analysis supports existing evidence that mRNA-1273 has greater short-term reactogenicity than BNT162b2. However, most observed ARs were local and mild in intensity, confirming the tolerable safety profile of the two vaccines. These results may be interpreted as a reassuring message for the medical and scientific community, even if vaccinated participants who agreed to participate may not be fully representative of the general population.

Data suggest a link between increased reactogenicity and greater immunogenicity, as suggested by the fact that younger age and previous exposure to SARS-CoV-2 were consistently associated with an increased incidence of ARs, while a reduced immune status was linked to reduced vaccine reactogenicity and better tolerability. The effect of sex must be further examined. The correlation of reported ARs and immunologic parameters could provide further insight into the role of immunogenicity. This is a question our group is currently trying to answer in a second project.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s40259-022-00543-9>.

Acknowledgements The authors confirm that all persons named in the Acknowledgement section have given written permission to be included in the manuscript. We thank the nursing and administrative teams who run and coordinate the vaccination campaign at Hospital Clinic of Barcelona and the collaborators of the VigilVacCOVID

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Declarations

Funding The study was made using the resources of the Hospital Clinic. No grants/funding were received for this study.

Conflict of interest None of the authors have any conflicts of interest to disclose.

Availability of data and material No data are available.

Ethical approval All procedures performed in studies involving human participants were carried out in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Research Ethics Committee of the Hospital Clinic (HCB/2021/0684 and HCB/2021/0685).

Consent A waiver of informed consent was approved for the project, since the surveillance program was considered part of normal medical care activity in the context of an innovative therapeutic strategy never tested before. However, an information leaflet on the program was made available to all vaccinated subjects. Participation in the surveillance was voluntary.

Author contributions The corresponding author (JSP) states that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. Joaquín Sáez-Peñataro (JSP), Gonzalo Calvo (GC), and Ferran Torres (FT) had full access to all of the data

in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: JSP, GC, FT, and Joan Bartra (JB). Acquisition, analysis, or interpretation of data: JSP, GC, FT, JB, Juan Bascuas (JBA), Anna Vilella (AV), Marta Tortajada (MT), Sebastiana Quesada (SQ), Elisenda González (EG), Ester López-Suñé (ELS), Antoni Castells (AC), Sandra Serrano (SS), Concepción Camacho (CC), and Antoni Trilla (AT). Drafting of the manuscript: JSP, GC, FT, and JBA. Critical revision of the manuscript for important intellectual content: JSP, GC, FT, JB, JBA, AV, MT, SQ, EG, ELS, AC, SS, CC, and AT. Statistical analysis: FT. Data management and EDC: SS. The lead author (JSP) confirms that the article is an honest, accurate, and transparent account of the study being reported and there are no important aspects of the study that have been omitted or any discrepancies from the study as planned have been reported and explained.

Code availability Not applicable.

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ELECTRONIC SUPPLEMENTARY MATERIAL

Journal: Biodrugs

Tolerability and reactogenicity profile of mRNA SAR-CoV-2 vaccines from a mass vaccination campaign in a tertiary hospital: Between-vaccine and between-population prospective, observational study (VigilVacCOVID Study)

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CONTENTS

Tables:

eTable 1 Baseline characteristics of participants according to IPTW (comparison between HCP)

eTable 2 Comparison between HCP: Distribution of solicited and unsolicited AR per vaccine group (BNT162b2 and mRNA-1273) and dose (raw and IPTW data)

eTable 3 Distribution of severe AR per vaccine group (BNT162b2 and mRNA-1273) and dose

eTable 4 Distribution of severe AR per vaccine group (BNT162b2 and mRNA-1273) and dose, as per specific types of AR

eTable 5 Mean and median Likert Score values per vaccine group (BNT162b2 and mRNA-1273) and dose

eTable 6 Mean and median Likert Score values per vaccine group (BNT162b2 and mRNA-1273) and dose, as per specific types of AR

eTable 7 Distribution of solicited and unsolicited AR per group (HCP and SOTR) and dose

eTable 8 Distribution of severe AR per group (HCP and SOTR) and dose

eTable 9 Distribution of severe AR per group (HCP and SOTR) and dose, as per specific types of AR

eTable 10 Mean and median Likert Score values per group (HCP and SOTR) and dose

eTable 11 Mean and median Likert Score values per group (HCP and SOTR) and dose, as per specific types of AR

eTable 12 Multivariate logistic regression analysis with covariates. Exploratory analyses with any severe AR

eTable 13 Predefined list of AR

eTable 14 List of AR of special interest

eTable 15 STROBE Checklist

eFigure 1 Questionnaire

eFigure 2 BNT162b2 vs mRNA-1273. Distribution of AR per vaccine group and dose

eFigure 3 SOTR vs HCP (mRNA-1273). Distribution of AR per vaccine group and dose

eTable 1: Baseline characteristics according to vaccine exposure in HCP (raw and IPTW data)

Baseline covariate	Raw analysis				IPTW analysis			
	BNT162b2 (n=3564) No. (%)	mRNA-1273 (n=1524) No (%)	p-value	^a SD (%)	BNT162b2 (n= 3536.92) No. (%)	mRNA-1273 (n= 1695.61) No (%)	p-value	SD (%)
	n= 5088				n= 5232.53			
Number of doses								
1 dose	3564 (100)	1524 (100)		0	3537(100.0)	1696 (100.0)		0
2 doses	2790 (78.3)	1331 (87.3)	<0.001	24.2	2846 (80.5)	1379 (81.3)	0.455	2.2
Gender								
Male	1044 (29.3)	461 (30.2)		2.1	1030 (29.1)	480 (28.3)		1.8
Female	2520 (70.7)	1063 (69.8)	0.494		2507 (70.9)	1215 (71.7)	0.547	
Age (yeAR)								
Mean (SD)	41.2 (12.6)	43.0 (14.2)	<0.001	13.9	41.62(13.04)	42.65(13.92)	0.012	7.7
Median (P25, P75)	41.00 (30.0, 52.0)	43.00 (30.0, 55.0)			41 (30.00, 53.00)	42.00(31.00,54.0)		
Age strata (n, %)								
18-30	974 (27.3)	395 (25.9)		14.0	958 (27.1)	407 (24.0)		7.3
31-40	794 (22.3)	289 (19.0)	<0.001		754 (21.3)	371 (21.9)	0.122	
41-50	806 (22.6)	316 (20.7)			776 (21.9)	373 (22.0)		
51-65	942 (26.4)	470 (30.8)			981 (27.7)	515 (30.4)		
>65	48 (1.3)	54 (3.5)			68 (1.9)	29 (1.7)		
Occupational SAR-CoV-2 contact								
In contact with SAR-CoV-2 patients	1644 (46.1)	484 (31.8)	<0.001	29.0	1477 (41.8)	605 (35.7)	0.001	12.3
Without contact with SAR-CoV-2 patients	1915 (53.7)	1038 (31.8)			2056 (58.1)	1089 (64.2)		
^bPrevious SAR-CoV-2 infection								
Yes	354 (9.9)	262 (17.2)	<0.001	21.3	407 (11.5)	215 (12.7)	0.224	3.6
No	3210 (90.1)	1262 (82.8)			3130 (88.5)	1481 (87.3)		

Baseline covariate	Raw analysis				IPTW analysis			
	BNT162b2 (n=3564) No. (%)	mRNA-1273 (n=1524) No (%)	p-value	^a SD (%)	BNT162b2 (n= 3536.92) No. (%)	mRNA-1273 (n= 1695.61) No (%)	p-value	SD (%)
	n= 5088				n= 5232.53			
Comorbidities ^c Any comorbidity Arterial hypertension Diabetes mellitus Heart failure Chronic bronchitis Asthma Rheumatic/immune-mediated disease	1267 (35.5)	512 (33.6)	0.404	4.2	1235 (34.9)	619 (36.5)	0.471	4.2
	122 (3.4)	61 (4)	0.309	3.1	129 (3.6)	55 (3.2)	0.430	2.4
	38 (1.1)	18 (1.2)	0.719	1.1	37 (1.0)	20 (1.2)	0.686	1.2
	10 (0.3)	2 (0.1)	0.314	3.3	12 (0.3)	2 (0.1)	0.161	4.5
	7 (0.2)	6 (0.4)	0.202	3.6	7 (0.2)	4 (0.3)	0.613	1.5
	126 (3.5)	46 (3)	0.350	2.9	122 (3.5)	67 (3.9)	0.374	2.6
	33 (0.9)	12 (0.8)	0.629	1.5	31 (0.9)	10 (0.6)	0.275	3.3
Drug allergies	294 (8.2)	113 (7.4)	0.315	3.1	283 (8.0)	151 (8.9)	0.273	3.2
Food allergies	154 (4.3)	54 (3.5)	0.199	4.0	141 (4.0)	55 (3.2)	0.179	4.0

HCP: Healthcare professionals; ^aSTD= Standardized differences; ^bDiagnosis by RCP, antigen test and antibody positive serology; ^cNumber of vaccinees with at least one comorbidity

eTable 2 Comparison between HCP: Distribution of solicited and unsolicited AR per vaccine group and dose

Raw analysis				^a p-value	IPTW analysis				^a p-value	
BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			
Total AR		Total AR			Total AR		Total AR			
AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
Solicited adverse reactions										
SOC: General disorders and administration site conditions										
Injection site pain <i>PT: Injection site pain</i>	1248 (35)		766 (50.3)		<0.001	1228 (34.7)		838 (49.4)		<0.001
	1010 (28.3)	519 (14.6)	569 (37.3)	374 (24.5)		991 (28.0)	522 (14.7)	609 (35.9)	433 (25.5)	
	274 (7.7)	735 (20.6)	223 (14.6)	372 (24.4)		274 (7.7)	738 (20.9)	213 (12.6)	383 (22.6)	
Fatigue/asthenia <i>PT: Fatigue</i>	910 (25.5)		532 (34.9)		<0.001	915 (25.9)		533 (31.4)		<0.001
	841 (23.6)	769 (21.6)	216 (14.2)	671 (44.0)		851 (24.0)	737 (43.5)			
	114 (3.2)	769 (21.6)	216 (14.2)	671 (44.0)		119 (3.4)	779 (22.0)	185 (10.9)	653 (38.5)	
Fever <i>PT: Pyrexia</i>	529 (14.8)		400 (26.2)		<0.001	532 (15.0)		387 (22.8)		<0.001
	85 (2.4)	464 (13.0)	133 (8.7)	297 (19.5)		85 (2.4)	466 (13.2)	133 (7.8)	276 (16.3)	
	326 (9.1)	341 (22.4)	3205 (90.6)	1361 (80.2)		40 (1.1)	298 (8.4)	78 (4.6)	282 (16.6)	
Malaise <i>PT: Malaise</i>	326 (9.1)		341 (22.4)		<0.001	3205 (90.6)		1361 (80.2)		<0.001
	40 (1.1)	292 (8.2)	81 (5.3)	284 (18.6)		40 (1.1)	298 (8.4)	78 (4.6)	282 (16.6)	
	102 (2.9)	145 (9.5)	101 (2.9)	139 (8.2)						
Injection site redness	102 (2.9)		145 (9.5)			101 (2.9)		139 (8.2)		

Adverse reactions (PT MedDRA term)	Raw analysis				^a p- value	IPTW analysis				^a p- value
	BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)		
	Total AR		Total AR			Total AR		Total AR		
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	
	57 (1.6)	45 (1.3)	87 (5.7)	70 (4.6)		56 (1.6)	44 (1.2)	78 (4.6)	72 (4.3)	
<i>PT:</i> <i>Application site redness</i>					<0.001					<0.001
SOC: Musculoskeletal and connective tissue disorders										
Arm pain <i>PT:</i> <i>Pain in extremity</i>	938 (26.3)		624 (40.9)		<0.001	941 (26.6)		665 (39.2)		<0.001
	586 (16.4)	509 (14.3)	476 (31.2)	277 (18.2)		587 (16.6)	513 (14.5)	506 (29.8)	311 (18.4)	
Muscle pain <i>PT:</i> <i>Myalgia</i>	647 (18.2)		337 (22.1)		0.001	638 (18.0)		357 (21.0)		0.0092
	83 (2.3)	584 (16.4)	94 (6.2)	266 (17.5)		81 (2.3)	578 (16.4)	87 (5.1)	288 (17.0)	
Joint pain <i>PT:</i> <i>Arthralgia</i>	266 (7.5)		203 (13.3)		<0.001	265 (7.5)		207 (12.2)		<0.001
	14 (0.4)	257 (7.2)	49 (3.2)	165 (10.8)		15 (0.4)	256 (7.2)	46 (2.7)	178 (10.5)	
Shoulder pain <i>PT:</i> <i>Musculoskeletal pain</i>	81 (2.3)		93 (6.1)		<0.001	77 (2.2)		123 (7.2)		<0.001
	56 (1.6)	34 (1.0)	36 (2.4)	59 (3.9)		54 (1.5)	33 (0.9)	73 (4.3)	51 (3.0)	

Raw analysis				^a p-value		IPTW analysis				^a p-value			
Adverse reactions (PT MedDRA term)						BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)					
Total AR						Total AR		Total AR					
AR after 1 st dose		AR after 2 nd dose				AR after 1 st dose		AR after 2 nd dose					
SOC: Injury, poisoning and procedural complications													
Injection site swelling PT: Application site swelling		167 (4.7)		228 (15.0)		<0.001		167 (4.7)		247 (14.5)		<0.0001	
		98 (2.7)	79 (2.2)	137 (9.0)	113 (7.4)			98 (2.8)	80 (2.3)	134 (7.9)	136 (8.0)		
SOC: Vaccination site pruritus													
Injection site pruritus PT: Vaccination site pruritus		30 (0.8)		87 (5.7)		<0.001		31 (0.9)		81 (4.8)		<0.0001	
		18 (0.5)	14 (0.4)	55 (3.6)	38 (2.5)			19 (0.5)	14 (0.4)	48 (2.8)	39 (2.3)		
SOC: Nervous system disorders													
Headache PT: Headache		847 (23.8)		511 (33.5)		<0.001		850 (24.0)		547 (32.3)		<0.0001	
		269 (7.5)	648 (18.2)	216 (14.2)	383 (25.1)			276 (7.8)	648 (18.3)	237 (14.0)	390 (23.0)		
Facial paralysis PT: Facial paralysis		1 (0.0)		0 (0.0)		0.513		1 (0.0)		0 (0.0)		0.5559	
		1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)			1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		
Insomnia PT: Insomnia		33 (0.9)		15 (1.0)		0.844		32 (0.9)		12 (0.7)		0.5108	
		11 (0.3)	23 (0.6)	3 (0.2)	13 (0.9)			11 (0.3)	22 (0.6)	3 (0.2)	10 (0.6)		

Adverse reactions (PT MedDRA term)	Raw analysis				^a p- value	IPTW analysis				^a p- value
	BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)		
	Total AR		Total AR			Total AR		Total AR		
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	
	SOC: Gastrointestinal disorders									
Nausea PT: Nausea	131 (3.7)		97 (6.4)		<0.001	132 (3.7)		97 (5.7)		0.0010
	35 (1.0)	99 (2.8)	43 (2.8)	61 (4.0)		36 (1.0)	99 (2.8)	41 (2.4)	62 (3.7)	
Diarrhea PT: Diarrhoea	111 (3.1)		36 (2.4)		0.142	107 (3.0)		37 (2.2)		0.0933
	38 (1.1)	79 (2.2)	13 (0.9)	23 (1.5)		34 (1.0)	78 (2.2)	15 (0.9)	22 (1.3)	
Vomiting PT: Vomiting	50 (1.4)		41 (2.7)		0.002	49 (1.4)		37 (2.2)		0.0375
	14 (0.4)	38 (1.1)	4 (0.3)	38 (2.5)		13 (0.4)	38 (1.1)	4 (0.2)	34 (2.0)	
Unsolicited adverse reactions										
SOC: Musculoskeletal and connective tissue disorders										
Other musculoskeletal disorders PT: Musculoskeletal disorder	85 (2.4)		41 (2.7)		0.521	90 (2.6)		43 (2.5)		0.9554
	28 (0.8)	59 (1.7)	18 (1.2)	25 (1.6)		30 (0.8)	63 (1.8)	23 (1.4)	22 (1.3)	
SOC: Skin and subcutaneous tissue disorders										
Petechia, ecchymosis PT: Ecchymosis	12 (0.3)		10 (0.7)		0.112	12 (0.4)		9 (0.5)		0.2974
	8 (0.2)	4 (0.1)	5 (0.3)	5 (0.3)		8 (0.2)	4 (0.1)	4 (0.2)	5 (0.3)	

Adverse reactions (PT MedDRA term)	Raw analysis				^a p- value	IPTW analysis				^a p- value
	BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)		
	Total AR		Total AR			Total AR		Total AR		
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	
	Total AR		Total AR			Total AR		Total AR		
SOC: Nervous system disorders										
Cognitive alteration <i>PT:</i> <i>Cognitive disorder</i>	6 (0.2)		5 (0.3)		0.261	6 (0.2)		4 (0.3)		0.5547
	5 (0.1)	1 (0.0)	2 (0.1)	3 (0.2)		5 (0.1)	1 (0.0)	1 (0.1)	3 (0.2)	
Alterations of smell and taste <i>PT:</i> <i>Parosmia</i>	8 (0.2)		3 (0.2)		0.846	7 (0.2)		5 (0.3)		0.5154
	5 (0.1)	3 (0.1)	2 (0.1)	1 (0.1)		4 (0.1)	3 (0.1)	4 (0.2)	1 (0.0)	
Paresthesia and hyperesthesia <i>PT:</i> <i>Dysaesthesia</i>	25 (0.7)		10 (0.7)		0.858	25 (0.7)		11 (0.7)		0.8788
	12 (0.3)	15 (0.4)	5 (0.3)	5 (0.3)		12 (0.3)	15 (0.4)	7 (0.4)	4 (0.3)	
Presyncope, syncope and vasovagal syncope <i>PT:</i> <i>Syncope</i>	5 (0.1)		5 (0.3)		0.166	5 (0.1)		4 (0.3)		0.3375
	0 (0.0)	5 (0.1)	2 (0.1)	3 (0.2)		0 (0.0)	5 (0.1)	2 (0.1)	3 (0.2)	
Sleepiness, hypersomnia <i>PT:</i> <i>Hypersomnia</i>	16 (0.4)		11 (0.7)		0.219	16 (0.4)		10 (0.6)		0.4227
	5 (0.1)	11 (0.3)	3 (0.2)	8 (0.5)		5 (0.1)	11 (0.3)	2 (0.1)	8 (0.5)	
Instability sensation, vertigo, sickness <i>PT:</i> <i>Dizziness</i>	72 (2.0)		51 (3.3)		0.005	77 (2.2)		51 (3.0)		0.0681
	22 (0.6)	50 (1.4)	24 (1.6)	30 (2.0)		24 (0.7)	53 (1.5)	28 (1.7)	25 (1.5)	
Tremor	5 (0.1)		5 (0.3)		0.166	5 (0.1)		4 (0.2)		0.3531

Adverse reactions (PT MedDRA term)	Raw analysis				^a p- value	IPTW analysis				^a p- value
	BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)		
	Total AR		Total AR			Total AR		Total AR		
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	
	2 (0.1)	3 (0.1)	2 (0.1)	3 (0.2)		2 (0.0)	3 (0.1)	1 (0.1)	3 (0.2)	
SOC: Gastrointestinal disorders										
Gastrointestinal disorders PT: Gastrointestinal disorder	37 (1.0)		14 (0.9)		0.695	37 (1.1)		14 (0.8)		0.4518
	12 (0.3)	25 (0.7)	5 (0.3)	9 (0.6)		12 (0.3)	25 (0.7)	4 (0.2)	10 (0.6)	
SOC: Respiratory, thoracic and mediastinal disorders										
Asthma PT: Asthma	2 (0.1)		3 (0.2)		0.142	2 (0.1)		3 (0.2)		0.2494
	0 (0.0)	1 (0.0)	2 (0.1)	1 (0.1)		0 (0.0)	1 (0.0)	2 (0.1)	1 (0.1)	
Rhinitis, nasal discharge PT: Rhinitis	24 (0.7)		10 (0.7)		0.945	23 (0.7)		9 (0.5)		0.5470
	13 (0.4)	12 (0.3)	4 (0.3)	6 (0.4)		12 (0.3)	12 (0.4)	4 (0.3)	4 (0.3)	
SOC: Investigations										
	10 (0.3)		6 (0.4)		0.509	10 (0.3)		4 (0.3)		0.8960

Adverse reactions (PT MedDRA term)	Raw analysis				^a p- value	IPTW analysis				^a p- value
	BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)		
	Total AR		Total AR			Total AR		Total AR		
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	
	5 (0.1)		5 (0.1)			5 (0.1)		5 (0.1)		
Hypertension, hypotension PT: Blood pressure abnormal	4 (0.3)					1 (0.0)		3 (0.2)		
SOC: General disorders and administration site conditions										
Influenza-like symptoms PT:	40 (1.1)		18 (1.2)		0.857	38 (1.1)		17 (1.0)		0.7628
Influenza like illness	11 (0.3)	30 (0.8)	12 (0.8)	7 (0.5)		10 (0.3)	29 (0.8)	12 (0.7)	6 (0.4)	
Sensation of heat, sensation of cold PT: Temperature regulation disorder	5 (0.1)		5 (0.3)		0.166	5 (0.1)		6 (0.4)		0.0943
Chest pain PT:	3 (0.1)	2 (0.1)	2 (0.1)	3 (0.2)		3 (0.1)	2 (0.1)	3 (0.2)	3 (0.2)	
Chest pain	7 (0.2)		1 (0.1)		0.281	7 (0.2)		1 (0.0)		0.1378
Chest pain	0 (0)	7 (0.2)	0 (0)	1 (0.1)		0 (0.0)	7 (0.2)	0 (0.0)	1 (0.0)	
Hiporexia, anorexia PT:	2 (0.1)		4 (0.3)		0.049	2 (0.1)		3 (0.2)		0.2346
Decreased appetite	0 (0.0)	2 (0.1)	1 (0.1)	3 (0.2)		0 (0.0)	2 (0.1)	1 (0.0)	2 (0.1)	
	10 (0.3)		7 (0.5)		0.312	10 (0.3)		6 (0.4)		0.5246

Adverse reactions (PT MedDRA term)	Raw analysis				^a p- value	IPTW analysis				^a p- value					
	BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)			BNT162b2 (n= 3564) n (%)		mRNA-1273 (n=1524) n (%)							
	Total AR		Total AR			Total AR		Total AR							
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose						
	3 (0.1)		7 (0.2)			4 (0.3)		4 (0.3)			3 (0.1)		7 (0.2)		4 (0.2)
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>						SOC: Ear and labyrinth disorders									
PT: <i>Ear pain</i>						4 (0.1)		1 (0.1)		0.627	4 (0.1)		1 (0.0)		0.3365
						1 (0.0)	3 (0.1)	1 (0.1)	0 (0.0)		2 (0.0)	3 (0.1)	1 (0.0)	0 (0.0)	
						SOC: Skin and subcutaneous tissue disorders									
General pruritus PT: <i>Pruritus</i>						11 (0.3)		1 (0.1)		0.102	13 (0.4)		2 (0.1)		0.0893
						7 (0.2)	4 (0.1)	0 (0.0)	1 (0.1)		8 (0.2)	4 (0.1)	0 (0.0)	2 (0.1)	
Sweat PT: <i>Cold sweat</i>						16 (0.4)		9 (0.6)		0.508	16 (0.4)		7 (0.4)		0.9251
						2 (0.1)	14 (0.4)	3 (0.2)	6 (0.4)		2 (0.1)	13 (0.4)	2 (0.1)	5 (0.3)	
						SOC: Immune system disorders									

Adverse reactions (PT MedDRA term)	Raw analysis						^a p- value	IPTW analysis						^a p- value
	BNT162b2 (n= 3564) n (%)			mRNA-1273 (n=1524) n (%)				BNT162b2 (n= 3564) n (%)			mRNA-1273 (n=1524) n (%)			
	Total AR			Total AR				Total AR			Total AR			
	AR after 1 st dose	AR after 2 nd dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	AR after 2 nd dose		AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose	
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: <i>Hypersensitivity</i>	36 (1.0)			32 (2.1)			34 (1.0)			28 (1.7)			0.0276	
	15 (0.4)	21 (0.6)		16 (1.0)	23 (1.5)		15 (0.4)	19 (0.5)	13 (0.8)	20 (1.2)				
	0.002													
SOC: Cardiac disorders														
Tachycardia PT: <i>Tachycardia</i>	11 (0.3)			5 (0.3)			10 (0.3)			3 (0.2)			0.5564	
	6 (0.2)	5 (0.1)		0 (0.0)	5 (0.3)		5 (0.2)	5 (0.1)	0 (0.0)	3 (0.2)				
SOC: Blood and lymphatic system disorders														
Lymphadenopathy PT: <i>Lymphadenopathy</i>	118 (3.3)			82 (5.4)			116 (3.3)			103 (6.1)			<0.0001	
	24 (0.7)	98 (2.7)		39 (2.6)	44 (2.9)		24 (0.7)	96 (2.7)	52 (3.1)	42 (2.5)				
SOC: Infections and infestations														
Herpetic infection PT: <i>Herpes virus infection</i>	10 (0.3)			4 (0.3)			9 (0.3)			3 (0.2)			0.473	
	6 (0.2)	5 (0.1)		4 (0.3)	0 (0.0)		6 (0.2)	5 (0.1)	3 (0.2)	0 (0.0)				
Distribution of proportions of solicited and unsolicited AR are displayed by vaccine and dose; ^a chi-square test; HCP: healthcare professionals; AR: adverse reaction														

eTable 3 Distribution of severe AR per vaccine group (BNT162b2 and mRNA-1273) and dose

HCP vaccinated with BNT162b2 (n= 3536.92) No. (%)		HCP vaccinated with mRNA-1273 (n=1695.61) No. (%)		^a p-value
Grade 3 AR both doses (No., %)		Grade 3 AR both doses (No., %)		
1192 (33.7)		957 (56.4)		<0.0001
Grade 3 AR after 1 st dose (No., %)	Grade 3 AR after 2 nd dose (No., %)	Grade 3 AR after 1 st dose (No., %)	Grade 3 AR after 2 nd dose (No., %)	
512 (14.5)	891 (25.2)	566 (33.4)	652 (38.4)	

Proportions of vaccinated participants experiencing grade 3 AR, for each vaccinated group and dose (IPTW data); HCP: healthcare professionals; AR: adverse reaction; ^achi-square test

eTable 4 Distribution of severe AR per vaccine group (BNT162b2 and mRNA-1273) and dose, as per specific types of AR

Grade 3 AR proportions per AR type (No, %)					
Adverse reaction (PT MedDRA term)	HCP vaccinated with BNT162b2 (n= 3536.92) No. (%)		HCP vaccinated with mRNA-1273 (n=1695.61) No. (%)		p-value
	Grade 3 events both doses (No. (%))		Grade 3 events both doses (No. (%))		
	Grade 3 events after 1 st dose	Grade 3 events after 2 nd dose	Grade 3 events after 1 st dose	Grade 3 events after 2 nd dose	
	Grade 3 events after 1 st dose	Grade 3 events after 2 nd dose	Grade 3 events after 1 st dose	Grade 3 events after 2 nd dose	
Injection site pain <i>PT: Injection site pain</i>	264 (7.5)		276 (16.3)		<0.001
	176 (5.0)	109 (3.1)	174 (10.3)	125 (7.4)	
Arm pain <i>PT: Pain in extremity</i>	284 (8.0)		339 (20.0)		<0.001
	151 (4.3)	156 (4.4)	247 (14.5)	138 (8.1)	
Fatigue/asthenia <i>PT: Fatigue</i>	438 (12.4)		276 (16.3)		<0.001
	107 (3.0)	354 (10.0)	82 (4.8)	200 (11.8)	
Headache <i>PT: Headache</i>	355 (10.0)		268 (15.8)		<0.001
	100 (2.8)	271 (7.7)	99 (5.9)	184 (10.9)	
Muscle pain <i>PT: Myalgia</i>	295 (8.3)		198 (11.7)		0.001
	26 (0.7)	272 (7.7)	39 (2.3)	167 (9.8)	
Malaise <i>PT: Malaise</i>	245 (6.9)		210 (12.4)		<0.001
	22 (0.6)	224 (6.3)	64 (3.8)	153 (9.0)	
Joint pain <i>PT: Arthralgia</i>	139 (3.9)		117 (6.9)		<0.001
	4 (0.1)	137 (3.9)	16 (1.0)	102 (6.0)	
	31 (0.9)		78 (4.6)		<0.001

Injection site swelling <i>PT:</i> <i>Application site swelling</i>	17 (0.5)	16 (0.5)	48 (2.8)	35 (2.1)	
Injection site redness <i>PT:</i> <i>Application site redness</i>	19 (0.5)	10 (0.3)	55 (3.3)	31 (1.8)	<0.001
Shoulder pain <i>PT:</i> <i>Musculoskeletal pain</i>	14 (0.4)	5 (0.2)	46 (2.7)	19 (1.1)	<0.001
Injection site pruritus <i>PT:</i> <i>Vaccination site pruritus</i>	8 (0.2)	4 (0.1)	23 (1.4)	13 (0.7)	<0.001

Severity of each AR per vaccine group and dose according to IPTW, measured as proportions of vaccinated participants experiencing grade 3 events of each type of AR; HCP: healthcare professionals; AR: adverse reaction

eTable 5 Mean and median Likert Score values per vaccine group (BNT162b2 and mRNA-1273) and dose

HCP vaccinated with BNT162b2 (n= 3536,92) No. (%)		HCP vaccinated with mRNA-1273 (n=1695,61) No. (%)	
Mean and median LS both doses		Mean and median LS both doses	
4.45 (3.09) 5.00 (1.00, 7.00)		6.28 (3.04) 7.00 (5.00, 8.00)	
Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose
2.68 (2.91) 2.00 (0.00, 5.00)	3.28 (3.32) 3.00 (0.00, 6.00)	4.65 (3.40) 5.00 (2.00,7.00)	4.67 (3.43) 5.00 (0.00, 7.50)

LS: Likert Score; Mean and median LS values are represented for each vaccinated group and dose; HCP: healthcare professionals

eTable 6 Mean and median Likert Score values per vaccine group (BNT162b2 and mRNA-1273) and dose, as per specific types of AR

Adverse reaction (PT MedDRA term)	HCP vaccinated with BNT162b2 (n= 3536.92) No. (%)		HCP vaccinated with mRNA-1273 (n=1695.61) No. (%)	
	Mean and median LS both doses (Mean (SD), Median (IQR))		Mean and median LS both doses (Mean (SD), Median (IQR))	
	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose
Injection site pain <i>PT:</i> <i>Injection site pain</i>	4.65 (1.98) 5.00 (3.00, 6.00)		5.36 (2.10) 5.00 (4.00, 7.00)	
	4.45 (1.95) 4.00 (3.00, 6.00)	4.59 (1.97) 4.00 (3.00, 6.00)	5.27 (2.09) 5.00 (4.00, 7.00)	5.01 (2.08) 5.00 (3.00, 7.00)
Arm pain <i>PT:</i> <i>Pain in extremity</i>	5.31 (1.95) 5.00 (4.00, 7.00)		6.08 (2.00) 6.00 (5.00, 8.00)	
	5.09 (1.94) 5.00 (4.00, 7.00)	5.30 (1.96) 5.00 (4.00, 7.00)	5.95 (1.97) 6.00 (5.00, 7.00)	5.93 (2.05) 6.00 (4.00, 8.00)
Fatigue/asthenia <i>PT:</i> <i>Fatigue</i>	6.23 (1.85) 6.00 (5.00, 8.00)		6.43 (1.77) 7.00 (5.00, 8.00)	
	5.92 (1.71) 6.00 (5.00, 7.00)	6.19 (1.90) 6.00 (5.00, 8.00)	5.81 (1.69) 6.00 (5.00, 7.00)	6.53 (1.83) 7.00 (5.00, 8.00)
Headache <i>PT:</i> <i>Headache</i>	5.96 (1.94) 6.00 (5.00, 7.00)		6.16 (1.96) 6.00 (5.00, 8.00)	
	5.57 (2.16) 6.00 (4.00, 7.00)	6.00 (1.83) 6.00 (5.00, 7.00)	5.77 (1.93) 6.00 (5.00, 7.00)	6.19 (1.94) 6.00 (5.00, 8.00)
Muscle pain <i>PT:</i> <i>Myalgia</i>	6.13 (1.86) 6.00 (5.00, 8.00)		6.53 (2.50) 7.00 (5.00, 8.00)	
	5.37 (2.09) 5.00 (4.00, 7.00)	6.19 (1.82) 6.00 (5.00, 8.00)	6.12 (1.73) 6.00 (5.00, 7.00)	6.59 (2.68) 7.00 (5.00, 8.00)
Malaise <i>PT:</i>	6.22 (1.85) 6.00 (5.00, 8.00)		6.54 (3.49) 7.00 (5.00, 8.00)	

Adverse reaction (PT MedDRA term)	HCP vaccinated with BNT162b2 (n= 3536.92) No. (%)		HCP vaccinated with mRNA-1273 (n=1695.61) No. (%)	
	Mean and median LS both doses (Mean (SD), Median (IQR))		Mean and median LS both doses (Mean (SD), Median (IQR))	
	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose
<i>Malaise</i>	5.58 (1.88) 5.00 (4.00, 7.00)	6.25 (1.85) 6.00 (5.00, 8.00)	6.50 (5.42) 6.00 (5.00, 7.00)	6.44 (1.87) 7.00 (5.00, 8.00)
	6.47 (1.84) 7.00 (5.00, 8.00)		6.49 (1.99) 7.00 (5.00, 8.00)	
Joint pain <i>PT:</i> <i>Arthralgia</i>	5.15 (1.57) 5.00 (4.00, 7.00)	6.53 (1.83) 7.00 (5.00, 8.00)	5.98 (2.14) 6.00 (4.00, 8.00)	6.52 (1.97) 7.00 (5.00, 8.00)
	4.86 (1.86) 5.00 (3.00, 6.00)		5.56 (2.01) 6.00 (4.00, 7.00)	
Injection site swelling <i>PT:</i> <i>Application site</i> <i>swelling</i>	4.71 (1.82) 5.00 (3.00, 6.00)	4.97 (1.94) 5.00 (3.00, 6.00)	5.50 (1.98) 6.00 (4.00, 7.00)	5.44 (2.01) 5.00 (4.00, 7.00)
	4.75 (1.85) 5.00 (3.00, 6.00)		5.80 (2.19) 6.00 (4.00, 8.00)	
Injection site redness <i>PT:</i> <i>Application site</i> <i>redness</i>	4.39 (1.90) 4.00 (3.00, 6.00)	5.14 (1.75) 5.00 (4.00, 6.00)	5.59 (2.23) 6.00 (4.00, 7.00)	5.98 (2.05) 6.00 (5.00, 8.00)
	4.51 (1.90) 4.00 (3.00, 6.00)		5.74 (1.99) 6.00 (4.00, 7.00)	
Shoulder pain <i>PT:</i> <i>Musculoskeletal</i> <i>pain</i>	4.04 (1.86) 4.00 (3.00, 5.00)	5.50 (2.17) 5.00 (4.00, 7.00)	5.50 (2.17) 5.00 (4.00, 7.00)	5.93 (2.05) 6.00 (4.00, 8.00)
	4.48 (2.11) 4.00 (3.00, 7.00)		5.23 (1.93) 5.00 (4.00, 7.00)	
Injection site pruritus <i>PT:</i> <i>Vaccination site</i> <i>pruritus</i>	4.53 (1.59) 4.00 (3.00, 6.00)	4.36 (2.59) 3.50 (3.00, 7.00)	5.35 (1.77) 5.50 (4.50, 7.00)	5.11 (2.04) 5.00 (3.00, 7.00)

HCP: healthcare professionals; AR: adverse reaction; LS: Likert Score; Mean and median LS values are represented for each vaccinated group and AR type

eTable 7 Distribution of solicited and unsolicited AR per group (Healthcare professionals (HCP) and solid-organ transplant recipients (SOTR)) and dose

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	^a p-value
	Total AR		Total AR			
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain PT: <i>Injection site pain</i>	558 (43.3)		766 (50.3)		1324 (47.1)	0.002
	452 (35.1)	255 (19.8)	569 (37.3)	374 (24.5)		
Fatigue/asthenia PT: <i>Fatigue</i>	251 (19.5)		532 (34.9)		783 (27.8)	<0.001
	153 (11.9)	135 (10.5)	223 (14.6)	372 (24.4)		
Fever PT: <i>Pyrexia</i>	202 (15.7)		774 (50.8)		976 (34.7)	<0.001
	99 (7.7)	133 (10.3)	216 (14.2)	671 (44.0)		
Malaise PT: <i>Malaise</i>	113 (8.8)		400 (26.2)		513 (18.2)	<0.001
	58 (4.5)	71 (5.5)	133 (8.7)	297 (19.5)		
Chills PT: <i>Chills</i>	109 (8.5)		341 (22.4)		450 (16.0)	<0.001
	52 (4.0)	69 (5.4)	81 (5.3)	284 (18.6)		
Injection site redness PT: <i>Application site redness</i>	28 (2.2)		145 (9.5)		173 (6.2)	<0.001
	13 (1.0)	15 (1.2)	87 (5.7)	70 (4.6)		
SOC: Musculoskeletal and connective tissue disorders						

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	a p-value
	Total AR		Total AR			
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
Arm pain PT: Pain in extremity	345 (26.8)		624 (40.9)		969 (34.4)	<0.0001
	270 (20.9)	153 (11.9)	476 (31.2)	277 (18.2)		
Muscle pain PT: Myalgia	62 (4.8)		337 (22.1)		399 (14.2)	<0.001
	37 (2.9)	30 (2.3)	94 (6.2)	266 (17.5)		
Joint pain PT: Arthralgia	39 (3.0)		203 (13.3)		242 (8.6)	<0.001
	23 (1.8)	21 (1.6)	49 (3.2)	16 (10.8)		
Shoulder pain PT: Musculoskeletal pain	86 (6.7)		93 (6.1)		179 (6.4)	0.538
	19 (1.5)	71 (5.5)	36 (2.4)	59 (3.9)		
SOC: Injury, poisoning and procedural complications						
Injection site swelling PT: Application site swelling	61 (4.7)		228 (15.0)		289(10.3)	<0.001
	43 (3.3)	24 (1.9)	137 (9.0)	113 (7.4)		
SOC: Vaccination site pruritus						
Injection site pruritus PT: Vaccination site pruritus	21 (1.6)		87 (5.7)		108(3.8%)	<0.001
	8 (0.6)	13 (1.0)	55 (3.6)	38 (2.5)		
SOC: Nervous system disorders						

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	^a p-value
	Total AR		Total AR			
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
	511 (33.5)					
Headache <i>PT: Headache</i>	105 (8.1)	88 (6.8)	216 (14.2)	383 (25.1)	678 (24.1)	<0.001
	3 (0.2)		15 (1.0)		18 (0.6)	0.013
Insomnia <i>PT: Insomnia</i>	0 (0.0)		3 (0.2)	13 (0.9)		
SOC: Gastrointestinal disorders						
Nausea <i>PT: Nausea</i>	25 (1.9)		97 (6.4)		122 (4.3)	<0.001
	15 (1.2)	12 (0.9)	43 (2.8)	61 (4.0)		
Diarrhea <i>PT: Diarrhoea</i>	27 (2.1)		36 (2.4)		63 (2.2)	0.633
	12 (0.9)	17 (1.3)	13 (0.9)	23 (1.5)		
Vomiting <i>PT: Vomiting</i>	24 (1.9)		41 (2.7)		65 (2.3)	0.145
	13 (1.0)	15 (1.2)	4 (0.3)	38 (2.5)		
Unsolicited adverse reactions						
SOC: Musculoskeletal and connective tissue disorders						
Other musculoskeletal disorders <i>PT: Musculoskeletal disorder</i>	10 (0.8)		41 (2.7)		51 (1.8)	0.001
	8 (0.6)	2 (0.2)	18 (1.2)	25 (1.6)		
SOC: Skin and subcutaneous tissue disorders						
	7 (0.5)		10 (0.7)		17 (0.6)	0.699

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	a p-value
	Total AR		Total AR			
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
	5 (0.4)	2 (0.2)	5 (0.3)	5 (0.3)		
Petechia, ecchymosis PT: <i>Ecchymosis</i>						
SOC: Nervous system disorders						
Cognitive alteration PT: <i>Cognitive disorder</i>	8 (0.6)		5 (0.3)		13 (0.5)	0.254
Alterations of smell and taste PT: <i>Parosmia</i>	8 (0.6)	0 (0.0)	2 (0.1)	3 (0.2)		
	2 (0.2)		3 (0.2)		5 (0.2)	0.794
	1 (0.1)	1 (0.1)	2 (0.1)	1 (0.1)		
Paresthesia and hyperesthesia PT: <i>Dysaesthesia</i>	2 (0.2)		10 (0.7)		12 (0.4)	0.042
	1 (0.1)	0 (0.0)	5 (0.3)	5 (0.3)		
Presyncope, syncope and vasovagal syncope PT: <i>Syncope</i>	0 (0.0)	0 (0.0)	5 (0.3)	3 (0.2)	5 (0.2)	0.039
Sleepiness, hypersomnia PT: <i>Hypersomnia</i>	9 (0.7)		11 (0.7)		20 (0.7)	0.941
	7 (0.5)	4 (0.3)	3 (0.2)	8 (0.5)		
	35 (2.7)		51 (3.3)		86 (3.1)	0.333

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	a p-value
	Total AR		Total AR			
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
Instability sensation, vertigo, sickness PT: Dizziness	17 (1.3)	21 (1.6)	24 (1.6)	30 (2.0)		
	2 (0.2)	2 (0.2)	0 (0.0)	2 (0.1)	5 (0.3)	3 (0.2)
SOC: Gastrointestinal disorders						
Gastrointestinal disorders PT: Gastrointestinal disorder	9 (0.7)		14 (0.9)			
	3 (0.2)	6 (0.5)	5 (0.3)	9 (0.6)	23 (0.8)	0.518
SOC: Respiratory, thoracic and mediastinal disorders						
Asthma PT: Asthma	0 (0.0)	0 (0.0)	2 (0.1)	1 (0.1)	3 (0.1)	0.111
	0 (0.0)					
Rhinitis, nasal discharge PT: Rhinitis	8 (0.6)		10 (0.7)			
	4 (0.3)	4 (0.3)	4 (0.3)	6 (0.4)	18 (0.6)	0.906
SOC: Investigations						
Hypertension, hypotension PT: Blood pressure abnormal	4 (0.3)		6 (0.4)			
	2 (0.2)	2 (0.2)	2 (0.1)	4 (0.3)	10 (0.4)	0.711

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	a p-value	
	Total AR		Total AR				
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose			
SOC: General disorders and administration site conditions							
Influenza-like symptoms PT: <i>Influenza like illness</i>	4 (0.3)		18 (1.2)		22 (0.8)	0.009	
	3 (0.2)	1 (0.1)	12 (0.8)	7 (0.5)			
	Sensation of heat, sensation of cold PT: <i>Temperature regulation disorder</i>	0 (0.0)		6 (0.4)		6 (0.2)	0.024
		0 (0.0)	0 (0.0)	3 (0.2)	3 (0.2)		
Chest pain PT: <i>Chest pain</i>	3 (0.2)		1 (0.1)		4 (0.1)	0.241	
	0 (0)	2 (0.1)	0 (0)	1 (0.1)			
Hiporexia, anorexia PT: <i>Decreased appetite</i>	2 (0.2)		4 (0.3)		6 (0.2)	0.539	
	1 (0.1)	1 (0.1)	1 (0.1)	3 (0.2)			
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>	0 (0.0)		7 (0.5)		7 (0.2)	0.015	
	0 (0.0)	0 (0.0)	4 (0.3)	4 (0.3)			
SOC: Ear and labyrinth disorders							
PT: Ear pain PT: <i>Ear pain</i>	1 (0.1)		1 (0.1)		2 (0.1)	0.906	
	0 (0.0)	1 (0.1)	1 (0.1)	0 (0.0)			

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	a p-value
	Total AR		Total AR			
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
	SOC: Skin and subcutaneous tissue disorders					
General pruritus PT: <i>Pruritus</i>	0 (0.0)		1 (0.1)		1 (0.0)	0.358
	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)		
Sweat PT: <i>Cold sweat</i>	1 (0.1)		9 (0.6)		10 (0.4)	0.023
	1 (0.1)	0 (0.0)	3 (0.2)	6 (0.4)		
SOC: Immune system disorders						
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: <i>Hypersensitivity</i>	1 (0.1)		32 (2.1)		33 (1.2)	<0.001
	0 (0.0)	1 (0.1)	16 (1.0)	23 (1.5)		
SOC: Cardiac disorders						
Tachycardia PT: <i>Tachycardia</i>	1 (0.1)		5 (0.3)		6 (0.2)	0.151
	1 (0.1)	0 (0.0)	0 (0.0)	5 (0.3)		
SOC: Blood and lymphatic system disorders						
Lymphadenopathy PT: <i>Lymphadenopathy</i>	10 (0.8)		82 (5.4)		92 (3.3)	<0.001
	6 (0.5)	5 (0.4)	39 (2.6)	44 (2.9)		
SOC: Infections and infestations						
Herpetic infection	0 (0.0)		4 (0.3)		4 (0.1)	0.066

Adverse reaction (verbatim, PT MedDRA term)	SOTR (n=1289) No. (%)		HCP (n=1524) No. (%)		Total (n=2813) No. (%)	^a p-value
	Total AR					
	AR after 1 st dose	AR after 2 nd dose	AR after 1 st dose	AR after 2 nd dose		
	0 (0.0)	0 (0.0)	4 (0.3)	0 (0.0)		
PT: <i>Herpes virus infection</i>						

Distribution of proportions of solicited and unsolicited AR are displayed by vaccine and dose. ^achi-square test; HCP: healthcare professionals; SOTR: solid-organ transplant recipients; AR: adverse reaction

eTable 8 Distribution of severe AR per group (Healthcare professionals (HCP) and solid-organ transplant recipients (SOTR)) and dose

SOTR (n=1289) No. (%)		HCP vaccinated with mRNA-1273 (n=1524) No. (%)		^a p-value
Grade 3 AR both doses No. (%)		Grade 3 AR both doses No. (%)		p-value
315 (24.4)		886 (58.1)		^a <0.001
Grade 3 AR after 1 st dose No. (%)	Grade 3 AR after 2 nd dose No. (%)	Grade 3 AR after 1 st dose No. (%)	Grade 3 AR after 2 nd dose No. (%)	
243 (18.9)	139 (10.8)	527 (34.6)	620 (40.7)	

HCP: healthcare professionals; SOTR: solid-organ transplant recipients; AR: adverse reaction. Proportions of vaccinated participants experiencing grade 3 AR, for each vaccinated group and dose; ^achi-square test

eTable 9 Distribution of severe AR per group (Healthcare professionals (HCP) and solid-organ transplant recipients (SOTR)) and dose, as per specific types of AR

Grade 3 AR proportions per AR type (No., %)					
Adverse reaction (PT MedDRA term)	Grade 3 events both doses (No. (%))		Grade 3 events both doses (No. (%))		a-p-value
	Grade 3 events after 1 st dose (No. (%))	Grade 3 events after 2 nd dose (No. (%))	Grade 3 events after 1 st dose (No. (%))	Grade 3 events after 2 nd dose (No. (%))	
Injection site pain <i>PT: Injection site pain</i>	116 (9.0)		242 (15.9)		<0.001
	97 (7.5)	26 (2.0)	167 (11.0)	99 (6.5)	
Arm pain <i>PT:</i>	107 (8.3)		304 (19.9)		<0.001
	81 (6.3)	33 (2.6)	220 (14.4)	119 (7.8)	
<i>Pain in extremity</i>					<0.001
Fatigue/asthenia <i>PT: Fatigue</i>	79 (6.1)		276 (18.1)		
	55 (4.3)	37 (2.9)	81 (5.3)	203 (13.3)	
Headache <i>PT:</i>	48 (3.7)		242 (15.9)		<0.001
	29 (2.2)	22 (1.7)	80 (5.2)	181 (11.9)	
<i>Headache</i>					<0.001
Muscle pain <i>PT:</i>	24 (1.9)		175 (11.5)		
	12 (0.9)	13 (1.0)	44 (2.9)	140 (9.2)	<0.001
<i>Myalgia</i>					
Malaise <i>PT: Malaise</i>	36 (2.8)		208 (13.6)		<0.001
	15 (1.2)	25 (1.9)	55 (3.6)	160 (10.5)	
Joint pain <i>PT: Arthralgia</i>	14 (1.1)		110 (7.2)		<0.001
	8 (0.6)	9 (0.7)	22 (1.4)	91 (6.0)	
Injection site swelling <i>PT:</i>	15 (1.1)		75 (4.9)		<0.001
	12 (0.9)	4 (0.3)	45 (3.0)	34 (2.2)	
<i>Application site swelling</i>					<0.001
Injection site redness <i>PT:</i>	5 (0.4)		56 (3.7)		
	3 (0.2)	2 (0.2)	31 (2.0)	28 (1.8)	<0.001
<i>Application site redness</i>					
Shoulder pain <i>PT:</i>	20 (1.6)		33 (2.2)		0.233
	2 (0.2)	18 (1.4)	12 (0.8)	20 (1.3)	
<i>Musculoskeletal pain</i>					0.233
Injection site pruritus	1 (0.1)		25 (1.6)		

<i>PT:</i> <i>Vaccination site pruritus</i>	0 (0.0)	1 (0.1)	15 (1.0)	11 (0.7)	<0.001
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HCP: healthcare professionals; SOTR: solid-organ transplant recipients. Severity of each AR per vaccine group and dose, measured as proportions of vaccinated participants experiencing grade 3 events of each type of AR; ^achi-square test

eTable 10 Mean and median Likert Score values per group (Healthcare professionals (HCP) and solid-organ transplant recipients (SOTR)) and dose

SOTR vaccinated with mRNA-1273 (n=1289) No. (%)		HCP vaccinated with mRNA-1273 (n=1524) No. (%)	
Mean and median LS both doses		Mean and median LS both doses	
4.06 (2.96) 4.00 (1.00, 6.00)		6.28 (3.04) 7.00 (5.00, 8.00)	
Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose
3.37 (2.99) 4.00 (0.00, 6.00)	2.35 (2.79) 0.00 (0.00, 4.00)	4.65 (3.40) 5.00 (2.00, 7.00)	4.67 (3.43) 5.00 (0.00, 7.50)

HCP: healthcare professionals; SOTR: solid-organ transplant recipients; LS: Likert Score; Mean and median LS values are represented for each vaccinated group and dose

eTable 11 Mean and median Likert Score values per group (Healthcare professionals (HCP) and solid-organ transplant recipients (SOTR)) and dose, as per specific types of AR

Adverse reaction (PT)	SOTR vaccinated with mRNA-1273 (n=1289) No. (%)		HCP vaccinated with mRNA-1273 (n=1524) No. (%)	
	Mean and median LS both doses (Mean (SD), Median (IQR))		Mean and median LS both doses (Mean (SD), Median (IQR))	
	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose
Injection site pain <i>PT:</i> <i>Injection site pain</i>	4.61 (2.11) 4.00 (3.00, 6.00)		5.36 (2.10) 5.00 (4.00, 7.00)	
	4.67 (2.10) 5.00 (3.00, 6.00)	3.95 (1.89) 4.00 (3.00, 5.00)	5.27 (2.09) 5.00 (4.00, 7.00)	5.01 (2.08) 5.00 (3.00, 7.00)
Arm pain <i>PT:</i> <i>Pain in extremity</i>	5.36 (2.03) 5.00 (4.00, 7.00)		6.08 (2.00) 6.00 (5.00, 8.00)	
	5.36 (1.98) 5.00 (4.00, 7.00)	4.72 (2.05) 4.00 (3.00, 6.00)	5.95 (1.97) 6.00 (5.00, 7.00)	5.93 (2.05) 6.00 (4.00, 8.00)
Fatigue/astenia <i>PT:</i> <i>Fatigue</i>	5.44 (1.96) 5.00 (4.00, 7.00)		6.43 (1.77) 7.00 (5.00, 8.00)	
	5.69 (1.93) 6.00 (4.00, 7.00)	5.11 (2.00) 5.00 (4.00, 7.00)	5.81 (1.69) 6.00 (5.00, 7.00)	6.53 (1.83) 7.00 (5.00, 8.00)
Headache <i>PT:</i> <i>Headache</i>	5.30 (2.05) 5.00 (4.00, 7.00)		6.16 (1.96) 6.00 (5.00, 8.00)	
	5.07 (2.04) 5.00 (4.00, 7.00)	5.22 (2.01) 5.00 (4.00, 6.50)	5.77 (1.93) 6.00 (5.00, 7.00)	6.19 (1.94) 6.00 (5.00, 8.00)
Muscle pain <i>PT:</i> <i>Myalgia</i>	5.73 (2.01) 6.00 (4.00, 7.00)		6.53 (2.50) 7.00 (5.00, 8.00)	
	5.62 (1.95) 6.00 (4.00, 7.00)	5.67 (2.11) 6.00 (4.00, 7.00)	6.12 (1.73) 6.00 (5.00, 7.00)	6.59 (2.68) 7.00 (5.00, 8.00)
Malaise <i>PT:</i> <i>Malaise</i>	5.55 (2.09) 5.00 (4.00, 7.00)		6.54 (3.49) 7.00 (5.00, 8.00)	
	5.30 (2.01) 5.00 (4.00, 7.00)	5.64 (2.14) 5.00 (4.00, 8.00)	6.50 (5.42) 6.00 (5.00, 7.00)	6.44 (1.87) 7.00 (5.00, 8.00)

Adverse reaction (PT)	SOTR vaccinated with mRNA-1273 (n=1289) No. (%)		HCP vaccinated with mRNA-1273 (n=1524) No. (%)	
	Mean and median LS both doses (Mean (SD), Median (IQR))		Mean and median LS both doses (Mean (SD), Median (IQR))	
	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose	Mean and median LS after 1 st dose	Mean and median LS after 2 nd dose
Joint pain <i>PT:</i> <i>Arthralgia</i>	6.00 (2.00) 6.00 (4.00, 7.50)		6.49 (1.99) 7.00 (5.00, 8.00)	
	5.75 (2.02) 5.50 (4.00, 7.50)	6.43 (1.91) 6.00 (5.00, 8.00)	5.98 (2.14) 6.00 (4.00, 8.00)	6.52 (1.97) 7.00 (5.00, 8.00)
Injection site swelling <i>PT:</i> <i>Application site</i> <i>swelling</i>	4.53 (2.24) 4.50 (3.00, 6.00)		5.56 (2.01) 6.00 (4.00, 7.00)	
	4.83 (2.26) 5.00 (3.00, 7.00)	3.88 (1.98) 4.00 (2.00, 5.00)	5.50 (1.98) 6.00 (4.00, 7.00)	5.44 (2.01) 5.00 (4.00, 7.00)
Injection site redness <i>PT:</i> <i>Application site</i> <i>redness</i>	4.22 (2.38) 4.00 (2.00, 5.00)		5.80 (2.19) 6.00 (4.00, 8.00)	
	4.54 (2.93) 4.00 (2.00, 5.00)	3.71 (1.82) 3.00 (2.00, 5.00)	5.59 (2.23) 6.00 (4.00, 7.00)	5.98 (2.05) 6.00 (5.00, 8.00)
Shoulder pain <i>PT:</i> <i>Musculoskeletal</i> <i>pain</i>	5.09 (1.95) 5.00 (4.00, 6.00)		5.74 (1.99) 6.00 (4.00, 7.00)	
	4.53 (1.61) 5.00 (4.00, 5.00)	5.14 (2.02) 5.00 (4.00, 7.00)	5.50 (2.17) 5.00 (4.00, 7.00)	5.69 (2.02) 6.00 (4.00, 8.00)
Injection site pruritus <i>PT:</i> <i>Vaccination site</i> <i>pruritus</i>	4.00 (1.56) 4.00 (3.00, 5.00)		5.23 (1.93) 5.00 (4.00, 7.00)	
	4.57 (1.13) 4.00 (4.00, 6.00)	3.67 (1.72) 3.00 (2.50, 4.50)	5.35 (1.77) 5.50 (4.50, 7.00)	5.11 (2.04) 5.00 (3.00, 7.00)

HCP: healthcare professionals; SOTR: solid-organ transplant recipients; IQR: 25-75% interquartile range; LS: Likert Score; Mean and median LS values are represented for each vaccinated group and AR type

eTable 12 Multivariate logistic regression analysis with covariates. Exploratory analyses with any severe AR

Comparison HCP (mRNA-1273 vs BNT162b2). Any severe AR			
Type of analysis	Covariates	p-value	OR (95% CI)
Covariate-adjusted analysis	Vaccine Type mRNA-1273 vs BNT162b2	<0.0001	2.83 (2.48 to 3.23)
	Drug allergies	0.0598	1.23 (0.99 to 1.53)
	Age	<0.0001	0.99 (0.98 to 0.99)
	Previous SAR-CoV-2 Infection	0.0003	1.47 (1.81 to 1.19)
	Gender (Female vs male)	<0.0001	2.83 (2.46 to 3.26)
Vaccination with mRNA-1273: Comparison HCP vs SOTR. Any severe AR			
Type of analysis	Covariates	p-value	OR (95% CI)
Covariate-adjusted analysis	Drug allergies	0.0036	1.78 (1.21 to 2.61)
	Age	<0.0001	0.96 (0.95 to 0.97)
	Previous SAR-CoV-2 Infection	0.0758	1.69 (3.03 to 0.94)
	Gender (Female vs male)	<0.0001	2.25 (1.72 - 2.95)

Exploratory covariate-adjusted analysis considering severe AR. OR with 95% CIs are represented; HCP: healthcare professionals; SOTR: solid-organ transplant recipients; AR: adverse reaction

eTable 13 Adverse reactions of special interest defined according to the predefined list of adverse events published by the Regional Autonomic Pharmacovigilance Centre and according to the Brighton Collaboration criteria:

Anaphylaxis
Arrhythmia
Cardiac failure
Stress cardiomyopathy
Coronary disease
Myocarditis
Sudden death
Death of any cause
Guillain-Barré syndrome
Acute disseminated encephalomyelitis
Transversal myelitis
Facial paralysis
Cutaneous vasculitis
Erythema multiforme
Microangiopathy
Coagulation disorders (thromboembolism and hemorrhages)
Idiopathic thrombocytopenia
Acute aseptic arthritis
Acute respiratory distress
COVID-19 vaccine-associated enhanced disease (VAED)
COVID-19 vaccine-associated enhanced respiratory disease (VAERD)
Anosmia
Ageusia / Hypogeusia
Acute liver injury
Acute renal injury

References:

Black SB, Law B, Chen RT, Dekker CL, Sturkenboom M, Huang WT, Gurwith M PG. The critical role of background rates of possible adverse events in the assessment of COVID-19 vaccine safety. *Vaccine*. 2021;39(19):2712–8.

Dodd CN, Willame C SM. Background rates of Adverse Events of Special Interest for monitoring COVID-19 vaccines EUPAS37273.

Collaboration B. Priority List of Adverse Events of Special Interest: COVID-19 [Internet]. [cited 2021 May 24]. Available from: <https://brightoncollaboration.us/priority-list-aesi-covid/>

eTable 14 List of pre-defined adverse reactions

Injection site pain

Injection site swelling

Injection site pruritus

Injection site redness

Arm pain

Shoulder pain

Malaise

Fatigue

Muscle pain

Joint pain

Headache

Insomnia

Difficulty sleeping

Shivering

Nausea

Vomiting

Diarrhea

Facial paralysis

Fever

eTable 15 STROBE Checklist

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

	Item No	Recommendation	Page No*
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	7
Objectives	3	State specific objectives, including any prespecified hypotheses	11
Methods			
Study design	4	Present key elements of study design early in the paper	8-11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	8-9
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11-12
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	12
Bias	9	Describe any efforts to address potential sources of bias	12
Study size	10	Explain how the study size was arrived at	11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	11-12
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	11-12
		(b) Describe any methods used to examine subgroups and interactions	
		(c) Explain how missing data were addressed	
		(d) If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	13
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest	13
Outcome data	15*	Report numbers of outcome events or summary measures	13-15
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	13-15
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12-15
Discussion			
Key results	18	Summarise key results with reference to study objectives	16-18
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	18
Generalisability	21	Discuss the generalisability (external validity) of the study results	18
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	5

*Pages where the different items of the STROBE checklist are described

Reference: <https://www.strobe-statement.org/>

eFigure 1 Questionnaire

ADVERSE REACTIONS											
D1 - Date of vaccination: __/__/__											
D2 Vaccine: BNT162b2 / mRNA-1273											
D3 – Did you have any adverse reaction after receiving the vaccine dose?											
☐ YES ☐ NO											
ADVERSE REACTIONS RECORD											
D4 –Adverse reaction intensity :											
<i>Please indicate intensity 0 (lower) to 10 (higher):</i>											
	0	1	2	3	4	5	6	7	8	9	10
D4.1 - Injection site pain	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.2 - Injection site swelling	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.3 - Injection site pruritus	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.4 - Injection site redness	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.5 - Arm pain	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.6 - Shoulder pain	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.7 - Malaise	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.8 - Fatigue	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.9 - Muscle pain	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D4.10 - Joint pain	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
D34.11 - Headache	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
<i>Please, indicate if you developed any of the following adverse reactions after receiving the vaccine dose:</i>											
	Yes	No									
D4.12 - Insomnia	☐	☐									
D4.13 - Difficulty sleeping	☐	☐									
D4.14 - Shivers	☐	☐									
D4.15 - Nausea	☐	☐									
D4.16 - Vomiting	☐	☐									
D4.17 - Diarrhea	☐	☐									
D4.18 - Partial face paralysis	☐	☐									
D4.19 – Have you had fever after the vaccine dose?											
☐ YES ☐ NO											
D5 - Start and end date of reported adverse reactions in question D4 (in case they have finalized):											

		Start date	End date
D5.1 -	Injection site pain	/ /2021	/ /2021
D5.2 -	Injection site swelling	/ /2021	/ /2021
D5.3 -	Injection site pruritus	/ /2021	/ /2021
D5.4 -	Injection site redness	/ /2021	/ /2021
D5.5 -	Arm pain	/ /2021	/ /2021
D5.6 -	Shoulder pain	/ /2021	/ /2021
D5.7 -	Malaise	/ /2021	/ /2021
D5.8 -	Fatigue	/ /2021	/ /2021
D5.9 -	Muscle pain	/ /2021	/ /2021
D5.10 -	Joint pain	/ /2021	/ /2021
D5.11 -	Headache	/ /2021	/ /2021
D5.12 -	Insomnia	/ /2021	/ /2021
D5.13 -	Difficulty sleeping	/ /2021	/ /2021
D5.14 -	Shivering	/ /2021	/ /2021
D5.15 -	Nausea	/ /2021	/ /2021
D5.16 -	Vomiting	/ /2021	/ /2021
D5.17 -	Diarrhea	/ /2021	/ /2021
D5.18 -	Facial paralysis	/ /2021	/ /2021
D5.19 -	Fever	/ /2021	/ /2021

D6 – Have you had any adverse reaction not included in the previous list?

☐ YES

☐ NO →GO TO D7

D6.1 – Other adverse reactions: please, indicate start and end date for each one

Adverse reaction #1

Description:

Start date:

End date:

Adverse reaction #2

Description:

Start date:

End date:

Adverse reaction #3

Description:

Start date:

End date:

Adverse reaction #4

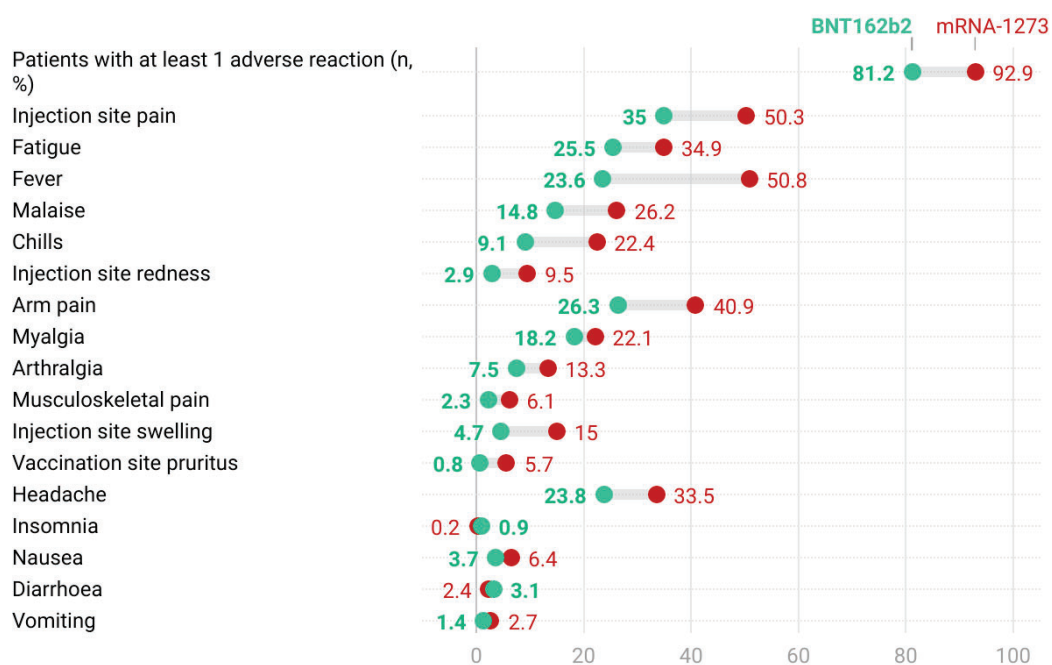
Description:

Start date:

End date:

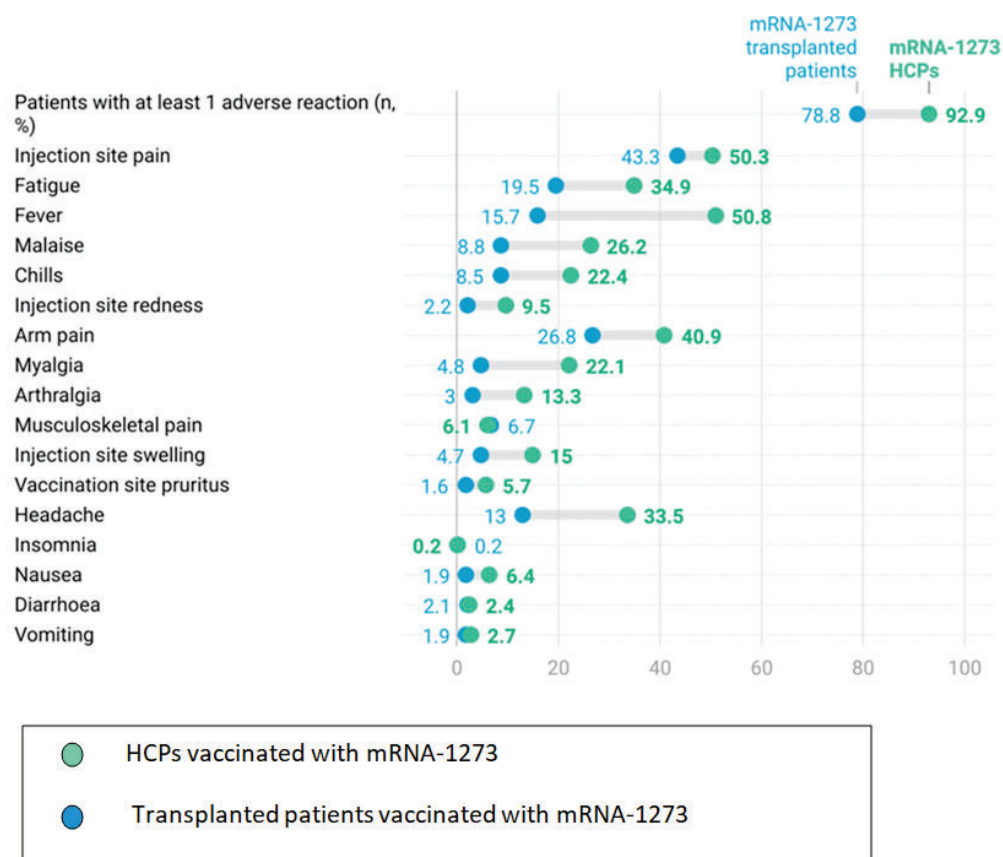
D7 – Interviewers' comments

eFigure 2 BNT162b2 vs mRNA-1273. Distribution of ARs per vaccine group and dose



Adverse reaction distribution per vaccine type (mRNA-1273 vs BNT162b2). Frequencies are displayed for each type of adverse reaction; AR: adverse reactions

eFigure 3 Transplanted patients vs HCPs (mRNA-1273). Distribution of ARs per vaccine group and dose



Adverse reaction distribution per vaccine type (Transplanted patients vs HCPs, both vaccinated with mRNA-1273). Frequencies are displayed for each type of adverse reaction; HCPs: healthcare professionals

Article

Association between Reactogenicity and Immunogenicity in a Vaccinated Cohort with Two mRNA SARS-CoV-2 Vaccines at a High-Complexity Reference Hospital: A *Post Hoc* Analysis on Immunology Aspects of a Prospective Cohort Study

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Citation: Sáez-Peñataro, J.; Calvo, G.; Bascuas, J.; Mosquera, M.M.; Marcos, M.Á.; Egri, N.; Torres, F. Association between Reactogenicity and Immunogenicity in a Vaccinated Cohort with Two mRNA SARS-CoV-2 Vaccines at a High-Complexity Reference Hospital: A *Post Hoc* Analysis on Immunology Aspects of a Prospective Cohort Study. *Vaccines* **2024**, *12*, 665. <https://doi.org/10.3390/vaccines12060665>

Academic Editor: Giuseppe La Torre

Received: 7 May 2024

Revised: 14 June 2024

Accepted: 15 June 2024

Published: 17 June 2024



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Abstract: Enhancing our comprehension of mRNA vaccines may facilitate the future design of novel vaccines aimed at augmenting immune protection while minimising reactogenic responses. Before this design is carried out, it is important to determine whether adaptive immunity correlates with the reactogenicity profile of vaccines. We studied a large cohort that was vaccinated with mRNA vaccines to answer this question. This was an observational study with real-world data. Reactogenicity data were obtained from the VigilVacCOVID study. Immunogenicity (humoral and cellular) data were retrieved from health records. One main population ($n = 215$) and two subpopulations were defined (subpopulation 1, $n = 3563$; subpopulation 2, $n = 597$). Sensitivity analyses were performed with subpopulations 1 and 2 to explore the consistency of results. We analysed the association of the intensity and types of adverse reactions with the development and quantity of elicited antibody titres. As an exploratory analysis in subpopulation 1, we assessed the association between reactogenicity and cellular immunogenicity. A higher incidence of fever, malaise, and myalgia including severe cases was significantly associated with the development and quantity of positive antibody titres. No significant findings were observed with cellular immunity. We observed a positive association between immunogenicity and reactogenicity. These findings can be relevant for the future development of our understanding of how mRNA vaccines function.

Keywords: mRNA vaccines; reactogenicity; immunogenicity; pharmacovigilance; real-world data

1. Introduction

The short-term safety and efficacy of SARS-CoV-2 mRNA vaccines have been widely characterised in pivotal clinical trials [1,2]. After carrying out pivotal clinical trials, several large scale “real-world” studies were conducted, reporting that SARS-CoV-2 vaccines were safe [3–5]. In these studies, the most frequently reported adverse reactions (ARs) were local (e.g., pain at the injection site) or systemic ARs (e.g., fever, myalgia, or malaise) [1,2]. These were usually self-limited and manageable ARs, which further reinforces the positive benefit–risk balance of the currently available SARS-CoV-2 vaccines.

However, given the emergence of several variants of concern, it is highly likely that new vaccine platforms that target new age strata, new antigens, and different booster doses will continue to be developed.

Undoubtedly, the ideal vaccine construct should effectively mobilize both the innate and adaptive immune responses without provoking systemic inflammation, which could potentially lead to serious ARs. One remaining question is whether vaccine-associated ARs can be uncoupled from the elicited immune response in an ideal vaccine construct [6,7]. Various authors have described binding and neutralizing antibodies as potential immune correlates of protection for SARS-CoV-2 vaccines [8,9]. The question that follows from their research is whether this adaptive immunity is, to some extent, correlated with the reactogenicity profile of vaccines in current vaccine constructs.

Recent studies have suggested that a higher reported frequency of ARs after vaccination could be associated, to some extent, with an increase in antibody titres [10,11]. Krammer et al. reported that individuals with pre-existing SARS-CoV-2 immunity showed a significant spike antibody immunogenicity and experienced more severe reactogenicity after the first dose compared to individuals without pre-existing antibodies [11]. Additionally, Debes et al. reported that spike IgG antibodies were higher in vaccinees with prior SARS-CoV-2 infection and those that reported clinically significant reactions after vaccination [12]. However, to date, the potential link between immunogenicity and reactogenicity has yielded contradictory results [13–15]. Therefore, the association between reactogenicity and immunogenicity after the first and second doses remains to be confirmed in larger vaccinated cohorts, especially in a setting that can be extrapolatable to clinical practice (i.e., in a heterogeneous and more representative population beyond health-care professionals (HCPs)).

Overall, a deeper understanding of the immunological correlates of reactogenicity can assist in the future development of newer vaccine constructs through which immune protection can be boosted without hindering a proportional increase in reactogenicity. This becomes increasingly important given the expected increase in mRNA technology development in several clinical indications beyond SARS-CoV-2 vaccines [16].

In our prior VigilVacCOVID study, we observed a correlation between reactogenicity and immunogenicity [17]. This was evidenced by the consistent association of a higher incidence of AR with younger age and prior exposure to SARS-CoV-2, which is also in line with other published reports [18]. Conversely, reduced vaccine reactogenicity was linked to a compromised immune status, leading to improved tolerability. Following this rationale, we took advantage of the reactogenicity data collected within the framework of the study to analyse the immunogenicity data and substantiate the hypothesis of a potential link between reactogenicity and immunogenicity in a large, vaccinated cohort under the conditions of clinical practice.

The VigilvacCOVID study was an observational, single-centre post-authorisation study aimed at characterising short-term reactogenicity after vaccination with mRNA-1273 and BNT162b2 vaccines in two different populations: HCPs and solid organ transplant recipients (SOTRs). The study was designed in December 2020, and reactogenicity data were obtained during the vaccination campaign conducted at Hospital Clínic of Barcelona (HCB) from 7 January, when a vaccination campaign began in the HCB, until 30 April 2021. The first vaccinee group were HCPs. The type of vaccine was determined by national guidance and availability depending on local supply. Accordingly, at the beginning of the vaccination campaign, BNT162b2 was the only vaccine available for HCPs. The vaccination plan was amended on January 22nd with the inclusion of mRNA-1273 for HCPs, and from March 2nd onwards, mRNA-1273 was also administered to SOTRs. Reactogenicity was collected through a structured questionnaire that was delivered to each vaccinee through a telephone interview after receiving each vaccine dose.

2. Materials and Methods

2.1. Study Design and Collected Data

This was an observational, unicentric study with real-world data (RWD) that was implemented as a post hoc subanalysis of the VigilVacCOVID study [17]. The population of the VigilVacCOVID study included HCPs vaccinated with BNT162b2 and mRNA-1273 vaccines and SOTRs vaccinated with mRNA-1273. The study was reported following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for cross-sectional studies [19]. The current post hoc subanalysis was approved by the Ethics Committee of HCB as a major amendment of the VigilVacCOVID study (references: HCB/2021/0684-amendment 1 and HCB/2021/0685-amendment 1). An informed consent waiver was approved by the Ethics Committee given the retrospective nature of the data collection and review, with no interventions performed on patients.

Reactogenicity and tolerability results had been previously obtained during the vaccination campaign in the context of the VigilVacCOVID study. The characteristics of the implemented surveillance program, the methodology of the safety data collection, and the overall reactogenicity profile assessed in the main VigilVacCOVID study are described elsewhere [17].

For the current study, we obtained the immunogenicity results of the above-mentioned participants from the electronic health records of HCB, with the collaboration of the Microbiology and Immunology Departments. The immunogenicity data included humoral (i.e., antibody titres) and cellular immunological results. Humoral immunogenicity had been assessed via the analysis of neutralising and anti-spike antibodies (IgG + IgM), based on the semiquantitative measurement of antibody titres on a scale ranging from 0 to >10. Results of >1.0 were considered positive antibody titres [20]. IgG titres were measured on samples that tested positive in the Cobas platform (Cobas; Roche Molecular Systems, Branchburg, NJ, USA) in a subset of patients, as per local clinical practice protocols [21]. IgG was measured only in a subset of the total vaccinated population (N = 322 vaccinees). Cellular immunogenicity was measured as the development of T cell-virus-specific responses as assessed via the interferon-gamma ELISPOT assay applied to isolated peripheral blood mononuclear cells (PBMCs) [22]. Given that cellular immunogenicity assessment was not routine clinical practice at the time of the vaccination campaign, data on cellular immunity were only available for a subset of vaccinees (N = 59 vaccinees). The complete method description is provided in the Supplementary Materials.

2.2. Statistical Analysis

2.2.1. Sample Size Calculation and Planned Analyses

The VigilVacCOVID cohort comprised HCPs vaccinated with BNT162b2 and mRNA-1273 vaccines and SOTRs vaccinated with mRNA-1273. It included a total of 6865 subjects, of whom 5088 responded to the questionnaire and thereby provided reactogenicity data for the current study. Of these, immunogenicity data were available for 3563 participants.

Given that a prospective analysis of immunogenicity had not been planned during our previous VigilVacCOVID study and considering the retrospective and real-world nature of data collection, there were some constraints that we had to take into consideration for the analysis of results. On one hand, the method previously used to measure humoral immunogenicity in clinical practice was semiquantitative and did not distinguish between infection-related (nucleocapsid protein (NCP) IgG antibodies) and vaccine-related (neutralising IgG antibodies to the receptor-binding protein of the S1 subunit of the spike protein) neutralising antibodies. SARS-CoV-2 tests were not routinely performed before and after vaccination. Likewise, in this initial population with available immunogenicity data (N = 3563), some immunity assessments had been performed either within a 4-month period after vaccination or prior to vaccination. We considered that the positive antibody titres of these distant measurements were not representative of ARs occurring immediately after receiving vaccine doses, nor were they necessarily related to vaccination. For instance, the possibility exists that some of the included participants developed immuno-

genicity before or after vaccination because of SARS-CoV-2 infection and not specifically due to vaccination.

To adjust these potential biases, we decided to perform two sensitivity analyses (see flowchart in Figure 1). We first refined the initial population by restricting the immunogenicity assessment timeframe to 4 weeks, and we defined subpopulation 2 for sensitivity analysis 2 (N = 597). Furthermore, we excluded vaccinees who had immunogenicity data prior to vaccination (N = 215). This was considered the most sensitive and least-biased population, and for this reason, it was considered the main population for analysis. We subsequently redefined the initial population (N = 3563) as subpopulation 1, and we considered the analysis of this population as sensitivity analysis 1. The main aim of this sensitivity analysis was to assess the consistency of results across analyses with a higher sample size. Baseline characteristics of subpopulations 1 and 2 are displayed in the Supplementary Materials (Tables S7 and S17), and the baseline characteristics of the main population are depicted in Table 1.

Table 1. Characteristics of the participants (main analysis).

Covariates	Total Population (N = 215)
Age	
N	215
Mean (SD)	41.13 (12.71)
Gender	
Male (n, %)	70 (32.6)
Female (n, %)	145 (67.4)
Occupational SARS-CoV-2 contact	
In contact with SARS-CoV-2 patients	105 (48.8)
Without contact with SARS-CoV-2 patients	110 (51.2)
^a Previous SARS-CoV-2 infection	
Yes	78 (36.3)
No	137 (63.7)
Comorbidities	
^b Any comorbidity	83 (38.6)
Arterial hypertension	12 (5.6)
Diabetes mellitus	3 (1.4)
Heart failure	1 (0.5)
Asthma	8 (3.7)
Rheumatic/immune-mediated disease	1 (0.5)
Drug allergies	17 (7.9)
Food allergies	10 (4.7)
Type of administered vaccine	
BNT16b2	
First dose:	194 (90.2)
Second dose:	138 (64.2)
mRNA-1273	
First dose:	21 (9.8)
Second dose:	17 (7.9)
Humoral immunogenicity (antibody positiveness on semiquantitative antibody titres)	
Number of vaccinees with positive antibody titres (titres of >1.0) (n, %)	151 (70)
Number of vaccinees with negative antibody titres (titres of <1.0) (n, %)	64 (30)

Table 1. Cont.

Covariates	Total Population (N = 215)
Humoral immunogenicity (antibody semiquantitative titres above/below median)	
Median value of antibody titres (P25, P75) *	7.69 (0.73,10.00)
Number of vaccinees with antibody titres above median (n, %)	93 (43%)
Number of vaccinees with antibody titres below median (n, %)	122 (57%)

Covariables of studied population. ^a Diagnosis via RCP, antigen test, and antibody positive serology. ^b Number of vaccinees with at least one comorbidity; * On a semiquantitative scale ranging from 0 to >10.

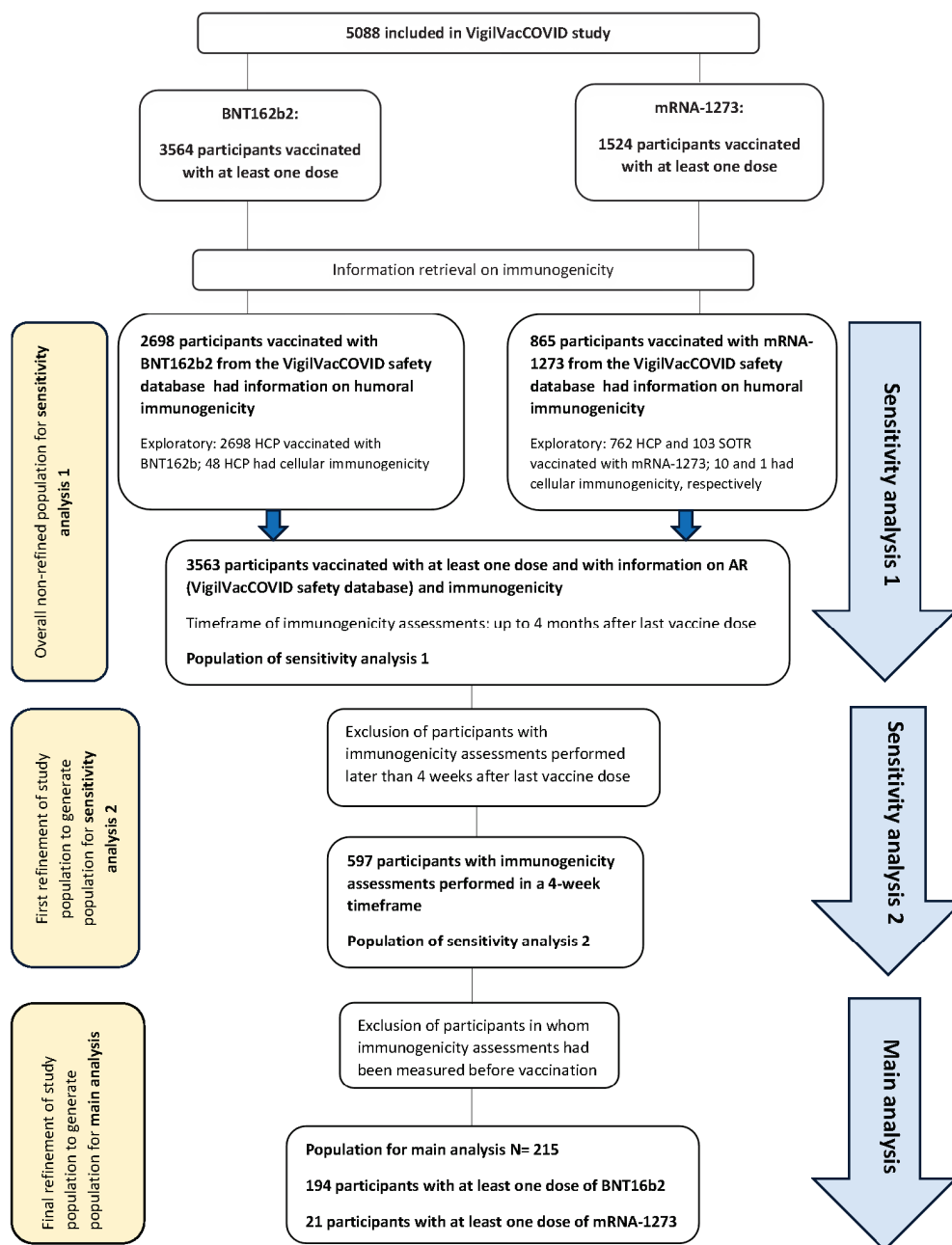


Figure 1. Flowchart of the study displaying the populations for the main analysis and sensitivity analyses.

2.2.2. Study Endpoints

The primary endpoint was to assess the association between reactogenicity after vaccination and the development of humoral immunogenicity in the main population. Reactogenicity was defined in terms of the proportion of vaccinees with mild, moderate, and severe ARs for each immunogenicity category and in terms of AR intensity.

The key secondary endpoint was to assess the association between specific solicited and unsolicited AR and the development of immunogenicity.

As exploratory endpoints, we assessed the same reactogenicity and humoral immunogenicity endpoints as in sensitivity analyses 1 and 2. In addition, cellular immunogenicity was assessed as an exploratory endpoint in subpopulation 1. Cellular immunogenicity was not assessed in the main population, nor in subpopulation 2, given the lack of sample size and available data on cellular immunogenicity.

Finally, reactogenicity and humoral immunogenicity were assessed, in a descriptive and exploratory manner, in different subgroups of subpopulation 1, to assess potential trends that could suggest a difference with regard to the type of administered vaccine (mRNA-1273 vs. BTN162b2) or the vaccinated population (HCP vs. SOTR). Due to the sample size limitations and its exploratory nature, this descriptive analysis was only performed on subpopulation 1 and only considered the humoral immunogenicity results.

2.2.3. Statistical Analysis

Categorical variables were analysed as frequencies and percentages, whereas continuous variables were described as the mean $\pm$ standard deviation or median (25–75% interquartile range), as deemed suitable. Categorical data underwent comparison via the chi-square test or the Fisher's exact test, as applicable. Continuous variables were assessed using Mann–Whitney testing.

Reactogenicity data were expressed in terms of the solicited and unsolicited AR rate (n, %), types of solicited and unsolicited ARs, intensity distribution of solicited ARs according to three grades of maximum intensity (mild, moderate, severe), and the mean and median Likert score (LS) values of the solicited ARs. Intensity was assessed as the proportion of vaccinees with mild, moderate, and severe ARs for each immunogenicity category (positive or negative). In addition, the mean (SD) Likert score (LS) (with values ranging from 0 to 10) of the intensity of the developed ARs was assessed for each immunogenicity category. Finally, the distribution of AR intensity in each immunogenicity category was analysed with the maximum intensity grade of AR experienced after vaccination and the distribution (%) of vaccinees in each maximum grade according to whether they tested positive or negative in the immunogenicity tests. These analyses were performed after the first, second, or any vaccine dose. Severe ARs were defined by an LS score of 7–10. Intensity was measured for solicited ARs, which were the primary events for study. Due to the subjectivity of some solicited ARs and the questionnaire-based methodology of the study, intensity was not measured for insomnia, nausea, diarrhoea, and vomiting, or for unsolicited ARs that had been collected through a free text option.

Given that specific IgG titres were only measured in 322 vaccinees, and on a different platform than the one used for total antibody titres, we decided to use only the total semiquantitative antibody titres for the immunogenicity analysis and not IgG titres. For the analysis of immunogenicity, we defined antibody positiveness considering a cut-off of >1.0 (positive humoral immunogenicity above a cut-off of >1.0 ; for the cut-off and detection method, see the Materials and Methods and Supplementary Materials). Given that antibody titres were measured with a semiquantitative method on a scale ranging from 0 to >10 , we calculated the median value of the titres' level (second quartile, Q2), and then analysed the distribution of vaccinees above and below the median to carry out a semiquantitative assessment that considered the extent of elicited humoral immunogenicity. Results were expressed as absolute and relative numbers of vaccinees with the reported AR and negative antibody titres or titres below the median value vs. vaccinees with the reported AR and positive antibody titres or titres above the median value, with *p*-values.

Cellular immunogenicity was not assessed in the main population, nor in subpopulation 2, given the lack of sample size and available data on cellular immunogenicity ($N = 59$) and because this was considered as an exploratory analysis.

We applied a two-sided significance level of 5%. All calculations were performed using SAS v9.4 software (Cary, NC, USA). In addition, we conducted exploratory analyses using a less conservative significance level of 10% to explore, in a descriptive manner, any potential relationships that could be of clinical interest despite not meeting the standard 5% threshold.

3. Results

3.1. Immunogenicity and Reactogenicity Results

The overall results are depicted in Tables 2–4. The individual absolute and relative numbers of each assessed variable can be found in the tables in the Supplementary Materials. Figures 2–4 depict the association between immunogenicity and reactogenicity (as a percentage of solicited ARs). For the purposes of clarity and to highlight the main results of the analyses, only those ARs that showed a significant association are displayed. The complete graphic results of Tables 2–4 are shown in the Supplementary Materials, Figures S1–S3.

3.1.1. Main Analysis (Main Population, $N = 215$)

Immunogenicity Assessed as Positive/Negative Antibody Titres

We did not observe any significant association with the maximum intensity grades or LS (mean, SD). Regarding specific ARs, we observed a significant association between the development of severe malaise after any dose and the development of positive antibody titres against SARS-CoV-2 (vaccinees with malaise: 1 (1.6%) with negative antibody titres, 15 (9.9%) with positive antibody titres, $p = 0.033$) (Figure 2, Table 2, and Tables S1–S3 in the Supplementary Materials).

Immunogenicity Assessed as Antibody Titres above/below Median

No significant associations were observed in the intensity analysis (Table 2 and Table S4 in the Supplementary Materials).

Regarding specific ARs, we observed a significant association between the development of fever (after first dose: 5 (5.4%) vs. 19 (15.6%), $p = 0.019$; after any dose: 0 (0.0%) vs. 7 (5.7%), $p = 0.019$; after second dose: 0 (0.0%) vs. 5 (4.1%), $p = 0.048$), arm pain (after first dose: 10 (10.8%) vs. 26 (21.3%), $p = 0.019$), lymphadenopathy (after any dose: 0 (0.0%) vs. 6 (4.9%), $p = 0.030$; after second dose: 0 (0.0%) vs. 5 (4.1%), $p = 0.048$), and the measurement of semiquantitative antibody titres above the median. These ARs developed after any vaccine dose and did not demonstrate a specific predominance in frequency over the first or second doses (Figure 2, Table 2, and Table S5 in the Supplementary Materials).

In terms of severe ARs, we observed a significant association with the development of severe fever (after any dose: 0 (0.0%) vs. 7 (5.7%), $p = 0.019$; after second dose: 0 (0.0%) vs. 5 (4.1%), $p = 0.048$) (Table 2 and Table S6 in the Supplementary Materials).

Table 2. Results of the main analysis.

	Main Analysis					
	N = 215	Antibody Positiveness		Antibody Titres		p-Value
		Negative Antibody Titres (n = 64)	Positive Antibody Titres (n = 151)	Antibody Titres below Median * (n = 93)	Antibody Titres above Median * (n = 122)	
Proportion of AR (% of vaccinees with an AR)	Any mild AR after 1st dose (%)	19 (29.7)	28 (18.5)	22 (23.7)	25 (20.5)	0.578
	Malaise after any dose (%)	4 (6.3)	24 (15.9)	9 (9.7)	19 (15.6)	0.203
	Malaise after 2nd dose (%)	3 (4.7)	19 (12.6)	8 (8.6)	14 (11.5)	0.491
	Severe malaise after any dose (%)	1 (1.6)	15 (9.9)	4 (4.3)	12 (9.8)	0.126
	Severe malaise after 2nd dose (%)	1 (1.6)	13 (8.6)	4 (4.3)	10 (8.2)	0.251
	Fever after 1st dose (%)	4 (6.3)	20 (13.2)	5 (5.4)	19 (15.6)	0.019
	Severe fever after any dose (%)	0 (0.0)	7 (4.6)	0 (0.0)	7 (5.7)	0.019
	Severe fever after 2nd dose (%)	0 (0.0)	5 (3.3)	0 (0.0)	5 (4.1)	0.048
	Severe fatigue after any dose (%)	9 (14.1)	10 (6.6)	13 (14.0)	6 (4.9)	0.020
	Arm pain after any dose (%)	15 (23.4)	38 (25.2)	17 (18.3)	36 (29.5)	0.058 ⁿ
Proportion of specific AR (% of vaccinees with an AR)	Arm pain after 1st dose (%)	9 (14.1)	27 (17.9)	10 (10.8)	26 (21.3)	0.040
	Lymphadenopathy after any dose (%)	0 (0.0)	6 (4.0)	0 (0.0)	6 (4.9)	0.030
	Lymphadenopathy after 2nd dose (%)	0 (0.0)	5 (3.3)	0 (0.0)	5 (4.1)	0.048
	Severe myalgia after 1st dose (%)	2 (3.1)	7 (4.6)	0 (0.0)	4 (3.3)	0.078 ⁿ
	Severe injection site swelling after any dose (%)	0 (0.0)	4 (2.6)	0 (0.0)	4 (3.3)	0.078 ⁿ
	Severe injection site swelling after 1st dose (%)	0 (0.0)	4 (2.6)	0 (0.0)	4 (3.3)	0.078 ⁿ

NS: Non-significant; $p \leq 0.05$: statistically significant; statistically significant results that are consistent in the three analyses (overall immunogenicity analysis) are shown in bold type; ⁿ ARs for which a positive nominal trend was observed; * Median value of antibody titres (P25, P75) = 7.69 (0.73, 10.00) on a semiquantitative scale ranging from 0 to >10.

Table 3. Results of sensitivity analysis 1.

Sensitivity Analysis 1						
N = 3563	Antibody Positiveness			Antibody Titres		
	Negative Antibody Titres (n = 76)	Positive Antibody Titres (n = 3487)	p-Value	Antibody Titres below Median * (n = 440)	Antibody Titres above Median * (n = 3123)	p-Value
Intensity (maximum intensity grade; % vaccinees at each grade)	Grade 1 after any vaccine dose (%)	15 (23.8)	350 (12.2)	62 (16.9)	303 (11.8)	
	Grade 2 after any vaccine dose (%)	23 (36.5)	1132 (39.3)	142 (38.7)	1013 (39.3)	0.017
	Grade 3 after any vaccine dose (%)	25 (39.7)	1397 (48.5)	163 (44.4)	1259 (48.9)	
	Grade 1 after 2nd vaccine dose (%)	12 (27.3)	261 (11.6)	47 (17.0)	226 (11.2)	
	Grade 2 after 2nd vaccine dose (%)	19 (43.2)	949 (42.3)	121 (43.8)	847 (42.1)	0.006
	Grade 3 after 2nd vaccine dose (%)	13 (29.5)	1036 (46.1)	108 (39.1)	941 (46.7)	
Intensity (mean, SD Likert score)	After any dose (mean, SD)	5.48 (2.47)	6.15 (2.36)	5.91 (2.17)	6.16 (2.38)	0.067 ⁿ
	After 2nd dose (mean, SD)	4.95 (2.37)	6.10 (2.06)	5.64 (2.14)	6.14 (2.05)	0.003
	Any AR after 1st dose (%)	56 (73.7)	2222 (63.7)	295 (67.0)	1983 (63.5)	0.147
	Any AR after 2nd dose (%)	45 (59.2)	2335 (67.0)	291 (66.1)	2089 (66.9)	0.753
Proportion of ARs (% of vaccinees with an AR)	Any mild AR after any dose (%)	29 (38.2)	1089 (31.2)	168 (38.2)	950 (30.4)	0.001
	Any mild AR after 1st dose (%)	21 (27.6)	667 (19.1)	102 (23.2)	586 (18.8)	0.028
	Any mild AR after 2nd dose (%)	17 (22.4)	622 (17.8)	99 (22.5)	540 (17.3)	0.008
	Any severe AR after 2nd dose (%)	13 (17.1)	1036 (29.7)	108 (24.5)	941 (30.1)	0.016
	Fever after 2nd dose (%)	11 (14.5)	974 (27.9)	114 (25.9)	871 (27.9)	0.380
	Malaise after any dose (%)	7 (9.2)	622 (17.8)	68 (15.5)	561 (18.0)	0.200
Proportion of specific ARs (% of vaccinees with an AR)	Malaise after 2nd dose (%)	4 (5.3)	517 (14.8)	56 (12.7)	465 (14.9)	0.230
	Severe malaise after any dose (%)	1 (1.2)	305 (8.8)	32 (7.3)	274 (8.8)	0.290
	Severe malaise after 2nd dose (%)	1 (1.2)	258 (7.4)	25 (5.7)	234 (7.5)	0.170
	Severe arm pain after 2nd dose (%)	1 (1.3)	187 (5.4)	15 (3.4)	173 (5.5)	0.060 ⁿ
	Chills after 2nd dose (%)	2 (2.6)	385 (11.0)	35 (8.0)	352 (11.3)	0.040

Table 3. Cont.

Sensitivity Analysis 1							
N = 3563	Antibody Positiveness			Antibody Titres			
	Negative Antibody Titres (n = 76)	Positive Antibody Titres (n = 3487)	p-Value	Antibody Titres below Median * (n = 440)	Antibody Titres above Median * (n = 3123)	p-Value	
	Myalgia after any dose (%)	5 (6.6)	709 (20.3)	0.003	94 (21.4)	620 (19.9)	0.460
	Myalgia after 2nd dose (%)	4 (5.3)	621 (17.8)	0.004	79 (18.0)	546 (17.5)	0.810
	Severe myalgia after 2nd dose (%)	2 (2.6)	296 (8.5)	0.070 ⁿ	29 (6.6)	269 (8.6)	0.150
	Headache after any dose (%)	11 (14.5)	967 (27.7)	0.010	118 (26.8)	860 (27.5)	0.750
	Headache after 2nd dose (%)	8 (10.5)	750 (21.5)	0.020	89 (20.2)	669 (21.4)	0.570
	Severe headache after any dose (%)	3 (3.9)	419 (12.0)	0.030	39 (8.9)	383 (12.3)	0.040
	Severe headache after 2nd dose (%)	2 (2.6)	331 (9.5)	0.040	28 (6.4)	305 (9.8)	0.020

Table 4. Results of sensitivity analysis 2.

Sensitivity Analysis 2						
Intensity (maximum intensity grade; % vaccinees at each grade) Intensity (mean, SD Likert score)	Antibody Positiveness			Antibody Titres		
	Negative Antibody Titres (n = 273)	Positive Antibody Titres (n = 324)	p-Value	Antibody Titres below Median * (n = 288)	Antibody Titres above Median * (n = 309)	p-Value
	Grade 1 after 1st vaccine dose (%)	48 (17.6)	39 (12.0)	50 (26.2)	37 (17.5)	0.072 ⁿ
	Grade 2 after 1st vaccine dose (%)	79 (28.9)	94 (29.0)	81 (42.4)	92 (43.4)	
	Grade 3 after 1st vaccine dose (%)	53 (19.4)	90 (27.8)	60 (31.4)	83 (39.2)	
	After 1st dose (mean, SD)	3.37 (2.99)	3.83 (3.21)	5.28 (2.18)	5.65 (2.09)	0.083 ⁿ
	After 2nd dose (mean, SD)	3.63 (3.31)	3.01 (3.23)	5.87 (2.13)	5.77 (2.02)	0.570

Table 4. Cont.

Sensitivity Analysis 2							
N = 597	Antibody Positiveness			Antibody Titres			
	Negative Antibody Titres (n = 273)	Positive Antibody Titres (n = 324)	p-Value	Antibody Titres below Median * (n = 288)	Antibody Titres above Median * (n = 309)	p-Value	
Proportion of ARs (% of vaccinees with)	Any AR after 2nd dose (%)	187 (68.5)	188 (58.0)	0.008	193 (67.0)	182 (58.9)	0.040
	Any moderate AR after 1st dose (%)	99 (36.3)	139 (42.9)	0.099	103 (35.8)	135 (43.7)	0.048
	Any moderate AR after 2nd dose (%)	126 (46.2)	124 (38.3)	0.052 ⁿ	129 (44.8)	121 (39.2)	0.163
	Any severe AR after 1st dose (%)	53 (19.4)	90 (27.8)	0.017	60 (20.8)	83 (26.9)	0.085 ⁿ
	Fatigue after 1st dose (%)	21 (7.7)	20 (6.2)	0.465	24 (8.3)	43 (13.9)	0.031
	Fever after 1st dose (%)	16 (5.9)	73 (22.5)	<0.001	19 (6.6)	70 (22.7)	<0.001
	Severe fever after 1st dose (%)	0 (0.0)	11 (3.4)	0.002	1 (0.3)	10 (3.2)	0.009
	Malaise after any dose (%)	37 (13.6)	76 (23.5)	0.002	41 (14.2)	72 (23.3)	0.004
	Malaise after 1st dose (%)	5 (1.8)	36 (11.1)	<0.001	7 (2.4)	34 (11.0)	<0.001
	Severe malaise after any dose (%)	17 (6.2)	42 (13.0)	0.006	19 (6.6)	40 (12.9)	0.009
Proportion of specific ARs (% of vaccinees with)	Severe malaise after 1st dose (%)	3 (1.1)	18 (5.6)	0.003	5 (1.7)	16 (5.2)	0.023
	Chills after 1st dose (%)	2 (0.7)	25 (7.7)	<0.001	4 (1.4)	23 (7.4)	0.004
	Myalgia after 1st dose (%)	9 (3.3)	36 (11.1)	0.003	9 (3.1)	36 (11.7)	<0.001
	Severe myalgia after 1st dose (%)	3 (1.0)	18 (5.8)	0.003	3 (1.0)	18 (5.8)	0.002
	Other musculoskeletal disorders after 1st dose (%)	2 (0.7)	9 (2.8)	0.064 ⁿ	3 (1.0)	8 (2.6)	0.160
	Arthralgia after 1st dose (%)	0 (0.0)	10 (3.1)	0.034	1 (0.3)	9 (2.9)	0.015
	Severe arthralgia after 1st dose (%)	0 (0.0)	6 (1.9)	0.024	1 (0.3)	5 (1.6)	0.120
	Headache after 1st dose (%)	24 (8.8)	48 (14.8)	0.024	26 (9.0)	46 (14.9)	0.028
	Severe headache after 1st dose (%)	6 (2.2)	20 (6.2)	0.018	6 (2.1)	20 (6.5)	0.009

NS; Non-significant; $p \leq 0.05$; statistically significant; statistically significant results that are consistent in the three analyses (overall immunogenicity analysis) are shown in bold type; ⁿ AR for which a positive nominal trend was observed; * Median value of antibody titres (P25, P75) = 2.53 (0.47, 10.00) on a semiquantitative scale ranging from 0 to >10.

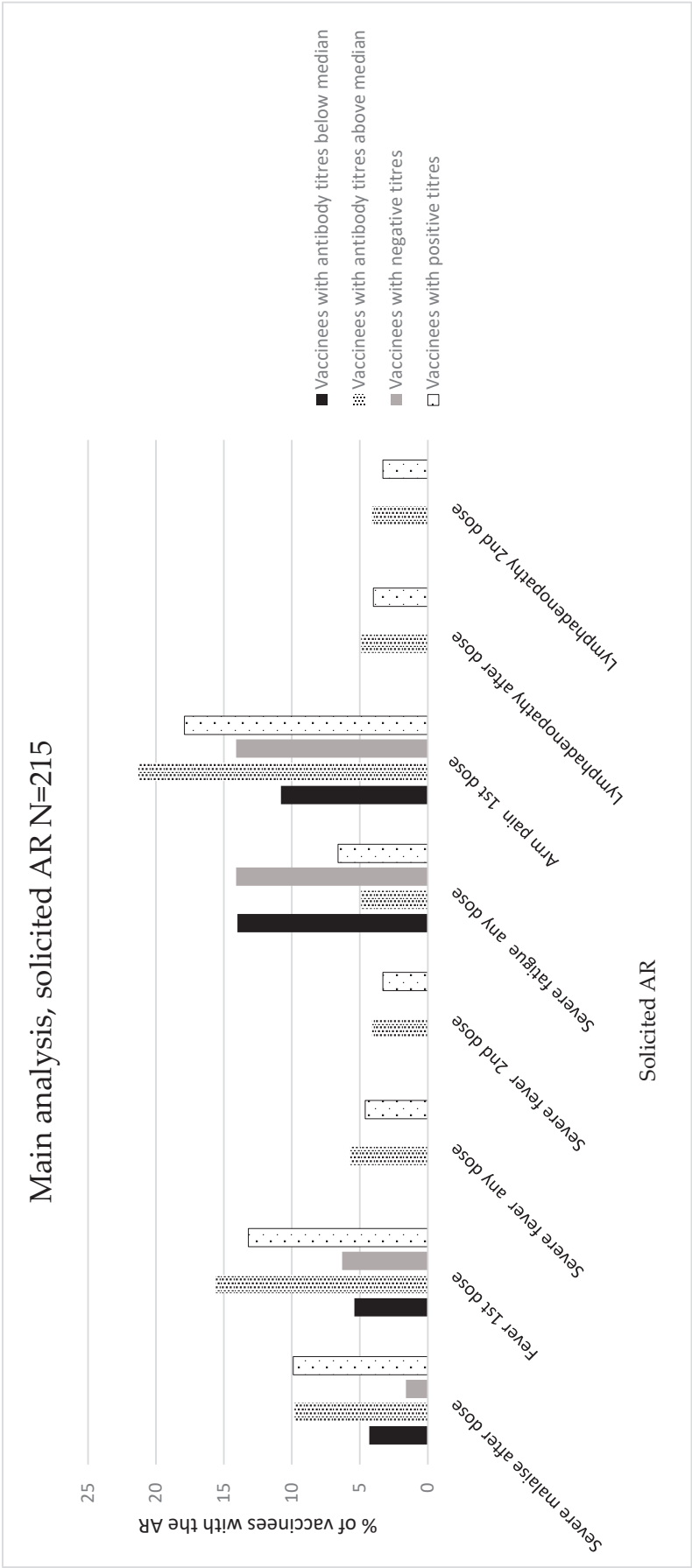


Figure 2. Immunogenicity and reactogenicity results of the main population. Bar diagram of the results in Table 2. Immunogenicity is expressed as antibody positiveness (positive/negative titres) and antibody titres (antibody titres below/above median) data (%). Reactogenicity results are represented as a percentage of vaccinees developing each solicited AR.

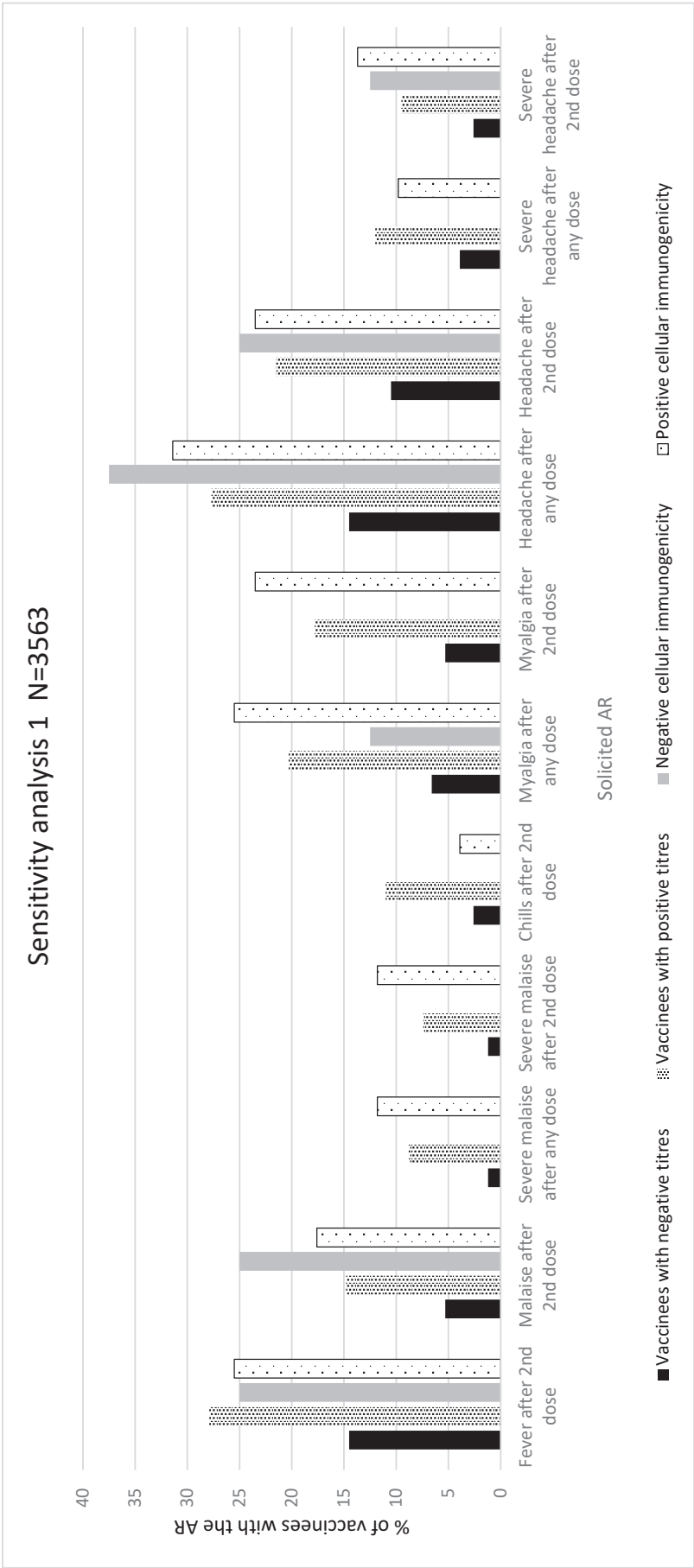


Figure 3. Immunogenicity and reactogenicity results of sensitivity analysis 1. Bar diagram of the results in Table 3. Immunogenicity is expressed as antibody positiveness (positive/negative titres) and cellular immunogenicity (positive/negative) data (%). Reactogenicity results are represented as a percentage of vaccinees developing each solicited AR.

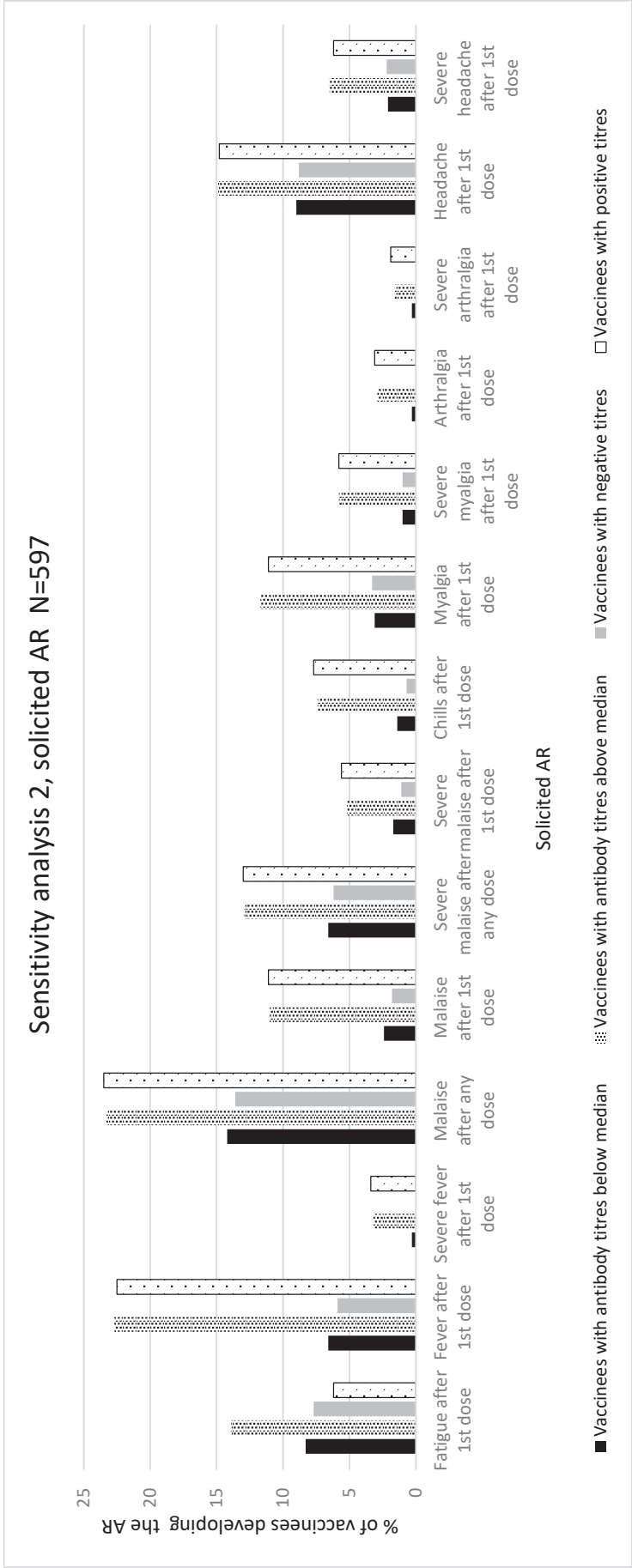


Figure 4. Immunogenicity and reactogenicity results of sensitivity analysis 2. Bar diagram of the results in Table 4. Immunogenicity is expressed as antibody positiveness (positive/negative titres) and antibody titres (antibody titres below/above median) data (%). Reactogenicity results are represented as a percentage of vaccinees developing each solicited AR.

3.1.2. Sensitivity Analysis 1 (N = 3563)

Immunogenicity Assessed as Positive/Negative Antibody Titres

A significant association was found in the distribution of ARs of grades 1–3 after any vaccine dose (grade 1: 15 (23.8% vs. 350 (12.2%); grade 2: 23 (36.5%) vs. 1132 (39.3%); grade 3: 25 (39.7%) vs. 1397 (48.5%), $p = 0.020$) and after a second vaccine dose (grade 1: 12 (27.3%) vs. 261 (11.6%); grade 2: 19 (43.2%) vs. 949 (42.3%); grade 3: 13 (29.5%) vs. 1036 (46.1%, $p = 0.003$)). We also found a significant association with intensity measured by LS (mean, SD) after any vaccine dose (5.48 (2.47%) vs. 6.15 (2.36%), $p = 0.031$) and after a second vaccine dose (4.95 (2.37%) vs. 6.10 (2.06%), $p = 0.001$). Likewise, there was a significantly higher proportion of severe ARs after a second dose (13 (17.1%) vs. 1036 (29.7%), $p = 0.017$) (Table 3, and Table S8 in the Supplementary Materials).

Regarding specific ARs, we observed a significantly higher proportion of fever after a second dose (11 (14.5%) vs. 974 (27.9%), $p = 0.010$), malaise after any dose (7 (9.2%) vs. 622 (17.8%), $p = 0.050$) and after a second dose (4 (5.3%) vs. 517 (14.8%), $p = 0.020$), chills after a second dose (2 (2.6%) vs. 385 (11.0%), $p = 0.020$), myalgia after any dose (5 (6.6%) vs. 709 (20.3%), $p = 0.003$) and after a second dose (4 (5.3%) vs. 621 (17.8%), $p = 0.004$), and headache after any dose (11 (14.5%) vs. 967 (27.7%), $p = 0.010$) and after a second dose (8 (10.5%) vs. 750 (21.5%), $p = 0.020$), (Table S9 in the Supplementary Materials). Regarding severe specific ARs, a higher proportion of these were observed for severe malaise after any dose (1 (1.2%) vs. 305 (8.8%), $p = 0.010$), severe malaise after a second dose (1 (1.2%) vs. 258 (7.4%), $p = 0.030$), severe headache after any dose (3 (3.9%) vs. 419 (12.0%), $p = 0.030$), and severe headache after a second dose (2 (2.6%) vs. 331 (9.5%), $p = 0.040$) (Figure 3, Table 3, and Table S10 in the Supplementary Materials).

Immunogenicity Assessed as Antibody Titres above/below Median

We observed a significant association for ARs of grades 1–3, LS (mean, SD), and the development of any AR after a second vaccine dose (Table S11 in the Supplementary Materials).

As for specific ARs, we observed a significantly higher proportion of chills after a second dose (35 (8.0%) vs. 352 (11.3%), $p = 0.040$) as well as severe headache after any dose (39 (8.9%) vs. 383 (12.3%), $p = 0.040$) and severe headache after a second dose (28 (6.4%) vs. 305 (9.8%), $p = 0.020$) in vaccinees with antibody titres above the median cut-off (Table 3 and Tables S12 and S13 in the Supplementary Materials).

Cellular Immunogenicity

A positive trend of a higher frequency of vaccinees with positive cellular immunogenicity was observed for some solicited ARs such as malaise, chills, arm pain, myalgia, and severe headache (Figure 3 and Tables S14–S16 in the Supplementary Materials). However, none of these associations were statistically significant. The analysis of intensity did not show any differences between vaccinees with negative and positive cellular immunogenicity.

Descriptive Analysis According to Type of Vaccine and Population

Regarding vaccine type (mRNA-1273 vs. BNT162b2), data obtained from HCPs vaccinated with both types of vaccine showed similar immunogenicity rates (98.5% vs. 99% of vaccinees with positive antibody titres, respectively). However, the reactogenicity seemed to be greater with mRNA-1273 vaccines than with BNT162b2 (the proportion of any AR after vaccination was 97.4% vs. 81.7%, respectively), especially concerning severe AR rates (61.8% vs. 34.2%, respectively). Increased reactogenicity with mRNA-1273 vaccines was also observed in the analysis of solicited ARs (Table S24 and Figure S1).

We also observed a difference in terms of immunogenicity and reactogenicity between the HCPs and SOTRs after vaccination with mRNA-1273 vaccines. Accordingly, we observed a decreased rate of immunogenicity in SOTRs as opposed to HCPs (57% vs. 98.5% of vaccinees with positive antibody titres, respectively) and a decreased reactogenicity rate (proportion of vaccinees with at least one AR, 87.4% vs. 97.4%, respectively; proportion of

vaccinees with at least one severe AR, 28.2% vs. 61.8%, respectively). The same trend was observed with the solicited ARs (Table S24, Figure S2).

3.1.3. Sensitivity Analysis 2 (N = 597)

Immunogenicity Assessed as Positive/Negative Antibody Titres

Although there was a positive trend, we did not find a significant association with ARs of grades 1–3 after the first vaccine dose. There was a significant association with intensity measured as the LS after a second dose (3.63 (3.31%) vs. 3.01 (3.23%), $p = 0.001$). In addition, we observed a positive association between the development of any AR after a second dose (187 (68.5%) vs. 188 (58.0%), $p = 0.008$) and the development of any severe AR after the first dose (53 (19.4%) vs. 90 (27.8%), $p = 0.017$) (Table 4 and Table S18 in the Supplementary Materials).

As for the distribution of specific ARs, we observed a significant association with fever after a first dose (16 (5.9%) vs. 73 (22.5%), $p \leq 0.001$), malaise after any dose (37 (13.6%) vs. 76 (23.5%), $p \leq 0.002$) and after a first dose (5 (1.8%) vs. 36 (11.1%), $p \leq 0.001$), chills after a first dose (2 (0.7%) vs. 25 (7.7%), $p \leq 0.001$), myalgia after a first dose (9 (3.3%) vs. 36 (11.1%), $p = 0.003$), arthralgia after a first dose (0 (0.0%) vs. 10 (3.1%), $p = 0.034$), and headache after a first dose (24 (8.8%) vs. 48 (14.8%), $p = 0.024$) (Table 2 and Table S19 in the Supplementary Materials). Regarding severe ARs, severe fever after a first dose (0 (0.0%) vs. 11 (3.4%), $p = 0.002$), severe malaise after any dose (17 (6.2%) vs. 42 (13.0%), $p = 0.006$) and after a first dose (3 (1.1%) vs. 18 (5.6%), $p = 0.003$), severe myalgia after a first dose (3 (1.0%) vs. 18 (5.8%), $p = 0.003$), severe arthralgia after a first dose (0 (0.0%) vs. 6 (1.9%), $p = 0.024$), and severe headache after a first dose (6 (2.2%) vs. 20 (6.2%), $p = 0.018$) showed a significant association with the development of positive antibody titres (Figure 4, Table 4, and Table S20 in the Supplementary Materials).

Immunogenicity Assessed as Antibody Titres above/below Median

We did not observe any significant association with the maximum intensity grades or with LS (mean, SD). We observed a significant association with the proportion of ARs after a second dose (193 (67.0%) vs. 182 (58.9%), $p = 0.040$) and the proportion of any moderate AR after the first dose (103 (35.8%) vs. 135 (43.7%), $p = 0.048$) (Table 4 and Table S21 in the Supplementary Materials).

A positive association was also observed for some specific ARs such as fatigue after the first dose (24 (8.3%) vs. 43 (13.9%), $p = 0.031$), fever after the first dose (19 (6.6%) vs. 70 (22.7%), $p \leq 0.001$), malaise after any dose (41 (14.2%) vs. 72 (23.3%), $p = 0.004$) and after the first dose (7 (2.4%) vs. 34 (11.0%), $p \leq 0.001$), chills after a first dose (4 (1.4%) vs. 23 (7.4%), $p = 0.004$), myalgia after a first dose (9 (3.1%) vs. 36 (11.7%), $p \leq 0.001$), arthralgia after the first dose (1 (0.3%) vs. 9 (2.9%), $p = 0.015$), and headache after a first dose (26 (9.0%) vs. 46 (14.9%), $p = 0.028$). Regarding severe ARs, we observed a significant association for severe fever after the first dose (1 (0.3%) vs. 10 (3.2%), $p = 0.009$), severe malaise after any dose (19 (6.6%) vs. 40 (12.9%), $p = 0.009$) and after the first dose (5 (1.7%) vs. 16 (5.2%), $p = 0.023$), severe myalgia after the first dose (3 (1.0%) vs. 18 (5.8%), $p = 0.002$), severe arthralgia after the first dose (1 (0.3%) vs. 5 (1.6%), $p = 0.120$), and severe headache after a first dose (6 (2.1%) vs. 20 (6.5%), $p = 0.009$) (Table 4 and Tables S22–S23 in the Supplementary Materials).

3.1.4. Overall Reactogenicity and Immunogenicity Analysis

Tables 2–4 depict ARs for which statistical significance or a trend towards significance (with a more conservative alpha level of 10%) was observed. After a consistency assessment among the different analyses, a higher proportion of fever, malaise, and myalgia including severe episodes of these ARs was observed among vaccinees that developed positive antibody titres in the three analyses. The proportion of vaccinees with myalgia was statistically significant in sensitivity analyses 1 and 2, and a trend towards significance (alpha level of <10%) was observed for severe myalgia when antibody positiveness was considered in the main analysis. These ARs developed after any vaccine dose in the primary

analysis and sensitivity analysis 1 and did not demonstrate a specific predominance in frequency over the first or second doses, whereas most of the statistically significant ARs in sensitivity analysis 2 were observed after any dose or after a second vaccine dose. Figures 2–4 show an association between reactogenicity (as expressed by solicited ARs) and immunogenicity that seemed consistent across the three performed analyses.

Some ARs such as chills and headache were significant in sensitivity analyses 1 and 2 but were not significant in the main analysis. Similarly, the association between the intensity of ARs and immunogenicity was not consistent either.

Of note, in some of these ARs (e.g., injection site pain, injection site swelling, fatigue, hypersensitivity, gastrointestinal disorders), a significant association was observed, but this association was observed in a higher proportion of vaccinees from the negative immunogenicity group, thereby showing a negative association. The individual absolute and relative numbers of these ARs can be found in the tables in the Supplementary Materials (Tables S1–S23).

4. Discussion

We believe our study provides novel insights into the enquiry of the hypothesis of the potential link between immunogenicity and reactogenicity in mRNA vaccines including the following: (i) an exploration of both humoral and cellular immunogenicity, (ii) a methodology for assessing both the development and quantity of immunogenicity, (iii) the inclusion of a large vaccinated cohort composed of HCPs and immunocompromised patients that can be more reflective of typical vaccination practices, (iv) the assessment of two mRNA vaccines, and (v) the evaluation of a wide range of local and systemic reactions with the additional consideration of symptom intensity.

Overall, we observed a consistent significant association between fever, malaise, and myalgia and the development of elicited humoral immunogenicity. According to our data, these results also seem to be associated with the quantity of immunogenicity developed. In the sensitivity analyses performed, we also observed a significant association between chills, headache, injection site pain, arm pain, arthralgia, and elicited immunogenicity.

On the whole, our results are consistent with those reported in other studies by various authors. Interestingly, most of these previous works studied HCPs vaccinated with BNT162b2 vaccines. Speletas et al. studied the intensity and duration of immunogenicity in 511 individuals who were vaccinated with two doses of the BNT162b2 vaccine. Taking into account the impact of adverse side effects on the strength of IgG antibody responses, a multivariate analysis showed that on day 21, only increased age had a significant effect, with no impact observed from AR. However, individuals who experienced fever and muscle pain after the second dose had notably higher IgG levels on day 42. Interestingly, this effect had disappeared by day 90 and day 180 following vaccination [23].

Yamamoto et al. assessed the immunogenicity and reactogenicity in 100 HCPs who had been vaccinated with BNT162b2. Participants who exhibited fever showed notably elevated spike IgG titres 7 days after the second vaccination dose compared to those who reported no fever or only mild fever. Although spike IgG titres tended to decline over time for all participants, those who had a high fever still maintained higher titres than those who experienced no or mild fever, even at both 39 and 60–74 days post-vaccination [24]. Other studies have also found a positive association between local and systemic ARs and the development of immunogenicity after vaccination with BNT162b2 [25,26].

Immunogenicity and reactogenicity after vaccination with mRNA-1273 vaccines have been explored less than with BNT162b2. Matsumoto et al. assessed the dynamics of immunogenicity in 47 healthy individuals vaccinated with a third dose of mRNA-1273 at 3 days, 7 days, and 1 month post-vaccination. The investigators found a significant association between fever and the development of antibody titres [27]. Other SARS-CoV-2 vaccines have also been studied in terms of immunogenicity and reactogenicity, without consistent results. Yun Lim et al. assessed a prospective cohort of HCPs vaccinated with

ChAdOx1 and BNT162b2. In their study, only a positive association with local ARs was observed [28].

Alternatively, other authors have found negative results after vaccination with ChAdOx1 and BNT162b2, with sample sizes of 67 HCP [29], 80 HCP [15], and 135 healthy adults [30], while others have only observed some association with AR intensity but not with AR frequency [14], a weak correlation [31], or an association only in males but not in female vaccinees [32].

With regard to AR intensity, while we found some significant differences in the sensitivity analyses, we did not observe any difference in the main population of the study, as was the case in most of the previously described studies. Hence, a positive association with AR intensity cannot be firmly inferred.

Compared to previously reported results, our study analysed a wider study population (HCP and SOTR), two mRNA vaccines (BNT162b2 vs. mRNA-1273), and both immunity arms (humoral and cellular). In the explanatory analysis, we observed decreased immunogenicity and reactogenicity in SOTRs vaccinated with mRNA-1273 in contrast to HCPs who had also been vaccinated with mRNA-1273. While reactogenicity was higher with mRNA-1273 in the vaccinated HCPs, and taking into consideration that certain reactogenicities may stem from vaccine composition rather than immunogenicity itself, the differences observed in reactogenicity between HCPs and SOTRs provide evidence for the role of immunogenicity. These data, together with the results in the overall population, provide support for the confirmation of our initial hypothesis formulated following the results obtained in our first study.

Notwithstanding, our study has several limitations. Firstly, our study was based on reactogenicity data from the VigilVacCOVID study. Therefore, the AR data may have limitations inherent to the observational and non-randomised nature of the VigilVacCOVID study such as recall bias and potential confounders. In addition, AR data were obtained through a structured questionnaire. This can be a subjective method for the assessment of certain ARs such as fatigue or malaise. Furthermore, the method employed for assessing humoral immunogenicity in clinical practice was semiquantitative and did not differentiate between infection-related and vaccine-induced neutralising antibodies. Routine SARS-CoV-2 testing was not conducted pre- and post-vaccination. Additionally, some immunogenicity measurements were obtained months after vaccination, while others were obtained prior to vaccination, potentially not reflecting ARs immediately following vaccination. Therefore, we cannot exclude the possibility that some participants may have developed immunogenicity due to SARS-CoV-2 infection rather than vaccination. However, we performed two sensitivity analyses to account for these potential biases; this reinforced the consistency of our main results.

Of note, the first sensitivity analysis included all vaccinees followed-up in the VigilVacCOVID study with immunogenicity and reactogenicity data (3563 vaccinees); these data were collected in a 4-month timeframe. Despite the limitations related to the 4-month study timeframe, one should take into consideration that previous authors have found an association between ARs and immunogenicity even at 74 days post-vaccination [24]. Therefore, we consider that the results obtained in the 4-month timeframe, with a higher sample size of 3563 vaccinees, are still informative. The results of cellular immunogenicity were negative, both in the rate and intensity of ARs and in the assessment of solicited and unsolicited AR. We acknowledge that our sample size for this assessment was rather small ($n = 59$ vaccinees with testing performed), given that cellular immunogenicity was not standard practice for all vaccinees at the time of the vaccination campaign. Most likely because of the sample size, we could observe some positive trends in some solicited ARs, but none were statistically significant. Although Speletas et al. elucidated a clear correlation between humoral and cellular responses [23], few studies have assessed both humoral and cellular immunogenicity, and those that did measure the T cell responses included similar sample sizes [15,28,32] with negative results. Our results, in terms of cellular immunogenicity, were statistically non-significant. However, we observed a positive trend,

with 86% of vaccinees testing positive for cellular immunogenicity amongst those with positive antibody titres. A relevant aspect to consider is that humoral immunogenicity is a surrogate marker of the T cell response; in fact, there is no immune memory without T lymphocytes and, although it is not frequent, there can be one without antibodies or B lymphocytes [33]. Although the humoral response can be considered a valid marker of the association between reactogenicity and immunogenicity, it is relevant to assess the two arms of adaptive immunity (humoral and cellular immunogenicity) whenever possible, especially in those patients without an antibody response and/or at risk of severe COVID-19. Lack of standardised protocols for the concomitant assessment of the two arms of adaptive immunity in the standard practice of clinical centres can be a limitation for the collection of RWD regarding cellular immunogenicity, and humoral immunogenicity can be a valid marker in these scenarios. Nonetheless, further studies with larger sample sizes and where reactogenicity and cellular immunogenicity are assessed are necessary, especially with the likely development of new vaccines based on the mRNA platform.

On the other hand, a significant association was observed for some ARs, but this association was found among a higher proportion of vaccinees in the negative immunogenicity group. The clinical meaning of this finding is unclear to us, and it should be explored in future studies where systemic and local ARs are thoroughly assessed.

Moreover, immunogenicity assessments were based solely on the total antibody titres (IgM + IgG), and specific IgG titres were only measured in a subset of vaccinees. While we are aware of the limitations related to data sources, data type, and the timing of determinations, we would like to highlight that these limitations are due to RWD acquisition processes. Our data are reflective of the standard practice during the period when the vaccination campaign was established rapidly amid the pressures of SARS-CoV-2 fears, and safety follow-ups were also organised rapidly to provide vaccinees with exhaustive vaccine surveillance. Immunogenicity assessments were not standard practice at the time and, as such, could not be carried out during the vaccinations. Because the data were obtained within the limitations of standard practice, we consider the results to be representative, and that these as results can be generalised to the overall population who are routinely vaccinated at healthcare centres. The methodology of our study reflects data acquisition and processing within a real-world context in standard clinical practice, which should be instructive for the development of further RWD studies, especially those that are performed within the context of mass vaccination campaigns during emergency periods with the use of data from clinical practices.

Translating our results into clinical practice, fever, myalgia, and malaise are systemic symptoms that develop rapidly after vaccination and can be relatively easily detected. Furthermore, these symptoms are relatively frequent (the European Medicines Agency has identified 1.7 million spontaneous reports of suspected ARs in almost 768 million vaccine doses administered in Europe [34], of which fever and myalgia are two of the most reported ARs in the European database). The reporting of these symptoms may be indicative of the prospective development of immunogenicity after vaccination. Likewise, SOTRs may be asymptomatic after vaccination and may have lower chances of developing immunogenicity. The main focus of our study was to assess a potential link between reactogenicity and immunogenicity. These are preliminary results that cannot be translated directly into clinical practice. That is to say, immunogenicity is a heterogeneous multimodal clinical entity that can be explained by several factors other than reactogenicity such as genomic data or other types of clinical data, among others. The construction and validation of a future prediction signature may be helpful when planning immunogenicity testing programs, vaccination campaigns, and closer monitoring programs in special patient subgroups in clinical care centres. Therefore, we consider that the data of our study, which revealed an association between immunogenicity and reactogenicity, can contribute to the creation of this signature in further dedicated studies.

Conversely, the EMA Pharmacovigilance Risk Assessment Committee (PRAC) began an analysis of rare cases of myocarditis and pericarditis after vaccination with BNT162b2 [35]

following early reports of cases of myocarditis after vaccination with BNT162b2 [36]. The safety signal of myocarditis and pericarditis was finally validated and included in the Summary of Product Characteristics (SmPC) of the vaccines [37]. The overall incidence of myocarditis and pericarditis is very low. Although figures varied according to the different series [38–40], published evidence shows that these events tend to be rare or very rare. Therefore, with 5088 subjects, the VigilVacCOVID study was not powered enough to detect rare cases such as myocarditis or pericarditis, but adequate for obtaining a short-term reactogenicity profile. The current study, in line with the VigilVacCOVID study, aimed to characterise the association between short-term reactogenicity and developed immunogenicity.

In addition, apart from myocarditis and pericarditis, no other safety signals related to cardiovascular complications have been validated to date. Cardiovascular events such as myocardial infarctions, strokes, embolism, and others have been studied, and while some studies have suggested increased reporting rates of these events [41,42], others have not found such an association [36,43–47]. While some authors have previously suggested that mRNA vaccines have been associated with increased reports of adverse cardiovascular reactions than other types of SARS-CoV-2 vaccines, it is true that the strongest association remains thus far with myocarditis and pericarditis, and the evidence regarding the association of SARS-CoV-2 vaccines with cardiovascular outcomes still remains to be elucidated [48]. In our previous VigilVacCOVID study, we detected some cases of syncope, hypertension, hypotension, chest pain, cold sweat, and tachycardia as unsolicited ARs; similar findings were previously reported as potential cardiovascular adverse effects in other studies [41]. These results confirm the sensitivity of our study to detect and report ARs developed after vaccination, in line with what has been previously published. However, since the study was designed to obtain a short-term reactogenicity profile, we were not able to confirm whether these symptoms were finally confirmed through a cardiovascular diagnosis, and whether there was a causal link with the vaccine. Therefore, in the current analysis, we did not observe an association between these events and immunogenicity.

Finally, our study suggests a positive association between reactogenicity and the development and quantity of immunogenicity after vaccination. This association seems to be linked to the development of anti-spike protective immunogenicity and should not be generalised to other types of adaptive immunity. For instance, recent reports have suggested that autoantibodies targeting type I IFNs develop after vaccination with mRNA SARS-CoV-2 vaccines but not with viral vector-based vaccines [49,50]. While these autoantibodies have been associated with adverse reactions in other vaccines such as the live-attenuated yellow vaccine [51], such an association has not been observed in SARS-CoV-2 vaccines [52,53].

5. Conclusions

In conclusion, our study detected a positive association between reactogenicity (defined as the onset of systemic symptoms such as fever, myalgia, and malaise) and the development and quantity of immunogenicity after vaccination. In addition to their high prevalence, these ARs are early, easily detectable symptoms post-vaccination and are indicative of the development of potential immunogenicity. These results can be valuable for the future construction of immunogenicity prediction signatures. Moreover, we believe that our results can be impactful in advancing our understanding of the immunological correlates of mRNA vaccines and, consequently, in the design of future mRNA vaccine constructs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/vaccines12060665/s1>, Measurement of antibodies and T cell response. Analytical methods; Table S1: Assessment of antibody positiveness (positive/negative titres); Table S2: Assessment of antibody positiveness (positive/negative titres): solicited and unsolicited ARs; Table S3: Assessment of antibody positiveness (positive/negative titres): severe solicited and unsolicited ARs; Table S4: Assessment of antibody titres (antibody titres above/below median); Table S5: Assessment of antibody titres (antibody titres above/below median): solicited and unsolicited ARs; Table S6: Assessment of antibody titres (antibody titres above/below median): severe solicited ARs; Figure S1: Summary of results of immunogenicity and reactogenicity of the main population; Table S7:

Characteristics of subpopulation 1; Table S8: Assessment of antibody positiveness (positive/negative titres); Table S9: Assessment of antibody positiveness (positive/negative titres): solicited and unsolicited ARs; Table S10: Assessment of antibody positiveness (positive/negative titres): severe solicited ARs; Table S11: Assessment of antibody titres (antibody titres above/below median); Table S12: Assessment of antibody titres (antibody titres above/below median): solicited and unsolicited ARs; Table S13: Assessment of antibody titres (antibody titres above/below median): severe solicited ARs; Table S14: Cellular immunogenicity assessment; Table S15: Cellular immunogenicity assessment: solicited and unsolicited ARs; Table S16: Cellular immunogenicity assessment: severe solicited ARs; Figure S2: Summary of results of the immunogenicity and reactogenicity of sensitivity analysis 1; Table S17: Characteristics of subpopulation 2; Table S18: Assessment of antibody positiveness (positive/negative titres); Table S19: Assessment of antibody positiveness (positive/negative titres): solicited and unsolicited ARs; Table S20: Assessment of antibody positiveness (positive/negative titres): severe solicited ARs; Table S21: Assessment of antibody titres (antibody titres above/below median); Table S22: Assessment of antibody titres (antibody titres above/below median): solicited and unsolicited ARs; Table S23: Assessment of antibody titres (antibody titres above/below median): severe solicited ARs; Figure S3: Summary of results of the immunogenicity and reactogenicity of sensitivity analysis 2; Table S24: Reactogenicity and immunogenicity by vaccine and population types; Figure S4: Comparison of vaccine types; Figure S5: Comparison of population types; Table S25: STROBE Checklist.

Author Contributions: The corresponding author (J.S.-P.) attests that all of the listed authors meet authorship criteria and that no others meeting the criteria have been omitted. J.S.-P., F.T., J.B. and G.C. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: J.S.-P., F.T., J.B. and G.C. Acquisition, analysis, or interpretation of data: J.S.-P., G.C., F.T., J.B., M.Á.M., M.M.M. and N.E. Drafting of the manuscript: J.S.-P., G.C., F.T., J.B., M.Á.M., M.M.M. and N.E. Critical revision of the manuscript for significant intellectual content: J.S.-P., G.C., F.T., J.B., M.Á.M., M.M.M. and N.E. Statistical analysis: F.T. The lead author (J.S.-P.) confirms that the manuscript is an honest, accurate, and transparent account of the study being reported, there are no important aspects of the study that have been omitted, and that any discrepancies from the study as planned have been reported and explained. All authors have read and agreed to the published version of the manuscript.

Funding: The study was performed Hospital Clínic's own resources. No grants/funding were received for this study.

Institutional Review Board Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was approved by the Research Ethics Committee of Hospital Clínic (HCB/2021/0684-amendment 1 and HCB/2021/0685-amendment 1, approval date: 7 March 2022).

Informed Consent Statement: An informed consent waiver was approved by the Ethics Committee given the retrospective nature of data collection and review, with no interventions performed on patients.

Data Availability Statement: Complete data are available in the Supplementary Materials. Raw data are available from the corresponding author upon reasonable request.

Acknowledgments: The authors confirm that all persons named in the Acknowledgements section have provided written permission to be included in the manuscript. We are thankful to the valuable support provided by Sandra Serrano, Victor Sapena, and Juan Bascuas in the data extraction. We are also thankful to the interviewer's team (IT) that made this work possible by their support in the VigilVacCOVID study: Juan Bascuas, Coordinator of the IT; Luis Aparicio; Susanna Bañuelos; Pilar Cano; Vanessa de Dios; Anna Massó; Celia Puente; Carlos Sánchez; and Martina Villarreal.

Conflicts of Interest: None of the authors have any conflicts of interest to disclose.

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ELECTRONIC SUPPLEMENTARY MATERIAL

Journal: Vaccines

Association between reactogenicity and immunogenicity in a vaccinated cohort with two mRNA SARS-CoV-2 vaccines at a high-complexity reference hospital: a *post-hoc* analysis on immunology aspects of a prospective cohort study

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CONTENTS

Tables:

i) Measurement of antibodies and T-cell response. Analytical methods

ii) Main analysis and sensitivity analyses:

ii.i Main analysis:

Table S1: Assessment of antibody positiveness (positive-negative titres)

Table S2: Assessment of antibody positiveness (positive-negative titres): solicited and unsolicited AR

Table S3: Assessment of antibody positiveness (positive-negative titres): severe solicited AR

Table S4: Assessment of antibody titres (antibody titres above-below median)

Table S5: Assessment of antibody titres (antibody titres above-below median): solicited and unsolicited AR

Table S6: Assessment of antibody titres (antibody titres above-below median): severe solicited AR

Figure S1: Summary of results of immunogenicity and reactogenicity of the main population

ii.ii Sensitivity analysis 1:

Table S7: Characteristics of subpopulation 1

Table S8: Assessment of antibody positiveness (positive-negative titres)

Table S9: Assessment of antibody positiveness (positive-negative titres): solicited and unsolicited AR

Table S10: Assessment of antibody positiveness (positive-negative titres): severe solicited AR

Table S11: Assessment of antibody titres (antibody titres above-below median)

Table S12: Assessment of antibody titres (antibody titres above-below median): solicited and unsolicited AR

Table S13: Assessment of antibody titres (antibody titres above-below median): severe solicited AR

Table S14: Cellular immunogenicity assessment

Table S15: Cellular immunogenicity assessment: solicited and unsolicited AR

Table S16: Cellular immunogenicity assessment: severe solicited AR

Figure S2: Summary of results of immunogenicity and reactogenicity of sensitivity analysis 1

ii.iii Sensitivity analysis 2:

Table S17: Characteristics of subpopulation 2

Table S18: Assessment of antibody positiveness (positive-negative titres)

Table S19: Assessment of antibody positiveness (positive-negative titres): solicited and unsolicited AR

Table S20: Assessment of antibody positiveness (positive-negative titres): severe solicited AR

Table S21: Assessment of antibody titres (antibody titres above-below median):

Table S22: Assessment of antibody titres (antibody titres above-below median): solicited and unsolicited AR

Table S23: Assessment of antibody titres (antibody titres above-below median): severe solicited AR

Figure S3: Summary of results of immunogenicity and reactogenicity of sensitivity analysis 2

ii.iv Exploratory analysis:

Table S24: Reactogenicity and immunogenicity by vaccine and population types

Figure S4: Comparison of vaccine types

Figure S5: Comparison of population types

iv Table S25: STROBE Checklist

Measurement of antibodies and T-cell response. Analytical methods

Humoral immunogenicity

Levels of neutralizing and anti-spike antibodies (IgG + IgM) were measured on the Cobas platform (Roche©), based on semiquantitative measurement of antibody titres through serological enhanced “electrochemiluminiscence”, on a scale that ranges from 0 to >10. Results >1.0 were considered positive antibody titres. IgG titres were measured on samples that tested positive in the Cobas platform in a subset of patients, as per local clinical practice protocols (Akashi Y et al, 2022). During the vaccination campaign the measurement method changed, and IgG titres were measured in the Atellica Analyzer (Siemens Healthineers©) (Florin L et al, 2021), with a quantitative scale that ranged from 0.5 to >150 U/ml. IgG were measured only in a subset of the total vaccinated population (N=322 vaccinees).

Cellular immunogenicity

SARS-CoV-2-specific T-cell response was measured by interferon-gamma ELISPOT assay measured from isolated PBMCs, as described by Egri N et al (Egri N et al., 2022).

In summary, venous blood samples were obtained from the forearm utilizing a butterfly needle connected to heparinized and serum-containing tubes. Peripheral blood mononuclear cells (PBMCs) were extracted from the blood samples through Ficoll-Paque density gradient centrifugation. Stimulation experiments were performed using 2×10^5 PBMCs cultured in X-VIVO™15 medium supplemented with 10% heat-inactivated AB serum and PepTivator® SARS-CoV-2 Prot_S and N peptide pools at a concentration of mg/ml (Miltenyi Bio-tec®). The diluent utilized was PBS+DMSO with a final DMSO concentration of 1%. For the ELISpot negative control, X-VIVO 15 medium with 20% DMSO to a final concentration of 1% was employed. Negative control wells were devoid of peptides, while positive control wells contained mAb CD3-2 from the kit. Cells were then cultured overnight (16–20 h) at 37°C with 5% CO₂ in precoated anti-IFN-γ MSIP white plates (Human IFNγ ELISpotPRO kit (ALP) (Mabtech) Ref: 3420-2AST-2, Mabtech®). Following incubation, plates were washed five times with PBS (Sigma-Aldrich) and incubated for 2 h at room temperature with horseradish peroxidase (HRP)-conjugated anti-IFN-γ detection antibody (1 mg/ml; clone mAb-7B6-1; Mabtech®).

After five additional washes with PBS, tetra-methylbenzidine (TMB) substrate was added, and spots were enumerated using an automated ELISpot Reader System (Autoimmun Diagnostika GmbH®). Positive peptide-specific responses were quantified by subtracting spots in unstimulated wells from peptide-stimulated wells, with results expressed as SFU (Spot Forming Units)/2x10⁵ PBMCs. SARS-CoV-2-specific spots were determined by spot increment, defined as stimulated spot numbers ≥ 6 SFU/2 $\times$ 10⁵ PBMCs. This threshold was established by calculating the mean $\pm$ 2 standard deviations in a cohort of healthy donors obtained prior to the onset of the SARS-CoV-2 pandemic. Spot counting was conducted automatically and was manually verified in all instances.

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ii. Main analysis and sensitivity analyses

ii.i Main analysis

Table S2 Assessment of antibody positiveness (positive-negative titres): solicited and unsolicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain <i>PT: Injection site pain</i> Fatigue/asthenia <i>PT:</i> <i>Fatigue</i>	31 (48.4)	11 (17.2)	56 (37.1)		0.121	
	23 (35.9)		47 (31.1)	21 (13.9)	0.491	
	16 (25.0)		27 (17.9)		0.233	
	4 (6.3)	12 (18.8)	8 (5.3)	21 (13.9)	0.781	
Fever <i>PT:</i> <i>Pyrexia</i>	17 (26.6)		46 (30.5)		0.566	
	4 (6.3)	13 (20.3)	20 (13.2)	35 (23.2)	0.136	
Malaise <i>PT:</i> <i>Malaise</i>	4 (6.3)		24 (15.9)		0.055	
	1 (1.6)	3 (4.7)	4 (2.6)	19 (12.6)	0.629	
Chills <i>PT:</i> <i>Chills</i>	3 (4.7)		11 (7.3)		0.480	
	0 (0)	3 (4.7)	4 (2.6)	7 (4.6)	0.189	
Injection site redness <i>PT:</i> <i>Application site redness</i>	3 (4.7)		4 (2.6)		0.441	
	2 (3.1)	2 (3.1)	2 (1.3)	2 (1.3)	0.372	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Negative antibody titres		Positive antibody titres		
	After any dose		After any dose		
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	
SOC: Musculoskeletal and connective tissue disorders					
Arm pain PT: Pain in extremity	15 (23.4)		38 (25.2)		0.788
	9 (14.1)	8 (12.5)	27 (17.9)	14 (9.3)	0.493
	12 (18.8)		29 (19.2)		0.938
	2 (3.1)	10 (15.6)	7 (4.6)	24 (15.9)	0.613
Muscle pain PT: Myalgia	5 (7.8)		13 (8.6)		0.847
	0 (0)	5 (7.8)	1 (0.7)	12 (7.9)	0.514
	3 (4.7)		6 (4.0)		0.811
	2 (3.1)	1 (1.6)	4 (2.6)	1 (0.7)	0.770
SOC: Injury, poisoning and procedural complications					
Injection site swelling PT: Application site swelling	5 (7.8)		10 (6.6)		0.754
	3 (4.7)	2 (3.1)	9 (6.0)	2 (1.3)	0.710
	SOC: Vaccination site pruritus				0.372
Injection site pruritus PT: Vaccination site pruritus	1 (1.6)		2 (1.3)		0.892
	0 (0)	1 (1.6)	0 (0)	2 (1.3)	NA
					0.892

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Negative antibody titres		Positive antibody titres		
	After any dose		After any dose		
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	
SOC: Nervous system disorders					
Headache <i>PT: Headache</i>	19 (29.7)		40 (26.5)		0.631
	8 (12.5)	13 (20.3)	19 (12.6)	23 (15.2)	0.987 0.362
Facial paralysis <i>PT: Facial paralysis</i>	0 (0)		0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA NA
Insomnia <i>PT: Insomnia</i>	2 (3.1)		2 (1.3)		0.372
	0 (0)	2 (3.1)	0 (0)	2 (1.3)	NA 0.372
SOC: Gastrointestinal disorders					
Nausea <i>PT: Nausea</i>	2 (3.1)		5 (3.3)		0.944
	1 (1.6)	1 (1.6)	4 (2.6)	1 (0.7)	0.629 0.530
Diarrhea <i>PT: Diarrhoea</i>	1 (1.6)		2 (1.3)		0.892
	1 (1.6)	0 (0)	1 (0.7)	1 (0.7)	0.530 0.514
Vomiting <i>PT: Vomiting</i>	0 (0)		0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity						^a p-value
	Negative antibody titres			Positive antibody titres			
	After any dose			After any dose			
	After 1 st dose	After 2 nd dose		After 1 st dose	After 2 nd dose		
Unsolicited adverse reactions							
SOC: Musculoskeletal and connective tissue disorders							
Other musculoskeletal disorders PT: Musculoskeletal disorder	3 (4.7)		4 (2.6)		0.441		
	1 (1.6)	2 (3.1)	0 (0)	4 (2.6)	0.124	0.846	
SOC: Skin and subcutaneous tissue disorders							
Petechia, ecchymosis PT: Ecchymosis	0 (0)		0 (0)		NA		
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	
SOC: Nervous system disorders							
Cognitive alteration PT: Cognitive disorder	0 (0)		0 (0)		NA		
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	
Alterations of smell and taste PT: Parosmia	0 (0)		1 (0.7)		0.514		
	0 (0)	0 (0)	0 (0)	1 (0.7)	NA	0.514	
Paresthesia and hyperesthesia PT: Dysaesthesia	1 (1.6)		2 (1.3)		0.892		
	0 (0)	1 (1.6)	2 (1.3)	0 (0)	0.355	0.124	
Presyncope, syncope and vasovagal syncope PT: Syncope	0 (0)		0 (0)		NA		
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	
Sleepiness, hypersomnia PT: Hypersomnia	0 (0)		0 (0)		NA		
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity						a p-value
	Negative antibody titres		Positive antibody titres				
	After any dose		After any dose				
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	
	1 (1.6)		2 (1.3)				
Instability sensation, vertigo, sickness PT: Dizziness	0 (0)	1 (1.6)	1 (0.7)		1 (0.7)	0.514	0.530
	0 (0)		0 (0)		0 (0)	NA	
Tremor PT: Tremor	0 (0)	0 (0)	0 (0)		0 (0)	NA	NA
	SOC: Gastrointestinal disorders						
Gastrointestinal disorders PT: Gastrointestinal disorder	0 (0)		1 (0.7)		0.514		
	0 (0)	0 (0)	1 (0.7)		0 (0)	0.514	NA
SOC: Respiratory, thoracic and mediastinal disorders							
Asthma PT: Asthma	0 (0)		0 (0)		NA		
	0 (0)	0 (0)	0 (0)		0 (0)	NA	NA
Rhinitis, nasal discharge PT: Rhinitis	0 (0)		1 (0.7)		0.514		
	0 (0)	0 (0)	0 (0)		1 (0.7)	NA	0.514

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Negative antibody titres		Positive antibody titres		
	After any dose		After any dose		
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	
SOC: <i>Investigations</i>					
Hypertension, hypotension PT: <i>Blood pressure abnormal</i>	2 (3.1)		0 (0)		0.029
	0 (0)	2 (3.1)	0 (0)	0 (0)	NA
SOC: <i>General disorders and administration site conditions</i>					
Influenza-like symptoms PT: <i>Influenza like illness</i>	0 (0)		0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA
Sensation of heat, sensation of cold PT: <i>Temperature regulation disorder</i>	0 (0)		2 (1.3)		0.355
	0 (0)	0 (0)	1 (0.7)	1 (0.7)	0.514
Chest pain PT: <i>Chest pain</i>	0 (0)		0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA
Hiporexia, anorexia PT: <i>Decreased appetite</i>	0 (0)		0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>	0 (0)		0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity						^a p-value
	Negative antibody titres			Positive antibody titres			
	After any dose			After any dose			
	After 1 st dose	After 2 nd dose		After 1 st dose	After 2 nd dose		
SOC: Ear and labyrinth disorders							
PT: Ear pain	0 (0)		0 (0)		NA		
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	
SOC: Skin and subcutaneous tissue disorders							
General pruritus PT: Pruritus	2 (3.1)		0 (0)		0.029		
	1 (1.6)	1 (1.6)	0 (0)	0 (0)	0.124	0.124	
Sweat PT: Cold sweat	0 (0)		0 (0)		NA		
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	
SOC: Immune system disorders							
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: Hypersensitivity	1 (1.6)		1 (0.7)		0.530		
	0 (0)	1 (1.6)	1 (0.7)	0 (0)	0.514	0.124	
SOC: Cardiac disorders							
Tachycardia PT: Tachycardia	0 (0)		1 (0.7)		0.514		
	0 (0)	0 (0)	0 (0)	1 (0.7)	NA	0.514	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					a p-value
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	SOC: Blood and lymphatic system disorders					
Lymphadenopathy PT: <i>Lymphadenopathy</i>	0 (0)	0 (0)	6 (4.0)	5 (3.3)	0.106	
SOC: Infections and infestations						
Herpetic infection PT: <i>Herpes virus infection</i>	0 (0)	0 (0)	0 (0)	0 (0)	NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	

Distribution of proportions of solicited and unsolicited AR are displayed by elicited qualitative humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable.

Table S3 Assessment of antibody positiveness (positive-negative titres): severe solicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain PT: <i>Injection site pain</i> Fatigue/asthenia PT: <i>Fatigue</i>	3 (4.7)		15 (9.9)		0.204	
	3 (4.7)	0 (0)	13 (8.6)	3 (2.0)	0.316	0.256
	9 (14.1)		10 (6.6)		0.079	
	3 (4.7)	6 (9.4)	1 (0.7)	9 (6.0)	0.046	0.369
Fever PT: <i>Pyrexia</i> Malaise PT: <i>Malaise</i>	0 (0)		7 (4.6)		0.080	
	0 (0)	0 (0)	3 (2.0)	5 (3.3)	0.136	0.141
	1 (1.6)		15 (9.9)		0.033	
	0 (0)	1 (1.6)	1 (0.7)	13 (8.6)	0.514	0.056
Chills PT: <i>Chills</i>	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
Injection site redness PT: <i>Application site redness</i>	0 (0)		1 (0.7)		0.514	
	0 (0)	0 (0)	1 (0.7)	0 (0)	0.514	NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					a p-value
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	SOC: Musculoskeletal and connective tissue disorders					
Arm pain PT: Pain in extremity	8 (12.5)		15 (9.9)		0.578	
	6 (9.4)	2 (3.1)	10 (6.6)	5 (3.3)	0.482	
	0.944					
Muscle pain PT: Myalgia	12 (18.8)		29 (19.2)		0.938	
	2 (3.1)	10 (15.6)	7 (4.6)	24 (15.9)	0.613	
	0.961					
Joint pain PT: Arthralgia	3 (4.7)		10 (6.6)		0.586	
	0 (0)	3 (4.7)	0 (0)	10 (6.6)	NA	
	0.586					
Shoulder pain PT: Musculoskeletal pain	0 (0)		2 (1.3)		0.355	
	0 (0)	0 (0)	1 (0.7)	0 (0)	0.514	
	NA					
SOC: Injury, poisoning and procedural complications						
Injection site swelling PT: Application site swelling	0 (0)		4 (2.6)		0.189	
	0 (0)	0 (0)	4 (2.6)	0 (0)	0.189	
	NA					
SOC: Vaccination site pruritus						
Injection site pruritus PT: Vaccination site pruritus	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	
	NA					

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Negative antibody titres		Positive antibody titres		
	After any dose		After any dose		
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	
SOC: Nervous system disorders					
Headache PT: Headache	6 (9.4)		17 (11.3)		0.683
	2 (3.1)	5 (7.8)	6 (4.0)	11 (7.3)	0.764
					0.893

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited qualitative humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable

Table S5 Assessment of antibody titres (antibody titres above-below median): solicited and unsolicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain <i>PT: Injection site pain</i> Fatigue/asthenia <i>PT:</i> <i>Fatigue</i> Fever <i>PT:</i> <i>Pyrexia</i> Malaise <i>PT:</i> <i>Malaise</i> Chills <i>PT:</i> <i>Chills</i> Injection site redness <i>PT:</i> <i>Application site redness</i>	29 (31.2)	39 (41.9)	41 (33.6)	48 (39.3)	0.707	0.701
	23 (24.7)	16 (17.2)	20 (16.4)	16 (13.1)	0.130	0.404
	5 (5.4)	18 (19.4)	7 (5.7)	15 (12.3)	0.909	0.155
	24 (25.8)		39 (32.0)		0.326	
	5 (5.4)	19 (20.4)	19 (15.6)	29 (23.8)	0.019	0.560
	9 (9.7)		19 (15.6)		0.203	
	1 (1.1)	8 (8.6)	4 (3.3)	14 (11.5)	0.288	0.491
	7 (7.5)		7 (5.7)		0.598	
	1 (1.1)	6 (6.5)	3 (2.5)	4 (3.3)	0.457	0.274
	3 (3.2)		4 (3.3)		0.983	
	2 (2.2)	2 (2.2)	2 (1.6)	2 (1.6)	0.784	0.784

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)		
	After any dose		After any dose		
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	
SOC: Musculoskeletal and connective tissue disorders					
Arm pain <i>PT:</i> <i>Pain in extremity</i>	17 (18.3)		36 (29.5)		0.058
	10 (10.8)	9 (9.7)	26 (21.3)	13 (10.7)	0.040 0.815
	17 (18.3)		24 (19.7)		0.797
	2 (2.2)	15 (16.1)	7 (5.7)	19 (15.6)	0.193 0.912
Muscle pain <i>PT:</i> <i>Myalgia</i>	10 (10.8)		8 (6.6)		0.271
	0 (0)	10 (10.8)	1 (0.8)	7 (5.7)	0.382 0.177
	5 (5.4)		4 (3.3)		0.447
Shoulder pain <i>PT:</i> <i>Musculoskeletal pain</i>	4 (4.3)	1 (1.1)	2 (1.6)	1 (0.8)	0.240 0.847
	SOC: Injury, poisoning and procedural complications				
Injection site swelling <i>PT:</i> <i>Application site swelling</i>	5 (5.4)		10 (8.2)		0.421
	3 (3.2)	2 (2.2)	9 (7.4)	2 (1.6)	0.189 0.784
SOC: Vaccination site pruritus					
Injection site pruritus <i>PT:</i> <i>Vaccination site pruritus</i>	1 (1.1)		2 (1.6)		0.727
	0 (0)	1 (1.1)	0 (0)	2 (1.6)	NA 0.727

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	SOC: Nervous system disorders					
Headache PT: Headache	29 (31.2)		30 (24.6)		0.283	
	13 (14.0)	19 (20.4)	14 (11.5)	17 (13.9)	0.583	
	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	
Facial paralysis PT: Facial paralysis	2 (2.2)		2 (1.6)		0.784	
	0 (0)	2 (2.2)	0 (0)	2 (1.6)	NA	
	0 (0)		0 (0)		NA	
	0 (0)	2 (2.2)	0 (0)	2 (1.6)	0.784	
SOC: Gastrointestinal disorders						
Nausea PT: Nausea	2 (2.2)		5 (4.1)		0.425	
	1 (1.1)	1 (1.1)	4 (3.3)	1 (0.8)	0.288	
	1 (1.1)		2 (1.6)		0.727	
	1 (1.1)	0 (0)	1 (0.8)	1 (0.8)	0.847	
Diarrhea PT: Diarrhoea	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	
	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	Unsolicited adverse reactions					
SOC: Musculoskeletal and connective tissue disorders						
Other musculoskeletal disorders <i>PT: Musculoskeletal disorder</i>	4 (4.3)		3 (2.5)			0.451
	1 (1.1)	3 (3.2)	0 (0)	3 (2.5)		0.251
SOC: Skin and subcutaneous tissue disorders						
Petechia, ecchymosis <i>PT: Ecchymosis</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)		NA
SOC: Nervous system disorders						
Cognitive alteration <i>PT: Cognitive disorder</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)		NA
Alterations of smell and taste <i>PT: Parosmia</i>	0 (0)		1 (0.8)			0.382
	0 (0)	0 (0)	0 (0)	1 (0.8)		NA
Paresthesia and hyperesthesia <i>PT: Dysaesthesia</i>	1 (1.1)		2 (1.6)			0.727
	0 (0)	1 (1.1)	2 (1.6)	0 (0)		0.215
Presyncope, syncope and vasovagal syncope <i>PT: Syncope</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)		NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
SOC: Investigations						
Hypertension, hypotension PT: Blood pressure abnormal	2 (2.2)		0 (0)			0.104
	0 (0)	2 (2.2)	0 (0)	0 (0)	0 (0)	NA 0.104
SOC: General disorders and administration site conditions						
Influenza-like symptoms PT: Influenza like illness	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA NA
Sensation of heat, sensation of cold PT: Temperature regulation disorder	1 (1.1)		1 (0.8)			0.847
	1 (1.1)	0 (0)	0 (0)	0 (0)	1 (0.8)	0.251 0.382
Chest pain PT: Chest pain	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA NA
Hiporexia, anorexia PT: Decreased appetite	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA NA
Inflammation in extremities other than the vaccinated arm PT: Inflammation	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
SOC: Ear and labyrinth disorders						
PT: Ear pain	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
SOC: Skin and subcutaneous tissue disorders						
General pruritus PT: Pruritus	2 (2.2)		0 (0)			0.104
	1 (1.1)	1 (1.1)	0 (0)	0 (0)	0 (0)	0.251
Sweat PT: Cold sweat	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
SOC: Immune system disorders						
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: Hypersensitivity	1 (1.1)		1 (0.8)			0.847
	0 (0)	1 (1.1)	1 (0.8)	0 (0)	0 (0)	0.251
SOC: Cardiac disorders						
Tachycardia PT: Tachycardia	0 (0)		1 (0.8)			0.382
	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.8)	0.382

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
	SOC: Blood and lymphatic system disorders					
Lymphadenopathy PT: <i>Lymphadenopathy</i>	0 (0)		6 (4.9)		0.030	
	0 (0)	0 (0)	1 (0.8)	5 (4.1)	0.382	0.048
SOC: Infections and infestations						
Herpetic infection PT: <i>Herpes virus infection</i>	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited quantitative humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, *Median value of antibody titres (P25, P75)= 7.69 (0.73,10.00) on a semiquantitative scale ranging from 0 to >10

Table S6 Assessment of antibody titres (antibody titres above-below median): severe solicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Humoral immunogenicity		Humoral immunogenicity		
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)		
	After any dose		After any dose		
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	
Solicited adverse reactions					
SOC: General disorders and administration site conditions					
Injection site pain <i>PT: Injection site pain</i>	6 (6.5)		12 (9.8)		0.375
	6 (6.5)	0 (0)	10 (8.2)	3 (2.5)	0.629 0.128
Fatigue/asthenia <i>PT: Fatigue</i>	13 (14.0)		6 (4.9)		0.020
	3 (3.2)	10 (10.8)	1 (0.8)	5 (4.1)	0.196 0.058
Fever <i>PT: Pyrexia</i>	0 (0)		7 (5.7)		0.019
	0 (0)	0 (0)	3 (2.5)	5 (4.1)	0.128 0.048
Malaise <i>PT: Malaise</i>	4 (4.3)		12 (9.8)		0.126
	0 (0)	4 (4.3)	1 (0.8)	10 (8.2)	0.382 0.251
Injection site redness <i>PT: Application site redness</i>	0 (0)		1 (0.8)		0.382
	0 (0)	0 (0)	1 (0.8)	0 (0)	0.382 NA
SOC: Musculoskeletal and connective tissue disorders					

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Humoral immunogenicity		Humoral immunogenicity			
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
Arm pain PT: Pain in extremity	8 (8.6)		15 (12.3)			0.385
	6 (6.5)	2 (2.2)	10 (8.2)	5 (4.1)		0.629
	7 (7.5)		11 (9.0)			0.696
	0 (0)	7 (7.5)	4 (3.3)	8 (6.6)		0.078
Muscle pain PT: Myalgia	8 (8.6)		5 (4.1)			0.170
	0 (0)	8 (8.6)	0 (0)	5 (4.1)		NA
	1 (1.1)		1 (0.8)			0.847
	1 (1.1)	0 (0)	0 (0)	1 (0.8)		0.251
SOC: Injury, poisoning and procedural complications						
Injection site swelling PT: Application site swelling	0 (0)		4 (3.3)			0.078
	0 (0)	0 (0)	4 (3.3)	0 (0)		0.078
SOC: Vaccination site pruritus						
Injection site pruritus PT: Vaccination site pruritus	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)		NA
SOC: Nervous system disorders						
Headache	11 (11.8)		12 (9.8)			0.640

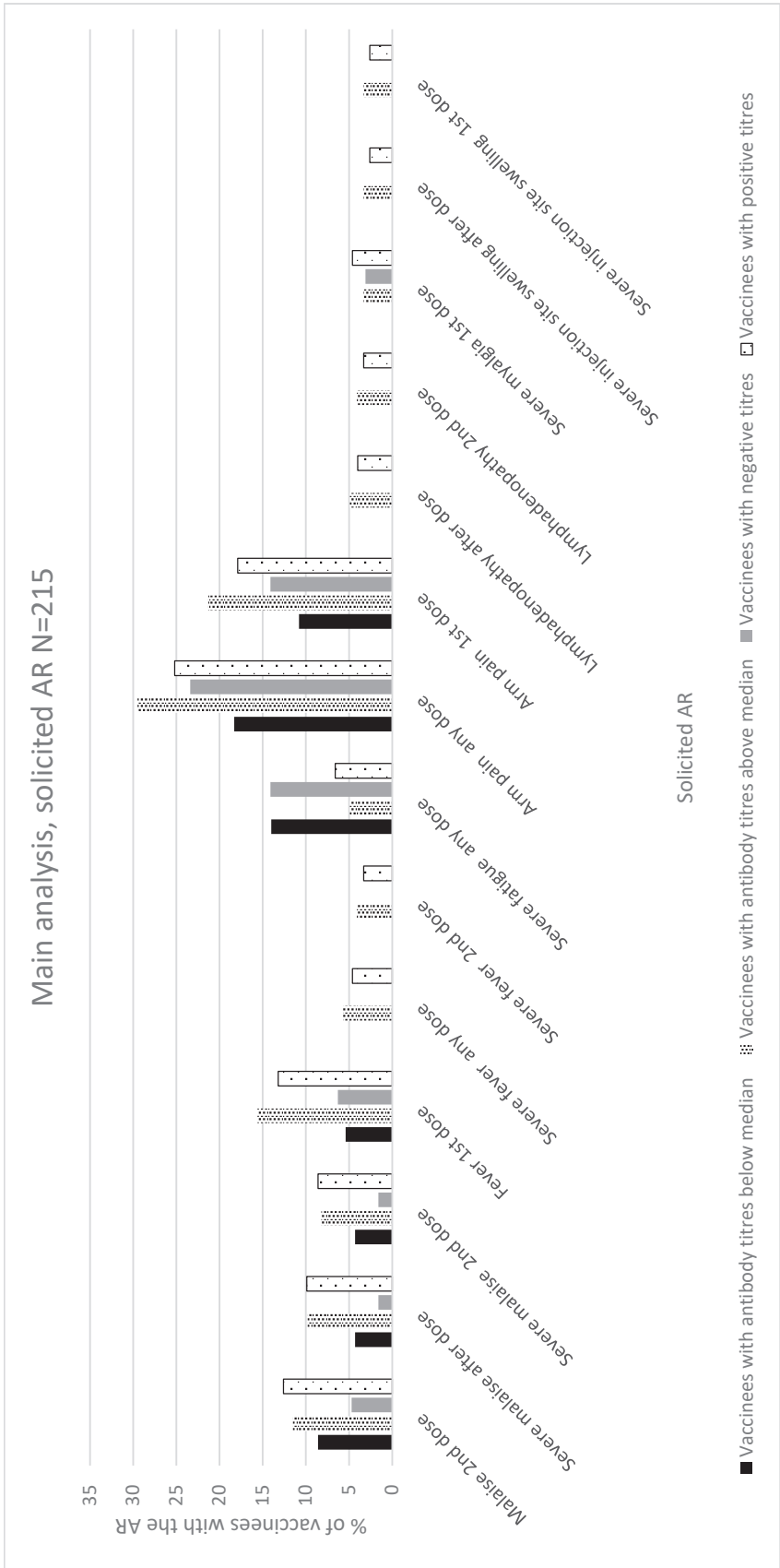
Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Humoral immunogenicity		Humoral immunogenicity		0.738	0.276
	Antibody titres below median* (n= 93)		Antibody titres above median* (n= 122)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose		
	<i>PT:</i> <i>Headache</i>	3 (3.2)	9 (9.7)	5 (4.1)	7 (5.7)	

PT:

Headache

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited qualitative humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, * Median value of antibody titres (P25, P75)= 7.69 (0.73,10.00) on a semiquantitative scale ranging from 0 to >10

Figure S1: Summary of results of immunogenicity and reactogenicity of the main population



Bar diagram of results of main analysis. Immunogenicity is expressed as antibody positiveness (positive-negative titres) and antibody titres below-above median) data (%). Reactogenicity results are represented as % of vaccinees developing each solicited AR

ii.ii Sensitivity analysis 1

Table S7: Characteristics of subpopulation 1

Covariates	Total population (N= 3563)
Age	
N	3563
Mean (SD)	41.84 (12.79)
Median (P25, P75)	42.00 (30.00, 53.00)
min, max	18.00, 81.00
Gender	
Male (n, %)	976 (27.4)
Female (n, %)	2587 (72.6)
Occupational SARS-CoV-2 contact	
In contact with SARS-CoV-2 patients	1501 (42.1)
Without contact with SARS-CoV-2 patients	1957 (54.9)
^aPrevious SARS-CoV-2 infection	
Yes	444 (12.5)
No	3119 (87.5)
Comorbidities	
^b Any comorbidity	1305 (36.6)
Arterial hypertension	141 (4.0)
Diabetes mellitus	53 (1.5)
Heart failure	13 (0.4)
Chronic bronchitis	12 (0.3)
Asthma	126 (3.5)
Rheumatic/immune-mediated disease	33 (0.9)
Drug allergies	302 (8.5)
Food allergies	146 (4.1)
Type of administered vaccine	
BNT16b2	
First dose:	2698 (75.7)
Second dose:	2167 (60.8)
mRNA-1273	
First dose:	865 (24.3)
Second dose:	773 (21.7)
^cPopulation type	
HCP	3460
SOTR	103
Humoral immunogenicity (semiquantitative ab titres)	
Positive	3487 (97.9)
Negative	76 (2.1)
IgG titres	326
Positive	322 (98.8)
Negative	4 (1.2)
Cellular immunogenicity	59
Positive	51 (86)
Negative	8 (14)

Covariables of studied population. ^aDiagnosis by RCP, antigen test and antibody positive serology; ^bNumber of vaccinees with at least one comorbidity; ^cHCPs: Healthcare professionals; SOTR: solid-organ transplant recipients. Results are provided as absolute and relative (%) numbers for each variable

Table S8: Assessment of antibody positiveness (positive-negative titres)

Variable	Antibody positiveness											
	Negative antibody titres (n= 76)						Positive antibody titres (n= 3487)					
	After 1 st vaccine dose	p- value	After 2 nd vaccine dose	p- value	After any dose	p-value	After 1 st vaccine dose	p-value	After 2 nd vaccine dose	p- value	After any dose	p- value
Proportions of AR												
Development of any AR (n, %)	56 (73.7)	0.074	45 (59.2)	0.156	64 (84.2)	0.810	2222 (63.7)	0.074	2335 (67.0)	0.156	2971 (85.2)	0.810
Development of any mild AR (n, %)	21 (27.6)	0.063	17 (22.4)	0.308	29 (38.2)	0.198	667 (19.1)	0.063	622 (17.8)	0.308	1089 (31.2)	0.198
Development of any moderate AR (n, %)	26 (34.2)	0.950	27 (35.5)	0.113	42 (55.3)	0.321	1181 (33.9)	0.950	1557 (44.7)	0.113	2123 (60.9)	0.321
Development of any severe AR (n, %)	17 (22.4)	0.520	13 (17.1)	0.017	25 (32.9)	0.207	677 (19.4)	0.520	1036 (29.7)	0.017	1397 (40.1)	0.207
Maximum intensity												
Grade 1 (n, %)	18 (33.3)	0.164	12 (27.3)	0.003	15 (23.8)	0.020	480 (22.9)	0.164	261 (11.6)	0.003	350 (12.2)	0.020
Grade 2 (n, %)	19 (35.2)		19 (43.2)		23 (36.5)		943 (44.9)		949 (42.3)		1132 (39.3)	
Grade 3 (n, %)	17 (31.5)		13 (29.5)		25 (39.7)		677 (32.2)		1036 (46.1)		1397 (48.5)	
Likert score												
Mean (SD)	4.96 (2.41)	0.241	4.95 (2.37)	0.001	5.48 (2.47)	0.031	5.34 (2.46)	0.241	6.10 (2.06)	0.004	6.15 (2.36)	0.031

Antibody titres according to proportions (rate) of AR and intensity of AR. AR: adverse reaction; Ab: antibody; in bold type: statistically significant results.
Results are provided as absolute and relative (%) numbers for each variable

Table S9: Assessment of antibody positiveness (positive-negative titres): solicited and unsolicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				a p-value
	Negative antibody titres		Positive antibody titres		
	After any dose		After any dose		
	Solicited adverse reactions				
SOC: General disorders and administration site conditions					
Injection site pain PT: <i>Injection site pain</i>	30 (39.5)	35 (46.1)	1348 (38.7)		0.190
		13 (17.1)	1058 (30.3)	603 (17.3)	0.090
	Fatigue/asthenia PT:	17 (22.4)	988 (28.3)		0.253
Fatigue PT:	7 (9.2)	11 (14.5)	223 (14.6)	782 (22.4)	0.100
					0.940
	Fever PT:	17 (22.4)	1095 (31.4)		0.090
Pyrexia PT:	9 (11.8)	11 (14.5)	216 (6.2)	974 (27.9)	0.050
					0.010
	Malaise PT:	7 (9.2)	622 (17.8)		0.050
Malaise PT:	3 (3.9)	4 (5.3)	138 (4.0)	517 (14.8)	0.990
					0.020
	Chills PT:	5 (6.6)	448 (12.8)		0.100
Chills PT:	3 (3.9)	2 (2.6)	83 (2.4)	385 (11.0)	0.380
					0.020
	Injection site redness PT:	2 (2.6)	155 (4.4)		0.450
Application site redness PT:	1 (1.3)	1 (1.3)	90 (2.6)	72 (2.1)	0.490
					0.650
SOC: Musculoskeletal and connective tissue disorders					
	24 (31.6)		1048 (30.1)		0.770

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
Arm pain <i>PT:</i> <i>Pain in extremity</i>	17 (22.4)	13 (17.1)	690 (19.8)	548 (15.7)	0.580	0.740
Muscle pain <i>PT:</i> <i>Myalgia</i>	5 (6.6)		709 (20.3)		0.003	
	2 (2.6)	4 (5.3)	117 (3.4)	621 (17.8)	0.730	0.004
Joint pain <i>PT:</i> <i>Arthralgia</i>	4 (5.3)		328 (9.4)		0.220	
	0 (0)	4 (5.3)	32 (0.9)	307 (8.8)	0.400	0.280
Shoulder pain <i>PT:</i> <i>Musculoskeletal pain</i>	8 (10.5)		106 (3.0)		0.002	
	1 (1.3)	7 (9.2)	61 (1.7)	52 (1.5)	0.770	<0.001
SOC: Injury, poisoning and procedural complications						
Injection site swelling <i>PT:</i>	5 (6.6)		246 (7.1)		0.870	
Application site swelling	5 (6.6)	0 (0)	144 (4.1)	124 (3.6)	0.290	0.090
SOC: Vaccination site pruritus						
Injection site pruritus <i>PT:</i>	0 (0)		73 (2.1)		0.200	
Vaccination site pruritus	0 (0)	0 (0)	40 (1.1)	39 (1.1)	0.350	0.350
SOC: Nervous system disorders						
Headache <i>PT:</i> <i>Headache</i>	11 (14.5)		967 (27.7)		0.010	
	5 (6.6)	8 (10.5)	330 (9.5)	750 (21.5)	0.390	0.020

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					*p-value
	Negative antibody titres		Positive antibody titres			
			After any dose			
	After any dose		After any dose			
Facial paralysis <i>PT:</i> <i>Facial paralysis</i>	0 (0)	0 (0)	1 (0)	0 (0)	0.880	
Insomnia <i>PT:</i> <i>Insomnia</i>	0 (0)	0 (0)	35 (1.0)		0.380	
	0 (0)	0 (0)	9 (0.3)	26 (0.7)	0.660	
SOC: Gastrointestinal disorders						
Nausea <i>PT:</i> <i>Nausea</i>	2 (2.6)		164 (4.7)		0.400	
	0 (0)	2 (2.6)	53 (1.5)	119 (3.4)	0.280	
Diarrhea <i>PT:</i> <i>Diarrhoea</i>	3 (3.9)		107 (3.1)		0.660	
	1 (1.3)	2 (2.6)	35 (1.0)	73 (2.1)	0.790	
Vomiting <i>PT:</i> <i>Vomiting</i>	2 (2.6)		61 (1.7)		0.560	
	0 (0)	2 (2.6)	15 (0.4)	50 (1.4)	0.570	
Unsolicited adverse reactions						
SOC: Musculoskeletal and connective tissue disorders						
Other musculoskeletal disorders <i>PT: Musculoskeletal disorder</i>	0 (0)		90 (2.6)		0.160	
	0 (0)	0 (0)	32 (0.9)	62 (1.8)	0.400	
SOC: Skin and subcutaneous tissue disorders						
Petechia, ecchymosis	0 (0)		14 (0.4)		0.580	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				*p-value	
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	0 (0)	0 (0)	10 (0.3)	4 (0.1)	0.640	0.770
PT: <i>Ecchymosis</i>						
SOC: <i>Nervous system disorders</i>						
Cognitive alteration <i>PT: Cognitive disorder</i>	1 (1.3)		5 (0.1)		0.010	
Alterations of smell and taste <i>PT: Parosmia</i>	1 (1.3)	0 (0)	3 (0.1)	2 (0.1)	0.002	0.830
	0 (0)		8 (0.2)		0.680	
	0 (0)	0 (0)	5 (0.1)	3 (0.1)	0.740	0.800
Paresthesia and hyperesthesia <i>PT: Dysaesthesia</i>	0 (0)		27 (0.8)		0.440	
	0 (0)	0 (0)	13 (0.4)	16 (0.5)	0.590	0.550
	0 (0)		7 (0.2)		0.700	
Presyncope, syncope and vasovagal syncope <i>PT: Syncope</i>	0 (0)	0 (0)	2 (0.1)	5 (0.1)	0.830	0.740
	0 (0)		16 (0.5)		0.550	
	0 (0)	0 (0)	3 (0.1)	13 (0.4)	0.800	0.590
Sleepiness, hypersomnia <i>PT: Hypersomnia</i>	4 (5.3)		86 (2.5)		0.120	
Instability sensation, vertigo, sickness <i>PT: Dizziness</i>	0 (0)	4 (5.3)	30 (0.9)	58 (1.7)	0.420	0.020
	0 (0)		7 (0.2)		0.70	
	0 (0)		7 (0.2)		0.70	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	0 (0)	0 (0)	2 (0.1)	5 (0.1)	0.830	0.740
SOC: Gastrointestinal disorders						
Gastrointestinal disorders PT: Gastrointestinal disorder	1 (1.3)		35 (1.0)		0.790	
	0 (0)	1 (1.3)	12 (0.3)	22 (0.6)	0.610	0.460
SOC: Respiratory, thoracic and mediastinal disorders						
Asthma PT: Asthma	0 (0)		4 (0.1)		0.770	
	0 (0)	0 (0)	1 (0)	2 (0.1)	0.880	0.830
Rhinitis, nasal discharge PT: Rhinitis	0 (0)		26 (0.7)		0.450	
	0 (0)	0 (0)	11 (0.3)	16 (0.5)	0.620	0.550
SOC: Investigations						
Hypertension, hypotension PT: Blood pressure abnormal	1 (1.3)		12 (0.3)		0.160	
	0 (0)	1 (1.3)	4 (0.1)	7 (0.2)	0.770	0.040
SOC: General disorders and administration site conditions						

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Negative antibody titres		Positive antibody titres		
	After any dose		After any dose		
Influenza-like symptoms PT: <i>Influenza like illness</i>	0 (0)	0 (0)	16 (0.5)	32 (0.9)	0.310
Sensation of heat, sensation of cold PT: <i>Temperature regulation disorder</i>	1 (1.3)	0 (0)	2 (0.1)	5 (0.1)	0.010
Chest pain PT: <i>Chest pain</i>	0 (0)	0 (0)	0 (0)	8 (0.2)	0.680
Hiporexia, anorexia PT: <i>Decreased appetite</i>	0 (0)	0 (0)	0 (0)	4 (0.1)	0.770
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>	0 (0)	0 (0)	5 (0.1)	7 (0.2)	0.700
SOC: <i>Ear and labyrinth disorders</i>					
PT: <i>Ear pain</i>	0 (0)	0 (0)	1 (0)	2 (0.1)	0.800
	0 (0)	0 (0)			0.880
SOC: <i>Skin and subcutaneous tissue disorders</i>					

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	1 (1.3)		7 (0.2)			
General pruritus PT: <i>Pruritus</i>	0 (0)	1 (1.3)	4 (0.1)	3 (0.1)	0.770	0.002
Sweat PT:	0 (0)		16 (0.5)		0.550	
<i>Cold sweat</i>	0 (0)	0 (0)	2 (0.1)	14 (0.4)	0.830	0.580
SOC: Immune system disorders						
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: <i>Hypersensitivity</i>	1 (1.3)		49 (1.4)		0.950	
	0 (0)	1 (1.3)	24 (0.7)	31 (0.9)	0.470	0.700
SOC: Cardiac disorders						
Tachycardia PT: <i>Tachycardia</i>	0 (0)		11 (0.3)		0.620	
	0 (0)	0 (0)	5 (0.1)	6 (0.2)	0.740	0.720
SOC: Blood and lymphatic system disorders						
Lymphadenopathy PT: <i>Lymphadenopathy</i>	4 (5.3)		142 (4.1)		0.600	
	2 (2.6)	2 (2.6)	39 (1.1)	107 (3.1)	0.220	0.830
SOC: Infections and infestations						

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
Herpetic infection PT: <i>Herpes virus infection</i>	0 (0)		10 (0.3)		0.640	
	0 (0)	0 (0)	6 (0.2)	4 (0.1)	0.720	0.770

Distribution of proportions of solicited and unsolicited AR are displayed by elicited immunogenicity (humoral and cellular) and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable

Table S10: Assessment of antibody positiveness (positive-negative titres): severe solicited AR

Severe adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Negative antibody titres		Positive antibody titres		
	After any dose		After any dose		
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	
	Solicited adverse reactions				
SOC: General disorders and administration site conditions					
Injection site pain PT: <i>Injection site pain</i> Fatigue/asthenia PT: <i>Fatigue</i>	11 (14.5)		338 (9.7)		0.170
	10 (13.2)	1 (1.3)	229 (6.6)	138 (4.0)	0.020
	7 (9.2)		483 (13.9)		0.250
	1 (1.3)	6 (7.9)	114 (3.3)	389 (11.2)	0.340
Fever PT: <i>Pyrexia</i> Malaise PT: <i>Malaise</i>	2 (2.6)		110 (3.2)		0.800
	0 (0)	2 (2.6)	19 (0.5)	92 (2.6)	0.520
	1 (1.3)		305 (8.7)		0.020
	0 (0)	1 (1.3)	53 (1.5)	258 (7.4)	0.280
Injection site redness PT: <i>Application site redness</i>	1 (1.3)		39 (1.1)		0.870
	0 (0)	1 (1.3)	21 (0.6)	20 (0.6)	0.500
	SOC: Musculoskeletal and connective tissue disorders				
	5 (6.6)		392 (11.2)		0.200
Arm pain PT: <i>Pain in extremity</i>	4 (5.3)	1 (1.3)	238 (6.8)	187 (5.4)	0.590
					0.120

Severe adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres		Positive antibody titres			
			After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	3 (3.9)		333 (9.5)			
Muscle pain <i>PT: Myalgia</i>	1 (1.3)	2 (2.6)	45 (1.3)	296 (8.5)	0.980	0.070
Joint pain <i>PT: Arthralgia</i>	2 (2.6)		184 (5.3)		0.310	
Shoulder pain <i>PT: Musculoskeletal pain</i>	0 (0)	2 (2.6)	17 (0.5)	171 (4.9)	0.540	0.360
	1 (1.3)		28 (0.8)		0.620	
	0 (0)	1 (1.3)	11 (0.3)	16 (0.5)	0.620	0.280
SOC: Injury, poisoning and procedural complications						
Injection site swelling <i>PT: Application site swelling</i>	1 (1.3)		70 (2.0)		0.670	
	1 (1.3)	0 (0)	43 (1.2)	33 (0.9)	0.950	0.390
SOC: Vaccination site pruritus						
Injection site pruritus <i>PT: Vaccination site pruritus</i>	0 (0)		18 (0.5)		0.530	
	0 (0)	0 (0)	8 (0.2)	11 (0.3)	0.680	0.620

Severe adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Negative antibody titres		Positive antibody titres			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
SOC: Nervous system disorders						
Headache <i>PT:</i> <i>Headache</i>	3 (3.9)		419 (12.0)		0.030	
	1 (1.3)	2 (2.6)	115 (3.3)	331 (9.5)		
						0.340

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited immunogenicity (humoral and cellular) and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable

Table S11: Assessment of antibody titres (antibody titres above-below median):

Variable	Antibody titres below median* (n= 440)						Antibody titres above median * (n= 3123)					
	After 1 st vaccine dose	p- value	After 2 nd vaccine dose	p- value	After any dose	p-value	After 1 st vaccine dose	p-value	After 2 nd vaccine dose	p- value	After any dose	p- value
Proportions of AR												
Development of any AR (n, %)	295 (67.0)	0.147	291 (66.1)	0.753	382 (86.8)	0.302	1983 (63.5)	0.147	2089 (66.9)	0.753	2653 (85.0)	0.302
Development of any mild AR (n, %)	102 (23.2)	0.028	99 (22.5)	0.008	168 (38.2)	0.001	586 (18.8)	0.028	540 (17.3)	0.008	950 (30.4)	0.001
Development of any moderate AR (n, %)	147 (33.4)	0.825	188 (42.7)	0.436	257 (58.4)	0.280	1060 (33.9)	0.825	1396 (44.7)	0.436	1908 (61.1)	0.280
Development of any severe AR (n, %)	90 (20.5)	0.581	108 (24.5)	0.016	163 (37.0)	0.190	604 (19.3)	0.581	941 (30.1)	0.016	1259 (40.3)	0.190
Maximum intensity												
Grade 1 (n, %)	75 (26.9)	0.223	47 (17.0)	0.006	62 (16.9)	0.017	423 (22.6)	0.223	226 (11.2)	0.006	303 (11.8)	0.017
Grade 2 (n, %)	114 (40.9)		121 (43.8)		142 (38.7)		848 (45.2)		847 (42.1)		1013 (39.3)	
Grade 3 (n, %)	90 (32.3)		108 (39.1)		163 (44.4)		604 (32.2)		941 (46.7)		1259 (48.9)	
Likert score Mean (SD)	5.27 (2.21)	0.685	5.64 (2.14)	0.003	5.91 (2.17)	0.067	5.34 (2.50)	0.685	6.14 (2.05)	0.003	6.16 (2.38)	0.067

Antibody titres (in median titres </> median) according to proportions (rate) of AR and intensity of AR. AR: adverse reaction; Ab: antibody; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, * Median value of antibody titres (P25, P75)= 10.00 (10.00, 10.00) on a semiquantitative scale ranging from 0 to >10

Table S12: Assessment of antibody titres (antibody titres above-below median): solicited and unsolicited AR

Adverse reactions (PT MedDRA term)	Development of any AR				^a p-value
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)		
	After any dose		After any dose		
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	
Solicited adverse reactions					
SOC: General disorders and administration site conditions					
Injection site pain <i>PT: Injection site pain</i>	192 (43.6)		1191 (38.1)		0.030
	145 (33.0)	82 (18.6)	943 (30.2)	534 (17.1)	0.240
	112 (25.5)		893 (28.6)		0.170
	41 (9.3)	85 (19.3)	279 (8.9)	708 (22.7)	0.790
Fatigue/asthenia <i>PT: Fatigue</i>	139 (31.6)		973 (31.2)		0.850
	37 (8.4)	114 (25.9)	188 (6.0)	871 (27.9)	0.050
	68 (15.5)		561 (18.0)		0.200
	16 (3.6)	56 (12.7)	125 (4.0)	465 (14.9)	0.710
Malaise <i>PT: Malaise</i>	45 (10.2)		408 (13.1)		0.090
	10 (2.3)	35 (8.0)	76 (2.4)	352 (11.3)	0.840
	17 (3.9)		140 (4.5)		0.550
	10 (2.3)		8 (1.8)	81 (2.6)	65 (2.1)
Injection site redness <i>PT: Application site redness</i>					0.700
					0.720

Adverse reactions (PT MedDRA term)	Development of any AR					a p-value
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
SOC: Musculoskeletal and connective tissue disorders						
Arm pain PT: Pain in extremity	126 (28.6)		946 (30.3)			0.480
	84 (19.1)	69 (15.7)	623 (19.9)	492 (15.8)	0.670	0.970
	94 (21.4)		620 (19.9)			0.460
Muscle pain PT: Myalgia	17 (3.9)	79 (18.0)	102 (3.3)	546 (17.5)	0.510	0.810
	43 (9.8)		289 (9.3)			0.730
Joint pain PT: Arthralgia	5 (1.1)	38 (8.6)	27 (0.9)	273 (8.7)	0.570	0.940
	21 (4.8)		93 (3.0)			0.040
Shoulder pain PT: Musculoskeletal pain	8 (1.8)	13 (3.0)	54 (1.7)	46 (1.5)	0.890	0.020
	SOC: Injury, poisoning and procedural complications					
Injection site swelling PT: Application site swelling	37 (8.4)		214 (6.9)			0.230
	25 (5.7)	14 (3.2)	124 (4.0)	110 (3.5)	0.090	0.720

Adverse reactions (PT MedDRA term)	Development of any AR					a p-value	
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)				
	After any dose		After any dose				
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose		
SOC: Vaccination site pruritus							
Injection site pruritus PT: Vaccination site pruritus	11 (2.5)		62 (2.0)			0.480	
	6 (1.4)	5 (1.1)	34 (1.1)	34 (1.1)		0.610	0.930
SOC: Nervous system disorders							
Headache PT: Headache	118 (26.8)		860 (27.5)			0.750	
	38 (8.6)	89 (20.2)	297 (9.5)	669 (21.4)		0.560	0.570
Facial paralysis PT: Facial paralysis	0 (0)		1 (0)			0.710	
	0 (0)	0 (0)	1 (0)	0 (0)		0.710	NA
Insomnia PT: Insomnia	7 (1.6)		28 (0.9)			0.170	
	1 (0.2)	6 (1.4)	8 (0.3)	20 (0.6)		0.910	0.100
SOC: Gastrointestinal disorders							
Nausea PT: Nausea	25 (5.7)		141 (4.5)			0.280	
	13 (3.0)	12 (2.7)	40 (1.3)	109 (3.5)		0.010	0.410

Adverse reactions (PT MedDRA term)	Development of any AR					^a p-value
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
Diarrhea <i>PT: Diarrhoea</i> Vomiting <i>PT: Vomiting</i>	13 (3.0)		97 (3.1)			0.860
	3 (0.7)	10 (2.3)	33 (1.1)	65 (2.1)		0.460
	8 (1.8)		55 (1.8)			0.930
	1 (0.2)	7 (1.6)	14 (0.4)	45 (1.4)		0.500
Unsolicited adverse reactions						
SOC: Musculoskeletal and connective tissue disorders						
Other musculoskeletal disorders <i>PT: Musculoskeletal disorder</i>	10 (2.3)		80 (2.6)			0.720
	3 (0.7)	7 (1.6)	29 (0.9)	55 (1.8)		0.610
SOC: Skin and subcutaneous tissue disorders						
Petechia, ecchymosis <i>PT: Ecchymosis</i>	1 (0.2)		13 (0.4)			0.560
	1 (0.2)	0 (0)	9 (0.3)	4 (0.1)		0.820
SOC: Nervous system disorders						
Cognitive alteration <i>PT: Cognitive disorder</i>	1 (0.2)		5 (0.2)			0.750
	1 (0.2)	0 (0)	3 (0.1)	2 (0.1)		0.440
						0.600

Adverse reactions (PT MedDRA term)	Development of any AR					a p-value
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
Alterations of smell and taste PT: Parosmia	2 (0.5)		6 (0.2)			0.280
	1 (0.2)	1 (0.2)	4 (0.1)	2 (0.1)		0.600 0.270
Paresthesia and hyperesthesia PT: Dysaesthesia	5 (1.1)		22 (0.7)			0.330
	2 (0.5)	3 (0.7)	11 (0.4)	13 (0.4)		0.740 0.440
Presyncope, syncope and vasovagal syncope PT: Syncope	1 (0.2)		6 (0.2)			0.880
	0 (0)	1 (0.2)	2 (0.1)	4 (0.1)		0.560 0.600
Sleepiness, hypersomnia PT: Hypersomnia	0 (0)		16 (0.5)			0.130
	0 (0)	0 (0)	3 (0.1)	13 (0.4)		0.520 0.180
Instability sensation, vertigo, sickness PT: Dizziness	8 (1.8)		82 (2.6)			0.310
	2 (0.5)	7 (1.6)	28 (0.9)	55 (1.8)		0.340 0.800
Tremor PT: Tremor	0 (0)		7 (0.2)			0.320
	0 (0)	0 (0)	2 (0.1)	5 (0.1)		0.600 0.400
SOC: Gastrointestinal disorders						

Adverse reactions (PT MedDRA term)	Development of any AR					^a p-value
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
Gastrointestinal disorders <i>PT:</i> <i>Gastrointestinal disorder</i>	7 (1.6)		29 (0.9)			0.190
	1 (0.2)	5 (1.1)	11 (0.4)	18 (0.6)	0.670	0.170
	SOC: Respiratory, thoracic and mediastinal disorders					
Asthma <i>PT:</i> <i>Asthma</i>	1 (0.2)		3 (0.1)			0.440
	1 (0.2)	0 (0)	0 (0)	2 (0.1)	0.010	0.600
	2 (0.5)		24 (0.8)			0.47
Rhinitis, nasal discharge <i>PT:</i> <i>Rhinitis</i>	2 (0.5)	0 (0)	9 (0.3)	16 (0.5)	0.560	0.130
	SOC: Investigations					

Adverse reactions (PT MedDRA term)	Development of any AR					^a p-value
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
Hypertension, hypotension PT: Blood pressure abnormal	3 (0.7)		10 (0.3)			0.240
	0 (0)	3 (0.7)	4 (0.1)	5 (0.2)		0.450
	SOC: General disorders and administration site conditions					0.030
Influenza-like symptoms PT: Influenza like illness	4 (0.9)		43 (1.4)			0.420
	2 (0.5)	3 (0.7)	14 (0.4)	29 (0.9)		0.990
	3 (0.7)		10 (0.3)			0.610
Sensation of heat, sensation of cold PT: Temperature regulation disorder	0 (0)	3 (0.7)	4 (0.1)	5 (0.2)		0.240
	0 (0)		8 (0.3)			0.450
	0 (0)	0 (0)	0 (0)	8 (0.3)		0.290
Chest pain PT: Chest pain	0 (0)		4 (0.1)			0.290
	0 (0)	0 (0)	0 (0)	8 (0.3)		NA
	0 (0)		4 (0.1)			0.290
Hiporexia, anorexia PT: Decreased appetite	0 (0)	0 (0)	0 (0)	4 (0.1)		0.450
	0 (0)		4 (0.1)			NA
	2 (0.5)		9 (0.3)			0.450
	0.560					

Adverse reactions (PT MedDRA term)	Development of any AR				^a p-value	
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose		
	0 (0)	2 (0.5)	5 (0.2)	5 (0.2)		
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>	SOC: Ear and labyrinth disorders					
PT: <i>Ear pain</i>	0 (0)		3 (0.1)		0.520	
	0 (0)	0 (0)	1 (0)	2 (0.1)	0.710	0.600
SOC: Skin and subcutaneous tissue disorders						
General pruritus PT: <i>Pruritus</i>	2 (0.5)		6 (0.2)		0.280	
	1 (0.2)	1 (0.2)	3 (0.1)	3 (0.1)	0.440	0.440
	2 (0.5)		14 (0.4)		0.990	
Sweat PT: <i>Cold sweat</i>	0 (0)	2 (0.5)	2 (0.1)	12 (0.4)	0.600	0.830
SOC: Immune system disorders						
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: <i>Hypersensitivity</i>	8 (1.8)		42 (1.3)		0.430	
	1 (0.2)	7 (1.6)	23 (0.7)	25 (0.8)	0.220	0.100

Adverse reactions (PT MedDRA term)	Development of any AR					a p-value
	Antibody titres below median* (n=440)		Antibody titres below median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
SOC: Cardiac disorders						
Tachycardia PT: <i>Tachycardia</i>	0 (0)		11 (0.4)			0.210
	0 (0)	0 (0)	5 (0.2)	6 (0.2)		0.400 0.360
SOC: Blood and lymphatic system disorders						
Lymphadenopathy PT: <i>Lymphadenopathy</i>	20 (4.5)		126 (4.0)			0.610
	7 (1.6)	14 (3.2)	34 (1.1)	95 (3.0)		0.360 0.870
SOC: Infections and infestations						
Herpetic infection PT: <i>Herpes virus infection</i>	1 (0.2)		9 (0.3)			0.820
	1 (0.2)	0 (0)	5 (0.2)	4 (0.1)		0.750 0.450

Distribution of proportions of non-severe solicited and unsolicited AR are displayed by elicited humoral immunogenicity (mean Ab titres $\leq$ Q2) and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, *Median value of antibody titres (P25, P75)= 10.00 (10.00, 10.00) on a semiquantitative scale ranging from 0 to >10

Table S13: Assessment of antibody titres (antibody titres above-below median): severe solicited AR

Adverse reactions (PT MedDRA term)	Development of any severe AR					^a p-value
	Antibody titres below median* (n=440)		Antibody titres above median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose		
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain PT: <i>Injection site pain</i>	47 (10.7)		302 (9.7)		0.500	
	33 (7.5)	14 (3.2)	206 (6.6)	125 (4.0)	0.480	0.410
	49 (11.1)		441 (14.1)		0.090	
	11 (2.5)	40 (9.1)	104 (3.3)	355 (11.4)	0.360	0.150
	10 (2.3)		102 (3.3)		0.260	
Fever PT: <i>Pyrexia</i>	8 (1.8)		17 (0.5)		0.810	
	2 (0.5)		86 (2.8)		0.250	
	32 (7.3)		274 (8.8)		0.290	
	7 (1.6)	25 (5.7)	46 (1.5)	234 (7.5)	0.850	0.170
	NA		NA		NA	
Chills PT: <i>Chills</i>	NA		NA		NA	
	NA		NA		NA	
	6 (1.4)		34 (1.1)		0.610	
	4 (0.9)	2 (0.5)	17 (0.5)	19 (0.6)	0.350	0.690
	Injection site redness PT: <i>Application site redness</i>					

Adverse reactions (PT MedDRA term)	Development of any severe AR					^a p-value
	Antibody titres below median* (n=440)		Antibody titres above median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
	SOC: Musculoskeletal and connective tissue disorders					
Arm pain PT: Pain in extremity	43 (9.8)		354 (11.3)			0.330
	30 (6.8)	15 (3.4)	212 (6.8)	173 (5.5)	0.980	0.060
	35 (8.0)		301 (9.6)			0.260
Muscle pain PT: Myalgia	5 (1.1)	29 (6.6)	41 (1.3)	269 (8.6)	0.760	0.150
	25 (5.7)		161 (5.2)			0.640
	3 (0.7)	22 (5.0)	14 (0.4)	151 (4.8)	0.510	0.880
Shoulder pain PT: Musculoskeletal pain	3 (0.7)		26 (0.8)		0.740	
	2 (0.5)	1 (0.2)	9 (0.3)	16 (0.5)	0.560	0.420
	SOC: Injury, poisoning and procedural complications					
Injection site swelling PT: Application site swelling	12 (2.7)		59 (1.9)			0.240
	10 (2.3)	2 (0.5)	34 (1.1)	31 (1.0)	0.030	0.270

Adverse reactions (PT MedDRA term)	Development of any severe AR				^a p-value	
	Antibody titres below median* (n=440)		Antibody titres above median* (n=3123)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose		
	SOC: Vaccination site pruritus					
Injection site pruritus PT: Vaccination site pruritus	0 (0)		18 (0.6)		0.110	
	0 (0)		0 (0)		0.290	
SOC: Nervous system disorders						
Headache PT: Headache	39 (8.9)		383 (12.3)		0.040	
	11 (2.5)		105 (3.4)		0.340	
	28 (6.4)		305 (9.8)		0.020	

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited humoral immunogenicity (mean Ab titres $\leq$ / $>$ Q2) and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, *Median value of antibody titres (P25, P75)= 10.00 (10.00, 10.00) on a semiquantitative scale ranging from 0 to >10

Table S14: Cellular immunogenicity assessment

Variable	Negative cellular immunogenicity (n= 8)					Positive cellular immunogenicity (n= 51)						
	After 1 st vaccine dose	p- value	After 2 nd vaccine dose	p- value	After any dose	p- value	After 1 st vaccine dose	p-value	After 2 nd vaccine dose	p- value	After any dose	p- value
Proportions of AR												
Development of any AR (n, %)	6 (75.0)	0.715 9	6 (75.0)	0.715 9	6 (75.0)	0.135 5	35 (68.6)	0.7159	35 (68.6)	0.7159	47 (92.2)	0.1355
Development of any mild AR (n, %)	1 (12.5)	0.815 7	3 (37.5)	0.194 6	4 (50.0)	0.246 6	8 (15.7)	0.8157	9 (17.6)	0.1946	15 (29.4)	0.2466
Development of any moderate AR (n, %)	3 (37.5)	0.989 4	4 (50.0)	0.716 2	5 (62.5)	0.989 4	19 (37.3)	0.9894	22 (43.1)	0.7162	32 (62.7)	0.9894
Development of any severe AR (n, %)	3 (37.5)	0.256 3	1 (12.5)	0.169 1	3 (37.5)	0.478 3	10 (19.6)	0.2563	19 (37.3)	0.1691	26 (51.0)	0.4783
Maximum intensity												
Grade 1 (n, %)	1 (16.7)	0.639 4	1 (20.0)	0.342 3	1 (16.7)	0.906 7	7 (21.2)	0.6394	5 (14.3)	0.3423	5 (10.9)	0.9067
Grade 2 (n, %)	2 (33.3)		3 (60.0)		2 (33.3)		16 (48.5)		11 (31.4)		15 (32.6)	
Grade 3 (n, %)	3 (50.0)		1 (20.0)		3 (50.0)		10 (30.3)		19 (54.3)		26 (56.5)	
Likert score Mean (SD)	5.67 (1.97)	0.755	5.20 (2.28)	0.256	5.48 (2.47)	0.467	5.32 (1.89)	0.755	6.32 (1.85)	0.256	6.45 (1.73)	0.467

Cellular immunogenicity according to proportions (rate) of AR and intensity of AR. AR: adverse reaction; Ab: antibody; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable

Table S15: Cellular immunogenicity assessment: solicited and unsolicited AR

Adverse reactions (PT MedDRA term)	Cellular immunogenicity						^a p-value
	Negative			Positive			
	After any dose			After any dose			
	After 1 st dose			After 1 st dose			
	After 2 nd dose			After 2 nd dose			
Solicited adverse reactions							
SOC: General disorders and administration site conditions							
Injection site pain <i>PT: Injection site pain</i>	1 (12.5)		23 (45.1)		0.080		
	0 (0)	1 (12.5)	17 (33.3)	14 (27.5)	0.050	0.370	
	3 (37.5)		14 (27.5)		0.560		
	2 (25.0)	3 (37.5)	3 (5.9)	12 (23.5)	0.070	0.400	
Fever <i>PT: Pyrexia</i>	2 (25.0)		17 (33.3)		0.639		
	1 (12.5)	2 (25.0)	5 (9.8)	13 (25.5)	0.810	0.980	
	2 (25.0)		10 (19.6)		0.720		
	0 (0)	2 (25.0)	2 (3.9)	9 (17.6)	0.570	0.620	
Chills <i>PT: Chills</i>	1 (12.5)		7 (13.7)		0.925		
	1 (12.5)	0 (0)	5 (9.8)	2 (3.9)	0.810	0.570	
	0 (0)		2 (3.9)		0.570		
	0 (0)	0 (0)	1 (2.0)	1 (2.0)	0.690	0.690	

Adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
SOC: Musculoskeletal and connective tissue disorders						
Arm pain <i>PT:</i> <i>Pain in extremity</i>	3 (37.5)		15 (29.4)		0.640	
	3 (37.5)	1 (12.5)	8 (15.7)	7 (13.7)	0.140	0.930
	1 (12.5)		13 (25.5)		0.420	
Muscle pain <i>PT:</i> <i>Myalgia</i>	1 (12.5)	0 (0)	2 (3.9)	12 (23.5)	0.300	0.120
	0 (0)		7 (13.7)		0.260	
	0 (0)	0 (0)	0 (0)	7 (13.7)	NA	0.260
Shoulder pain <i>PT:</i> <i>Musculoskeletal pain</i>	1 (12.5)		1 (2.0)		0.130	
	1 (12.5)	0 (0)	0 (0)	1 (2.0)	0.010	0.700
	SOC: Injury, poisoning and procedural complications					
Injection site swelling <i>PT:</i> <i>Application site swelling</i>	0 (0)		4 (7.8)		0.410	
	0 (0)	0 (0)	3 (5.9)	2 (3.9)	0.480	0.570

Adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
SOC: Vaccination site pruritus						
Injection site pruritus PT: Vaccination site pruritus	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
SOC: Nervous system disorders						
Headache PT: Headache	3 (37.5)		16 (31.4)			0.730
	2 (25.0)	2 (25.0)	6 (11.8)	12 (23.5)	0.310	0.930
Facial paralysis PT: Facial paralysis	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
Insomnia PT: Insomnia	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
SOC: Gastrointestinal disorders						
Nausea PT: Nausea	0 (0)		3 (5.9)			0.480
	0 (0)	0 (0)	1 (2.0)	2 (3.9)	0.700	0.600
Diarrhea PT: Diarrhoea	1 (12.5)		0 (0)			0.010
	1 (12.5)	78 (2.2)	0 (0)	0 (0)	0.010	NA
Vomiting PT: Vomiting	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA

Adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
Unsolicited adverse reactions						
SOC: Musculoskeletal and connective tissue disorders						
Other musculoskeletal disorders <i>PT: Musculoskeletal disorder</i>	1 (12.5)		1 (2.0)		0.130	
	0 (0)	1 (12.5)	0 (0)	1 (2.0)	NA	0.130
SOC: Skin and subcutaneous tissue disorders						
Petechia, ecchymosis <i>PT: Ecchymosis</i>	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
SOC: Nervous system disorders						
Cognitive alteration <i>PT: Cognitive disorder</i>	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
Alterations of smell and taste <i>PT: Parosmia</i>	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
Paresthesia and hyperesthesia <i>PT: Dysaesthesia</i>	0 (0)		0 (0)		NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA

Adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	0 (0)		0 (0)			
Rhinitis, nasal discharge PT: Rhinitis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
	SOC: Investigations					NA
Hypertension, hypotension PT: Blood pressure abnormal	0 (0)			0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
SOC: General disorders and administration site conditions						
Influenza-like symptoms PT: Influenza like illness	0 (0)			1 (2.0)		0.700
	0 (0)	0 (0)		1 (2.0)	0 (0)	0.700
Sensation of heat, sensation of cold PT: Temperature regulation disorder	0 (0)			0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Chest pain PT: Chest pain	0 (0)			0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
Hiporexia, anorexia PT: Decreased appetite	0 (0)			0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA

Adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value	
	Negative		Positive				
			After any dose				
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose		
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
SOC: Ear and labyrinth disorders							
PT: <i>Ear pain</i>	0 (0)		0 (0)			NA	
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
SOC: Skin and subcutaneous tissue disorders							
General pruritus PT: <i>Pruritus</i>	0 (0)		0 (0)			NA	
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
Sweat PT: <i>Cold sweat</i>	0 (0)		0 (0)			NA	
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
SOC: Immune system disorders							
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: <i>Hypersensitivity</i>	0 (0)		0 (0)			NA	
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA

Adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
SOC: <i>Cardiac disorders</i>						
Tachycardia PT: <i>Tachycardia</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
SOC: <i>Blood and lymphatic system disorders</i>						
Lymphadenopathy PT: <i>Lymphadenopathy</i>	0 (0)		2 (3.9)			0.570
	0 (0)	0 (0)	1 (2.0)	1 (2.0)	1 (2.0)	0.700
SOC: <i>Infections and infestations</i>						
Herpetic infection PT: <i>Herpes virus infection</i>	0 (0)		1 (2.0)			0.700
	0 (0)	0 (0)	1 (2.0)	0 (0)	0 (0)	NA

Distribution of proportions of non-severe solicited and unsolicited AR are displayed by elicited cellular immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant. Results are provided as absolute and relative (%) numbers for each variable

Table S16: Cellular immunogenicity assessment: severe solicited AR

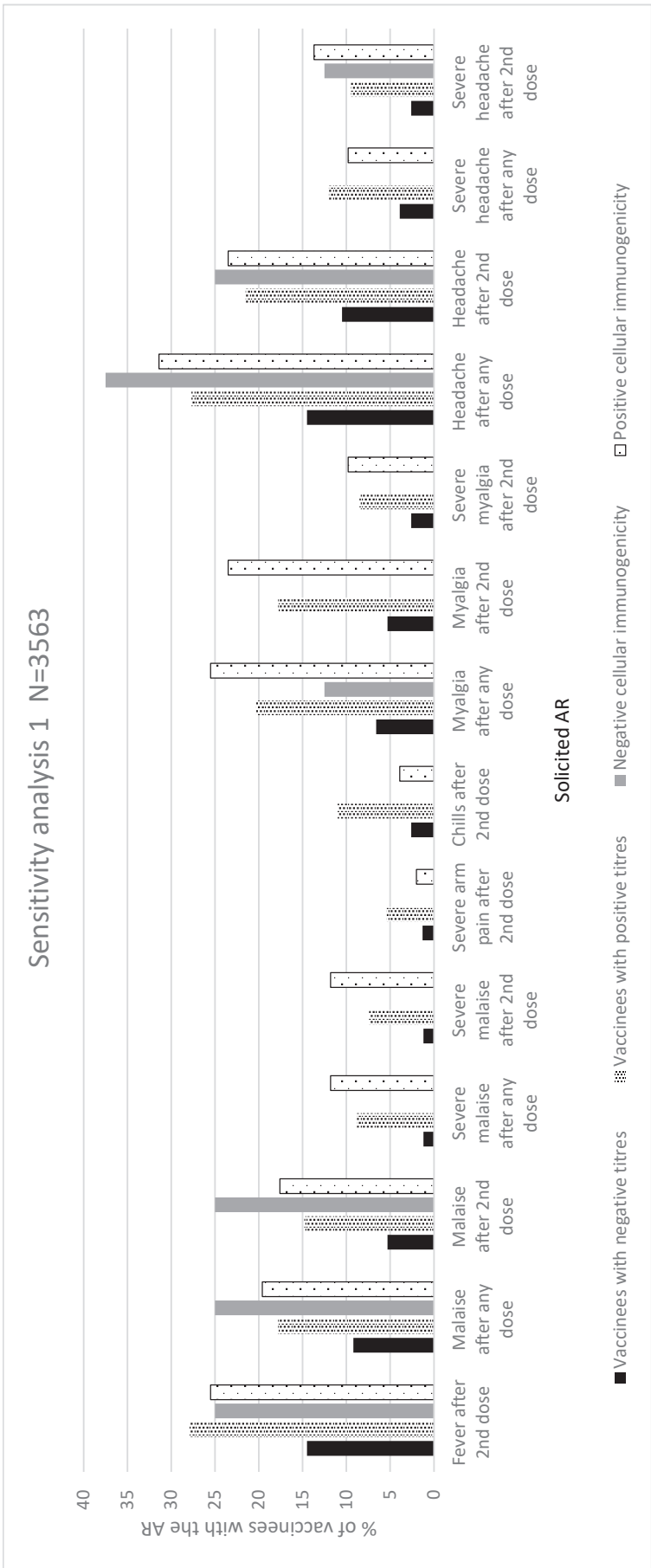
Severe adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain PT: <i>Injection site pain</i> Fatigue/asthenia PT: <i>Fatigue</i>	0 (0)	0 (0)	2 (3.9)	7 (13.7)	0.570	0.260
	1 (12.5)	0 (0)	10 (19.6)			0.350
	1 (12.5)	0 (0)	2 (3.9)	9 (17.6)	0.300	0.200
	0 (0)	0 (0)	0 (0)			NA
Fever PT: <i>Pyrexia</i>	0 (0)	0 (0)	0 (0)	0 (0)		NA
	0 (0)	0 (0)	0 (0)	0 (0)		
	0 (0)	0 (0)	0 (0)	0 (0)		
Malaise PT: <i>Malaise</i>	0 (0)	0 (0)	0 (0)	6 (11.8)		0.310
	0 (0)	0 (0)	0 (0)	6 (11.8)	NA	0.310
	0 (0)	0 (0)	1 (2.0)			0.700
Injection site redness PT: <i>Application site redness</i>	0 (0)	0 (0)	1 (2.0)	0 (0)	0.70	NA
	SOC: Musculoskeletal and connective tissue disorders					

Severe adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
Arm pain PT: Pain in extremity	2 (25.0)		5 (9.8)			0.220
	2 (25.0)	0 (0)	4 (7.8)	1 (2.0)	0.140	
	0 (0)		6 (11.8)			0.310
	0 (0)	0 (0)	1 (2.0)	5 (9.8)	0.700	
Muscle pain PT: Myalgia	0 (0)		5 (9.8)			0.350
	0 (0)	0 (0)	0 (0)	5 (9.8)	0.350	
	0 (0)		5 (9.8)			0.350
	0 (0)	0 (0)	0 (0)	5 (9.8)	0.350	
Joint pain PT: Arthralgia	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	5 (9.8)	0.350	
	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	5 (9.8)	0.350	
Shoulder pain PT: Musculoskeletal pain	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0.350	
	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0.350	
SOC: Injury, poisoning and procedural complications						

Severe adverse reactions (PT MedDRA term)	Cellular immunogenicity					^a p-value
	Negative		Positive			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
Injection site swelling <i>PT:</i> <i>Application site swelling</i>	0 (0)		2 (3.9)			0.570
	0 (0)	0 (0)	2 (3.9)	0 (0)	0 (0)	
	SOC: Vaccination site pruritus					
Injection site pruritus <i>PT:</i> <i>Vaccination site pruritus</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
	SOC: Nervous system disorders					
Headache <i>PT:</i> <i>Headache</i>	1 (12.5)		8 (15.7)			0.820
	0 (0)	1 (12.5)	1 (2.0)	7 (13.7)	0.700	0.930

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited cellular immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant. Results are provided as absolute and relative (%) numbers for each variable

Figure S2: Summary of results of immunogenicity and reactogenicity of sensitivity analysis 1



Bar diagram of results of main analysis. Immunogenicity is expressed as antibody positiveness (positive-negative titres) and cellular immunogenicity (positive-negative) data (%). Reactogenicity results are represented as % of vaccinees developing each solicited AR

ii.iii Sensitivity analysis 2

Table S17: Characteristics of subpopulation 2

Covariates	Total population (N=597)
Age	
N	3563
Mean (SD)	39.42 (12.69)
Median (P25, P75)	38.00 (28.00 , 50.00)
min, max	18.00 , 69.00
Gender	
Male (n, %)	173 (29.0)
Female (n, %)	424 (71.0)
Occupational SARS-CoV-2 contact	
In contact with SARS-CoV-2 patients	282 (47.2)
Without contact with SARS-CoV-2 patients	315 (52.8)
^aPrevious SARS-CoV-2 infection	
Yes	269 (45.1)
No	328 (54.9)
Comorbidities	
^b Any comorbidity	212 (35.5)
Arterial hypertension	25 (4.2)
Diabetes mellitus	9 (1.5)
Heart failure	2 (0.3)
Chronic bronchitis	2 (0.3)
Asthma	19 (3.2)
Rheumatic/immune-mediated disease	6 (1.0)
Drug allergies	49 (8.2)
Food allergies	25 (4.2)
Type of administered vaccine	
BNT16b2	
First dose:	446 (74.7)
Second dose:	341 (57.1)
mRNA-1273	
First dose:	151 (25.3)
Second dose:	120 (20.1)
Humoral immunogenicity (semiquantitative ab titres)	
Positive	324 (54)
Negative	273 (46)
IgG titres	
Positive	164 (99.4)
Negative	2 (1.2)

Covariables of studied population. ^aDiagnosis by RCP, antigen test and antibody positive serology; ^bNumber of vaccinees with at least one comorbidity. Results are provided as absolute and relative (%) numbers for each variable

Table S18: Assessment of antibody positiveness (positive-negative titres)

Variable	Antibody positiveness										
	Negative antibody titres (n= 273)						Positive antibody titres (n= 324)				
	After 1 st vaccine dose	p- value	After 2 nd vaccine dose	p- value	After any dose	p-value	After 1 st vaccine dose	After 2 nd vaccine dose	p- value	After any dose	p- value
Proportions of AR											
Development of any AR (n, %)	189 (69.2)	0.221	187 (68.5)	0.008	238 (87.2)	0.445	239 (73.8)	188 (58.0)	0.008	289 (89.2)	0.445
Development of any mild AR (n, %)	68 (24.9)	0.296	60 (22.0)	0.100	108 (39.6)	0.115	69 (21.3)	54 (16.7)	0.100	108 (33.3)	0.115
Development of any moderate AR (n, %)	99 (36.3)	0.099	126 (46.2)	0.052	165 (60.4)	0.690	139 (42.9)	124 (38.3)	0.052	201 (62.0)	0.690
Development of any severe AR (n, %)	53 (19.4)	0.017	72 (26.4)	0.411	99 (36.3)	0.204	90 (27.8)	76 (23.5)	0.411	134 (41.4)	0.204
Maximum intensity											
Grade 0 (n, %)	93 (34.1)	0.050	98 (35.9)	0.265	45 (16.5)	0.587	101 (31.2)	142 (43.8)	0.265	47 (14.5)	0.587
Grade 1 (n, %)	48 (17.6)		26 (9.5)		35 (12.8)		39 (12.0)	25 (7.7)		35 (10.8)	
Grade 2 (n, %)	79 (28.9)		77 (28.2)		94 (34.4)		94 (29.0)	81 (25.0)		108 (33.3)	
Grade 3 (n, %)	53 (19.4)		72 (26.4)		99 (36.3)		90 (27.8)	76 (23.5)		134 (41.4)	
Likert score											
Mean (SD)	3.37 (2.99)	0.083	3.63 (3.31)	0.001	4.93 (2.99)	0.772	3.83 (3.21)	3.01 (3.23)	0.001	5.03 (2.95)	0.772

Antibody titres according to proportions (rate) of AR and intensity of AR. AR: adverse reaction; Ab: antibody; in bold type: statistically significant. Results are provided as absolute and relative (%) numbers for each variable

Table S19: Assessment of antibody positiveness (positive-negative titres): solicited and unsolicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain <i>PT: Injection site pain</i>	134 (49.1)		119 (36.7)		0.002	
	103 (37.7)	50 (18.3)	92 (28.4)	50 (15.4)	0.015	0.347
Fatigue/asthenia <i>PT: Fatigue</i>	31 (11.4)		23 (7.1)		0.071	
	21 (7.7)	11 (4.0)	20 (6.2)	5 (1.5)	0.465	0.061
Fever <i>PT: Pyrexia</i>	91 (33.3)		121 (37.3)		0.308	
	16 (5.9)	82 (30.0)	73 (22.5)	79 (24.4)	<0.001	0.121
Malaise <i>PT: Malaise</i>	37 (13.6)		76 (23.5)		0.002	
	5 (1.8)	34 (12.5)	36 (11.1)	49 (15.1)	<0.001	0.348
Chills <i>PT: Chills</i>	27 (9.9)		45 (13.9)		0.135	
	2 (0.7)	25 (9.2)	25 (7.7)	24 (7.4)	<0.001	0.438

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
Injection site redness <i>PT:</i> <i>Application site redness</i>	15 (5.5)		11 (3.4)			0.211
	10 (3.7)	6 (2.2)	8 (2.5)	3 (0.9)		0.395
0.204						
SOC: Musculoskeletal and connective tissue disorders						
Arm pain <i>PT:</i> <i>Pain in extremity</i>	81 (29.7)		90 (27.8)			0.610
	49 (17.9)	47 (17.2)	73 (22.5)	31 (9.6)		0.167
Muscle pain <i>PT:</i> <i>Myalgia</i>	66 (24.2)		81 (25.0)			0.816
	9 (3.3)	58 (21.2)	36 (11.1)	55 (17.0)		0.003
Joint pain <i>PT:</i> <i>Arthralgia</i>	25 (9.2)		27 (8.3)			0.722
	0 (0)	25 (9.2)	10 (3.1)	20 (6.2)		0.034
Shoulder pain <i>PT:</i> <i>Musculoskeletal pain</i>	7 (2.6)		10 (3.1)			0.702
	5 (1.8)	2 (0.7)	4 (1.2)	5 (1.5)		0.551
0.359						
SOC: Injury, poisoning and procedural complications						

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
Injection site swelling <i>PT:</i> <i>Application site swelling</i>	31 (11.4)		23 (7.1)			0.071
	21 (7.7)	11 (4.0)	20 (6.2)	5 (1.5)		
SOC: Vaccination site pruritus						
Injection site pruritus <i>PT:</i> <i>Vaccination site pruritus</i>	8 (2.9)		4 (1.2)			0.141
	5 (1.8)	3 (1.1)	2 (0.6)	2 (0.6)		
SOC: Nervous system disorders						
Headache <i>PT:</i> <i>Headache</i>	79 (28.9)		92 (28.4)			0.884
	24 (8.8)	61 (22.3)	48 (14.8)	60 (18.5)		
Facial paralysis <i>PT:</i> <i>Facial paralysis</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)		
Insomnia <i>PT:</i> <i>Insomnia</i>	6 (2.2)		5 (1.5)			0.554
	1 (0.4)	5 (1.8)	1 (0.3)	4 (1.2)		
SOC: Gastrointestinal disorders						
Nausea	16 (5.9)		16 (4.9)			0.6181

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
	8 (2.9)	8 (2.9)	11 (3.4)	5 (1.5)		
PT: Nausea	8 (2.9)		9 (2.8)		0.911	
Diarrhea PT: Diarrhoea	3 (1.1)	5 (1.8)	4 (1.2)	5 (1.5)	0.878	0.785
Vomiting PT: Vomiting	7 (2.6)		3 (0.9)		0.120	
	1 (0.4)	6 (2.2)	1 (0.3)	2 (0.6)	0.903	0.094
Unsolicited adverse reactions						
SOC: Musculoskeletal and connective tissue disorders						
Other musculoskeletal disorders PT: Musculoskeletal disorder	2 (0.7)	7 (2.6)	5 (1.8)	9 (2.8)	0.064	0.182
	SOC: Skin and subcutaneous tissue disorders					
Petechia, ecchymosis PT: Ecchymosis	0 (0)	0 (0)	0 (0)	1 (0.3)	0.358	NA
	SOC: Nervous system disorders					
Cognitive alteration PT: Cognitive disorder	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
Alterations of smell and taste	1 (0.4)			1 (0.3)	0.903	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value	
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)				
	After any dose		After any dose				
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose		
	1 (0.4)	0 (0)	0 (0)	1 (0.3)	0.276		0.358
PT: Parosmia	3 (1.1)		4 (1.2)			0.878	
Paresthesia and hyperesthesia PT: Dysaesthesia	1 (0.4)	2 (0.7)	3 (0.9)	1 (0.3)	0.404	0.466	
Presyncope, syncope and vasovagal syncope PT: Syncope	1 (0.4)		0 (0)		0.276		
	0 (0)	1 (0.4)	0 (0)	0 (0)	NA	0.276	
Sleepiness, hypersomnia PT: Hypersomnia	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	
Instability sensation, vertigo, sickness PT: Dizziness	2 (0.7)	5 (1.8)	2 (0.6)	2 (0.6)	0.863	0.170	
	0 (0)		0 (0)		NA		
Tremor PT: Tremor	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA	
SOC: Gastrointestinal disorders							
Gastrointestinal disorders PT: Gastrointestinal disorder	1 (0.4)	5 (1.8)	2 (0.6)		0.170		
		4 (1.5)	1 (0.3)	0 (0)	0.903	0.029	
SOC: Respiratory, thoracic and mediastinal disorders							

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					a p-value
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
Asthma PT: <i>Asthma</i>	1 (0.4)		0 (0)			0.276
	1 (0.4)	0 (0)	0 (0)	0 (0)	0.276	NA
	2 (0.7)		2 (0.6)			0.863
Rhinitis, nasal discharge PT: <i>Rhinitis</i>	2 (0.7)	0 (0)	1 (0.3)	1 (0.3)	0.466	0.358
SOC: <i>Investigations</i>						
Hypertension, hypotension PT: <i>Blood pressure abnormal</i>	2 (0.7)		0 (0)			0.123
	0 (0)	2 (0.7)	0 (0)	0 (0)	NA	0.123
SOC: <i>General disorders and administration site conditions</i>						
Influenza-like symptoms PT: <i>Influenza like illness</i>	5 (1.8)		4 (1.2)			0.551
	2 (0.7)	4 (1.5)	2 (0.6)	2 (0.6)	0.863	0.301
Sensation of heat, sensation of cold PT: <i>Temperature regulation disorder</i>	0 (0)		1 (0.3)			0.358
	0 (0)	0 (0)	1 (0.3)	0 (0)	0.358	NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
Chest pain PT: <i>Chest pain</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
	NA	NA	NA	NA	NA	NA
Hiporexia, anorexia PT: <i>Decreased appetite</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
	NA	NA	NA	NA	NA	NA
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>	2 (0.7)		0 (0)			0.123
	0 (0)	2 (0.7)	0 (0)	0 (0)	0 (0)	0.123
	NA	NA	NA	NA	NA	0.123
SOC: Ear and labyrinth disorders						
PT: <i>Ear pain</i>	0 (0)		0 (0)			NA
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
	NA	NA	NA	NA	NA	NA
SOC: Skin and subcutaneous tissue disorders						
General pruritus PT: <i>Pruritus</i>	2 (0.7)		0 (0)			0.123
	1 (0.4)	0 (0)	1 (0.4)	0 (0)	0 (0)	0.276
	NA	NA	NA	NA	NA	0.276
Sweat	2 (0.7)		1 (0.3)			0.466

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
	0 (0)	2 (0.7)	1 (0.3)	0 (0)		
PT: <i>Cold sweat</i>					0.358	0.123
SOC: Immune system disorders						
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: <i>Hypersensitivity</i>	6 (2.2)		4 (1.2)		0.361	
	0 (0)	6 (2.2)	3 (0.9)	1 (0.3)	0.111	0.033
SOC: Cardiac disorders						
Tachycardia PT: <i>Tachycardia</i>	0 (0)		2 (0.6)		0.194	
	0 (0)	0 (0)	1 (0.3)	1 (0.3)	0.358	0.358
SOC: Blood and lymphatic system disorders						
Lymphadenopathy PT: <i>Lymphadenopathy</i>	16 (5.9)		9 (2.8)		0.061	
	6 (2.2)	11 (4.0)	3 (0.9)	6 (1.9)	0.204	0.111
SOC: Infections and infestations						
Herpetic infection PT: <i>Herpes virus infection</i>	1 (0.4)		1 (0.3)		0.903	
	1 (0.4)	0 (0)	1 (0.3)	0 (0)	0.903	NA

Distribution of proportions of non-severe solicited and unsolicited AR are displayed by elicited humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant. Results are provided as absolute and relative (%) numbers for each variable

Table S20: Assessment of antibody positiveness (positive-negative titres): severe solicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain <i>PT: Injection site pain</i>	26 (9.5)		30 (9.3)		0.912	
	17 (6.2)	8 (2.9)	20 (6.2)	11 (3.4)	0.978	0.747
Fatigue/asthenia <i>PT: Fatigue</i>	34 (12.5)		40 (12.3)		0.968	
	9 (3.3)	27 (9.9)	14 (4.3)	26 (8.0)	0.517	0.425
	7 (2.6)		17 (5.2)		0.096	
Fever <i>PT: Pyrexia</i>	0 (0)	7 (2.6)	11 (3.4)	7 (2.2)	0.002	0.746
Malaise <i>PT: Malaise</i>	17 (6.2)		42 (13.0)		0.006	
	3 (1.1)	14 (5.1)	18 (5.6)	26 (8.0)	0.003	0.159
Injection site redness <i>PT: Application site redness</i>	5 (1.7)		2 (0.6)		0.170	
	4 (1.4)	1 (0.3)	2 (0.6)	0 (0)	0.301	0.276

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose	After 2 nd dose	
SOC: Musculoskeletal and connective tissue disorders						
Arm pain <i>PT:</i> <i>Pain in extremity</i>	28 (10.3)		35 (10.8)			0.829
	19 (7.0)	11 (4.0)	28 (8.6)	8 (2.5)		0.447
	23 (8.0)		36 (11.7)			0.273
	3 (1.0)	20 (6.9)	18 (5.8)	21 (6.8)		0.003
Muscle pain <i>PT:</i> <i>Myalgia</i>						0.684
Joint pain <i>PT:</i> <i>Arthralgia</i>	14 (5.1)		17 (5.2)			0.948
	0 (0)	14 (5.1)	6 (1.9)	13 (4.0)		0.024
	1 (0.4)		2 (0.6)			0.666
	1 (0.4)	0 (0)	1 (0.3)	0 (0)		0.903
<i>Musculoskeletal pain</i>	SOC: Injury, poisoning and procedural complications					NA
Injection site swelling <i>PT:</i> <i>Application site swelling</i>	9 (3.3)		9 (2.8)			0.712
	8 (2.9)	1 (0.4)	8 (2.5)	1 (0.3)		0.728
	SOC: Vaccination site pruritus					0.903
Injection site pruritus <i>PT:</i> <i>Vaccination site pruritus</i>	0 (0)		1 (0.3)			0.358
	0 (0)	0 (0)	1 (0.3)	0 (0)		0.358
						NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Negative antibody titres (n= 273)		Positive antibody titres (n= 324)			
	After any dose		After any dose			
	After 1 st dose	After 2 nd dose	After 1 st dose	After 2 nd dose		
	SOC: Nervous system disorders					
Headache <i>PT:</i> <i>Headache</i>	24 (8.8)		40 (12.3)		0.162	
	6 (2.2)	19 (7.0)	20 (6.2)	21 (6.5)	0.018	0.816

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant. Results are provided as absolute and relative (%) numbers for each variable

Table S21: Assessment of antibody titres (antibody titres above-below median):

Variable	Antibody titres below median* (n= 288)					Antibody titres above median* (n= 309)				
	After 1 st vaccine dose	p-value	After 2 nd vaccine dose	p-value	After any dose	p-value	After 1 st vaccine dose	p-value	After 2 nd vaccine dose	p-value
Proportions of AR										
Development of any AR (n, %)	200 (69.4)	0.239	193 (67.0)	0.04 0	249 (86.5)	0.183	228 (73.8)	0.04 0	182 (58.9)	278 (90.0)
Development of any mild AR (n, %)	70 (24.3)	0.446	62 (21.5)	0.14 4	111 (38.5)	0.247	67 (21.7)	0.14 4	52 (16.8)	105 (34.0)
Development of any moderate AR (n, %)	103 (35.8)	0.048	129 (44.8)	0.16 3	172 (59.7)	0.443	135 (43.7)	0.16 3	121 (39.2)	194 (62.8)
Development of any severe AR (n, %)	60 (20.8)	0.085	75 (26.0)	0.49 4	107 (37.2)	0.364	83 (26.9)	0.49 4	73 (23.6)	126 (40.8)
Maximum intensity										
Grade 1 (n, %)	50 (26.2)	0.072	27 (14.9)	0.93 5	36 (15.1)	0.719	37 (17.5)	0.93 5	24 (13.6)	34 (12.8)
Grade 2 (n, %)	81 (42.4)		79 (43.6)		96 (40.2)		92 (43.4)		79 (44.9)	106 (39.8)
Grade 3 (n, %)	60 (31.4)		75 (41.4)		107 (44.8)		83 (39.2)		73 (41.5)	126 (47.4)
Likert score		0.083		0.57 0		0.592		0.57 0		
N	189		175		236		206		163	259
Mean (SD)	5.28 (2.18)		5.87 (2.13)		6.06 (2.15)		5.65 (2.09)		5.77 (2.02)	5.97 (2.07)
Median	5.00 (3.00, 7.00)		6.00 (4.00, 8.00)		6.00 (4.00, 8.00)		6.00 (4.00, 7.00)		6.00 (4.00, 7.00)	6.00 (4.00, 8.00)
(P25, P75) min,Max	{1.00, 10.00}		{2.00,10.00}		{1.00,10.00}		{0.00,10.00}		{2.00,10.00}	{0.00,10.00}

Antibody titres (in titres above and below median) according to proportions (rate) of AR and intensity of AR. AR: adverse reaction; Ab: antibody; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, Median value of antibody titres (P25, P75)= 2.53 (0.47 , 10.00) on a semiquantitative scale ranging from 0 to >10

Table S22: Assessment of antibody titres (antibody titres above-below median): solicited and unsolicited AR

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
	Solicited adverse reactions					
SOC: General disorders and administration site conditions						
Injection site pain <i>PT: Injection site pain</i>	141 (49.0)		112 (36.2)			0.002
	110 (38.2)	52 (18.1)	85 (27.5)	48 (15.5)		0.005
Fatigue/asthenia <i>PT: Fatigue</i>	74 (25.7)		86 (27.8)			0.556
	24 (8.3)	58 (20.1)	43 (13.9)	53 (17.2)		0.031
Fever <i>PT: Pyrexia</i>	95 (33.0)		117 (37.9)			0.213
	19 (6.6)	83 (28.8)	70 (22.7)	78 (25.2)		<0.001
Malaise <i>PT: Malaise</i>	41 (14.2)		72 (23.3)			0.004
	7 (2.4)	37 (12.8)	34 (11.0)	46 (14.9)		<0.001
Chills <i>PT: Chills</i>	29 (10.1)		43 (13.9)			0.149
	4 (1.4)	25 (8.7)	23 (7.4)	24 (7.8)		0.004
						0.685

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value	
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)				
	After any dose		After any dose				
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose		
	15 (5.2)		11 (3.6)				
Injection site redness <i>PT:</i> <i>Application site redness</i>	10 (3.5)	6 (2.1)	8 (2.6)	3 (1.0)	0.324	0.528	0.265
SOC: Musculoskeletal and connective tissue disorders							
Arm pain <i>PT:</i> <i>Pain in extremity</i>	85 (29.5)		86 (27.8)			0.650	
Muscle pain <i>PT:</i> <i>Myalgia</i>	52 (18.1)	49 (17.0)	70 (22.7)	29 (9.4)		0.164	0.006
	68 (23.6)		79 (25.6)			0.580	
	9 (3.1)	60 (20.8)	36 (11.7)	53 (17.2)		<0.001	0.251
Joint pain <i>PT:</i> <i>Arthralgia</i>	28 (9.7)		24 (7.8)			0.397	
Shoulder pain <i>PT:</i> <i>Musculoskeletal pain</i>	1 (0.3)	27 (9.4)	9 (2.9)	18 (5.8)		0.015	0.101
	9 (3.1)		8 (2.6)			0.694	
	6 (2.1)	3 (1.0)	3 (1.0)	4 (1.3)		0.265	0.774
SOC: Injury, poisoning and procedural complications							
Injection site swelling <i>PT:</i> <i>Application site swelling</i>	31 (10.8)		23 (7.4)			0.158	
	21 (7.3)	11 (3.8)	20 (6.5)	5 (1.6)		0.693	0.096
	SOC: Vaccination site pruritus						
	Injection site pruritus <i>PT:</i>	8 (2.8)		4 (1.3)			0.200
	5 (1.7)	3 (1.0)	2 (0.6)	2 (0.6)		0.217	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose		
Vaccination site pruritus						
SOC: Nervous system disorders						
Headache PT: Headache	83 (28.8)		88 (28.5)		0.927	
	26 (9.0)	64 (22.2)	46 (14.9)	57 (18.4)	0.028	0.252
	0 (0)		0 (0)		NA	
Facial paralysis PT: Facial paralysis	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
	6 (2.1)		5 (1.6)		0.673	
Insomnia PT: Insomnia	1 (0.3)	5 (1.7)	1 (0.3)	4 (1.3)	0.960	0.658
	SOC: Gastrointestinal disorders					
Nausea PT: Nausea	17 (5.9)		15 (4.9)		0.570	
	9 (3.1)	8 (2.8)	10 (3.2)	5 (1.6)	0.938	0.332
	9 (3.1)		8 (2.6)		0.694	
Diarrhea PT: Diarrhoea	3 (1.0)	6 (2.1)	4 (1.3)	4 (1.3)	0.774	0.453
	7 (2.4)		3 (1.0)		0.165	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value	
	Antibody titres below median* (n= 288)		Antibody titres above median * (n= 309)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose		
	1 (0.3)	6 (2.1)	1 (0.3)	2 (0.6)		
Unsolicited adverse reactions						
SOC: Musculoskeletal and connective tissue disorders						
Other musculoskeletal disorders PT: Musculoskeletal disorder	3 (1.0)	8 (2.8)	5 (1.7)	8 (2.6)	14 (4.5)	0.256
					7 (2.3)	0.160
SOC: Skin and subcutaneous tissue disorders						
Petechia, ecchymosis PT: Ecchymosis	0 (0)	0 (0)	0 (0)	1 (0.3)	1 (0.3)	0.334
					0 (0)	0.334
SOC: Nervous system disorders						
Cognitive alteration PT: Cognitive disorder	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
						NA
Alterations of smell and taste PT: Parosmia	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (0.3)	0.300
						0.960
Paresthesia and hyperesthesia	3 (1.0)			4 (1.3)		0.774

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
PT: Dysaesthesia	1 (0.3)	2 (0.7)	3 (1.0)	1 (0.3)	0.351	0.522
Presyncope, syncope and vasovagal syncope PT: Syncope	1 (0.3)		0 (0)		0.300	
	0 (0)	1 (0.3)	0 (0)	0 (0)	NA	0.300
Sleepiness, hypersomnia PT: Hypersomnia	0 (0)				NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
Instability sensation, vertigo, sickness PT: Dizziness	6 (2.1)		3 (1.0)		0.265	
	2 (0.7)	5 (1.7)	2 (0.6)	2 (0.6)	0.944	0.217
Tremor PT: Tremor	0 (0)				NA	
	0 (0)	0 (0)	0 (0)	0 (0)	NA	NA
SOC: Gastrointestinal disorders						
Gastrointestinal disorders PT: Gastrointestinal disorder	6 (2.1)		1 (0.3)		0.046	
	1 (0.3)	4 (1.4)	1 (0.3)	0 (0)	0.960	0.038
SOC: Respiratory, thoracic and mediastinal disorders						

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					a p-value
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
Asthma PT: Asthma	1 (0.3)		0 (0)			0.300
	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)	0.300 NA
Rhinitis, nasal discharge PT: Rhinitis	2 (0.7)		2 (0.6)			0.944
	2 (0.7)	0 (0)	1 (0.3)	1 (0.3)	1 (0.3)	0.522 0.334
SOC: Investigations						
Hypertension, hypotension PT: Blood pressure abnormal	2 (0.7)		0 (0)			0.142
	0 (0)	2 (0.7)	0 (0)	0 (0)	0 (0)	NA 0.142
SOC: General disorders and administration site conditions						
Influenza-like symptoms PT: Influenza like illness	5 (1.7)		4 (1.3)			0.658
	2 (0.7)	4 (1.4)	2 (0.6)	2 (0.6)	2 (0.6)	0.944 0.364
Sensation of heat, sensation of cold PT: Temperature regulation disorder	0 (0)		1 (0.3)			0.334
	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0.334 NA

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)			
	After any dose		After any dose			
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose	
Chest pain PT: <i>Chest pain</i>						
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
						NA
Hiporexia, anorexia PT: <i>Decreased appetite</i>						
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
						NA
Inflammation in extremities other than the vaccinated arm PT: <i>Inflammation</i>						
	2 (0.7)		0 (0)			0.142
	0 (0)	2 (0.7)	0 (0)	0 (0)	0 (0)	NA
SOC: <i>Ear and labyrinth disorders</i>						
PT: <i>Ear pain</i>						
	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	NA
						NA
SOC: <i>Skin and subcutaneous tissue disorders</i>						
General pruritus PT: <i>Pruritus</i>						
	2 (0.7)		0 (0)			0.142
	1 (0.4)	1 (0.4)	0 (0)	0 (0)	0 (0)	0.300
						0.300

Adverse reactions (PT MedDRA term)	Humoral immunogenicity					^a p-value		
	Antibody titres below median* (n= 288)		Antibody titres above median * (n= 309)					
	After any dose		After any dose					
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	After 2nd dose			
Sweat PT: <i>Cold sweat</i>	2 (0.7)		1 (0.3)			0.522		
	0 (0)	2 (0.7)	1 (0.3)	0 (0)		0.334	0.142	
SOC: Immune system disorders								
Immediate hypersensitivity, delayed hypersensitivity, exanthema, urticaria, rash PT: <i>Hypersensitivity</i>	6 (2.1)		4 (1.3)		0.453			
	0 (0)	6 (2.1)	3 (1.0)	1 (0.3)		0.094	0.046	
SOC: Cardiac disorders								
Tachycardia PT: <i>Tachycardia</i>	0 (0)		2 (0.6)			0.171		
	0 (0)	0 (0)	1 (0.3)		1 (0.3)	0.334	0.334	
SOC: Blood and lymphatic system disorders								
Lymphadenopathy PT: <i>Lymphadenopathy</i>	16 (5.6)		9 (2.9)			0.107		
	6 (2.1)	11 (3.8)	3 (1.0)	6 (1.9)		0.265	0.168	
SOC: Infections and infestations								
Herpetic infection PT:	1 (0.3)		1 (0.3)		0.960		0.960	NA
	1 (0.3)	0 (0)	1 (0.3)	0 (0)		0.960	NA	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)		
	After any dose		After any dose		
	After 1st dose	After 2nd dose	After 1st dose	After 2nd dose	
<i>Herpes virus infection</i>					

Herpes virus infection

Distribution of proportions of non-severe solicited and unsolicited AR are displayed by elicited humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, Median value of antibody titres (P25, P75)= 2.53 (0.47 , 10.00) on a semiquantitative scale ranging from 0 to >10

Table S23: Assessment of antibody titres (antibody titres above-below median): severe solicited AR

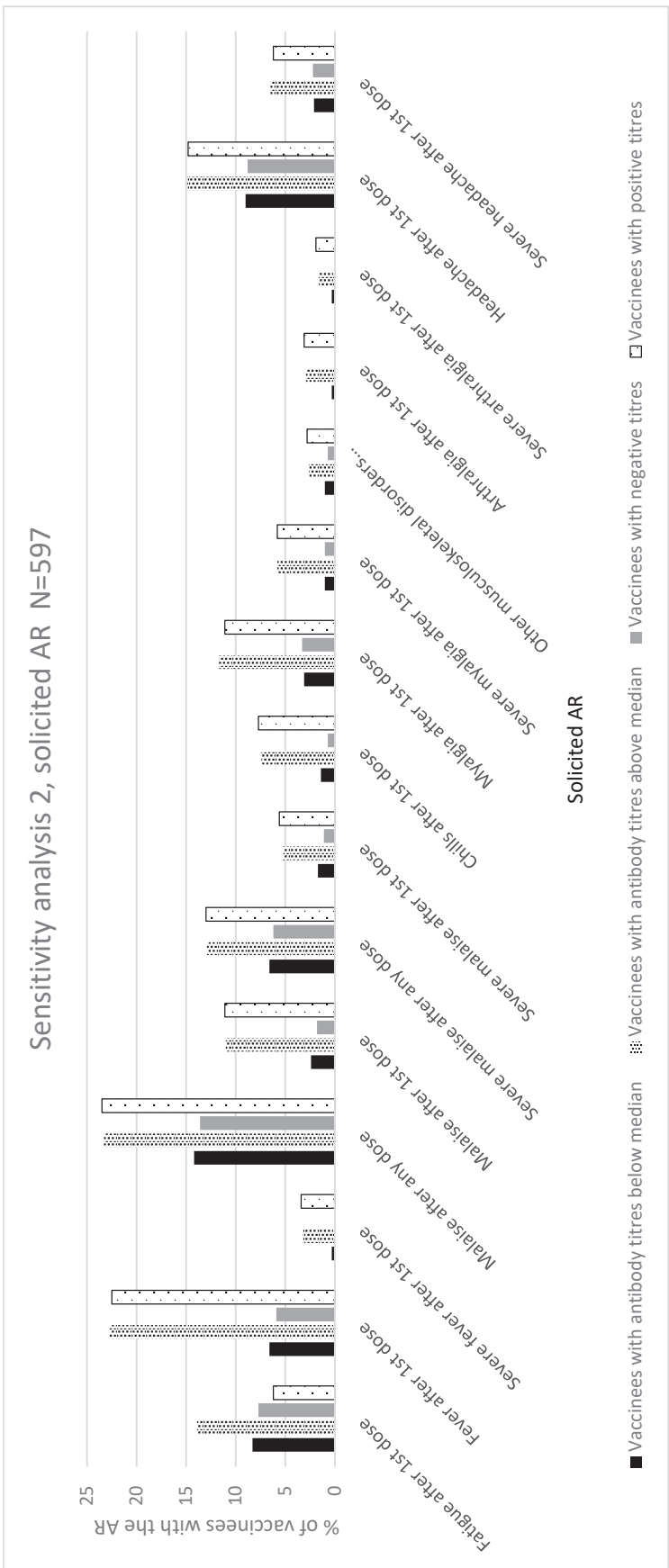
Adverse reactions (PT MedDRA term)	Humoral immunogenicity				^a p-value
	Humoral immunogenicity		Humoral immunogenicity		
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)		
	After any dose	After any dose	After any dose	After any dose	
	Solicited adverse reactions				
SOC: General disorders and administration site conditions					
Injection site pain PT: <i>Injection site pain</i>	31 (10.8)		25 (8.1)		0.263
	22 (7.6)	8 (2.8)	15 (4.9)	11 (3.6)	0.159
	35 (12.2)		39 (12.6)		0.587
Fatigue/asthenia PT: <i>Fatigue</i>	10 (3.5)	27 (9.4)	13 (4.2)	26 (8.4)	0.641
	8 (2.8)		16 (5.2)		0.680
	8 (2.8)		16 (5.2)		0.136
Fever PT: <i>Pyrexia</i>	1 (0.3)	7 (2.4)	10 (3.2)	7 (2.3)	0.009
	19 (6.6)		40 (12.9)		0.009
	5 (1.7)	15 (5.2)	16 (5.2)	25 (8.1)	0.023
Injection site redness PT: <i>Application site redness</i>	5 (1.7)		2 (0.6)		0.159
	5 (1.7)		2 (0.6)		0.217
	4 (1.4)	1 (0.3)	2 (0.6)	0 (0)	0.364
SOC: Musculoskeletal and connective tissue disorders					
Arm pain	30 (10.4)		33 (10.7)		0.917

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				a p-value	
	Humoral immunogenicity		Humoral immunogenicity			
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)			
	After any dose	After any dose	After any dose	After any dose		
PT: Pain in extremity	20 (6.9)	12 (4.2)	27 (8.7)	7 (2.3)	0.416	0.186
Muscle pain PT: Myalgia	23 (8.0)		36 (11.7)		0.134	
	3 (1.0)	20 (6.9)	18 (5.8)	21 (6.8)	0.002	0.943
Joint pain PT: Arthralgia	16 (5.6)		15 (4.9)		0.700	
	1 (0.3)	15 (5.2)	5 (1.6)	12 (3.9)	0.120	0.436
Shoulder pain PT:	2 (0.7)		1 (0.3)		0.522	
	2 (0.7)	0 (0)	0 (0)	0 (0)	0.142	NA
Musculoskeletal pain	SOC: Injury, poisoning and procedural complications					
Injection site swelling PT: Application site swelling	9 (3.1)		9 (2.9)		0.880	
	8 (2.8)	1 (0.3)	8 (2.6)	1 (0.3)	0.887	0.960
	SOC: Vaccination site pruritus					
Injection site pruritus PT: Vaccination site pruritus	0 (0)		1 (0.3)		0.334	
	0 (0)	0 (0)	1 (0.3)	0 (0)	0.334	NA
	SOC: Nervous system disorders					
Headache	26 (9.0)		38 (12.3)		0.200	

Adverse reactions (PT MedDRA term)	Humoral immunogenicity				a p-value	
	Humoral immunogenicity		Humoral immunogenicity		0.009	0.577
	Antibody titres below median* (n= 288)		Antibody titres above median* (n= 309)			
	After any dose	After any dose	After any dose	After any dose		
	6 (2.1)	21 (7.3)	20 (6.5)	19 (6.1)		
PT; Headache						

Distribution of proportions of severe solicited and unsolicited AR are displayed by elicited humoral immunogenicity and dose; ^achi-square test; AR: adverse reaction; SOC: System Organ Class; PT: Preferred term; NA: not applicable, p-value not calculable because of absence of valid values in both comparison groups; in bold type: statistically significant results. Results are provided as absolute and relative (%) numbers for each variable, Median value of antibody titres (P25, P75)= 2.53 (0.47 , 10.00) on a semiquantitative scale ranging from 0 to >10

Figure S3: Summary of results of immunogenicity and reactogenicity of sensitivity analysis 2



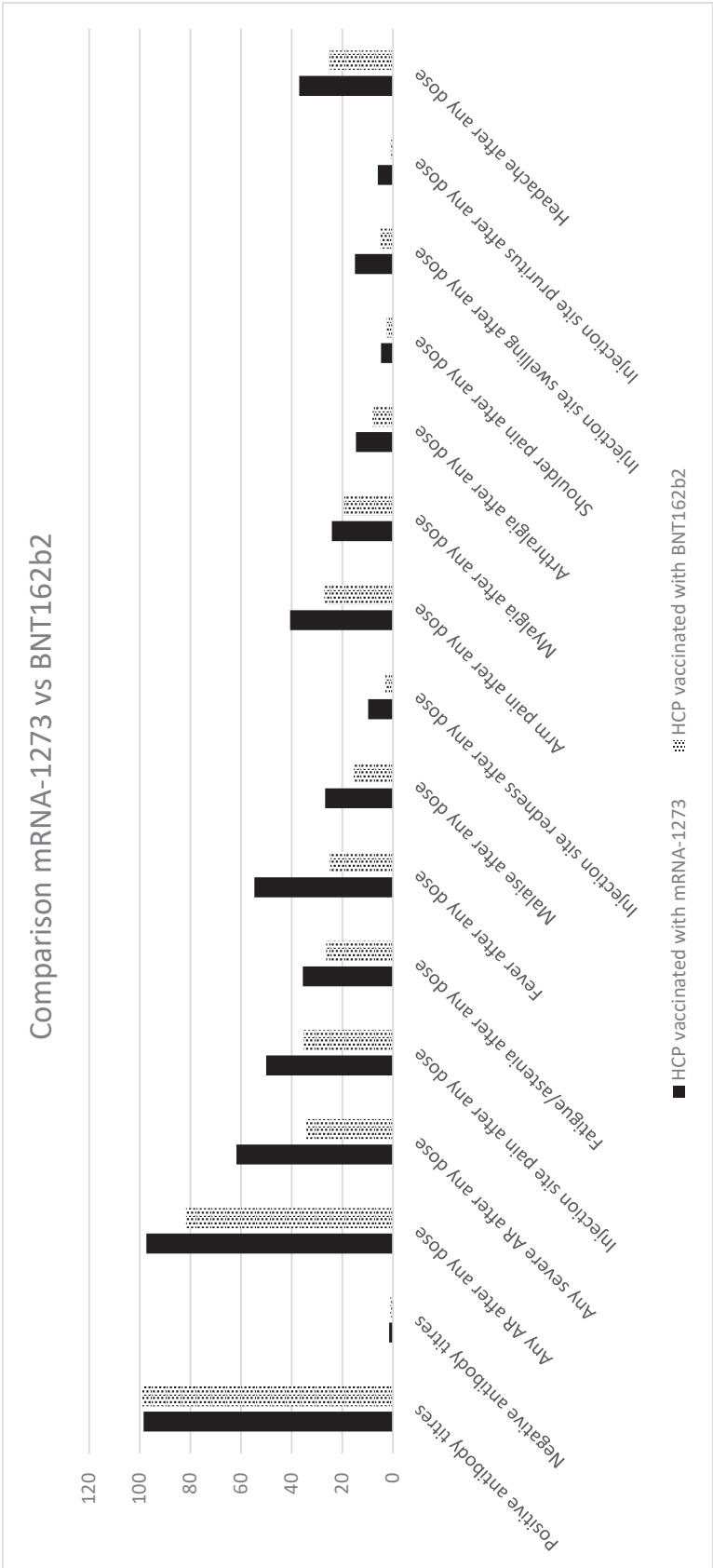
Bar diagram of results of main analysis. Immunogenicity is expressed as antibody positiveness (positive-negative titres) and cellular immunogenicity (positive-negative) data (%). Reactogenicity results are represented as % of vaccinees developing each solicited AR

Table S24: Reactogenicity and immunogenicity by vaccine and population types

Subpopulation 1	Vaccine type (mRNA-1273 vs BNT162b2)		Population type (HCP vs SOTR)		HCP		SOTR	
	mRNA-1273 All vaccinees N=865	BNT162b2 All vaccinees N=2698	HCP N=3460	SOTR N=103	HCP vaccinated with mRNA-1273 N=762	HCP vaccinated with BNT162b2 N=2698	SOTR vaccinated with mRNA-1273 N=103	SOTR vaccinated with BNT162b2
Immunogenicity (n, %)								
Qualitative immunogenicity	Positive titres	810 (94)	2677 (99)	59 (57)	746 (98.5)	2665 (99)	59 (57)	NA
	Negative titres	55 (6)	21 (1)	32 (1)	11 (1.5)	21 (1)	44 (43)	NA
Reactogenicity (n, %)								
Any AR after any dose	832 (96.2)	2203 (81.7)	2945 (85.1)	90 (87.4)	742 (97.4)	2203 (81.7)	90 (87.4)	NA
Any severe AR after any dose	500 (57.8)	922 (34.2)	1393 (40.3)	29 (28.2)	471 (61.8)	922 (34.2)	29 (28.2)	NA
Injection site pain	431 (49.8)	952 (35.3)	1334 (38.6)	49 (47.6)	382 (50.1)	952 (35.3)	49 (47.6)	NA
Fatigue/asthenia	292 (33.8)	713 (26.4)	984 (28.4)	21 (20.4)	271 (35.6)	713 (26.4)	21 (20.4)	NA
Fever	439 (50.8)	673 (24.9)	1090 (31.5)	22 (21.4)	417 (54.7)	673 (24.9)	22 (21.4)	NA
Malaise	212 (24.5)	417 (15.5)	621 (17.9)	8 (7.8)	204 (26.8)	417 (15.5)	8 (7.8)	NA
Injection site redness	77 (8.9)	80 (3.0)	155 (4.5)	2 (1.9)	75 (9.8)	80 (3.0)	2 (1.9)	NA
Arm pain	341 (39.4)	731 (27.1)	1040 (30.1)	32 (31.1)	309 (40.6)	731 (27.1)	32 (31.1)	NA
Myalgia	191 (22.1)	523 (19.4)	707 (20.4)	7 (6.8)	184 (24.1)	523 (19.4)	7 (6.8)	NA
Arthralgia	114 (13.2)	218 (8.1)	329 (9.5)	3 (2.9)	111 (14.6)	218 (8.1)	3 (2.9)	NA
Shoulder pain	46 (5.3)	68 (2.5)	104 (3.0)	10 (9.7)	36 (4.7)	68 (2.5)	10 (9.7)	NA
Injection site swelling	120 (13.9)	131 (4.9)	245 (7.1)	6 (5.8)	114 (15.0)	131 (4.9)	6 (5.8)	NA
Injection site pruritus	49 (5.7)	24 (0.9)	70 (2.0)	3 (2.9)	46 (6.0)	24 (0.9)	3 (2.9)	NA
Headache	297 (34.3)	681 (25.2)	963 (27.8)	15 (14.6)	282 (37.0)	681 (25.2)	15 (14.6)	NA

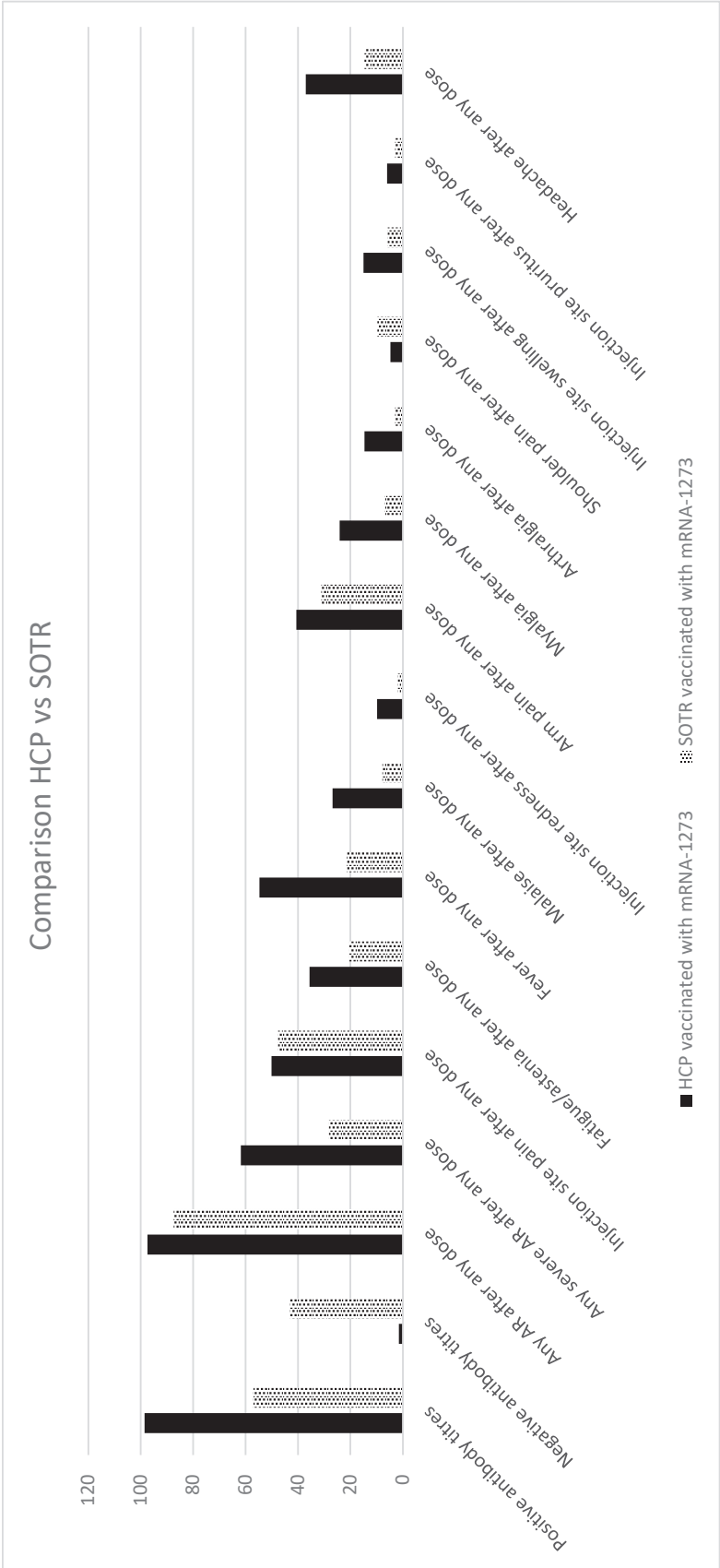
HCP: health-care professional; SOTR: solid-organ transplant recipient; NA: not applicable because all SOTR were vaccinated with mRNA-1273

Figure S4: Comparison of vaccine types



HCP: health-care professional; SOTR: solid-organ transplant recipient; AR: adverse reaction; positive/negative antibody titres: % of vaccinees with positive or negative titres; any AR, any severe AR and solicited AR: % of vaccinees with at least one event

Figure S5: Comparison of population types



HCP: health-care professional; SOTR: solid-organ transplant recipient; AR: adverse reaction; positive/negative antibody titres: % of vaccinees with positive or negative titres; any AR, any severe AR and solicited AR: % of vaccinees with at least one event

Table S25 STROBE Checklist

STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Item No		Recommendation	Page No*
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	7-8
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5-6
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants	5-6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-9
Bias	9	Describe any efforts to address potential sources of bias	7
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-9
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions	7-9

		(c) Explain how missing data were addressed		
		(d) If applicable, describe analytical methods taking account of sampling strategy		
		(e) Describe any sensitivity analyses		
Results				
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage	10	
		(c) Consider use of a flow diagram		
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest	10	
Outcome data	15*	Report numbers of outcome events or summary measures	10-12	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10-12	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10-12	

Discussion		
Key results	18 Summarise key results with reference to study objectives	13-15
Limitations	19 Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14-15
Interpretation	20 Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-16
Generalisability	21 Discuss the generalisability (external validity) of the study results	15-16
Other information		
Funding	22 Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Pages where the different items of the STROBE checklist are described

Reference: <https://www.strobe-statement.org/>

5 Discussion

The short-term safety of mRNA vaccines was reasonably well characterised by clinical trial data. However, real-world studies were needed to provide further supporting evidence of the safety profile of these vaccines. This evidence was essential, particularly at a time of great distress and anxiety caused by the pandemic, the initial uncertainties surrounding vaccination, and the fact that, for the first time in history, millions of people (the vast majority of the entire population in many countries) were expected to be vaccinated in a short period. In May 2020, the 42nd Global Advisory Committee on Vaccine Safety discussed the readiness of pharmacovigilance systems in anticipation of the upcoming COVID-19 vaccines (160). They recommended that all countries establish and activate the necessary infrastructure and capacity to monitor vaccine safety prior to the introduction of any COVID-19 vaccine.

Similarly, the WHO's COVID-19 vaccine safety surveillance manual emphasised the importance of monitoring and reporting adverse events following immunisation, as well as AESIs, alongside data management systems, safety communication, and the necessity of post-authorisation safety surveillance studies (161). Effective implementation of this surveillance required coordinated efforts at the local, national, regional, and global levels. Also, the implementation of a complete and integrated surveillance system required the provision of post-authorisation safety data from several sources, such as spontaneous reporting and active post-authorisation safety studies.

In line with the need to enrich post-authorisation safety data of vaccines in a “real-world” setting, the current thesis aims to provide a deeper understanding of important aspects related to mRNA vaccines that deserve further evaluation. Assessing the tolerability in a real-world setting, comparability of safety profiles, reactogenicity profile in different subgroups under study, and the association between post-vaccination reactogenicity and elicited immunogenicity.

To do so, an in-depth analysis of the comparability of the reactogenicity and tolerability profile of two mRNA COVID-19 vaccines (BNT162b2 and mRNA-1273) was performed in the VigilVacCOVID study. Reactogenicity was defined as the physical manifestation of an inflammatory response to vaccination, and included injection site pain, redness, swelling or induration at the injection site, as well as systemic symptoms, such as fever, myalgia, or headache (162). In line with this definition, reactogenicity was assessed by the frequency and

severity of solicited ARs. Additionally, tolerability was evaluated as a proxy of the general safety profile of the vaccines (163).

Of note, the term AEFI has been widely used in the literature; it is defined by the EMA as “any untoward medical occurrence following immunisation without necessarily having a causal relationship with the use of the vaccine”. In the literature, other synonyms have been used, such as “post-vaccination adverse events” (164). The determination of whether a causal relationship exists between an AEFI and vaccination is guided by the WHO protocols, which outline a four-step analytical process and algorithmic framework. From the outset, the evaluation considers all potential “alternative causes” that could account for the AEFI, thereby aiming to exclude the vaccine’s role. Even when there is biological plausibility and temporal correlation suggesting a causal link between the vaccine and the AEFI, the guidelines recommend examining any possible evidence that would refute the vaccine as the cause. This algorithmic approach raises several concerns, particularly when viewed through the lens of the multifactorial nature of vaccine-induced inflammatory and immune disorders. Given the large sample size analysed in this study and the high number of AEFIs evaluated, for the purposes of this study and to optimise the analysis, we considered all events occurring after vaccination with a temporally compatible sequence to be ARs.

Similarly, in May 2020, in anticipation of the development of COVID-19 vaccines, the Brighton Collaboration—in collaboration with the CEPI SPEAC—introduced a list of relevant AESIs to facilitate the analysis of clinical trial and post-marketing data. These AESIs were based on immune-mediated sequelae of COVID-19 or events commonly associated with many licensed vaccines that could potentially be observed in recipients of COVID-19 vaccines (165). In the VigilVacCOVID study, we established a predefined list of ARs that was agreed based on the safety profile described in the summary of product characteristics (SmPC) and the European Public Assessment Report (EPAR) of each vaccine product. In addition, some ARs of special interest from the list of the ACCESS (166) and SPEAC Projects (167), as well as Brighton Collaboration criteria (168), were also considered within the pre-defined list of ARs. However, given that the main objective was the characterisation of the short-term tolerability and reactogenicity profile, and most of the pre-defined AESIs were, in principle, not expected, we defined this list as “solicited ARs” and not AESIs. Therefore, from this point forward, we will refer to the terms ARs and solicited ARs.

Finally, the association between collected safety data and elicited immunological correlates, at humoral and cellular levels, was conducted considering the safety data obtained in the VigilVacCOVID study. The main results are discussed in the following sections.

5.1 Implementation and performance of a prospective and comparative pharmacovigilance system

A pivotal aspect of vaccine pharmacovigilance is the continuous monitoring of safety data from different sources (169). Since December 2020, nearly one billion doses of COVID-19 vaccines have been administered within the EU, marking it as the largest vaccination program ever conducted (170). Currently, there is an unprecedented amount of data that confirms the safety and efficacy of COVID-19 vaccines. Safety information has been provided by a number of pharmacovigilance sources (155,158,166,167), as well as real-world studies that have been published beyond the controlled context of clinical trials (171).

The WHO identifies two main approaches to pharmacovigilance: passive or spontaneous surveillance and active or intensive surveillance (172). Both methods represent two fundamental approaches to public health monitoring. Passive surveillance depends on voluntary reports submitted by hospitals, clinics, public health units, and other sources. While this approach is cost-effective and capable of covering extensive regions, and, as such, is highly valuable for generating hypotheses, detecting signals, and studying rare diseases, the data quality and timelines may vary, given its reliance on the voluntary participation of different institutions and individuals (173).

Besides, it is difficult to estimate rates and frequencies of ARs through spontaneous reporting given that the overall denominator of exposed individuals is unknown (172). Passive pharmacovigilance is also frequently criticised for underreporting, particularly in relation to the Weber effect, where the highest number of ARs are reported within the first two years after a medicine's launch, followed by a consistent decline (174–176). Conversely, active surveillance involves a dedicated team that systematically contacts healthcare providers or the general population to collect data on health conditions (177). This method is highly reliable, as it ensures the acquisition of accurate and timely information; however, it is associated with considerable costs.

Within active surveillance, cohort event-monitoring studies are recognised as an active surveillance method that facilitates the collection of detailed information regarding the temporal progression of ARs, providing a defined denominator for risk quantification. This approach is particularly effective in identifying reactogenic events, including those that do not require medical attention. The utility of cohort event monitoring as a complementary tool to existing spontaneous reporting systems was demonstrated during the H1N1 pandemic in various European countries. For example, a cohort event-monitoring study conducted in the Netherlands enabled tracking of the time course, onset, duration of ARs, and actions taken in response to adverse events, data which were underrepresented in published clinical trials (178).

The VigilVacCOVID study was an observational, prospective, unicentric post-authorisation study designed to characterise ARs occurring in the short term after vaccination. This study was part of the active surveillance program that had been developed by the Pharmacovigilance Program at the Hospital Clínic of Barcelona during the vaccination campaign on January 7th, 2021. The project was designed as an active cohort intensive monitoring study, a type of active surveillance monitoring initially described and implemented by the WHO (179). ARs were captured through a structured questionnaire that gathered relevant demographic data, medical background, and ARs occurring after each dose of the vaccination schedule. Vaccinees were interviewed through phone calls after each dose.

Some other real-world studies were published at the start of the vaccination campaign. Most of these studies were based on electronic health records or spontaneous adverse event reporting systems, such as large pharmacovigilance databases (VAERS) (171), voluntary, spontaneous notification through smartphone-based systems (like V-safe) (158), or both (180). Some studies evaluated post-vaccination safety profiles through validated self-administered questionnaires (181,182), but mainly in BNT162b2, which was the first mRNA vaccine to be authorised and used worldwide. Liu et al. analysed 26 safety studies in a recent systematic review and meta-analysis of real-world studies of SARS-CoV-2 vaccines (183). However, most of the 26 studies included were based on retrospective assessments of data or a single vaccine, and only a few small studies provided real-world comparative data.

Spontaneous reporting and retrospective data are of undeniable value, particularly those from large databases; nonetheless, active surveillance monitoring, with the comparison of two

mRNA vaccines, provides an enriching and useful complementary perspective. The protocol of a multi-country, three-phase study, including a cross-sectional survey to test for the short-term ARs of COVID-19 vaccines (the COVAST study), was published in the early phases of vaccine rollout (184). The second phase of this protocol planned to monitor booster dose ARs and, in the third phase, long-term vaccine safety and effectiveness. However, the first results were not published until 2023 (185).

The significance of our results lies in the provision of real-world evidence regarding the safety profile of two vaccines (BNT162b2 and mRNA-1273) and two populations—healthcare professionals (HCPs) and solid organ transplant recipients (SOTRs), in a prospective comparison that used an identical structured questionnaire for the evaluation of local and systemic ARs. These results were published at a time when uncertainties about the real-world safety of COVID-19 vaccines still persisted. Our results contributed to providing additional evidence regarding the short-term safety profile of the vaccines.

Furthermore, spontaneous reporting results depend on the type of reported ARs. In these studies, serious and severe ARs are frequently reported, whereas mild or moderate symptoms are not systematically reported. Active surveillance allows us to compute ARs with different severity grades. In our study, we could analyse solicited local and systemic ARs of different severity grades, establishing comparisons within BNT162b2 and mRNA-1273, and within HCPs and SOTRs.

Likewise, our results provide comparative data of 6377 assessed participants. This reasonably large sample size with a known denominator of vaccinated individuals enabled us to provide reliable incidence estimates of local and systemic ARs. Previous real-world studies, like those included in the review performed by Liu and colleagues (186), had sample sizes that ranged from 77 to 2041 vaccinated persons. Other studies focused on HCPs, such as those of Riad A et al. in the Czech Republic and Slovakia (181,182), with similar sample sizes to the studies included in Liu's revision (n=522 and n=922 participants, respectively). A larger study was conducted in Malta by Cuschieri and colleagues, enrolling 1480 HCPs vaccinated with BNT162b2 (187).

Subsequent more recent studies evaluated larger cohorts, such as the one performed by Saita M and colleagues in 3254 HCPs vaccinated with BNT162b2 (188). Likewise, another publication, designed as a cohort event-monitoring study and coordinated by the

Netherlands Pharmacovigilance Centre Lareb, provided comparative data on 22184 vaccinated participants, who were vaccinated with BNT162b2, mRNA-1273 and other types of vaccines, such as Vaxzevria® (189). Our study provided similar results in terms of proportions, severity, and types of reported ARs, which reinforces the reliability of our findings.

Similarly, in Europe, the "Covid Vaccine Monitor" (CVM) initiative, an active surveillance program spanning multiple nations, was launched in February 2021 (EU PAS number 42504). The primary objective of this project was to assess the safety of COVID-19 vaccines across the general population, as well as within specific groups of vulnerable individuals (including pregnant and breastfeeding women, children and adolescents, immunocompromised individuals, those with a history of allergies, and individuals with previous SARS-CoV-2 infection). Data on vaccine-related ARs were gathered through specialised web applications across 13 European countries. Derived from this project, Luxi N et al. published a prospective cohort study grounded on web-based questionnaires. In this study, 829 and 1,879 vaccinees vaccinated with BNT162b2 and mRNA-1273, respectively, were included in the analysis (190). ARs were higher after the second dose, with mRNA-1273 having a higher reactogenicity rate. Solicited ARs were mild, expected, and well-known and were mainly injection site reactions and systemic reactions related to the vaccine immune response, in line with that observed in pivotal trials and other studies, such as our VigilVacCOVID study.

Another multinational prospective cohort event-monitoring study was conducted with primary consented data collection in seven European countries through web-based questionnaires on self-reported ARs (191). Fatigue and headache were the most commonly reported solicited systemic ARs and injection site pain was the most common solicited local reaction. The rates of solicited and unsolicited, local and systemic ARs were similar to those reported in the VigilVacCOVID study.

5.1.1 Strengths and limitations

Overall, the main strength of the VigilVacCOVID study is the systematic and prospective comparison of two mRNA vaccines and two different populations. The design of the study was intensive cohort monitoring, which is a type of active pharmacovigilance study based on the interview of patients on a particular treatment (the cohort), for capturing problems (the events). This design is especially useful for providing real-world data on new medicines in routine clinical practice in the early post-marketing phase, thus being an early warning system (172). Indeed, cohort event-monitoring studies have several advantages (192,193), such as:

- The ability to generate a comprehensive profile of ARs.
- They are highly efficient in detecting early-stage signals.
- They allow us to correlate reactions with associated risk factors.
- They enable precise comparisons between different medical products to be conducted.

These characteristics of this study design were particularly useful in providing a prospective comparison of the two mRNA vaccines and the two populations, which were the focus of the first part of the project. Indeed, the use of safety outcomes reported by vaccinees enabled us to capture data on ARs that would otherwise not be captured in medical records because most vaccinated persons who have short-term, non-serious ARs, will not seek medical attention. Cohort event-monitoring studies have become a recognised approach for collecting rapid post-marketing safety data, particularly during the initial phase of vaccine deployment when extensive administrative data sources for retrospective analysis are either not yet accessible or are entirely unavailable, such as in certain countries (178,194,195).

Another strength of the VigilVacCOVID study is the large real-world cohort included. Previous authors have advised enrolling a cohort of 10,000 patients to provide a 95% probability of detecting an event with an incidence rate of 1:3,000, which would correspond to an uncommon or rare AR. In our study, we included a sample size of 6,377 vaccinees. This sample size can still be considered clinically relevant since it is assumed that a cohort of 3,000 patients gives a 95% chance of identifying a single AR with an incidence of 1:1,000 (179,196), which would correspond to a low-frequency AR. As mentioned before, most of the real-world studies conducted early in the rollout vaccination period had notably lower sample sizes.

Notwithstanding, cohort event-monitoring studies have some limitations (172), and this was also the case with the VigilVacCOVID study. Firstly, the method requires a higher level of human resources and necessitates the allocation of specialised personnel to conduct baseline assessments and follow-up interviews during the treatment process. Additionally, it incurs greater costs compared with spontaneous reporting. In the VigilVacCOVID study, it was necessary to form a team of nine professionals from the Medicines Area to provide support to the Pharmacovigilance Program. Besides, performing the interviews and the follow-up requires specific expertise in documenting adverse events and training is essential. Therefore, the interviewing team of the VigilVacCOVID was trained *ad-hoc* and they delivered the questionnaire through a phone call. Additionally, simulations with 10 dummy cases were performed to homogenise the delivery process amongst interviewers, and the process was supervised by specialised members of the Pharmacovigilance Program.

On the other hand, active pharmacovigilance studies may entail specific ethical considerations, as they involve a methodology that requires the collection of detailed personal data for the purpose of patient monitoring and follow-up. Certain studies might opt to obtain informed consent from each individual patient, though this process can be time-consuming. An alternative approach to acquiring informed consent is the 'opt-out principle,' in which information regarding the study is publicly disclosed. Patients are then given the opportunity to decide whether they wish to have their data included in the active pharmacovigilance program or not. These approaches have been endorsed by previous international ethical guidelines (197).

In the case of the VigilVacCOVID study, vaccine surveillance was part of a hospital care program. Both the vaccination program and vaccine surveillance were established in a very short period given the public health relevance of having as many people vaccinated as possible, at the earliest possible time. In addition, hundreds of persons were vaccinated per day at the vaccination centre. Therefore, written informed consent was not considered a feasible alternative. In its place, vaccinated persons were informed of this surveillance program with a leaflet before being vaccinated at the vaccination centre. The objectives and methods of the surveillance program were described in the leaflet. This information was explained again when calls were made after each dose. The survey was only delivered if the person agreed to it. The whole procedure was explained in the protocols that were assessed

and approved by the Ethics Committee of the Hospital Clínic (references: HCB/2021/0684 and HCB/2021/0685).

An additional potential limitation arising from this point is that participants can 'opt-out' and decline participation, which could pose challenges in achieving the necessary cohort size. Nonetheless, in the VigilVacCOVID study, we had an acceptance rate of 64% of HCPs and 77% of SOTRs, which can be considered acceptable. As is well known, cohort event-monitoring studies may be affected by selection bias due to selective non-response (193). Although the acceptance rate in our study was acceptable, we cannot exclude the possibility of this type of bias.

On the other hand, intensive cohort-event monitoring studies, with a cohort of 10,000 participants, can have a 95% chance of identifying a specific event that has an incidence of $\geq 1:3,000$ (i.e., an uncommon or rare event) (179,196). However, these types of studies may not have sufficient power to detect very rare ARs (with an incidence of $< 1/10,000$). With an $n=5088$, the VigilVacCOVID study was not sufficiently powered to detect very rare cases. Indeed, the study was not designed to detect rare or very rare ARs but to capture the comparative short-term reactogenicity and tolerability profile of BNT162b2 and mRNA-1273.

Additionally, the study was designed to capture only short-medium-term reactogenicity and ARs. Therefore, neither long-term ARs nor the long-term outcome of specific patients could be assessed.

Likewise, we did not perform a formal sample size calculation. We included all vaccinated HCPs and SOTRs who did not refuse to take part in the survey. Previously conducted studies have adopted similar approaches (181,190,191). It should be noted that some experts argue that sample size calculation and reassurance of statistical power are not necessarily “a must” in causal analyses of existing databases (198). Hernan MA considers that a restrictive attitude towards observational analyses is misguided, and cites three reasons: Firstly, when a causal question is important, it is preferable to have multiple studies with imprecise estimates than having no study at all. In this sense, refusing to conduct an observational study because “it’ll be underpowered” prevents the accumulation of scientific evidence. Secondly, there is a strong ethical argument for power—or, better, precision—calculations before conducting a randomised trial that may place patients at risk. This ethical argument is not transferable to

observational analyses of existing databases. Thirdly, truly exact calculations are impossible when adjustments for measurement error, selection bias, and confounding are required, especially in complex settings with time-varying treatments and confounders. In those cases, complex simulation studies would be needed to obtain approximate estimates of precision.

Nevertheless, the sample may still be considered a convenient sample of vaccinated participants in a particular setting of a specific Hospital, who agreed to participate in this study, and therefore they may not be fully representative of the general population. For instance, participants who engaged may have been more prone to report ARs.

Overall, both the vaccination program and vaccine surveillance were established in a very short period, given the public health relevance of having as many people vaccinated as possible at the earliest possible time. There was an important question to answer: the safety profile of COVID-19 vaccines in a real-world setting, and these studies, such as ours, despite their limitations and biases, still contribute to enriching existing knowledge on this type of vaccine, especially if these studies are taken together. Our data are reflective of usual practice in a period when the vaccination campaign was established rapidly amid the pressures of SARS-CoV-2 fears, and safety follow-up was also rapidly organised to serve vaccinees with exhaustive vaccine surveillance.

Despite the described limitations, the methodology of the study reflects data acquisition and processing within a real-world context in usual clinical practice, which should be instructive for the development of further real-world studies. This is particularly true for those that are performed within the context of mass vaccination campaigns during emergency periods with the use of clinical practice data.

5.1.2 Future perspectives

The implementation of mass vaccination programs has facilitated the conduct of real-world studies into vaccine safety within vaccinated populations. Such studies can supplement regular post-marketing surveillance conducted after post-authorisation commitments by offering additional data on safety signals. Furthermore, they can provide estimates of more frequent events, such as reactogenicity, in populations that differ from those included in clinical trials and normal clinical scenarios. In the case of COVID-19 vaccines, these real-world studies significantly influenced decision-making by regulatory agencies and

governments post-authorisation, owing to their unprecedented scale, early execution, and swift dissemination of findings (199).

As a matter of fact, during the pandemic, we were reminded of the importance of real-world evidence (RWE) to provide continuous impactful evidence on vaccine safety. Real-world data (RWD), coming from RWE, describe patient characteristics (including treatment utilisation and outcomes) in routine clinical practice. Therefore, the utilisation of RWE should be further pursued to better characterise the safety profile of SARS-CoV-2 vaccines beyond the regulated context of clinical trials under normal conditions of clinical use. However, RWE studies should be conducted with appropriate designs that generate valid and reliable evidence to answer a specific question, with transparent reporting and robust regulatory guidance and structures that promote an RWE research environment (200).

From a regulatory perspective, the main priorities are to **enable the use** of RWE and to **establish its value** for regulatory decision-making (201). Early regulatory efforts were implemented during the pandemic and the initial release of the first COVID-19 vaccines. The EMA and the European Medicines Regulatory Network (EMRN) started to work to establish a sustainable framework to enable the use and establish the value of RWE across different regulatory use cases, overseen by the Big Data Steering Group (BDSG). In line with the implication of the Agency in fostering RWE, the EMA commissioned 11 studies to consortia with expertise in observational research, including pharmacoepidemiological investigations utilising extensive European electronic healthcare databases, vaccine effectiveness assessments, and studies involving patient-reported data. By December 2022, six of these studies had been concluded, while five remained in progress (142). Furthermore, in 2021, the EMRN initiated the development of a distributed EU-wide RWD network, termed the Data Analytics and Real-World Interrogation Network (DARWIN EU) (202). DARWIN EU was officially launched in early 2022 with the establishment of its coordination centre, tasked with integrating data partners and facilitating the execution of studies requested by medicines regulators and, eventually, other relevant stakeholders.

In addition, in May 2022, the EMA and the European Centre for Disease Prevention and Control (ECDC) introduced the **EU Vaccine Monitoring Platform (VMP)**. This collaborative initiative aims to enhance the ongoing surveillance of vaccine safety and efficacy within the European Union. Through this platform, both agencies manage and

supervise independent, EU-funded post-authorisation studies on the utilisation, safety, and effectiveness of vaccines across EU member states.

The EMA released the first report on RWE in 2023. This report covered the time period from the receipt of the first pilot study requests (September 2021) up to the end of year one of DARWIN EU® (February 7, 2022) (202). Most of the research requests were aimed at generating evidence on drug/vaccine safety (22 requests, 36.1%). Similarly, most of these studies were descriptive cohort studies, such as the VigilVacCOVID study, and some were comparative, with more complex analyses performed. This report concluded on these key findings and needs for the future: i) RWE has the potential to enhance decision-making processes within diverse regulatory frameworks; ii) regulatory authorities require access to supplementary data sources, including secondary care databases, claims databases, and registries from various European countries; iii) it is critical to identify research requirements at the earliest possible stage to ensure that relevant studies can be completed in time to inform regulatory decisions; iv) a deeper understanding of the characteristics of data sources is necessary to improve the interpretation of RWE obtained from these sources; and v) there is a growing need to develop greater capability and infrastructure for generating RWE.

A second report, based on studies the EMA conducted using RWD between February 2023 and February 2024, has recently been published (202). This report shows a promising and favourable trend, with 40 studies, either completed or ongoing, including 13 studies on vaccine safety and effectiveness and related public health emergencies.

After the pandemic, guides on RWD and RWE were published. In 2024, a guide was made available on how the EMA can help generate RWE (203). This guide deals with: i) how stakeholders can request RWD studies from the EMA, ii) what types of studies can be performed, and iii) how the EMA can help identify resources to address research questions. The current landscape is continuously evolving as new data sources and analytical methods become increasingly accessible. An important point will be how this guidance is implemented in real life and how it evolves with subsequent changes in RWE and RWD methodologies.

Overall, these initiatives will help to access RWD for future studies and will stimulate the provision of RWE. Importantly, this regulatory framework, which is pivotal for RWE studies, should be further strengthened and developed in the future, updating to evolving research needs.

On the other hand, facilitating access to RWD establishes a critical foundation for conducting robust studies that produce evidence to support regulatory decision-making. Nonetheless, the role of RWE in this decision-making process, along with its evidentiary significance, continues to be a matter of ongoing discussion (201,204). The EMA published a “Reflection paper on use of real-world data in non-interventional studies to generate real-world evidence” in 2024. The open public consultation phase ended on August 31st, 2024 (205).

Although it is an important step forward in the integration of RWE into regulatory decision-making processes, the document has some limitations. For instance: i) the draft paper is mostly focused on methodology and the regulatory application of RWE, but it does not distinguish between pre-authorisation and post-authorisation procedures and applicable requirements in each case; ii) the scope and potential regulatory purposes, and how RWE can be integrated into regulatory processes, still need to be further specified in the document; iii) the recommendation paper is largely oriented towards industry, whereas academic studies, which are an important source of RWE and RWD, are barely mentioned; and iv) the document describes how to perform unique, convincing studies on RWD but not how various studies of different sorts could be proposed as another type of real-world evidence. Most probably, a single RWE study will not be good or large enough from a regulatory point of view to trigger regulatory changes, such as an extension of indication or a change in the SmPC. However, a combination of different studies can still contribute to enriching existing knowledge and be relevant from a regulatory point of view, especially if these studies are taken together. Guidance on this point should be further provided.

Overall, important steps have been taken towards the regulation and consolidation of well-conducted RWE studies. However, further work is still needed to better integrate RWD and RWE into regulatory decision-making alongside the evidence coming from more established sources, such as randomised clinical trials. In parallel, the conduct of prospective, real-world studies based on large cohorts of vaccinees should continue to enrich the current knowledge on the safety profile of vaccines. For future monitoring and future pandemics, the readiness of data collection infrastructures is important to implement cohort event monitoring and other types of real-world prospective studies. In the current pandemic, countries with established data collection infrastructures, supported by national government funding, were prepared to initiate enrolment at the onset of the vaccination campaign (191).

Active surveillance studies are complementary tools that should provide additional evidence to passive spontaneous reporting in pharmacovigilance databases, such as EV in Europe or VAERS in the US. Spontaneous reporting has an obviously important role, and together with data coming from active pharmacovigilance studies, it could be possible to explore potential safety signals and, in the long term, under continuous monitoring, rare ARs and ARs with a long time-to-onset.

5.2 Assessment of tolerability and reactogenicity in the VigilVacCOVID study

Overall, our analysis supports existing evidence that mRNA-1273 has greater short-term reactogenicity than BNT162b2. However, most of the observed ARs were local ARs and mild in intensity, thereby confirming a tolerable safety profile of both vaccines. In the following subsections, we will discuss these results in light of other published studies, potential underlying mechanisms, the main limitations and strengths of our results, and future directions in this line of research.

5.2.1 Comparison between BNT162b2 and mRNA-1273

Our first article provided evidence on a prospective comparison of two mRNA vaccines, BNT162b2 and mRNA-1273, administered under real-world conditions of use during the vaccination campaign at Hospital Clínic. These results were obtained through the active monitoring of a vaccinated cohort, which provided a denominator for the analysis of AR incidences within a well-defined vaccinated population. Further to the controlled context of clinical trials, our results provide additional supporting evidence of the reactogenicity and tolerability profile of mRNA vaccines, which were the first vaccines to be authorised or granted an EUA.

5.2.1.1 Reactogenicity

In our study, we reported a higher reactogenicity for mRNA-1273 (an increase of 11.7% in the rate of any observed AR compared to BNT162b2). Severe ARs were also reported more frequently with mRNA-1273 than with BNT162b2. While this higher reactogenicity rate is consistent with reports from the pivotal clinical trials (110,111), the rate of any AR that we found for the two vaccines was substantially higher than that reported in clinical trials (81.2%

vs 27% and 92.9% vs 87.8% for BNT162b2 and mRNA-1273 respectively). We cannot discard that these differences may be explained (at least partially) because of the different AR reporting methodologies that were used in the C4591001 clinical trial and the COVE study. For instance, as we described in the Introduction section, the rate of any AR in the C4591001 clinical trial was calculated from the overall study population, whereas local and systemic ARs were collected from a “reactogenicity” subset of vaccinated persons, which was a substantially smaller subpopulation.

This higher reactogenicity of mRNA-1273 has also been substantiated by several other large real-world cohort studies (180,189,191,206). Compared with pivotal clinical trials, the rate of any AR that we found in our study was different from other prospective observational studies. Table 9 shows the comparison between the VigilVacCOVID study, pivotal clinical trials of BNT162b2 and mRNA-1273 (C4591001 and COVE clinical trials, respectively), and other published large real-world cohorts. The comparison is made in terms of reactogenicity—defined as the rate (frequency) of any AR. Of note, many of the published large real-world cohorts do not consider the assessment of the rate of any AR. To make the comparison, we have included in the table the studies that consider this variable in the analysis, after the first vaccine dose (as it was reported in the pivotal clinical trials), and with similar populations. For instance, we have not included data from immunosuppressed or other special populations. The population included by Kant et al. comprised all Dutch residents older than 16 years of age who were vaccinated with a COVID-19 vaccine under the Dutch immunisation program during the study period from February to August 16 2021, thereby representing the general population. Likewise, Luxi N et al. also included all vaccinees that participated in the vaccination campaign in Italy. The authors included special cohorts, such as children and adolescents, pregnant or lactating women or immunocompromised patients. We only included data corresponding to the overall population, excluding these data from the table. Finally, the cohort of the VigilVacCOVID study was comprised of HCPs vaccinated with mRNA-1273 or BNT162b2.

As can be seen in the table, in all studies, the reactogenicity rate of mRNA-1273 is greater than that of BNT162b2. Our data regarding BNT162b2 and mRNA-1273 are comparable to those reported by Kant et al. and Luxi N et al., maybe even closer to the ones reported by Kant et al. The reactogenicity rate of BNT162b2 reported in the C4591001 clinical trial is clearly lower than that reported in real-world cohorts. As a matter of fact, the differences in

design and study populations should be considered when interpreting these comparative data. Besides, the table reflects an indirect non-adjusted comparison. Notwithstanding the differences, the data can still be understood as evidence of the robustness and consistency of our results.

Table 7. Comparison of reactogenicity rates between BNT162b2 and mRNA-1273

Proportion of participants experiencing at least one reactogenicity event (local or systemic) from dose 1	BNT162b2				mRNA-1273			
	C4591001 clinical trial (Polack FP et al.) N= 43448 (111)	VigilVacCO VID study N= 3564	Kant et al. N= 12888 (206)	Luxi N et al. N= 291 (190)	COVE clinical trial (Baden LR et al.) (110) N= 30420	VigilVacCO VID study N= 1524	Kant et al. N= 3426 (190)	Luxi N et al. N= 58 (190)
	5770 (26.7)	2054 (57.6)	5819 (45.2)	180 (61.9)	13319 (87.8)	1252 (82.2)	2799 (81.7)	49 (84.5)

Table 7. Data from pivotal clinical trials (C4591001 and COVE), as well as real-world cohorts, are represented. Table created by the author.

As regards the mechanisms that substantiate the observed differences in reactogenicity, despite the widespread use of mRNA COVID-19 vaccines, the mechanisms that induce immune responses associated with reactogenicity and immunogenicity remain largely unknown.

Currently, it is known that exogenous proteins encoded by mRNA vaccines can be internalised and processed by antigen-presenting cells, including monocytes/macrophages and dendritic cells, within tissues or draining lymph nodes. These proteins are then presented to specific CD4+ helper T cells through MHC class II molecules (207). The mechanisms by which mRNA vaccines are sensed by the human innate immune system are not completely clear yet. Since mRNA vaccines utilise single-stranded RNA, it was anticipated that the immune response would be activated through Toll-like receptor (TLR) 7/8. Instead, LNPs can induce immune activation by stimulating TLR2 and TLR4 and leading to NF- κ B activation and cytokine secretion.

Recent studies have indicated that mRNA vaccines primarily induce type I interferon (IFN-I) production via melanoma differentiation-associated gene 5 (MDA5) rather than TLR7, as well as the secretion of proinflammatory cytokines such as interleukin (IL)-1 β and IL-6. The production of IFN-I and these proinflammatory cytokines can enhance immune responses and increase VE, although they may also provoke adverse immunological effects (208,209). In murine dendritic cells and macrophages, the MDA5–IFNAR1 (interferon- α and interferon- β receptor subunit 1) signalling pathway played a crucial role in the production of IFN- α , the activation of innate immune cells, and the subsequent initiation of adaptive immune responses following vaccination with BNT162b2 (208).

Moreover, the nucleotide composition of RNA has previously been shown to influence its immune-stimulatory properties and reactogenicity profile (120). BNT162b2 and mRNA-1273 have the same RNA sequence, however, the latter has a higher content of RNA. This aspect may account for the observed differences in terms of reactogenicity between both vaccines. Conversely, the different content of pseudouridine (m1 Ψ) does not result in a significant difference in mRNA VE in mice, according to recent studies (137). These authors have also found that the 5' UTR of BNT162b2 mRNA is more efficient than that of mRNA-1273 in mRNA translation, while the opposite is true for the 3' UTR. The result is that both vaccine constructs work comparably at the level of UTRs.

Among the components of mRNA vaccines, LNPs, consisting of ionisable cationic lipids, cholesterol, phospholipids, and polyethylene glycol (PEG) have been employed to facilitate the effective transport of mRNA into the cytosol of target cells. LNPs have been shown to possess inherent adjuvant properties that facilitate the IL-6-dependent activation of potent follicular helper CD4⁺ T (T_{fh}) cells, germinal centre (GC) B cells, long-lived plasma cells, and memory B cells. These responses are linked to the production of durable and protective antibodies in murine models (209–211).

Other studies have shown that LNP containing mRNA and the ionisable lipid SM-102 (utilised in mRNA-1273) triggered the secretion of pyrogenic cytokines, including IL-1, IL-6, and tumour necrosis factor (TNF), from human peripheral blood mononuclear cells (211). These studies have shown that the IL-1 and interleukin 1 receptor antagonist (IL-1ra) axis modulates vaccine-induced systemic inflammation in a host-specific manner. In human immune cells, RNA-based vaccines stimulate the secretion of IL-1 cytokines, with IL-1 β being the primary form, a process influenced by both the RNA component and the lipid formulation of the vaccine. IL-1, in turn, triggers the induction of the broad spectrum of pro-inflammatory cytokines (including IL-6). Therefore, the IL-1 signalling pathway is crucial in initiating the innate immune response triggered by RNA vaccines. This effect was unexpectedly enhanced by specific lipids incorporated into vaccine formulations containing N1-methyl-pseudouridine-modified RNA, which is designed to minimise the activation of TLRs, in BNT162b2 and mRNA-1273.

Indeed, lipids have been demonstrated to elicit immune responses in the host following vaccine administration. While both the ALC-0315 cationic ionisable lipid—employed by BNT162b2—and SM-102—used by mRNA-1273—appear chemically similar, recent studies have shown that SM-102 outperforms ALC-0315 in intramuscular delivery of mRNA. Besides, SM-102 elicits comparable inflammatory responses but higher antibody production in mice compared with ALC-0315 (137).

Also, repeated exposure to PEG-based nanoparticles may provoke complement-mediated immune reactions, potentially compromising both the safety and efficacy profiles (212).

Broadly speaking, the different LNP composition of BNT162b2 and mRNA-1273 can, to some extent, explain the potential differences in reactogenicity reported in clinical studies.

5.2.1.2 Local and systemic adverse reactions

In this section, we will discuss the results obtained in terms of local and systemic solicited ARs and unsolicited ARs, considering other studies that have been published.

As regards solicited ARs, local reported events, such as injection site pain, arm pain, and systemic symptoms, such as fatigue, headache, and fever, were the most frequently reported solicited ARs for both BNT162b2 and mRNA-1273. Greater rates of injection site pain, fever, and arm pain were observed in the mRNA-1273 cohort compared with the BNT162b2 cohort. Likewise, severe ARs were reported more frequently with mRNA-1273 than with BNT162b2. In general, for both vaccines, severe systemic ARs were more frequent after the second dose. Overall, these results are in line with safety information already provided for BNT162b2 and mRNA-1273 in the respective SmpCs and previous observational cohorts (138,139).

However, whereas general trends in frequencies can be assumed to be similar across studies, there are some differences in the rates of specific local and systemic ARs. These differences are noted between the pivotal clinical trials of BNT162b2 and mRNA-1273, and real-world cohort studies. Tables 8-10 show the comparison between these pivotal clinical trials of BNT162b2 and mRNA-1273 (C4591001 and COVE, respectively, Table 8), other published large real-world cohorts, the VigilVacCOVID study (Table 9), and data from AR spontaneous reporting (Table 10). The comparison is made in terms of rates of tolerability (local and immediate reactions) and systemic reactions. Expressed rates correspond to those measured after the second vaccine dose, when overall higher rates of any AR are expected.

The study by Luxi N included all vaccinees that participated in the vaccination campaign in Italy. As in the former reactogenicity analysis, we only included data on local and systemic ARs corresponding to the overall population, excluding special populations, in the table. Finally, the study conducted by Menni et al. included vaccinees aged 18 years or older from the general population. This study compared BNT162n2 with ChAdOx1 nCoV-19 vaccine. Therefore, for comparative purposes with other studies, only data referring to BNT162b2 are included in Table 9. Kitagawa H et al. conducted their study in Hiroshima's University Hospital and included HCPs and university students. Finally, our cohort of the VigilVacCOVID study was comprised of HCPs vaccinated with mRNA-1273 or BNT162b2.

Of note, most studies, except the one performed by Kitagawa H and the VigilVacCOVID study, assessed seriousness but not severity. Seriousness was assessed as an AR being fatal, life-threatening, causing/prolonging hospitalisation, resulting in persistent or significant disability or incapacity, requiring intervention to prevent permanent damage, or causing congenital anomalies. Severity was assessed as mild, moderate, or severe by Kitagawa H and the VigilVacCOVID study. Furthermore, while pivotal clinical trials reported the severity of local and systemic ARs with age stratification, some observational studies only reported severity for the overall population. Therefore, for comparison purposes, local and systemic AR rates of observational studies are represented in Table 9 independently of age.

Upon examining the table, it is noteworthy that in pivotal clinical trials, systemic ARs exhibit a tendency towards a higher frequency than local ARs. This difference seems to be less pronounced in the data reported in real-world cohort studies, where frequencies of local ARs, especially injection site pain, are increased compared with clinical trials.

The study conducted by Kitagawa H et al. reported overall higher local and systemic AR rates compared with other observational studies and pivotal clinical trials. In observational studies, the rates of injection site pain and headache were, in general, lower than in clinical trials, except in the study of Kitagawa H et al., whereas the rates of myalgia, fever, nausea, and vomiting were slightly higher compared with clinical trials.

Comparing the VigilVacCOVID study with the other real-world studies, a similar proportion of ARs was reported, with some differences. A lower proportion of fatigue and myalgia were reported compared with the study of Luxi N et al. Conversely, a higher proportion of fever was reported in the VigilvacCOVID study compared with the rest of the studies, especially in the BNT162b2 group.

In some of the studies included in Table 9, chills and malaise were not assessed within solicited ARs. Other real-world studies did include these symptoms (191), finding slightly lower rates than in the VigilVacCOVID study.

Notably, some differences are also noted when comparing data derived from spontaneous reporting to large pharmacovigilance databases, such as the study performed by Rosenblum HG et al., performed with 340,522 VAERS reports of suspected ARs that were submitted from December 14, 2020, to June 14, 2021 (Table 10). This study reported overall higher

local and systemic AR rates, compared with other observational studies, especially injection site pain and fever, with rates very similar to those reported in the VigilVacCOVID study.

By and large, safety data from all sources show a clear trend towards mild to moderate ARs. The MedDRA SOC of “General disorders and administration site conditions” is clearly the most reported AR group, especially at the expense of injection site pain, fever, and fatigue. This group is followed in frequency by “Nervous System disorders”, mostly comprised of headache reports, and “Musculoskeletal and connective tissue disorders”, represented by myalgia and arm pain. This trend can be clearly observed in the data reported by the publicly available module of EV for both BNT162b2 and mRNA-1273 (Figures 9 and 10).

Of note, since 2012, EV data snapshots have been made publicly accessible and are updated on a weekly basis at <https://www.adrreports.eu/>. In response to anticipated public interest, this tool has been improved to provide customised access to data on all COVID-19 vaccines (213).

Figure 9. Spontaneous reports received in Eudravigilance for mRNA-1273 (Elasomeran)

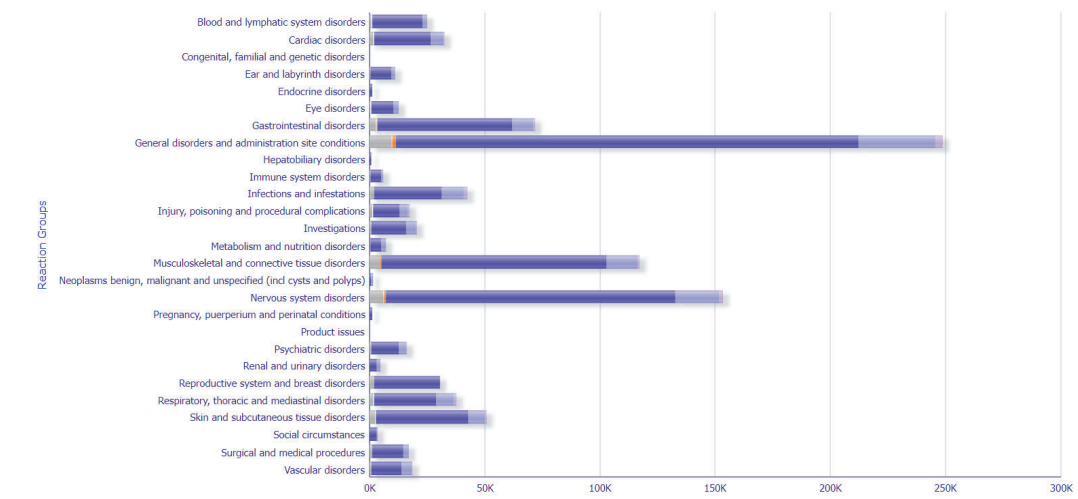


Figure 9. Spontaneous reports received for mRNA-1273 in Eudravigilance (cut-off date: October 25th, 2024). Reports are shown with their MedDRA SOC term (System Organ Class).

Figure 10. Spontaneous reports received in Eudravigilance for BNT162b2 (Tozinameran)

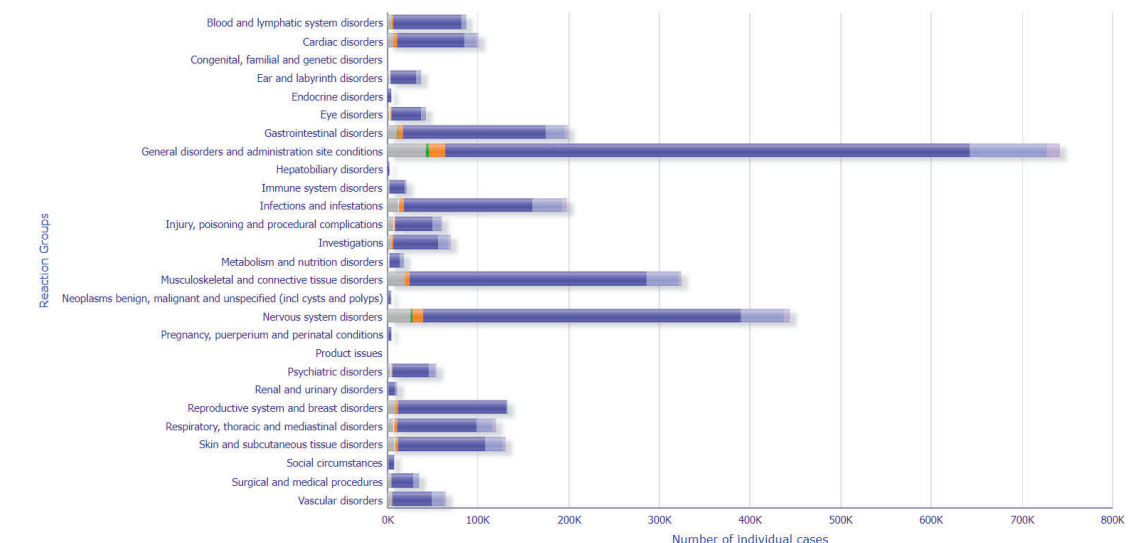


Figure 10. Spontaneous reports received for BNT162b2 in Eudravigilance (cut-off date: October 25th, 2024). Reports are shown with their MedDRA SOC term (System Organ Class).

Overall, most reported ARs were local and mild in intensity, confirming the tolerable safety profile of the two vaccines. These results can be interpreted as a reassuring message for the medical and scientific community, especially at a time when an adequate level of reactogenicity was expected to help optimise COVID-19 vaccine uptake and reduce vaccine hesitancy (214–216). However, some differences are observed when data from clinical trials, real-world cohort studies, and spontaneous reporting rates are compared. Differences in terms of fever and injection site pain are noteworthy, along with differences in the methodology of reporting local and systemic ARs. These discrepancies highlight the importance of performing continuous safety monitoring with different populations and different data sources as a way of enriching the amount and extent of safety data and the general knowledge regarding the safety profile of COVID-19 vaccines with complementary sources and methodologies.



Table 8. Tolerability and systemic reactions reported in pivotal clinical trials

Tolerability and systemic solicited reactions	BNT162b2 (111)			mRNA-1273 (110)		
	16-54 years		≥55 years	18-64 years		≥65 years
	Mild to moderate	Severe	Mild to moderate	Severe	Mild to moderate	Severe
Tolerability (Local and immediate reactions)						
Injection site pain	7.6%	1.2%	65.7%	0.5%	90.1%	4.6%
Erythema	5.4%	0.5%	6.8%	0.5%	9.0%	2.0%
Swelling	5.9%	0.3%	7.3%	0.2%	12.6%	1.7%
Systemic Reactions						
Headache	48.5%	3.2%	38.4%	0.5%	62.8%	5.0%
Fatigue	54.8%	4.6%	47.7%	2.8%	67.6%	10.6%
Myalgia	37.3%	2.2%	27.8%	1.0%	61.3%	10.0%
Nausea/Vomiting	1.7%	0.2%	0.6%	0.1%	21.3%	<0.1%
Fever	30.2%	1.2%	21.5%	0.3%	17.4%	1.6%

Table 8. Safety results reported in pivotal clinical trials. Adapted from Polack FP et al. (111) and Baden LR et al. (110).

Table 9. Tolerability and systemic reactions reported in real-world studies

Tolerability and systemic solicited reactions	VigilVacCOVID study				Luxi N et al. (190)		Menni C et al. (217)		Kitagawa H et al. (218)		
	BNT162b2		mRNA-1273		BNT162b2	mRNA-1273	BNT162b2	BNT162b2	BNT162b2		mRNA-1273
	Mild to moderate	Severe	Mild to moderate	Severe	NR	NR	NR	NR	Mild to moderate	Severe	Severe
Tolerability (Local and immediate reactions)											
Injection site pain	14.7%	3.1%	25.5%	7.4%	33.0%	56.1%	34.3%		86.4%	2.7%	5.5%
Erythema	8.4%	0.3%	16.6%	1.8%	6.8%	19.5%	7.2%		NR*	NR	NR
Swelling	0.5%	0.5%	8.0%	2.1%	11.9%	34.1%	9.8%		NR	NR	NR
Systemic Reactions											
Headache	18.0%	7.7%	23.0%	10.9%	19.9%	36.6%	13.2%		32.5%	5.5%	10.2%
Fatigue	20.9%	10.0%	22.9%	11.8%	29%	68.3%	14.4%		64.9%	10.4%	15.4%
Myalgia	16.4%	7.7%	17.0%	9.8%	24.4%	56.1%	5.0%		44.2%	5.6%	7.4%
Nausea/Vomiting	2.8%	NR	3.7%	NR	6.3%	12.2%	3.5%		4.7%	0.6%	1.3%
Fever	22.0%	NR	38.5%	NR	4.0%	34.1%	3.8%		57.3%	7.0%	24.8%

Table 9. Safety results from real-world studies. *NR: Not reported in the publication. Table created by the author.

Table 10. Tolerability and systemic reactions reported in spontaneous reporting pharmacovigilance databases

Tolerability and systemic solicited reactions		Rosenblum HG et al. (180)	
		BNT162b2	mRNA-1273
Tolerability (Local and immediate reactions)			
Injection site pain		62.8%	74.6%
Erythema		5.7%	17.2%
Swelling		10.6%	24.2%
Systemic Reactions			
Headache		40.7%	52.1%
Fatigue		50.5%	61.1%
Myalgia		37.2%	50.6%
Nausea/Vomiting		13.2%	18.8%
Fever		22.5%	37.2%

Table 10. Reported results in pharmacovigilance databases. Adapted from Rosenblum HG et al. (180).

Within the reported unsolicited ARs, contrarily to solicited ARs, we did not detect significant differences between vaccines or doses, except for hypersensitivity and lymphadenopathy, where we detected a significantly higher rate of events after vaccination with mRNA-1273, compared with BNT162b2. These events were more frequent after the second vaccine dose.

Regarding hypersensitivity, for analysis purposes, we pooled several symptoms under the MedDRA PT (preferred term) “hypersensitivity”, i.e., immediate hypersensitivity events, exanthema, urticaria, and skin rashes, amongst others. Other studies also reported hypersensitivity as a single AR, like the VigilVacCOVID study (191). However, these studies showed significantly lower event rates. El-Shitany NA et al. reported hypersensitivity in 8% of individuals after the first dose and 14.5% after the second dose of BNT162b2 (219). In this study, hypersensitivity rates were clearly higher than those reported in the VigilVacCOVID study for BNT162b2 (0.4% and 0.5% after the first and second dose, respectively in the VigilVacCOVID study).

Conversely, in other studies, hypersensitivity was reported as different and independent unsolicited ARs, such as urticaria, dermatitis, dermatitis allergic, erythema, periorbital swelling, rash pruritic, swelling face, amongst others (190). These discrepancies suggest that a different definition of hypersensitivity may have been used (199,227).

In our study, we did not detect any cases that could be suspicious of potential anaphylaxis, although we did not include specific questions about anaphylaxis. Other large real-world cohort studies did not report anaphylactic reactions either (190,191,206). Notably, both Raethke et al. and Kant et al. report one case of anaphylaxis with Janssen but none with BNT162b2 or mRNA-1273.

In general, anaphylactic reactions to mRNA vaccines have rarely been reported. In the pivotal clinical trials, local and hypersensitivity reactions were reported (110,111). In the COVE clinical trial, one case of anaphylaxis was reported in both the experimental and placebo arms. No cases of anaphylaxis were reported in the C4591001 clinical trial.

On December 8, 2020, following the administration of the first COVID-19 vaccine (BNT162b2), the UK reported three suspected cases of allergic reactions potentially linked to the vaccine. Two of these cases involved HCPs who experienced anaphylaxis within minutes of receiving the vaccine, both of whom responded promptly to epinephrine

treatment and made full recoveries. These individuals were known to have significant allergies, one to food and the other to medications. The third case presented with milder symptoms and did not require epinephrine intervention (220). According to a report by the New York Times on December 25th, a physician in Boston experienced an anaphylactic reaction to the mRNA-1273 vaccine shortly after the vaccination campaign began in the United States. The physician utilised his personal adrenaline autoinjector, which he carried for a shellfish allergy, and subsequently recovered without complications (221).

These events activated all alerts, and it was suggested that the occurrence of two cases of anaphylaxis on the second day of a vaccination campaign involving a new vaccine warranted careful investigation. Notwithstanding, as of January 10th, 2021, a total of 4,041,396 individuals had received the first dose of the mRNA-1273 vaccine, with 10 confirmed cases of anaphylaxis, all of which resulted in full recovery (222). After the rollout period of mRNA vaccines, and despite initial alerts, it has been acknowledged that anaphylaxis is a rare AR.

The Vaccine Safety Datalink is a collaborative initiative between the CDC's immunisation safety division and nine healthcare organisations. It has been continuously updated every week since 1990, with the primary aim of addressing critical vaccine safety concerns in large population groups. This study identified 33 cases of anaphylaxis out of 25,173,965 administered vaccine doses, establishing the commonly cited anaphylaxis rate of 1.31 cases per million doses (223).

Although the exact trigger remains undetermined, early reports suggested that polyethylene glycol 2000 (PEG-2000), an excipient present in the vaccine as part of a PEG-micellar carrier system, may be responsible for the observed anaphylactic reactions. PEGylation of LNPs is widely utilised to render stability and increase the plasma half-life. Currently, more than fifteen PEGylated drugs are authorised (224). Interestingly, PEGs have never been used in any vaccine; BNT162b2 and mRNA-1273 are the first to use this substance. PEGylated microsomes, utilised as the vaccine carrier, have the potential to induce anaphylactic responses in individuals with pre-existing PEG allergies (220,225); a phenomenon previously documented in the context of PEGylated drugs used for cancer therapy and the treatment of chronic diseases (226,227).

Allergic reactions to such PEGylated lipids are IgE-mediated (228,229). In addition to IgE, other antibody classes may induce similar symptoms or enhance an IgE-mediated response.

Non-IgE-mediated reactions, such as complement activation-related pseudoallergy (CARPA), have also been reported, particularly in the context of liposomes. CARPA is partly attributed to the binding of preexisting anti-PEG IgM to the liposomes with subsequent complement activation.

Besides, liposomes, widely used as drug delivery systems, have the potential to trigger complement system activation independently of PEGylation. This process occurs when liposome surface structures and charge non-specifically interact with complement proteins. These interactions can lead to the production of complement fragments such as **C3a, C4a, and C5a**, which are potent **anaphylatoxins**. These anaphylatoxins play a significant role in inducing allergic-like reactions, including hypersensitivity and inflammatory responses.

Cross-reactivity between PEG and its derivatives—structurally related polymers such as polysorbates—exists due to shared moieties. For this reason, Regulatory Authorities issued a caution for individuals allergic to polysorbates due to potential cross-reactivity (221). Besides, mRNA-1273 contains tromethamine, also called trometamol, an organic amine widely used in several medications for topical, enteral, or parenteral administration. The original formulation of BNT162b2 did not contain trometamol, although the updated formula did include this substance. Recently, the first case of anaphylaxis to trometamol as an excipient in a gadolinium-based contrast agent was reported (230).

The anaphylactic reactions observed following routine administration of the BNT162b2 vaccine led the UK's Medicines and Healthcare Products Regulatory Agency (MHRA) to issue a warning. This advisory body recommended that individuals with a history of severe allergic reactions to vaccines, medications, food, or any component of these specific vaccines should be advised against receiving them, and that no second dose should be administered to those who experienced anaphylaxis after the initial dose. This advice was applied in many countries.

Currently, restrictions for vaccine administration are issued only for individuals with hypersensitivity to the active ingredient or excipients in the approved vaccines. Those who have had a hypersensitive reaction to the first dose are also advised not to receive a second dose. In these cases, another vaccine can be selected, or passive immunisation with monoclonal antibodies, such as Evusheld (cilgavimab/tixagevimab), depending on national and regional recommendations (231). Some centres have elaborated protocols to identify

patients with previous severe allergic reactions to detect those in whom vaccines should be avoided (232).

In sum, allergic reactions have been widely reported. However, severe allergic reactions and anaphylaxis, despite initial alerts, have been rarely detected. The widespread global administration of COVID-19 vaccines to a large proportion of the population requires ongoing safety monitoring to better characterise and detect allergic responses.

Conversely, in the VigilVacCOVID study, we detected delayed hypersensitivity reactions in the form of inflamed arm, pain, and ecchymosis. Vaccinees were interviewed within one week after each vaccine dose. We aimed to remain within this timeframe, however, given the high number of vaccinees there were some delays in performing the interview. Therefore, these symptoms were sometimes captured within variable timeslots and different evolutionary stages. Accordingly, in some cases we were not able to assess whether the AR was a real delayed or immediate event, also considering that the interview was phone-based and not under onsite medical consultation. These cases were included under the MedDRA PTs “hypersensitivity”, “injection site swelling”, or “application site redness”, depending on the symptoms reported by the vaccinee, and we were not able to discern whether they corresponded to delayed type IV hypersensitivity reactions, or if these symptoms later progressed to a delayed cutaneous reaction.

Delayed cutaneous hypersensitivity reactions have also been reported by other authors (233–235). These reactions were morbilliform rashes (234), pityriasis rosea, pityriasis rubra pilaris, guttate psoriasis (235), purpuric lesions (236), pink plaques (237), erythematous plaques (238), and maculopapular rash (239). Magro C et al., in 2021, also reported eczematous dermatitis, interface dermatitis, and granulomatous inflammation symptoms without specifying the types of vaccines (235). Besides, some publications have reported the “COVID arm”, characterised by pain, redness, swelling, and itching at the injection site, fever, and chills with mild blistering at the site of injection. These symptoms were very severe in some cases, being a cause of workplace absenteeism (240). Agustin M et al. reviewed 18 studies with a total of 1129 participants after the first and second dose of mRNA-1273 (241). The authors reported that COVID arm was more common following the first vaccine dose, in the female and middle-aged group. Symptoms resolved within the seven days following treatment.

Noteworthy, in the VigilVacCOVID study, we found a higher proportion of injection site redness reactions after the first dose, compared with the second dose, with both BNT162b2 and mRNA-1273. Possibly, some of these cases were or later progressed to COVID arm. Likewise, some authors found a higher rate of delayed large local cutaneous reactions with mRNA-1273, compared with BNT162b2 (233). In our VigilVacCOVID study, we also found a higher proportion of injection site redness, arm pain, and injection site swelling in the group of participants vaccinated with mRNA-1273. Therefore, although we did not consider a specific PT of delayed cutaneous reaction, we were able to capture symptoms that may have corresponded to this type of AR.

Furthermore, in our study, we reported some cases of lymphadenopathy or lymph node enlargement after vaccination. Lymphadenopathy was previously described in pivotal clinical trials of mRNA vaccines. Other real-world studies have also reported cases of lymphadenopathy, at higher (181,182), lower (191), or comparable rates (190) to the VigilVacCOVID study. In the raw analysis, we found a slightly higher rate of lymphadenopathy with mRNA-1273, although these differences were blunted in the inverse probability of the treatment weighting (IPTW) analysis. Another comparative study also reported a higher proportion of lymphadenopathies in the mRNA-1273 group (191). In all these studies, including VigilVacCOVID, lymphadenopathy was more frequent after the second vaccine dose, which reinforces the idea that this AR is a clear clinical surrogate marker of immune activation following vaccine administration. Indeed, this can be considered a predictable AR, given that it has been frequently reported with other vaccines, such as the human papillomavirus and influenza vaccines (242–244).

In addition, we also captured some rare ARs, such as 14 cases of herpetic reactivation and one of facial paralysis. As regards herpes virus infection reactivation, previous publications have assessed a potential association between COVID-19 vaccines and this AR; however, most publications were based on case reports (245–247). A recent investigation conducted by Liu et al. examined the pathophysiological changes following COVID-19 vaccination. The study identified a decrease in CD8+ T cells, an elevation in the proportion of classical monocytes, enhanced NF- κ B signalling, and a diminished type I interferon response. Furthermore, during the initial 28 days following vaccine administration, the immune system remains in a vulnerable state (248). Since signalling through the Type I IFN receptor in

CD8⁺ T cells plays a crucial role in modulating memory cell responses to viral infections and preventing viral reactivation (248,249), some authors have proposed that these findings provide sufficient evidence to suggest that herpesvirus reactivation may occur after COVID-19 vaccination. However, literature reviews suggest that this AR is rare, and more research on this topic needs to be undertaken to elucidate a potential association and the underlying mechanism of AR generation (247).

With respect to facial paralysis, a systematic review including 37 studies concluded that Bell's palsy or facial paralysis, although rare, can be a plausible non-serious AR of COVID-19 vaccination (250). Most reported cases occurred after the first vaccine dose, as was the case in the AR that we reported in the VigilVacCOVID study.

Within unsolicited ARs, we reported other rare events, such as insomnia and hypersomnia, diarrhoea and other gastrointestinal symptoms, cognitive alterations, alterations of smell and taste, paraesthesia and hyperesthesia, syncope, vertigo, tremor, asthma, rhinitis, ear pain, hypertension and hypotension, influenza-like symptoms, sensation of heat and cold, chest pain, hyporexia, cold sweat, tachycardia, and other musculoskeletal disorders and inflammations affecting other parts of the body than the vaccinated arm. Most of these symptoms were also reported by active pharmacovigilance studies (190,191,206) and analyses of spontaneous reporting databases (180). Some authors have suggested that vaccination-derived symptoms mimic those generated by SARS-CoV-2 active infection (248). ARs such as influenza-like symptoms, gastrointestinal events, and alterations of smell and taste, also reported previously (251–253), can be explained under this hypothesis. Other symptoms such as tremor, tachycardia, hypotension and hypertension, or cold sweat, can be explained by the development of fever, or by pre-existing comorbidities (254). Likewise, ARs such as asthma reactivation can be explained by the immunological activation induced by the vaccine (255).

Notwithstanding, other ARs, such as insomnia, ear pain, or neurological symptoms, remain unexplained. Interestingly, a case study from a research group in Mexico documented a case of delirium following the administration of the first dose of BNT162b2. The delirium was marked by symptoms such as confusion, fluctuating levels of attention, anxiety, and a reversal of the sleep-wake cycle. Upon physical examination, the patient exhibited inattentiveness and oscillatory movements of the trunk. After excluding most other potential causes of delirium,

the patient showed gradual improvement over the subsequent 48 hours (256). In the VigilVacCOVID study, we reported 11 cases of cognitive alterations (in the forms of disorientation, memory loss, and others). Although it is difficult to confirm, some of these cases may share a common link with this reported case.

In conclusion, our results from the VigilVacCOVID study are comparable to other large real-world cohorts. The relevance of our data lies in the provision of real-world evidence on the safety profile of vaccination, in a prospective comparative study where two vaccines (BNT162b2 and mRNA-1273) were assessed to substantiate an exhaustive body of safety data regarding frequencies and severity of solicited and unsolicited ARs. The fact that we were able to detect rare ARs after vaccination, similar to other prospective large cohorts, reinforces the robustness of the design of our study to characterise the safety of mRNA vaccines in a real-world scenario.

5.3 Comparison between HCPs and SOTRs

Clinical trials are usually developed in a highly controlled experimental setting, with pre-determined and restrictive inclusion criteria that hardly reflect the general population, which is the main target of mass vaccination campaigns. For instance, in mRNA pivotal clinical trials, patients with chronic diseases were included only if the conditions were deemed stable by the investigators, and several subgroups of patients (i.e., transplanted, oncologic, and immunocompromised patients) were largely underrepresented. Thus, the safety and efficacy profile of vaccines was largely unknown at the time of authorisation of BNT162b2 and mRNA-1273.

It should be noted that hosts with altered immunity, including SOTRs, are at increased risk of complications and death due to SARS-CoV-2, with mortality rates up to 20% (257). For this reason, most countries have prioritised these patients in their vaccination programs (258). Understanding the safety and immunogenicity profile of vaccines in SOTRs has therefore become a meaningful clinical asset.

Experience with previously authorised vaccines has shown that transplanted patients elicit less robust immune responses to vaccines compared with nontransplant patients, regardless of vaccine type (259). Preliminary studies have demonstrated a reduced humoral immune response to BNT162b2 and mRNA-1273 mRNA vaccines in SOTRs after the first and

second dose—measured by anti-spike antibody responses—compared with healthy vaccinated persons. This decreased humoral response has been consistently reported in kidney, lung, heart, and liver transplant patients (257,259–265).

It could be hypothesised that this blunted immune response could also lead to a decreased AR rate, at least those that are immune-mediated; nonetheless, the safety profile of SARS-CoV-2 vaccines was not fully characterised when the VigilVacCOVID study was planned and designed, especially in underrepresented populations in clinical trials, compared with healthy, or relatively healthy vaccinees. Our study included a known denominator of vaccinated participants of two different populations (SOTR and HCP), both vaccinated with the same vaccine (mRNA-1273), with the main aim of characterising the safety profile of mRNA-1273 in these two populations in a real-world setting.

The vaccination campaign started in the Hospital Clínic, a tertiary Hospital, on January 7th, 2021. The campaign was implemented in two waves. In the first round, HCPs were vaccinated with BNT162b2 as it was the only available vaccine at the time. Once mRNA-1273 was authorised, it was used to vaccinate SOTRs. HCPs were also vaccinated with mRNA-1273 from March 2nd until April 15th, as per local authority guidance. The population of vaccinated transplanted patients included kidney, lung, heart, liver, and kidney-pancreas. Patients on dialysis were also included in the vaccination campaign. HCPs and SOTRs vaccinated with mRNA-1273 were recruited in the VigilVacCOVID study to assess the safety profile of vaccination in these two populations. Safety data of HCPs vaccinated with BNT162b2 and mRNA-1273 were used to determine the comparative reactogenicity and tolerability, as described in the previous subsection.

In the VigilVacCOVID study, a lower reactogenicity of mRNA-1273 was observed in the SOTR than in the HCP group in terms of a lower proportion of any AR. Likewise, SOTRs exhibited fewer local and systemic ARs, with reduced severity, evidenced by a smaller proportion of grade 3 ARs and lower Likert scores. Interestingly, severe systemic ARs, such as fatigue, headache, myalgia or arthralgia, were more frequent after the second dose in HCPs but not SOTRs. No relevant differences were observed after the second dose for severe or non-severe, local or systemic ARs in SOTRs.

Compared with the results reported by Baden LR et al. in the COVE clinical trial (Table 11), it is noteworthy that there is a lower proportion of both local and systemic ARs in HCPs in

the VigilVacCOVID study, except for fever. These differences can be attributed to differences in the included population, sample size, and the fact that VigilVacCOVID used data collected within a real-world scenario. Likewise, these differences may be attributed to the fact that, in clinical trials, all events were recorded regardless of their level of intensity, including those that were mild or clinically irrelevant. In contrast, the safety strategy in the VigilVacCOVID study focused primarily on events of certain clinical significance.

Despite these discrepancies, the SOTR group consistently showed lower reactogenicity (both in terms of any AR and any AR grade ≥ 3 after the second dose) and lower rates of local and systemic AR, compared with both the COVE clinical trial and the HCP cohort of the VigilVacCOVID study. These data reinforce the idea that SOTRs have lower reactogenicity after vaccination compared with non-immunosuppressed patients.

Table 11. mRNA-1273 results in the COVE clinical trial compared with real-world data from the VigilVacCOVID study

Safety endpoints	COVE clinical trial (110)	VigilVacCOVID SOTRs	VigilVacCOVID HCPs
Reactogenicity after dose 2			
Any AR	92.2%	54.2%	79.3%
Any AR grade ≥ 3	19.7%	10.8%	40.7%
Local and systemic AR after dose 2			
Pain	88.2%	19.8%	24.5%
Erythema	8.6%	1.2%	4.6%
Swelling	12.2%	1.9%	7.4%
Lymphadenopathy	14.2%	0.4%	2.9%
Fever	15.5%	10.3%	44%
Headache	58.6%	6.8%	25.1%
Fatigue	65.3%	10.5%	24.4%
Myalgia	58%	2.3%	17.5%
Arthralgia	42.8%	1.6%	10.8%
Nausea/vomiting	19%	0.9%	4%
Chills	44.2%	5.4%	18.6%

Table 11. Comparison of the results of the COVE clinical trial with those of the VigilVacCOVID study. AR: adverse reaction. Table created by the author.

Apart from the VigilVacCOVID study, other real-world cohorts have also explored the reactogenicity of COVID-19 vaccines in transplanted patients. In 2021, Boyarsky et al. assessed the reactogenicity of BNT162b2 and mRNA-1273 in the first group of SOTRs to be vaccinated against SARS-CoV-2 in the United States (266). They studied 187 SOTRs who received their first SARS-CoV-2 vaccination dose between December 16, 2020, and January 16, 2021. In their study, the most frequent local ARs were pain and swelling, whereas the most frequent systemic ARs were fatigue and headache. The reactogenicity profile was broadly consistent with expected vaccine reactogenicity, with mostly mild ARs and no reported cases of acute rejection, neurological diagnoses (Guillain-Barré syndrome, Bell's

Palsy, or neuropathy), or allergic reactions. Two participants reported the development of a new infection (one was acute-on-chronic pouchitis, the second influenza A) requiring treatment. As participants only received one dose, the authors were not able to assess differences in reactogenicity between doses.

Ou et al. studied the safety and reactogenicity of two doses of either BNT162b2 or mRNA-1273 in 741 SOTRs. The authors concluded that reactogenicity was similar to that reported in the original trials, and severe reactions were rare (267); however, one patient reported an incident of acute rejection after the second vaccine dose. As in the cohort published by Boyarsky et al., pain at the injection site was the most commonly reported local AR, whereas the most common systemic ARs were fatigue and headache. Notably, after the first dose, 23 SOTRs reported a new infection, including upper respiratory infection, urinary tract infection, skin abscess, and pneumonia. After dose 2, three participants indicated a new infection, including upper respiratory infection, urinary tract infection, and skin abscess. Regarding AR rates after each dose, all systemic ARs were more frequent after the second dose

Regarding specific SOTR groups, most real-world studies focused on the assessment of vaccine reactogenicity on kidney transplants, with a relative infra-representation of other types of organ transplantation. Cucchiari et al. studied 148 recipients of either kidney (n=133) or kidney-pancreas (n=15) grafts, all vaccinated with mRNA-1273 (268). The authors found that patient-reported side effects on a semiquantitative scale (none/mild/moderate/severe) were consistent with the pivotal trial, and no donor-specific antibodies were detected. Pain in the injection was the most frequent AR, affecting 86% and 75% of the vaccinated population after the first and the second dose, respectively. The second most commonly reported AR was fatigue, reported in 25% and 27% of population after the first and second dose, respectively. Like in the VigilVacCOVID study, there were no significant differences between the first and the second dose for all reported ARs.

Sanders JF et al. assessed the reactogenicity of two doses of mRNA-1273 in 162 participants with chronic kidney disease (CKD) stages G4/5 (eGFR < 30 mL/min/1.73m²), 159 participants on dialysis, 288 kidney transplant recipients, and 191 controls (269). Their results were in line with other similar studies, finding a lower rate of ARs in CKD patients, participants on dialysis, and kidney transplant recipients, compared with healthy controls.

Likewise, the most frequent local AR was pain, and the most frequent systemic ARs were fatigue, headache, and myalgia. A higher rate of ARs was found after second vaccine doses. Noteworthy, this study, published around the same time as the VigilVacCOVID study, was one of the first reports of a comparative assessment of transplanted patients and healthy controls.

Ou MT et al. assessed 609 kidney transplant recipients who were vaccinated with an mRNA vaccine, 24 (4%) of whom were on belatacept. Mild-to-moderate pain was the most frequent local AR for participants taking belatacept, while mild-to-moderate fatigue and headache were the most frequent systemic ARs (270). There were no significant differences in reactogenicity between patients taking belatacept or not. Non-SARS-CoV-2 infections were reported after the first and second dose in the group of patients not taking belatacept. Similarly, there was one report of hearing loss and another of transverse myelitis, both in the non-belatacept cohort, after the second vaccine dose.

Reactogenicity has also been assessed in other types of transplants. Itzhaki Ben Zadok O et al. studied the reactogenicity of BNT162b2 in 42 heart transplant recipients. Their findings were similar to other studies in other transplant recipients, reporting pain at the injection site as the most frequent AR, and fatigue and myalgia as the most common systemic reactions (265). Hallett AM et al. assessed the reactogenicity in 134 heart recipients and 103 lung recipients after receiving two doses of BNT162b2 or mRNA-1273. In line with other studies, pain at the injection site was the most commonly reported local reaction, while the most common systemic symptoms were fatigue and headache. Local ARs rates were lower after the second dose, whereas systemic ARs rates were higher after the second vaccine dose. Other studies have yielded similar results in heart and lung transplant recipients (271).

Rabinowich L et al. evaluated 71 liver transplant recipients and 21 controls after receiving two doses of BNT162b2 (263). Injection site reactions occurred at a similar frequency in both groups following the first and second dose. The only reported local symptom was mild-to-moderate pain at the injection site. The frequency of systemic ARs was similar in both groups after the first vaccine dose. Following the second dose, systemic ARs were reported less frequently among transplant recipients compared with controls. The most common systemic AR was fatigue, followed by headache and myalgia. Other studies have also reported

a lower frequency of systemic symptoms in liver transplant recipients compared with controls (272).

Conversely, reactogenicity has also been studied in patients with other comorbidities and immunosuppression risk factors. Shapiro Ben David S et al. assessed the BNT162b2 vaccine's short-term safety in two vaccine recipient populations after receiving a third booster dose: immunocompromised individuals and seniors aged 60 years or older. Immunocompromised participants included patients with haematologic or solid malignancies, patients on immunosuppressive treatment, SOTRs, and patients with diabetes and hypertension. Most immunocompromised (63.3%) and senior (67.5%) vaccinees reported injection site pain. Most immunocompromised patients and seniors (66.7% and 65.9%, respectively) did not report any systemic AR. Fatigue, myalgia, fever, and headache were the most frequently reported systemic ARs.

Also, the VAX4FRAIL study aimed at assessing immune responses to vaccination in a large trans-disease cohort of patients with haematological malignancies, solid tumours, and neurological and rheumatological diseases (273). Longitudinal, prospective evaluation of the safety profile was part of the study after vaccination with either BNT162b2 or mRNA-1273 in 566 patients. Lupo-Stanghellini MT et al. reported that 438 (77.3%) patients reported any grade adverse events after the first dose (62, 11.0% severe) and 373 (65.9%) after the second dose. The most frequently reported moderate-severe adverse events were pain at the injection site, fatigue, bone pain, and headache. As unsolicited ARs, patients reported chills, itching, gastrointestinal pain, myalgia, dizziness, drowsiness, sweats, confusion, and dysesthesia. A minority of patients also reported nausea, diarrhoea, insomnia, skin rash, and enlarged lymph nodes. Overall, 11 patients (1.9%) after the first dose and 7 (1.2%) after the second required postponement or suspension of the disease-specific treatment. No patients from the neurological or rheumatological disease cohorts required treatment delay. The authors concluded that mRNA-COVID-19 vaccination is safe in frail patients, with most ARs being manageable and with a minimum impact on the patient care path.

Other authors have further explored the safety of COVID-19 vaccines in patients with autoimmune diseases and under immunomodulatory treatment. Briggs FBS et al. provided real-world safety data in patients with multiple sclerosis under immunomodulatory treatment with disease-modifying therapies (274). The authors reported an overall reactogenicity rate

of 64%, similar to the rate observed in the general population. The most common reactions were pain at the injection site (54%), fatigue (34%), headache (28%), and malaise (21%). Patients on alpha4-integrin blocker or sphingosine-1-phosphate receptor modulator treatment had lower odds of reactions. Likewise, patients treated with a sphingosine-1-phosphate receptor modulator or fumarate were significantly less likely to report a reaction. Similar to previous studies, mRNA-1273 was more reactogenic than BNT162b2.

Bartels LE et al. explored the safety and reactogenicity of BNT162b2 among patients with systemic lupus erythematosus (n=128) and rheumatoid arthritis (n=154) (275). The authors reported that, compared with the BNT162b2 pivotal clinical trial, they observed a higher reactogenicity in terms of a higher rate of local ARs, fatigue, headache, muscle pain, and joint pain, although with comparable severity. Other studies have yielded similar results in patients with chronic rheumatic diseases (276), while comparative studies with mixed inflammatory conditions and healthy vaccinees have not found significant differences in reactogenicity in either group (277,278).

In general, all studies conducted on immunocompromised patients indicate that vaccination is safe, with a predominance of mild to moderate ARs. Some studies reported cases of infection following vaccination and a few cases of transplant rejection, although there is insufficient evidence to conclude that this represents a consistent safety signal. This is an important conclusion since limited knowledge about vaccine safety in this population may contribute to vaccine hesitancy. Indeed, A survey of populations prioritised for early vaccination found that more than 50% of respondents were either not likely or only somewhat likely to receive a vaccine (279). Transplant society guidelines strongly recommend COVID-19 vaccination in transplant candidates and recipients, however, the approach is heterogeneous across different countries and international transplant centres. A more extensive involvement of stakeholders is necessary to address global vaccine hesitancy and improve the acceptance of pre-transplant COVID-19 vaccination, thereby safeguarding the health of transplant recipients. These safety data may help alleviate vaccine hesitancy and refusal among transplant recipients and inform decision-making processes.

Notably, only a minority of studies compared transplant recipients and healthy vaccinees, and most enrolled small sample sizes. The VigilVacCOVID study performed an exhaustive comparative safety analysis of a large cohort of 1,289 transplanted patients and 1,524 HCPs.

The study showed a lower reactogenicity of SOTRs, which is in line with other published studies. While some works reported a higher AR rate after dose 2 (267,269), other studies showed fewer local ARs and higher rates of systemic ARs after the second dose (265), comparable local ARs and fewer systemic ARs after dose 2 (263), or no significant differences between doses (268). In the VigilVacCOVID study, we found lower rates of injection site pain, injection site swelling, fatigue, myalgia, arthralgia, and headache, with higher rates of fever, malaise, and chills after the second dose. Most probably, observed differences in the various studies referenced may be due to the different populations included, study settings, and additional baseline characteristics such as the immunosuppressor regime received. Hence, no clear conclusions regarding the frequency of AR rates after a second vaccine dose can be drawn. Conversely, what seems to be clear amongst studies is that pain is the most frequent local AR, and fatigue, headache, and fever are the most common systemic AR. This seems to be a common pattern, independently of the studied population. Interestingly, Ou MT et al. concluded that vaccinees that experienced moderate to severe pain or redness were more likely to develop an antibody response (267).

On the other hand, the rates of each individual local and systemic AR are lower than those reported for HCPs. The results of the VigilVacCOVID study align with these conclusions. Of note, patients with autoimmune disorders seem to develop increased, or at least comparable, reactogenicity compared to healthy vaccinees, with variable results according to the immunomodulatory treatment received. Further safety data are needed to elucidate potential differences in the various groups of patients with comorbidities, which may behave as different patient cohorts.

Furthermore, previous studies have identified factors associated with poor antibody response in SOTRs, such as older age, deceased donor status, antimetabolite use, recent rituximab exposure, and recent antithymocyte globulin exposure (280). In line with these findings, Ou MT et al. reported that younger and female vaccinees, as well as those under treatment with corticosteroids or regimens that included mTOR inhibitors (sirolimus, everolimus), compared with other treatment regimes, were more likely to experience local and systemic symptoms after vaccination (267). In the VigilVacCOVID study, we assessed the influence of age and sex on reactogenicity in the SOTR cohort; the results will be discussed in the next subsection. However, we did not evaluate the impact of the immunosuppressor regime on

reactogenicity. Given the design of the study—based on patient-reported events through telephonic interviews—we considered that it was difficult to obtain information on immunosuppressor treatment reliably.

Finally, few studies have assessed ARs other than solicited local and systemic ARs. In the VigilVacCOVID study, we evaluated different unsolicited events in the SOTR cohort, compared with HCPs. However, the number of events in each group was too small to reach any significant or meaningful conclusion.

5.3.1 Risk factors of reactogenicity

In the VigilVacCOVID study, we also assessed the association of risk factors with the development of reactogenicity. Explored risk factors included age, sex, type of vaccine, population group (HCP or SOTR), previous SARS-CoV-2 occupational contact, previous SARS-CoV-2 infection, and comorbidities (arterial hypertension, diabetes mellitus, rheumatic/immune-mediated disease, asthma, heart failure, chronic bronchitis, and previous drug allergies).

Regarding the comparison of BNT162b2 and mRNA-1273 in HCPs, the type of vaccine, age, sex, previous SARS-CoV-2 infection, and previous drug allergies were statistically significant factors in the multivariate analysis. Previous occupational contact was not significant. Concerning the comparison of HCPs and SOTRs vaccinated with mRNA-1273, the population group, age, sex, and previous SARS-CoV-2 infection were significant. Previous drug allergies were only significant in the multivariate analysis of severe ARs. The Receiver Operating Characteristic Area Under the Curve (ROC AUC) showed acceptable performance for the implemented multivariate models.

The association between age and reactogenicity was already indicated in the pivotal clinical trials. Subsequent real-world studies have confirmed this association and have suggested additional risk factors not proposed by previous pivotal trials, such as sex and previous SARS-CoV-2 infection (181,189,217). Prior research has suggested that age and sex are associated with differences in the immune response of vaccinated persons (162,281,282), and that the impact of age is likely due to the waning of innate immune defence mechanisms or immunosenescence mechanisms (189). These differential immune responses according to age and gender may translate into different reactogenicity rates. In this regard, Khoury J et

al. (283) reported that participants ≥ 50 years vaccinated with BNT162b2 had lower antibody titres than those < 30 years. Moreover, in elderly participants, higher titres were seen in females than in males. Likewise, Lustig Y et al. found that lower antibody concentrations were consistently associated with male sex, older age, immunosuppression, and other specific comorbidities, such as diabetes, hypertension, heart disease, and autoimmune diseases (284).

The results of the VigilVacCOVID study are in line with these results, signalling an association between age and reactogenicity. Noteworthy, a few previous studies had assessed the impact of age on specific types of local and systemic ARs (181,182). In the VigilVacCOVID study, considering the design and the sample size, we decided to analyse the association of age with overall reactogenicity in the multivariate analysis, without making specific analyses of local or systemic ARs. This is the approach adopted by most studies that have explored this hypothesis. Likewise, some studies have examined the impact of age according to different age strata (189). Similar to these studies, we found the odds of reactogenicity decreased with increasing age, considering age strata of 41-50, 51-65, and >65 years.

In the same way, previous studies evaluating the safety of vaccination in transplanted and immunosuppressed patients have evidenced an association between age and sex and reactogenicity (267,273), including the VigilVacCOVID study. The immune correlate of age and sex, which has been well substantiated in previous studies (285,286), can explain, at least partially, the lower reactogenicity observed in transplanted patients and vaccinees with previous comorbidities.

Other potential explanations underlying the impact of sex may be associated with genetic or hormonal differences. For instance, variations in skin thickness, blood flow, and the structure of the nervous system between men and women could contribute to a higher likelihood of injection-site inflammation in women. Additionally, sex hormones have been demonstrated to affect immune responses (162,287). Other underlying mechanisms, such as PK/PD and social and behavioural causes, must still be further elucidated. Indeed, sex-based differences do not seem to be circumscribed to vaccines but rather seem a general pattern also exhibited by other types of medicinal products (288,289). Further in-depth studies are needed to unveil the mechanisms underlying sex differences and assess the individual contribution of this potential factor to vaccine reactogenicity. Besides, sex-related factors refer to biological

differences between women and men, whereas gender-related factors refer to psychosocial, behavioural, or cultural differences. Further work is required to study this differentiation and how it impacts the assessment of vaccine reactogenicity.

Prior research has also indicated that SARS-CoV-2 previously infected individuals developed higher antibody titres to mRNA vaccines than non-pre-exposed individuals. Shields AM et al. observed that antibody responses were faster and of greater magnitude in HCPs previously exposed to SARS-CoV-2 after vaccination with a dose of BNT162b2 compared with non-pre-exposed HCPs (290). These results have been substantiated by other authors (291,292). Previous SARS-CoV-2 infection is also a trigger of higher immune response after vaccination in transplanted patients. Tylicki and colleagues demonstrated a 100% seroconversion rate in vaccinated kidney transplant patients with prior SARS-CoV-2 infection—compared with 45.78% in those without prior infection—after receiving two doses of either BNT162b2 or mRNA-1273 (293). However, while the association between age and sex, and reactogenicity has been explored in transplanted patients and frail vaccinees, the impact of previous SARS-CoV-2 infection on the safety profile remains poorly explored. In the VigilVacCOVID study, we demonstrated that, apart from age and sex, previous infection is also a risk factor for reactogenicity in transplanted patients.

From a mechanistic point of view, some authors have suggested that the fact that patients with a prior history of COVID-19 exhibited a higher incidence of ARs to the vaccination indicates a potential involvement of immunological memory responses to the S protein (207). In transgenic mice expressing ACE2, weight loss was observed for approximately one week following the second dose of the BNT162b2 vaccine, rather than after the first dose (294), further suggesting that memory responses may have contributed to the development of reactogenicity. Additionally, it has been proposed that the soluble S protein can disrupt endothelial cell function, thereby triggering pro-inflammatory cytokine production and modulating the renin-angiotensin system (295). These effects can be aggravated after the second vaccine dose, which is consistent with the higher rate of ARs observed after the second dose.

Interestingly, in the pivotal clinical trial of BNT162b2, solicited adverse events were less common in vaccine recipients who were SARS-CoV-2-positive at baseline (111), contrary to what subsequent studies have reported. This discrepancy highlights the importance of

conducting large-scale real-world studies following pivotal clinical trials to continuously characterise the safety profile and potential risk factors. In relation to chronic comorbidities and previous medical treatments, some prior studies reported an association (181,189), whilst others who also explored this hypothesis did not (182,217). Rolfes L et al. reported that some comorbidities were associated with reactogenicity, such as cardiac, respiratory, psychiatric, reproductive, eye, and neoplasm disorders (189). The authors discuss that for respiratory disorders and neoplasms, this may potentially be attributed to an increased predisposition to developing symptoms such as fever within these groups. These authors also reported that a body mass index (BMI) of 25.0 to 29.9 and over 30 was negatively associated with reactogenicity in general. BMI may be a potential factor influencing an individual's immune response. There might also be an association with the vaccine administration technique or needle length in persons with obesity (162). However, most studies of COVID-19 vaccines have not found a clear effect (296–298). For the time being, the comorbidities that have the clearest impact on reactogenicity are previous SARS-CoV-2 infection and drug allergies. With respect to other explored comorbidities, no solid conclusions can be drawn yet, and this topic should be further investigated.

Besides, other risk factors and explanations for reactogenicity have been proposed. For instance, a higher tolerance to pain and illness symptoms gained with life experience has been described in the literature (162). This argument has been purported to justify the impact of age on reactogenicity. Other authors have highlighted the potential impact of sleep quality, worry about adverse events, and education in the perception and reporting of ARs after vaccination (185).

Conversely, in the VigilVacCOVID study, we also observed an association between baseline drug allergies and the development of ARs after vaccination. In other studies, self-reported high-risk allergy history was associated with an increased risk of self-reported allergic reactions after mRNA COVID-19 vaccination (299), or with anaphylaxis (300). In line with these results, the Vaccine Safety Datalink provided interesting results, suggesting that a majority (85%) of individuals who experienced vaccine-related anaphylaxis had a preexisting atopic condition (223). Riad A et al. found an association between previous allergies and lymphadenopathy (182). Likewise, the authors reported a higher incidence of fatigue, fever, malaise, muscle pain, joint pain, and lymphadenopathy in asthmatic patients.

Interestingly, in the VigilVacCOVID study, we found an association between previous drug allergy and overall reactogenicity and not only with allergic ARs. In addition, in the multivariate analysis, this association was significant in HCPs, whereas in SOTRs, the association was only significant when severe ARs were considered in the definition of reactogenicity. These findings strongly suggest that immunogenic reactions can play a key role in the short-term tolerability of mRNA SARS-CoV-2 vaccines. Further research is needed to elucidate the mechanisms through which previous allergies can impact the development of overall reactogenicity in vaccinees.

Finally, we also analysed previous SARS-CoV-2 occupational contact, however, we did not find any significant association with reactogenicity. Riad A et al. found an association between some solicited ARs and having shorter work experience (181). This risk factor has been relatively unexplored in other studies.

5.3.2 Strengths and limitations

The findings of the VigilVacCOVID study indicate an elevated reactogenicity of mRNA-1273, characterised by a higher incidence of ARs, while both mRNA vaccines demonstrated an acceptable tolerability profile, supported by the predominance of mild and moderate ARs. In SOTRs, mRNA-1273 exhibited an improved reactogenicity and tolerability profile, reflected in a reduced incidence of ARs and a lower proportion of severe ARs.

The VigilVacCOVID study offers further prospective comparative data on both solicited and unsolicited ARs from a large cohort, thereby providing strong comparative evidence from two vaccines and two vaccinated populations receiving the same vaccine. Consistent with prior clinical trials and real-world studies, our findings indicate a predominance of mild, localised ARs, which serves as a reassuring indication of the safety of vaccination. This can be considered the main strength of the study.

Nevertheless, our study had several limitations linked to its observational and non-randomised nature, such as recall bias and potential confounders, that may persist even after applying balancing and adjustment methods. The first group of participants to be vaccinated were HCPs. The type of vaccine administered was determined by local authority guidelines and availability depending on supply at a national level. Initially, BNT162b2 was the only vaccine available for HCPs. The vaccination plan for HCPs was subsequently amended on

January 22nd, with the inclusion of mRNA-1273. Vaccination of SOTRs started on March 1st with mRNA-1273, as this vaccine was considered more reactogenic and immunogenic and hence more adequate for SOTRs. We cannot rule out differences in baseline characteristics between HCPs vaccinated in the first round and afterwards with mRNA-1273, especially considering that first-line HCPs were the first professionals to be vaccinated. To partially mitigate this bias and to assess baseline homogeneity, we used standardised differences (STD, differences between groups divided by pooled standard deviation). For the comparison between vaccines in HCPs (BNT162b2 and mRNA-1273), the IPTW approach was used to create a pseudo-population in which the two groups were balanced across baseline covariates. The stabilised weights were calculated using propensity scores to minimise standardised differences between arms. Covariate balance was assessed using STD with the goal of achieving absolute values <0.20 ($|<20\%|$), which are acceptable values of variability, as suggested by some authors (301,302). Sensitivity analyses were performed considering raw unadjusted data and covariate-adjusted data. STD were also calculated for the comparison between HCPs and SOTRs. However, IPTW was not applied to the HCP and SOTR groups comparisons, considering that these were very different populations (the key covariates being inherent to each group and non-modifiable), and the main outcome was precisely to describe whether the same vaccine was tolerated differently in both populations.

Our results regarding SOTRs are comparable to other previous studies. The VigilVacCOVID study did not take into account the immunosuppressive regimen of the patients. We acknowledge that this is a risk factor for lower reactogenicity. Additionally, our analysis focused on the overall SOTR population, which was highly heterogeneous as it included patients with various types of solid organ transplants. We understand that this fact may have also contributed to greater heterogeneity and could even have led to different impacts on reactogenicity depending on the type of transplant. However, we did not have a sample size sufficient to achieve adequate statistical power to analyse differences for each type of transplant.

As regards the analysis of risk factors, prior SARS-CoV-2 infection was assessed by directly asking the vaccinated individual if they had been diagnosed by CRP, antigen test, or had positive antibody serology. Apart from the potential impact of recall bias, we understand that there may have been some false positives or negatives, particularly among asymptomatic

individuals who were not tested. Unknown status was computed, for analysis purposes, as negative cases.

Two doses were the usual vaccine schedule at the time the study was conducted, and therefore, we were only able to assess tolerability and reactogenicity after two vaccine doses. We are aware that, following our study, booster doses were recommended for both the general population and immunosuppressed patients.

The structured questionnaire was delivered through telephonic interview after receiving each vaccine dose. We acknowledge that this AR assessment method is prone to subjectivity. However, this methodology has been successfully implemented in other similar studies, with the objective of assessing ARs following vaccination, some of them in large vaccinated cohorts (303,304). Moreover, seriousness was not systematically assessed for the following reasons: Firstly, ARs were captured through a structured questionnaire that was delivered through telephonic interview after receiving each vaccine dose, based on a predefined list of ARs that had been agreed based on the safety profile described in the SmPC and EPAR of each vaccine. These were considered solicited ARs. Electronic health records were not reviewed to assess whether the vaccinee had been hospitalised or received medical treatment, and these aspects were not systematically collected. Secondly, the study had been designed to capture the short-term reactivity of both mRNA vaccines and not the medical consequences occurring after vaccination. Likewise, given the high number of participants and recorded events, we did not formally assess causality, and we assumed that all reported events could potentially be associated with the vaccine or the vaccination process, such as vaccine administration. Notably, most of the referenced real-world studies did not formally assess causality.

Similarly, AR severity was assessed with a Likert scale (LS), with values ranging from 0 to 10, rated with instructions from the interviewer. Severe ARs were those scoring 7-10. The LS is a subjective method for assessing AR severity; nevertheless, previous studies have used this method, other numeric rating scores, or visual analogic scales (VAS) to assess patient-reported severity levels of AR symptoms (305), as a suitable method of evaluating patient or participant-perceived symptoms of potential ARs, especially for pain-related symptoms (306). Other studies have assessed the intensity or severity of local and systemic ARs through the number of ARs per participant (182). Other similar studies did not assess the severity of

each AR (181). We considered that the comparison of the severity of each AR, between vaccines and populations, was an important piece of information. Therefore, we decided to assess this variable. Besides, given that ARs were captured through telephonic interviews, we concluded that the best method to collect self-reported severity was through an LS under the guidance of the interviewer according to an agreed interview guideline.

A free-text option named “other” was also enabled in the questionnaire to collect other ARs not considered *a priori* in the drop-down list. To explore “other ARs” the interviewer asked an open question (e.g., Have you experienced other types of ARs?). Yes or no were recorded accordingly. If yes, the specific type of AR was recorded as a narrative description. These ARs were considered “unsolicited ARs”. For analysis purposes, we translated these unsolicited ARs into defined MedDRA terms. For instance, for hypersensitivity, we pooled several symptoms under the MedDRA PT “hypersensitivity”, i.e., immediate hypersensitivity events, exanthema, urticaria, and skin rashes, amongst others. This was also the case of delayed reactions, which were indeed more difficult to assess given the telephone-based approach of the interview. Inherently to this method, it is highly probable that these terms group different clinical entities.

Besides, to optimise the interview and prevent the respondent from becoming fatigued due to an excessively lengthy questionnaire, especially in SOTRs, we only assessed severity in solicited ARs. Indeed, for some unsolicited ARs, such as asthma or rhinitis, it was challenging to assess severity in a systematic manner. Likewise, we inquired about the severity of the fever, measured in degrees Celsius. However, since these results were reported directly by the participants and considering the possibility of recall bias, we deemed the data unreliable and, ultimately, did not include this variable in the severity assessment.

Furthermore, after completion of the VigilVacCOVID study, as a result of ongoing safety monitoring of COVID-19 vaccines, a total of 18 safety signals were prioritised and discussed by the PRAC, e.g., myocarditis/pericarditis or thrombosis with thrombocytopenia syndrome (TTS) (142). Given that the study of these safety signals had not even been initiated by the time the study was designed, nor during the period in which safety data were collected, these specific ARs were not considered in the list of ARs. Besides, we acknowledge that, being rare ARs, our sample size did not have enough statistical power to reliably detect these safety signals. Furthermore, as previously described, we established a predefined list of solicited

ARs based on the core safety information available at that moment, which was the safety profile described in pivotal clinical trials, as well as the SmPC and EPAR of each vaccine. In addition, ARs of special interest from the list of the ACCESS and SPEAC Projects and Brighton Collaboration criteria were considered. However, we did not include ARs such as myocarditis, transverse myelitis, or Guillain-Barré syndrome because, considering the core reference safety information when the study was designed, major complications or reactions that led the participant to hospital/emergency department or death within a short timeframe were, in principle, not expected. In addition, the diagnosis of these clinical entities would require a longer clinical follow-up that was not part of the study, which was designed to capture the short-term reactogenicity profile of BNT162b2 and mRNA-1273.

Notwithstanding, we were able to report some ARs that were subsequently labelled following PRAC's evaluation of summary and periodic safety reports throughout 2021-2022. These ARs are described in Table 12.

Table 12. Overview of adverse reactions associated with COVID-19 vaccines as reported in SSRs/PSURs during 2021–2022

Date of PRAC adoption	Vaccine	Added adverse reactions	SmPC section *	Source of safety information	Adverse reactions reported in the VigilVacCOVID study
11/Feb/2021	BNT162b2	Vomiting, diarrhoea	4.8	SSR	Nausea Diarrhoea Vomiting Gastrointestinal disorder
28/Nov/2021	BNT162b2	Diarrhoea	4.8	Variation in labelled indication	Diarrhoea Gastrointestinal disorder
02/Dec/2021	BNT162b2	Paraesthesia, hypoesthesia	4.8	SSR	Paraesthesia, dysaesthesia
02/Dec/2021	BNT162b2	Update of anxiety-related reactions	4.4	SSR	Syncope, dizziness, palpitations, increases in heart rate, alterations in blood pressure, paraesthesia, hypoesthesia, and sweating
13/Jan/2022	mRNA-1273	Paraesthesia	4.8	PSUR	Paraesthesia, dysaesthesia
10/Jun/2022	mRNA-1273	Urticaria	4.8	SSR	Exanthema, urticaria, rash

*Table 12. *Section of the SmPC in which the AR was added; 4.4 SmPC: Section 4.4- Special warnings and precautions for use; 4.8 SmPC: Section 4.8- Undesirable effects; PSUR: Periodic Safety Update Report; SSR: Summary Safety Report. Modified from Caplanusi I et al. (142)*

As can be seen in the table, some of these ARs were reported by the study before their official implementation in the SmPC. The ARs detected in the VigilVacCOVID study were reported to the regional centre of pharmacovigilance, according to current legislation and pharmacovigilance guidelines. Therefore, despite the described limitations, the study made a modest contribution to enhancing the characterisation of the safety profile of mRNA vaccines and enriching the detection of potential safety signals.

In contrast, cardiovascular ARs have been reported in subsequent post-authorisation safety studies. However, the results are conflicting. While some publications suggest increased reporting (307–309), other studies have not found a clear association (310–312). Currently, apart from myocarditis and pericarditis, no other safety signals related to cardiovascular complications have been validated so far. The question remains controversial and requires further elucidation; nonetheless, it is true that some authors have previously suggested that mRNA vaccines have been associated with more reports of cardiovascular adverse events than other types of SARS-CoV-2 vaccines (313). In our VigilVacCOVID study, we detected some cases of syncope, hypertension, hypotension, chest pain, cold sweat, and tachycardia as unsolicited ARs, which have been previously reported in other studies as potential cardiovascular adverse effects (311,312). These results confirm the sensitivity of our study to detect and report ARs developed after vaccination, aligning with that previously published.

Similarly, symptoms such as syncope, dizziness, palpitations, increased heart rate, alterations in blood pressure, paraesthesia, hypoesthesia, and sweating have been associated by some authors with anxiety-related reactions (199). These are not ARs caused by the vaccine formulation but are rather due to stress and anxiety associated with the vaccination process itself. Probably some of the related ARs that we reported in the VigilVacCOVID study are explained by physiological responses to the vaccine, including cases of fainting or syncope. In fact, section 4.4 of the SmPC of Comirnaty was updated in December 2021 to include an update of anxiety-related reactions (142).

Moreover, the safety follow-up performed by the Pharmacovigilance Program of the Hospital continued beyond the VigilVacCOVID study, aiming to provide reassurance regarding the short-term safety of COVID-19 vaccines during the initial phases of the vaccination campaign. Within the framework of the consolidated pharmacovigilance plan, we reported a case of autoimmune hepatitis in a patient vaccinated with mRNA-1273 (314). Autoimmune hepatitis was adopted as a safety signal by the PRAC on April 7th, 2022 (142).

5.4 Future perspectives

From the start of the pandemic to the present moment, 242 vaccine candidates have been tested in 821 vaccine trials, involving 80 countries in the clinical development of vaccine candidates. In addition, 50 vaccine candidates have been approved in at least one country (according to data available in the vaccine tracker (315), with the last database update on Dec 2, 2022).

In the EU, since the authorisation of the first mRNA vaccines against SARS-CoV-2, other vaccines have been granted a CMA and, afterwards, a full authorisation. These vaccines use different platforms other than mRNA. Vaxzevria from the MAH AstraZeneca (ChAdOx1-S, based on a modified chimpanzee adenovirus) received CMA from the EMA on January 29, 2021. Likewise, JCOVDEN from Janssen (based on the adenovirus Ad26.COV2-S) was granted a CMA on March 11, 2021. However, the marketing authorisation application (MAA) of both vaccines was later withdrawn at the request of the MAH (316,317). Other authorised vaccines in the EU are based on recombinant proteins, such as Bimervax (HIPRA Human Health S.L.U.) and Nuvaxovid (Novavax CZ, a.s.).

Booster doses of mRNA vaccines (third and fourth doses) have been administered worldwide. The overall safety profile of these booster doses has remained consistent with that seen after two doses (199). In addition, heterologous (mix and match) schemes have been applied in vaccination campaigns (318). A prime-boost vaccination approach refers to administering an initial (prime) dose followed by one or more booster doses of an immunogen. When the same immunogen is used for both the prime and booster doses, the strategy is termed homologous, whereas if different immunogens are employed, it is referred to as heterologous.

There is increasing global interest in the heterologous prime-boost COVID-19 vaccination regimen as a potential solution to challenges related to variable vaccine availability, severe ARs with prime vaccination, the emergence of new virulent strains, and diminishing immunity. This approach aims to elicit a strong immune response while maintaining a favourable safety profile regarding reactogenicity (318–320). Interest in heterologous COVID-19 vaccines increased due to severe ARs, such as thrombotic events with thrombocytopenia (VITT), seen with the ChAdOx1-S (AstraZeneca) and Ad26.COV2.S (Janssen) vaccines. Consequently, health authorities revised guidelines to include BNT162b2

(Pfizer BioNTech) as a booster for those initially vaccinated with ChAdOx1-S (318). Since then, many people worldwide have received heterologous booster doses.

Early results from some studies suggest that a ChAdOx1-prime with a BNT162b2-booster regimen may induce a robust immunological response with a tolerable reactogenicity profile (321–323). However, some studies have suggested that heterologous regimes induce greater systemic reactogenicity following the booster dose than homologous schemes. In a multi-centre, randomised study conducted in the United Kingdom, all four prime-boost combinations of the ChAdOx1-S and BNT162b2 vaccines were assessed at prime-boost intervals of 28 days and 84 days in 830 participants aged 50 and older. The study revealed that both heterologous prime-boost regimens (at both 28-day and 84-day intervals) resulted in increased systemic reactogenicity following the booster dose compared with their homologous counterparts. This was evidenced by higher incidence rates of fever, chills, fatigue, headache, joint pain, malaise, and muscle ache (324). Interestingly, these data were obtained in participants aged 50 years and older, a population that had yielded lower reactogenicity results in early clinical trials with homologous doses, thereby suggesting that older age was a protective factor of reactogenicity. It is, therefore, important to continue to characterise and understand the reactogenicity and safety profile of heterologous schemes.

Also importantly, various vaccines are suitable for completing primary immunisation after initial priming with BNT162b2 or other vaccines, thereby enabling swift global vaccine distribution and aiding in the acknowledgement of these regimens for vaccine certification purposes. The crucial point in this context is to select the construct that exhibits the most optimal profile of reactogenicity and safety and, ultimately, the most favourable risk-benefit balance, considering the protective immunogenicity generated by these vaccines. For instance, a clinical trial in the UK concluded that heterologous second dosing with mRNA-1273 but not NVX-CoV2373 (Novavax) increased transient systemic reactogenicity compared with homologous schedules. Other vaccines have also shown that NVX-CoV2373, when administered as a heterologous booster, has less reactogenicity than mRNA vaccines (325–327).

Along a different vein, original vaccines targeting SARS-CoV-2 were introduced in 2021. However, in November 2021, the Omicron variant of the virus emerged and quickly became the predominant strain. This variant further diversified into several sub-lineages. The

vaccines formulated during the initial phase of the pandemic demonstrated reduced efficacy against Omicron. Consequently, vaccine manufacturers have had to develop updated formulations to enhance protection. These **adapted vaccines** are intended to provide booster doses and to confer broader protection against different virus variants of concern (VOC) following initial vaccination (328,329). Adapted vaccines have been manufactured by adapting the mRNA sequence to the specific VOC. These specific sequences receive different names to differentiate them from the initial mRNA constructs. For instance, in the case of BNT162b2 (tozinameran), the mRNA construct has been adapted to recognise the new VOC Omicron BA.1. This mRNA construct is named riltzinameran. Other constructs have been synthesised for adapted vaccines to VOC, such as Omicron BA.4-5 (famtozinameran) and Omicron XBB.1.5 (raxtozinameran). All these new vaccines (except raxtozinameran) are also called bivalent vaccines since they combine the original vaccine with another mRNA vaccine aimed at Omicron subvariants. For instance, tozinameran has been combined with riltzinameran, which encodes for the spike protein of the Omicron BA.1 subvariant, and with famtozinameran, which encodes for the spike protein of the Omicron BA.4-5 subvariants. These bivalent vaccines have been granted provisional approval for use as booster doses.

Currently, the safety profile associated with bivalent vaccines is similar to that observed with tozinameran monotherapy. In individuals over 55 years of age, the reactogenicity profiles of the monovalent BNT162b2 Omicron BA.1 and the bivalent BNT162b2 Original/Omicron BA.1, administered as fourth-dose boosters at both dose levels, were comparable to those observed for the original vaccine when given as a third-dose booster (330). Similar results were noted for the monovalent vaccine in adults aged 18-55 years. The most common ARs reported in both age groups included pain at the injection site, fatigue, headache, myalgia, chills, and arthralgia. No new ARs were detected for either vaccine formulation. Preliminary clinical data at 7 and 30 days post-vaccination demonstrated that the bivalent Original/Omicron BA.4-5 vaccine was well-tolerated, with a safety profile consistent with that of the original vaccine (331).

Overall, no new safety signals have been identified with adapted vaccines, and the safety profile seems comparable to original vaccines (199,332). However, long-term safety data from real-world studies are still needed for bivalent vaccines. These data are of paramount

importance given the need to adapt vaccines and vaccinate the population against upcoming VOCs.

The mRNA constructs of mRNA-1273 (elasomeran) have also been adapted to new VOC. A bivalent version of the vaccine was created, incorporating elasomeran and imelasomeran, with the objective of enhancing immunity against the Omicron BA.1 subvariant. To increase protection against the Omicron BA.4-5 subvariants, elasomeran has since been combined with davesomeran, an mRNA-based vaccine that encodes the spike protein of the Omicron BA.4-5 subvariants (333). Likewise, andusomeran is a monovalent vaccine designed to protect against COVID-19 disease caused by Omicron XBB.1.5. As is the case with adapted vaccines derived from tozinameran (334). At the same time, some studies have assessed the safety of these vaccines (335–338), and the safety profile seems to be comparable to the original elasomeran; most of the data has largely been inferred from extensive clinical studies and real-world experience with earlier COVID-19 vaccines, especially at long-term. Therefore, safety data from continuous surveillance monitoring is needed in the future.

Furthermore, the indications of these mRNA vaccines have been extended to children. Both vaccines can be administered to children from six months of age (328) with lower doses of mRNA. After the authorisation of the BNT162b2 vaccine for adult populations, a clinical trial was conducted in participants aged 6 months to 11 years to evaluate age-appropriate doses of BNT162b2 for paediatric use. In the Phase I portion, children aged 5 to 11 years were administered two doses of either 10 µg, 20 µg, or 30 µg of BNT162b2 (NCT04816643). Due to the higher incidence of fever with the larger doses and the comparable neutralising antibody responses observed between the 10 and 20-µg doses, the 10-µg dose was chosen for further evaluation in the Phase II/III trial. For children aged six months to four years, participants received either 10 µg or 3 µg of BNT162b2. Given the increased frequency and severity of reactogenicity with the 10-µg dose compared with the 3-µg dose and the similar neutralising antibody profiles across dose levels as seen in older children, the 3-µg dose was selected for further evaluation (339,340).

The pivotal Phase II/III clinical trial of BNT162b2 also incorporated a group of 2,260 participants aged 12 to 15 years, with 1,131 receiving the BNT162b2 vaccine. The solicited local and systemic reactions were predominantly mild to moderate in intensity and occurred at a frequency comparable to that observed in participants aged 16 to 25 years. Of the 1,131

individuals who received the BNT162b2 vaccine, 786 were monitored for a period of at least four months following the administration of the second dose. The overall safety profile observed in this group remained consistent with that reported in participants aged 16 years and older (199).

The safety of BNT162b2 has also been explored in children under five years of age. In a cohort of 3,013 children aged six months to four years who were administered BNT162b2, the majority of local and systemic reactions were of mild to moderate severity, with no reports of grade 4 local reactions. The incidence of adverse events was comparable between recipients of BNT162b2 and those receiving the placebo (340). In another clinical trial, 3,040 children of two to five years of age and 1,762 children aged 6 to 23 months were randomly assigned to receive two 25- μ g injections of mRNA-1273; 1,008 children of two to five years of age and 593 children aged 6 to 23 months were randomly assigned to receive placebo. The occurrence of solicited local ARs was greater with mRNA-1273 compared with the placebo in both age groups. Additionally, within the mRNA-1273 group, the frequency of these reactions was higher following the second dose than after the first dose, which is in line with all previous studies with mRNA-1273. The majority of local ARs were classified as grade 1 or 2, with a duration of one to three days. Grade 3 events were infrequent overall but occurred more often following the second dose. The most commonly reported grade 3 reactions included erythema, swelling, and pain in children aged two to five years, and erythema and swelling in children aged 6 to 23 months. The authors concluded that in children aged 6 months to five years, a two-injection primary series of mRNA-1273 at a dose of 25 μ g had an acceptable safety profile. (341). Likewise, the TeenCOVE clinical trial concluded that the overall risk-benefit profile of mRNA-1273 remained favourable in adolescents, with durable 12-month immune responses against SARS-CoV-2 (342).

Overall, safety data in the paediatric population seem adequate and comparable to those reported in pivotal clinical trials in adults. These results were the basis to substantiate the extension of the original indication to this population. However, most real-world safety data, especially data related to long-term follow-up and reported safety signals, were obtained from the adult population during the initial stages of the vaccination rollout. Hence, further real-world data are needed for this population, which was initially underrepresented.

Other populations not initially represented in the pivotal clinical trials were studied during the vaccine rollout, e.g., pregnant women. Large real-world studies have yielded reassuring safety findings in this population, providing evidence that vaccination during pregnancy with the current mRNA vaccines does not elicit major adverse events and has similar reactogenicity profiles between pregnant women and the general population (343–346). The most common reported systemic adverse symptoms among pregnant women were tiredness, headache, myalgia, chills, nausea, and fever. Injection site pain was the most common local adverse event for both mRNA vaccines, in line with their known safety profile. Also, aligning with previous studies, the rate of systemic adverse events was higher following the second vaccine dose for both mRNA vaccines, and a higher frequency of adverse events was reported for the higher-concentration mRNA-1273 vaccine (100 µg) than for the BNT162b2 vaccine (30 µg).

No adverse perinatal outcomes have been associated with mRNA vaccination. Noteworthy, in some studies, myalgia, arthralgia, headache, injection site pain, and lymphadenopathy were less common among pregnant than non-pregnant BNT162b2-vaccinated women, whereas paraesthesia was more frequent in pregnant women. These data may suggest that pregnant women develop lower immunogenicity, however, several studies have shown that pregnant women elicit robust humoral immune responses to vaccination, with an IgG seropositivity of 100% following the second dose of an mRNA vaccine (347–349). Further long-term safety studies are needed in this population to characterise the safety profile of mRNA vaccines, the AR profile, and the association between these ARs and elicited immunogenicity. In addition, more safety data on vaccination during the first trimester are needed since most pregnant women who were included in the studies were in their second or third trimester. Further research is required to elucidate other remaining questions, such as the long-term safety profile of mRNA vaccines in the offspring; the optimal timing of COVID-19 vaccination during pregnancy to maximise protection against COVID-19 in the mother and foetus, as well as to minimise any adverse pregnancy outcomes; or the safety and immunogenicity profiles when COVID-19 vaccines are co-administered with other routine vaccines during pregnancy. In fact, beyond pregnant vaccinees, one of the key points of current debate is the co-administration of COVID-19 vaccines with other seasonal vaccines, such as the influenza vaccine.

The CDC recommended the updated 2024-2025 COVID-19 and 2024-2025 flu vaccines to protect against severe COVID-19 and flu (350). These vaccines can be administered at the same visit. This recommendation has also been issued by many governments and agencies worldwide. Previous studies have shown that concurrent administration of the bivalent COVID-19 booster and the inactivated influenza vaccine on the same day resulted in higher spike-specific IgG1 antibody responses at peak and six months compared with administration of these vaccines on separate days (351), thereby suggesting potential benefits of the concurrent administration of COVID-19 mRNA vaccines and the seasonal influenza vaccine. Conversely, other studies reported that simultaneous administration of a COVID-19 mRNA-booster dose and an influenza vaccine was associated with 8% to 11% increases, respectively, in systemic reactions compared with COVID-19 mRNA-booster alone (352). It is, therefore, important to continue to monitor the real-world safety and risk-benefit of these vaccination schedules, especially considering the importance they may have in terms of seasonal protection of the population.

On a different note, the safety profile of vaccination in transplant-recipient vaccinees, cancer patients, frail patients, and persons with other comorbidities should continue to be characterised. New vaccination schemes should be tested to stimulate protective immunogenicity while reducing reactogenicity symptoms that may be causative of disease burden. For example, there is emerging evidence suggesting that a heterologous prime-boost regimen may enhance the immune response in immunosuppressed individuals compared with a homologous regimen. In a study involving 40 transplant recipients, Schmidt et al. observed that heterologous vaccination (ChAdOx1 followed by an mRNA-based vaccine) resulted in a stronger antibody and CD4 T-cell response than a homologous regimen using mRNA-based COVID-19 vaccines (mRNA-1273 and BNT162b2) (353). It is important to evaluate whether these regimens, or other alternative vaccine combinations, not only elicit protective immune responses but can also be optimised to ensure an adequate safety, tolerability, and reactogenicity profile. For this purpose, it is important to have regulatory support and specific scientific and regulatory guidelines that guide and encourage the inclusion of these underrepresented patient populations in observational studies and clinical trials. Recently, the EMA published the “Addendum to the Guideline on clinical development of vaccines to address clinical trials in immunocompromised individuals”, which will probably be illustrative for this aim (354).

Moreover, the future applicability of COVID-19 vaccines could be enhanced by minimising associated ARs and mitigating the risk factors for reactogenicity linked to the use of modified mRNA and LNP. In this sense, as previously noted, PEGylation of LNPs is widely used to foster the stability and plasma half-life of LNPs. However, preexisting or *de-novo* anti-PEG antibodies can activate the complement system. Activation of the complement system can accelerate blood clearance (ABC) and significantly reduce the therapeutic efficacy of LNP-mRNA vaccines. Besides, anti-PEG antibodies have been linked to pseudo-allergic reactions. These hypersensitivity reactions are considered to occur through CARPA activation. This reaction is particularly challenging because it can happen independently of pre-existing antibodies and vary depending on the liposomal formulation, surface charge, and other physicochemical characteristics.

CARPA is a significant consideration in the clinical use of liposomal drugs due to its potential to cause ARs during treatment (355). Consequently, the development of PEG-free delivery systems is essential for the future advancement of mRNA vaccines. In this context, investigating the characteristics of diverse materials, including synthetic, natural, and zwitterionic polymers, is recommended to create more efficient and safer mRNA delivery systems. These substances have been acknowledged for their potential to deliver novel and promising medical applications in comparison to PEG. Nevertheless, it remains unclear whether they can offer therapeutic advantages without triggering pathological immune reactions (356).

Furthermore, it is also crucial for HCPs to recognise COVID-19 vaccine-related symptoms and establish multidisciplinary circuits that allow the detection of these severe clinical cases at the earliest possible opportunity through active and passive pharmacovigilance systems, conduct diagnostic workups with potential differential diagnoses, implement the appropriate patient treatment, and prepare the necessary regulatory reports. The Pharmacovigilance Program established at the Hospital Clínic is an illustrative example of how multidisciplinary efforts at a hospital level can contribute not only to the detection and treatment of cases, but also to the enrichment of current safety data on vaccines. It is important that these collaborative circuits, established in many other hospital and academic settings, which have the clinical perspective of the patient, work together with various international stakeholders

(private companies, regulators, decision-makers) to expand our current knowledge about vaccines in a continuous manner and to detect additional safety signals.

Given the considerable sequence homology between SARS-CoV-2 and SARS-CoV, one of the most serious concerns at the time of approval of the first mRNA COVID vaccines was the development of antibody-dependent enhancement (ADE), or its respiratory variation vaccine-associated enhanced respiratory disease (VAERD) (152,357). The main surveillance and pharmacovigilance systems worldwide were prepared to detect these cases. Besides, at a regulatory level, post-authorisation commitments in the European Union RMPs included studies to assess this specific risk (358). For the time being, no safety signals substantiating this risk have been identified. However, recent large-scale studies have suggested the existence of other potential safety signals and the need for further monitoring.

The Global COVID Vaccine Safety (GCoVS) Project (156), established in 2021 under the multinational Global Vaccine Data Network (GVDN®), has facilitated a comprehensive assessment of vaccine safety (156). The GCoVS Project is a global collaboration funded by the CDC, involving investigators and data sources from various countries, to monitor the safety of COVID-19 vaccines. Under this umbrella, Faksova K et al. conducted a global cohort study following the Observed vs Expected analyses (O/E) of COVID-19 AESIs, with data from 10 sites across eight countries participating in the GCoVS Project (359). The authors focused on the three vaccines with the highest number of doses administered: BNT162b2, mRNA-1273, and ChAdOx1. In total, participants included 99,068,901 vaccinated individuals. Data were obtained from healthcare records, comprised of either individual- or population-level data, depending on the availability at the study sites. Risk intervals used after each dose were 0-7 days, 8-21 days, 22-42 days, and 0-42 days. For each risk interval, 13 clinical outcomes representing AESIs of specific relevance were recorded. Clinical outcomes were selected from the list compiled by the Brighton Collaboration SPEAC Project (360).

The authors confirmed pre-established safety signals of COVID-19 vaccines, such as myocarditis, pericarditis, Guillain-Barré syndrome, and cerebral venous sinus thrombosis, in a multi-country context and with a large and diverse database. This study contributed to confirming previously identified rare ARs following COVID-19 vaccination and provided evidence of several other important outcomes, such as acute disseminated encephalomyelitis

(ADEM). Further investigation is warranted to confirm these associations and assess their clinical significance. This could be addressed by conducting association studies specific to individual outcomes by applying methodologies such as the self-controlled case series (SCCS), or other methods to assess causal inference to validate the association.

5.5 Reactogenicity and Immunogenicity

In the first part of the VigilVacCOVID study, we identified a correlation between reactogenicity and immunogenicity. This was demonstrated by the consistent finding that a higher occurrence of ARs was associated with younger age and prior exposure to SARS-CoV-2. In contrast, decreased vaccine reactogenicity was observed in individuals with compromised immune function, resulting in better tolerability. Based on this premise, we decided to implement this second part of the project in which we used the reactogenicity data collected during the study to analyse immunogenicity outcomes and support the hypothesis of a potential relationship between reactogenicity and immunogenicity in a large, vaccinated cohort under real-world clinical conditions. Below, we will discuss the results obtained in terms of the analysis of reactogenicity and elicited humoral and cellular immunogenicity.

5.5.1 Reactogenicity and elicited humoral and cellular immunogenicity

In the current era of heightened public concern regarding the potential adverse effects of vaccination, it is essential to focus on strategies that minimise both local and systemic reactions without compromising VE. This should be a key objective for the development of future vaccines. An optimal vaccine design must efficiently activate both the innate and adaptive immune responses without triggering systemic inflammation, which could result in untoward ARs. A key unresolved issue is whether the ARs associated with vaccination can be dissociated from the induced immune response in an ideal vaccine formulation (355,361).

Several researchers have identified binding and neutralising antibodies as potential immune correlates of protection in SARS-CoV-2 vaccines (362,363). Their findings raise the question of whether this immune response is, to some extent, linked to the reactogenicity profiles observed in current vaccine formulations. Indeed, vaccines that incorporate both aluminium and monophosphoryl lipid A (MPL) as adjuvants tend to exhibit increased reactogenicity,

raising the concerning possibility that boosting immune responses may be inherently linked to a corresponding rise in ARs (361). Several authors have observed that the increases linked to MPL were predominantly confined to localised side effects, such as transient pain, redness, and swelling at the injection site, rather than widespread systemic effects, with the exception of myalgia. Further analysis is required; however, this trend indicates the potential for dissociating favourable immunisation outcomes from systemic adverse effects (364,365). It is therefore important to understand the immunological basis of mRNA vaccines reactogenicity and to elucidate whether protective immunogenicity can also be dissociated, at least in part, from the elements contributing to reactogenicity in current mRNA vaccine composition.

In general terms, vaccine antigens are identified as potential pathogens by the pattern recognition receptors of the innate immune system, leading to the release of pyrogenic cytokines, including IL-1, IL-6, TNF- α , and prostaglandin E2. This triggers a cascade of immune responses following vaccination, which may be associated with local or systemic ARs in vaccinated individuals. However, previous research on the immunological correlates of reactogenicity in humans is limited and preliminary; it also yielded different results based on the type of vaccine and no definitive conclusions.

Zhuang CL et al. used data from three clinical trials of recombinant vaccines in a *post-hoc* analysis to assess the correlation between inflammation-related solicited ARs and immune responses after vaccination with the bivalent HPV-16/18 vaccine (366). The authors concluded that there was an association between inflammation-related ARs and a stronger immune response. Likewise, Burny W et al. noted that IL-6 and IFN- γ signalling were linked to systemic reactogenicity after the administration of the AS01B-adjuvanted vaccine. These signalling pathways resemble those previously observed in association with antibody and T-cell responses elicited by HBsAg-adjuvanted vaccines, indicating that comparable innate immune mechanisms might contribute to both adjuvant-induced reactogenicity and immunogenicity (367). Besides, in a previous study, a weak correlation was found between reactogenicity and immunogenicity following the herpes zoster vaccine (368).

The mRNA-1273 and BNT162b2 vaccines represent the first mRNA vaccines developed for human use, prompting concerns regarding their immunological correlates and potential for reactogenicity. Following the deployment of these mRNA vaccines, numerous studies have

examined the relationship between immunogenicity and reactogenicity, yielding variable and sometimes inconclusive findings, as was the case with previous vaccine platforms. Considering this gap, in the second part of the VigilVacCOVID study, we aimed to evaluate ARs and antibody titres following vaccination with both BNT162b2 and mRNA-1273 and determine the correlation between immunogenicity and reactogenicity. The results led to the second article of this thesis.

In the study, the main population for analysis comprised N=215 vaccinees. We defined two subpopulations for sensitivity analyses: subpopulation 1 for sensitivity analysis 1 (N=3563) and subpopulation 2 for sensitivity analysis 2 (N=215). Following a consistency evaluation across the various analyses, we found a higher proportion of fever, malaise, and myalgia, including severe episodes of these ARs, among vaccinees that developed positive antibody titres after vaccination. This association was not only significant in terms of positive-negative titres, but also in terms of the quantity of elicited immunogenicity, as assessed by antibody titres above/below median in the semiquantitative analysis.

Other authors have reported similar results. Speletas et al. studied the intensity and duration of immunogenicity in 511 individuals vaccinated with two doses of the BNT162b2 vaccine. Taking into account the impact of ARs on the strength of IgG antibody responses, individuals who experienced fever and myalgia after the second dose had notably higher IgG levels on day 42 (369). Likewise, Yamamoto et al. assessed immunogenicity and reactogenicity in 100 HCPs vaccinated with BNT162b2. Participants who exhibited fever showed notably elevated spike IgG titres seven days after the second vaccination dose compared with those who reported no fever or only mild fever (370). Spike IgG titres tended to decline over time for all participants, however, those who had a high fever still maintained higher titres than those who experienced no or mild fever, even at both 39 and 60-74 days post-vaccination.

Furthermore, Koike R et al. also found an association with fever in the longer term. The authors observed that age-adjusted antibody titres at three months after vaccination were 23.7% and 23.4% higher in patients with a fever than in those without after the first and second dose, respectively (371). In turn, Kobashi Y et al. studied 231 HCPs vaccinated with BNT162b2. The authors observed that the presence of ARs (fever, muscle and joint pain) after the second dose was associated with higher IgG antibody titres and neutralising activity

(372), thereby reporting a comparable profile of ARs to the VigilVacCOVID study, with a similar sample size to the main population for analysis. Notably, although the association with fever, malaise, and myalgia was consistent in the overall analysis of the VigilVacCOVID study, we also reported a significant association with chills, headache, arm pain, and arthralgia in the sensitivity analyses performed, in line with other studies, such as Kobashi Y et al. (372).

Compared with BNT162b2, fewer studies have investigated the association between immunogenicity and reactogenicity after vaccination with mRNA-1273. One of the largest to assess this hypothesis was that performed by Siangphoe U et al. in 2023. This study examined the relationship between immunogenicity and reactogenicity following two 100- μ g doses of mRNA-1273 in a total of 1,671 adolescent and adult participants (aged ≥ 12 years) from the primary immunogenicity cohorts of the blinded phases of the COVE (110) and TeenCOVE trials (342). The associations between immunogenicity up to day 57 and solicited ARs after the first and second mRNA-1273 injections were analysed using linear mixed-effects models, comparing participants with and without solicited ARs. The authors reported that antibody titres at day 57 were significantly higher in participants who experienced solicited systemic ARs after the second injection than those who did not and were associated with fever, chills, headache, fatigue, myalgia, and arthralgia; a safety profile comparable to that of the VigilVacCOVID study. The authors did not find significant associations with local ARs.

Similarly, few studies have made comparative assessments of the association of immunogenicity and reactogenicity between vaccines. Bannister WP et al. performed a comparative assessment of the immunogenicity and reactogenicity in 6,528 participants after vaccination with BNT162b2, mRNA-1273, and ChAdOx1, in the ENFORCE study (373). The authors observed an association between more severe ARs and stronger antibody response after the second vaccination. Similarly, Debes et al. had comparable results, with significantly higher spike IgG levels after the second vaccination in those who had clinically significant symptoms (fatigue, fever, chills) (374). Likewise, Choi MJ et al. observed, in 177 healthy participants vaccinated with mRNA-1273, that anti-S IgG antibody titres were not associated with local reactogenicity but were higher in participants who experienced systemic ARs (headache and myalgia) (375).

As regards the assessment of immunogenicity and reactogenicity in relation to the vaccine dose, in the VigilVacCOVID study, we did not observe a specific predominance or pattern of AR frequency in the first or second doses. However, most of the statistically significant ARs in sensitivity analysis 2 were observed after any dose or after the second vaccine dose. Results are also diverse in the literature. Takahashi W et al. reported that the antibody levels of participants with systemic ARs after the first dose were significantly higher than those without at both measurements in 470 HCPs vaccinated with BNT162b2. The authors obtained similar results after the second dose (376). In contrast, Jubishi D et al. assessed 887 participants vaccinated with BNT162b2, concluding that stronger reactions after the second dose were factors positively associated with the elevation of anti-spike IgG levels. Anti-spike IgG levels were significantly higher if ARs after the second dose were stronger than those after the first dose (377). Similarly, Levy I et al. found a significant correlation between an AR score and anti-RBD-IgG titres after the second but not the first vaccine dose in 831 HCPs vaccinated with BNT162b2 (378).

Some researchers have postulated that antigenic priming of the immune system after the first vaccine dose might contribute to increased reactogenicity following the subsequent antigenic exposure (378,379). Therefore, the hypothesis of immune priming and its relationship with immunogenicity and reactogenicity after the second dose has biological plausibility and previous confirmatory clinical data. Our results from the main analysis, and other published results (376), do not highlight a specific dose predominance; nevertheless, the fact that the analysis of subpopulation 2 (N=3563) showed an association with the second dose suggests that the discrepancy may be due to the limited sample size, especially considering that the studies that yielded a positive association towards second dose (377,378) did include greater sample sizes. Noteworthy, most of these studies included participants vaccinated with BNT162b2. Given that mRNA-1273 has been shown to be more reactogenic than BNT162b2, it would be valuable to assess dose predominance with mRNA-1273 in larger future cohorts.

Williams EC et al. observed that both local and systemic symptoms following the first and second dose were predictive of higher antibody titres after vaccination (380). Other studies have also found a positive association between local and systemic ARs and the development of immunogenicity after vaccination with BNT162b2 (379,381). Additional research, such as

that performed by Yun Lim et al., only found a positive association with local ARs (382). Notwithstanding, most of the reported studies, including the VigilVacCOVID study and others with large sample sizes, described an association with mostly systemic symptoms rather than local ARs.

From a mechanistic point of view, local ARs typically arise from the activation of immune cells in the local tissue, such as macrophages, dendritic cells, and mast cells, triggered by the adjuvant or LNPs used in mRNA vaccines to stabilise the mRNA. Systemic reactogenicity, on the other hand, is associated with the release of inflammatory mediators or products into the bloodstream or through the activation of the immune system by the protein serving as the antigen (e.g., spike protein) (383). These results are relevant since they may suggest that, although reducing the reactogenicity associated with adjuvants is important, future research should rather focus on identifying the biological bases responsible for generating adaptive immunity and reactogenicity, such as the mRNA construct, in order to develop constructs that are both immunogenic and minimally reactogenic.

Furthermore, in the VigilVacCOVID study, although sensitivity analyses revealed some significant differences in severe ARs, especially in subpopulation 2, we did not formally observe an association with severity in the primary study population. While some previous authors have suggested an association with AR severity (373,374,377), others have primarily reported an association with specific types of systemic ARs (369–372), rather than an association with AR severity. Therefore, a definitive positive association with AR severity or intensity cannot be firmly established.

Alternatively, other authors have found negative results after vaccination with ChAdOx1 and BNT162b2, with sample sizes of 67 HCPs (384), 80 HCPs (385), and 135 healthy adults (386), while others have only observed some association with AR intensity but not frequency (387), a weak correlation (388), or an association only in male but not female vaccinees (389). It can be assumed that, in these studies, the inclusion of a small sample size may have influenced the attainment of negative results. However, studies with larger sample sizes have also yielded negative results. Maeda K et al. evaluated 225 healthy individuals vaccinated with BNT162b2. The authors reported that the BNT162b2-elicited immune response had no significant association with ARs (390). Likewise, Coggins SA et al. analysed 206 persons vaccinated with BNT162b2. The authors found no correlation between vaccine-associated

symptom severity scores and vaccine-induced antibody titres one month after vaccination(391). Differences may be attributed to the studied population and limitations inherent to the study designs. Taking all studies together and the results afforded by large cohorts, we can conclude that there is a growing body of evidence supporting a positive association; however, the question still needs to be further assessed in large real-world cohorts.

As regards cellular immunogenicity, in the VigilVacCOVID study, a higher frequency of some solicited ARs, such as malaise, chills, arm pain, myalgia, and severe headache, was observed amongst vaccinees with positive cellular immunogenicity. However, the association was not statistically significant, probably because of the limited data available on cellular immunogenicity. Few studies have assessed both humoral and cellular immunogenicity. Choi H et al. analysed immunogenicity and reactogenic responses in 80 HCPs after a second dose of ChAdOx1 nCoV-19 (AZD1222). They found that the rash of the first shot and chills of the second shot were associated with humoral immunity. However, comprehensive ARs had no correlation with humoral and cellular immunity (385). Lim SY et al. also studied the immunogenicity and reactogenicity of AZD1222, together with BNT162b2, in 131 HCPs (382). The degrees of the local reactions after the first and second dose of AZD1222 were significantly associated with antibody titres, whereas those after the BNT162b2 vaccination were not. T-cell responses did not exhibit significant associations with either AZD1222 or BNT162b2. Likewise, Bauernfeind S et al. analysed immunogenic responses in 735 persons vaccinated with BNT162b2. Strikingly, the authors found a positive association between severe ARs and antibody titres in men but not in women. The authors did not find an association with cellular immunogenicity in either sex (389). However, they reported a higher proportion of positive ELISpot results in the AR group but could not show significance. They interpreted these results in the context of a small sample size to detect a slight difference. This could have been the case in the VigilVacCOVID study, with a smaller sample size of N=59 vaccinees with results on cellular immunogenicity.

Notably, few studies have examined both humoral and cellular immunogenicity in cohorts vaccinated with BNT162b2 and mRNA-1273, as was done in the VigilVacCOVID study. Further prospective cohorts with a larger sample size should be studied to confirm the potential association between reactogenicity and cellular immunogenicity and possible

differences between vaccines. Cellular immunity operates in conjunction with humoral immunity to generate an adequate immune response capable of controlling SARS-CoV-2 replication, and it has been identified as an indicator of prior infection or the severity of COVID-19. Speletas et al. (369) found a clear correlation between anti-S anti-SARS-CoV-2 T-cell immune responses and the height of antibody responses. A key consideration is that humoral immunogenicity serves as a surrogate marker of T-cell response; indeed, immune memory cannot exist without T lymphocytes, and although uncommon, it is possible to observe immune memory in the absence of antibodies or B lymphocytes (392). While the humoral response may be considered a valid indicator of the relationship between reactogenicity and immunogenicity, it is important to evaluate both arms of adaptive immunity (humoral and cellular immunogenicity) whenever feasible, particularly in patients lacking an antibody response and/or those at high risk for severe COVID-19. However, the lack of standardised protocols for the concomitant assessment of the two arms of adaptive immunity can be a limitation in some settings. Humoral immunogenicity can be a valid marker in these scenarios.

Notwithstanding, the precise role of cellular immunity and its relationship to humoral responses following vaccination has yet to be fully understood. Some studies of vaccine adjuvants suggest that there is a correlation between reactogenicity and humoral and cellular immune responses; however, the results are inconsistent (361,386,393). This association still remains to be elucidated in COVID-19 vaccines.

Very few studies have addressed the association between immunogenicity and reactogenicity from the perspective of vaccine comparison. In the VigilVacCOVID study, in addition to evaluating the association between immunogenicity and reactogenicity in the overall population, we performed an exploratory analysis to assess differences between vaccines (BNT162b2 vs mRNA-1273) and populations (HCPs and SOTR). We identified reduced immunogenicity and reactogenicity in SOTRs vaccinated with mRNA-1273 compared with HCPs who received the same vaccine. Reactogenicity was more pronounced in HCPs vaccinated with mRNA-1273. However, recognising that some reactogenic responses may arise from vaccine formulation rather than from immunogenicity alone, the disparities in reactogenicity between HCPs and SOTRs support our initial hypothesis of a potential association between immunogenicity and reactogenicity, which was formulated based on the

results from our first study. Regarding the exploratory analysis focused on vaccine types (mRNA-1273 vs BNT162b2), data obtained from HCPs vaccinated with both types of vaccine showed similar immunogenicity rates (98.5% vs 99% of vaccinees with positive antibody titres, respectively). However, reactogenicity seemed to be greater with mRNA-1273 vaccines than with BNT162b2 (the proportion of any ARs after vaccination was 97.4% vs 81.7%, respectively), especially concerning severe AR rates (61.8% vs 34.2%, respectively). These results may indicate that a proportion of observed reactogenicity is not due to elicited immunogenicity but to local inflammatory responses caused by the formulation (e.g., the LNP or excipients).

From an immunobiological point of view, in previous sections, we described that the immunogenicity and reactogenicity of COVID-19 mRNA vaccines are believed to be influenced by the MDA5–IFNAR1 signalling triggered by the mRNA component of the vaccine, the adjuvant properties of LNPs included in the vaccine formulation, and/or the S protein encoded by the vaccine (208,211,295). However, the immunobiological mechanisms that characterise the association between reactogenicity and immunogenicity still remain to be fully elucidated. On a preliminary basis, some researchers have reported that IL-15, IFN- γ , and IP-10 levels were correlated with SARS-CoV-2 spike antibody responses following BNT162b2 vaccination (394). Likewise, others revealed that IFN- γ and chemokines induced by IFN- γ (such as IP-10, MCP-1, and MIG) are associated with both neutralising antibody titres and systemic ARs (395). Therefore, it is likely that these cytokines are not effective as selective biomarkers for differentiating between the immunogenicity and reactogenicity of mRNA vaccines. Overall, the association between systemic ARs and adaptive immune responses still needs to be clarified (207).

On the other hand, the analysis of the activation of the innate immune system in the context of SARS-COV-2 vaccination has yielded interesting results. Arunachalam et al. showed that gene signatures associated with monocytes—induced by the BNT162b2 mRNA vaccine—correlated with neutralising antibody titres three weeks following the administration of the second dose of the BNT162b2 vaccine (396). Likewise, Syenina et al. highlighted the role of natural killer (NK) cells in the context of reactogenicity and immunogenicity. In their work, they found that a higher expression of genes associated with T and NK cell exhaustion and suppression at baseline before vaccination was positively correlated with the development of

moderately severe fatigue after BNT162b2 vaccination. Similarly, an increased expression of these genes in response to vaccination was associated with higher levels of innate immune activation one day post-vaccination (294).

It is interesting to further explore the role of NK cells and their association with reported systemic ARs. Indeed, some authors have observed that the dynamics of CD16⁺ NK cells appear to be associated with sustained and comprehensive antibody responses following both mRNA and recombinant protein COVID-19 vaccinations (207). In accordance with these findings, researchers have demonstrated that the early dynamics of NK/monocyte subsets (including CD16⁺ NK cells, CD56^{high} NK cells, and nonclassical monocytes), as well as dendritic cell subsets [DC3s and CD11c[−] Axl⁺ Siglec-6⁺ (AS)-DCs], along with NKT-like cells, serve as distinct cellular correlates for neutralising antibody titres, the severity of ARs, and both factors, respectively (395).

DC3s are recognised as a subset specifically involved in the production of pro-inflammatory cytokines (such as TNF, IL-1 β , IL-6, IL-12, and IL-23). Several of these cytokines possess pyrogenic properties and enhance inflammation by differentiating antigen-stimulated T cells into Th1 or Th17 subsets, potentially contributing to the exacerbation of systemic ARs (207). Additional research is required to enhance the efficacy and safety of mRNA vaccines, as definitive studies are still lacking, for instance, on the functional analysis of cellular subsets that seem to be key in the immunogenic/reactogenic balance, such as CD11c[−] AS-DCs.

5.5.2 Strengths and limitations

The study offers new insights into the potential link between immunogenicity and reactogenicity in mRNA vaccines, including the following: i) an exploration of both humoral and cellular immunogenicity, ii) a methodology for assessing both the development and quantity of immunogenicity, iii) the inclusion of a large vaccinated cohort composed of HCPs and immunocompromised patients that can be more reflective of typical vaccination practices, iv) the assessment of two mRNA vaccines, and v) the evaluation of a wide range of local and systemic reactions with the additional consideration of symptom intensity. These insights are the main strength of this second part of the VigilVacCOVID study.

Translating our findings into clinical practice, fever, myalgia, and malaise are systemic symptoms that arise rapidly following vaccination and can be readily identified. Additionally,

these symptoms occur with notable frequency, as evidenced by the EMA's identification of 1.7 million spontaneous reports of suspected ARs among nearly 768 million vaccine doses administered in Europe (141), with fever and myalgia being two of the most commonly reported ARs in the European database. The ideal future vaccine should be one that is immunogenic and poorly reactogenic, however, current vaccine constructs elicit several local and systemic ARs. The promising part is that these ARs are mild and transient, and some, such as fatigue, fever, and malaise, although they may afford temporary discomfort, are also associated with the development of protective immunity. In some settings with limited resources, such as middle-low-income countries with restricted access to immunological testing, the development of reactogenicity can be used as a proxy for immunogenicity. Moreover, we believe that our results can be impactful in advancing our understanding of the immunological correlates of mRNA vaccines and, consequently, in the design of future mRNA vaccine constructs. This is of growing significance due to the anticipated expansion of mRNA technology development across various clinical applications beyond SARS-CoV-2 vaccines (397).

Our study has several limitations. Firstly, safety data were based on reactogenicity results from the VigilVacCOVID study. Therefore, AR data may have limitations inherent to the observational and non-randomised nature of the VigilVacCOVID study, such as recall bias and potential confounders. In addition, AR data were obtained through a structured questionnaire, which can be a subjective method of assessment of certain ARs such as fatigue or malaise. Besides, in the first part of the VigilVacCOVID study, we inquired about the severity of the fever, but we did not include this variable in the severity assessment. We considered severe (grade 3) fever as a reported temperature higher than 38.5°C. On an exploratory basis and considering that fever is a marker of immune activation and systemic reactogenicity, we decided to include this variable in the analysis of this second part of the study.

Secondly, given that a prospective analysis of immunogenicity had not been planned during the first part of the VigilVacCOVID study and considering the retrospective and real-world nature of data collection, there were some design-related constraints that we had to take into consideration for the analysis of the results. On the one hand, the method used to measure humoral immunogenicity in clinical practice was semiquantitative and did not distinguish

between infection-related (nucleocapsid protein (NCP) IgG antibodies) and vaccine-related (neutralising IgG antibodies to the receptor-binding protein of the S1 subunit of the spike protein) neutralising antibodies. Likewise, SARS-CoV-2 tests were not routinely performed before and after vaccination.

On the other hand, in this initial population with available immunogenicity data (N=3563), some immunity assessments had been performed either within a four-month period after or prior to vaccination. We considered that positive antibody titres of these distant measurements were not representative of ARs occurring immediately after receiving vaccine doses, nor were they necessarily related to vaccination. For instance, the possibility existed that some of the included participants developed immunogenicity before or after vaccination because of SARS-CoV-2 infection and not specifically due to vaccination. To adjust these biases, we decided to perform two sensitivities. We first refined the initial population by restricting the immunogenicity assessment timeframe to four weeks, and we defined subpopulation 2 for sensitivity analysis 2 (N=597). Further, we excluded vaccinees who had immunogenicity data prior to vaccination (N=215). This was considered the most sensitive and least-biased population, and for this reason, it was considered the main population for analysis. We subsequently redefined the initial population (N=3,563) as subpopulation 1, and we considered the analysis of this population as sensitivity analysis 1. The main aim of this sensitivity analysis was to assess the consistency of results across analyses with a higher sample size. Despite the limitations related to the four-month study timeframe, one should take into consideration that previous authors had found an association between ARs and immunogenicity even at 74 days post-vaccination (370). Therefore, we consider that results obtained in a four-month timeframe in sensitivity analysis 2, with a higher sample size of 3,563 vaccinees, are still informative.

Furthermore, we observed that a higher frequency of vaccinees with positive cellular immunogenicity developed some solicited ARs, such as malaise, chills, arm pain, myalgia, and severe headache. However, the results were not significant, both in AR rate and intensity and in the assessment of solicited and unsolicited ARs. We acknowledge that our sample size for this assessment was rather small (n= 59 vaccinees with testing performed), given that cellular immunogenicity testing was not standard practice for all vaccinees at the time of the vaccination campaign.

As regards the immunogenicity assessment method, IgG titres were only measured in 322 vaccinees as this test was not standard practice at the start of the vaccination campaign, when immunogenicity data were retrieved. In addition, IgG titres were measured on a different platform than the one used for total antibody titres. Hence, we decided to use only total semiquantitative antibody titres (IgM + IgG) for the immunogenicity analysis and not IgG titres. For the immunogenicity analysis, we defined antibody positivity considering a cut-off of >1.0 . Given that antibody titres were measured with a semiquantitative method on a scale of 0 to >10 , we calculated the median value of the titre level (second quartile, Q2) and analysed the distribution of vaccinees above and below the median to conduct a semiquantitative assessment that considered the extent of elicited humoral immunogenicity. Therefore, our observations related to the association between reactogenicity and the extent or quantity of immunogenicity should be interpreted within the context of a semiquantitative scale.

Several previous studies have suggested that mRNA-1273 is slightly more effective than BNT162b2 in producing higher levels of antibodies (398–401). However, in the exploratory between-vaccine comparison conducted in the VigilVacCOVID study, data from HCPs vaccinated with both types of vaccines demonstrated similar immunogenicity rates (98.5% vs 99% of vaccinees with positive antibody titres, respectively), whereas reactogenicity appeared to be higher with mRNA-1273 compared with BNT162b2, particularly in terms of severe AR rates. This discrepancy with previous studies, observed in terms of immunogenicity levels, can be due to differences inherent to both HCP populations and to the aforementioned limitations regarding immunogenicity assessment and RWD analysis. It should also be noted that, in the opinion of some authors, literature published regarding differences in immunogenicity between both vaccines is limited, and the methods used are not directly comparable (402). Therefore, while it is true that the comparable immunogenicity observed in the VigilVacCOVID study may be partially due to inherent biases, future prospective and comparative real-world studies are needed to unveil potential differences in immunogenicity between mRNA-1273 and BNT162b2, as a first step to fully characterise differences in terms of the reactogenicity/immunogenicity balance.

Conversely, in the exploratory immunogenicity and reactogenicity analysis between populations, we observed a lower rate of immunogenicity in SOTRs compared with HCPs,

both vaccinated with mRNA-1273 (57% vs 98.5% of vaccinees with positive antibody titres, respectively), as well as a reduced rate of reactogenicity in SOTRs (proportion of vaccinees with at least one AR: 87.4% vs 97.4%, respectively; proportion of vaccinees with at least one severe AR: 28.2% vs 61.8%, respectively). These results support the hypothesis of an association between immunogenicity and reactogenicity.

However, the observation of similar immunogenicity results, with higher reactogenicity of mRNA-1273 in the analysis between vaccines, can question this hypothesis. Firstly, it should be noted that the analysis of vaccines and populations was only exploratory, and the main reactogenicity and immunogenicity analysis was performed on the overall population. Hence, these results should be interpreted as exploratory, and the contribution of different populations or different types of vaccines to overall immunogenicity and reactogenicity remains unclear. Notably enough, most previous studies were conducted in HCPs and immunocompetent vaccinees. Therefore, this question should be further addressed in future projects with larger cohorts of immunosuppressed patients. Secondly, the higher reactogenicity rate observed in HCPs, despite comparable immunogenicity, can be explained by this higher proportion being mainly produced at the expense of local inflammatory responses and local ARs rather than elicited adaptive immunity. In turn, the lower immunogenicity observed in SOTRs, associated with reduced reactogenicity, suggests that, in addition to a decrease in local reactions, adaptive immunity plays a role in the generation of reactogenicity. However, it is important to note that it is currently unclear which component of immunity (adaptive or local) is associated with clinically measured reactogenicity and, more importantly, what cellular and molecular mechanisms underlie this reactogenicity. It is crucial to delve deeper into this issue in future studies to elucidate the true association between immunogenicity and reactogenicity.

By and large, we can conclude that the data show a positive association between immunogenicity and reactogenicity in the overall population and, from an exploratory point of view, this association is also suggested in the comparison between HCPs and SOTRs, thus reinforcing the initial hypothesis. However, further analyses will need to be conducted to elucidate potential differences between populations and vaccine types in terms of immunogenicity and reactogenicity. Moreover, despite acknowledging the inherent limitations associated with data sources, data types, and the timing of determinations, we

emphasise that these constraints stem from the processes involved in the acquisition of RWD. Our data accurately represent the standard clinical practices in place during the period of rapid implementation of the vaccination campaign in response to the urgent circumstances of the SARS-CoV-2 pandemic. Similarly, safety monitoring protocols were promptly established to ensure comprehensive vaccine surveillance for recipients. Immunogenicity assessments, however, were not part of routine procedures at that time and, therefore, could not be conducted alongside vaccinations. Given that the data were collected within the constraints of standard practice, we believe the results are both representative and generalisable to the broader population receiving routine vaccinations in healthcare settings. The methodology employed in our study reflects the processes of data acquisition and analysis within a real-world clinical practice framework, offering valuable insights for the design of future RWD studies, particularly those conducted during emergency mass vaccination campaigns utilising data derived from routine clinical practice.

Finally, a significant association was observed for some ARs, however, this association occurred among a higher proportion of vaccinees in the negative immunogenicity group. The clinical significance of this finding is unclear to us, and it should be explored in future studies where systemic and local ARs are thoroughly assessed.

5.6 Future perspectives

Additional research is required to clarify the immunological mechanisms underlying the reactogenicity of mRNA vaccines and to determine whether protective immunogenicity can be, at least partially, separated from the factors that contribute to reactogenicity in the current formulations of mRNA vaccines. Studies on the immunological correlates of reactogenicity in humans are sparse and preliminary, producing variable outcomes depending on the vaccine type, with no definitive conclusions reached. It should be noted that each vaccine platform may elicit a different reactogenicity/immunogenicity balance, and results reported by other vaccine platforms, such as the HPV-16/18 (366) or herpes zoster vaccines (368), may not apply to new emergent vaccine platforms, like mRNA vaccines. In this sense, our study and others suggest a positive association between reactogenicity and the development and quantity of immunogenicity after vaccination. This association seems to be linked to the development of anti-spike protective immunogenicity (which is the type of immunogenicity measured in the studies) and should not be generalised to other types of adaptive immunity

or autoimmunity pathways. For instance, recent reports have suggested that autoantibodies targeting type I IFNs develop after vaccination with mRNA SARS-CoV-2 vaccines but not with viral vector-based vaccines (403,404). While these autoantibodies have been associated with ARs in other vaccines, such as the live-attenuated yellow vaccine (405), such an association has not been observed in SARS-CoV-2 vaccines (406,407).

In the same way, the results obtained with BNT162b2 may not directly apply to mRNA-1273. Few studies have examined the reactogenicity/immunogenicity balance in mRNA-1273, and fewer have performed comparative studies between mRNA-1273 and BNT162b2, and other types of vaccines. The information acquired so far is still informative and should guide the implementation of future prospective studies where this balance is characterised in a comparative manner. These studies will also need to assess the balance in the context of adapted vaccines to new VOCs, as well as heterologous vaccination schemes.

In the VigilVacCOVID study, we assessed, in an exploratory manner, a wider study population (HCPs and SOTRs), two mRNA vaccines (BNT162b2 vs mRNA-1273), and both immunity arms (humoral and cellular). However, little is currently known about the reactogenicity/immunogenicity balance in special populations, such as children, pregnant women, and patients with comorbidities, such as SOTRs and patients with autoimmune diseases. Of note, most studies assessing the reactogenicity/immunogenicity balance have focused on HCPs and other healthy participants. Therefore, further studies are needed with monovalent and bivalent-adapted vaccines. These data can be impactful in advancing our understanding of the immunological correlates of mRNA vaccines and, consequently, in the design of future mRNA vaccine constructs. Furthermore, as we stated in the previous subsection, future prospective and comparative real-world studies are required to uncover potential differences in immunogenicity between mRNA-1273 and BNT162b2.

Additionally, Jorda A et al. recently assessed the association between reactogenicity and immunogenicity after booster vaccination in 484 HCPs vaccinated with BNT162b2 (408). The authors observed a weak but statistically significant correlation between the severity of systemic symptoms and 28-day antibody levels. In their view, the absence of mild symptoms does not indicate an inadequate immune response, and the severity of symptoms cannot be used to predict the antibody response following booster vaccination with BNT162b2. Actually, the preliminary results of the VigilVacCOVID study and others, on their own, are

not sufficient to build a valid prediction signature. Importantly, immunogenicity is a heterogeneous multimodal clinical entity that can be explained by several susceptibility factors other than reactogenicity. Likewise, reactogenicity is also a multimodal clinical entity that cannot be explained alone by age, sex, or previous SARS-CoV-2 infection. Future studies are needed to identify different predictive risk factors of reactogenicity and immunogenicity and to use these factors to build and validate a prediction signature. This tool may be helpful when planning immunogenicity testing programs, vaccination campaigns, and closer monitoring programs in special patient subgroups in clinical care centres. The data of our study and others, which reveal an association between immunogenicity and reactogenicity, can contribute to the creation of this signature in further dedicated studies.

As mentioned in previous subsections, significant progress has been made towards the understanding of the biological basis of mRNA-mediated immunogenicity and reactogenicity. In previous studies, the role of MDA5–IFNAR1 signalling triggered by the mRNA component of the vaccine, the adjuvant properties of LNPs included in the vaccine formulation, and/or the S protein encoded by the vaccine has been purported. In addition, certain chemokines, such as IFN- γ , and groups of cells, like NK/monocyte subsets, DC subsets, and NK T-like cells, have been shown to be involved in mRNA vaccine effects (increasing neutralising antibody titres) and ARs. These results suggest that mRNA-induced high VE can be closely related to reactogenicity and ARs reported as post-vaccination symptoms. Notwithstanding, further work needs to be implemented to fully understand the biological basis of these processes. This knowledge is essential for the design of future vaccine constructs.

There are two concerns that need to be addressed in the future. Firstly, the toxicity of excipients and adjuvants like LNPs. Lipids have previously been demonstrated to stimulate host immune responses after vaccine administration. Moreover, immune reactivity to repeated administrations of PEG-based nanoparticles, driven by complement-mediated mechanisms, may have adverse effects on both safety and efficacy profiles, as we described in previous sections (212). The physical and chemical characteristics of LNPs, including their morphology and charge, influence their interactions with the immune system. Previous research demonstrated that LNPs, functioning as an inherent adjuvant, trigger robust antigen-specific CD4⁺ T follicular helper (TFH) cell and germinal centre (GC) B-cell

responses through the induction of IL-6 production. Notably, strong immunogenicity is frequently linked to reactogenicity, leading to both local and systemic inflammation (210). Further understanding of the molecular mechanisms by which adjuvants, such as lipid components, activate the immune system is crucial for ensuring the efficacy and safety of mRNA vaccines.

In previous sections, we stressed the fact that the development of PEG-free delivery systems could be a suitable pathway for the advancement of future mRNA vaccines. In this context, investigating the properties of diverse materials, including synthetic, natural, and zwitterionic polymers, is recommended for creating safer and more efficient mRNA delivery platforms. These materials have shown potential for innovative and promising medical applications, surpassing those of PEG. Nevertheless, it remains unclear whether they can offer therapeutic benefits without triggering adverse immune responses or other types of side effects. The development of LNPs with balanced immunogenicity and reactogenicity remains an active area of research.

Regarding RNA molecules, two technical strategies are commonly employed to mitigate the immunogenicity induced by exogenous RNA. The first approach involves chemically modifying mRNA molecules with pseudouridine (ψ) or N1-methylpseudouridine (m1 ψ), which enables the mRNA to evade innate immune detection triggered by the interaction of exogenous mRNA with TLR7. Secondly, chromatographic purification can mitigate immune responses and improve translational efficiency by removing double-stranded RNAs, including viral genome analogues, during intracellular mRNA production (355). However, the process may still require optimisation, as innate responses are still elicited despite chemical modifications, and the inflammatory cytokines generated have been shown to enhance adaptive immune responses. Thus, additional adjustments in the levels of base modifications may be needed in the future to achieve a balance between exogenous mRNA translation and innate immune activation in mRNA vaccination.

Moreover, to advance the investigation of the causes of ARs following mRNA vaccination, it is essential to develop mouse models capable of precisely replicating and assessing human adverse symptoms. One of the most important challenges to be resolved are the differences between humans and experimental animals and the development of an animal model that can mimic human responses in terms of immunogenicity and reactogenicity (207).

On the other hand, some authors have suggested that altering the route of vaccination may also affect the balance of immunogenicity and reactogenicity. In a murine model, subcutaneous vaccination with BNT162b2 led to reduced weight loss compared with intramuscular vaccination, which suggests a lower degree of reactogenicity in animal models. Significantly, antibody responses were not diminished by subcutaneous administration; in fact, this method may have enhanced the cytotoxic CD8⁺ T-cell response (294). The subcutaneous approach resulted in a higher level of neutralising antibodies in nonhuman primates vaccinated with the attenuated vaccinia virus compared with the intradermal and intramuscular routes. Conversely, polyfunctional CD8⁺ T-cell responses are more effectively elicited through intradermal or intramuscular injections (409). Thus, the vaccination route should be optimised based on the specific type of immunity intended to be stimulated. The reactogenicity/immunogenicity balance should be assessed in different vaccine administration models. Subcutaneous administration is an interesting model to be explored in future clinical trials.

While certain animal studies have shown that LNP-mRNA remains at the injection site for more than 24 hours post-injection, direct comparisons of trafficking kinetics, clearance, and proinflammatory cytokine expression between different mRNA vaccines and other platforms are necessary. Preliminary studies have observed some differences in vaccine persistence according to the platform used. For instance, protein-based vaccines utilise the saponin-derived Matrix-M adjuvant to improve the immunogenicity of the protein antigens. After the administration of the Matrix-M–adjuvanted vaccine, both saponins and the antigen become undetectable within 24 hours post-injection as they are transported to the draining lymph nodes, resulting in a significant reduction in local cytokine expression by 48 hours (410). This mean residence time can be higher for mRNA vaccines. Whether the time of persistence of the vaccine can influence, for example, the development of long-term immunogenicity and long-term ARs is a matter of debate.

5.7 General discussion

The COVID-19 pandemic has been unprecedented in numerous aspects, including numerous efforts and international collaborations to allow the rapid and safe development of the first SARS-CoV-2 vaccines. In addition, efforts have been undertaken to draw insights from prior pandemics and epidemics. The key lessons related to safety surveillance have focused on addressing public concerns about vaccine safety, emphasising the need for robust vaccine safety monitoring systems capable of delivering timely and effective communication. The standards for proving both efficacy and safety are generally more stringent than with other therapeutic medical products, owing to the distinct characteristics of the target population, which is a healthy one that takes the vaccine shot to prevent a severe and fatal disease. Besides, RNA-based vaccines were a new platform that was administered to millions of persons worldwide in a short period of time during the vaccination campaign. Therefore, various national and international regulatory authorities leveraged their established pharmacovigilance systems as the foundation for monitoring the safety of COVID-19 vaccines while also conducting public information campaigns.

Within pharmacovigilance systems, passive surveillance allows the collection and assessment of unsolicited reports of suspected adverse events, typically submitted as individual case safety reports (ICSRs) to a central database or health authority. This method forms the foundation of safety monitoring in immunisation programs, allowing for the identification of rare events, assessment of report clusters, and detection of safety signals that warrant further investigation. Nevertheless, spontaneous reporting is subject to various limitations. One significant issue is the absence of denominator data, which impedes the rapid estimation of vaccine uptake (i.e., the number of individuals vaccinated) within the target population. Furthermore, the lack of denominator data poses challenges when conducting signal-strengthening analyses, such as observed-to-expected (O/E) analyses, where the observed number of cases representing the signal is compared against an anticipated number of cases.

In the context of the COVID-19 vaccination campaign, passive surveillance needed to be supplemented by active surveillance and post-authorisation safety studies to estimate event rates and support risk assessment. Enhancements to existing systems were implemented, such as the introduction of mobile reporting applications to facilitate, encourage, and track spontaneous reporting. Additionally, in numerous regions, active surveillance measures were

either introduced or intensified. One of the most frequently implemented study types was cohort event monitoring. Previously, cohort event-monitoring studies have been used as an early warning system that interviews patients on a certain treatment (the cohort) for capturing problems (the events) with new medicines in public health programmes (172).

The Pharmacovigilance Program of the Hospital Clínic, as part of its services and clinical tasks, designed a pharmacovigilance plan to monitor the safety of vaccines administered to HCPs and patients at the Hospital. Within this pharmacovigilance plan, the Program decided to initiate VigilVacCOVID as a cohort event-monitoring study to provide real-world safety data on the two mRNA vaccines used at the time (BNT162b2 and mRNA-1273) and the two populations included in the vaccination campaign and the pharmacovigilance plan, HCPs and SOTR. The main objective of this study was to provide additional safety data in a real-world setting to enrich the data collected in current pharmacovigilance systems and surveillance monitoring programs.

In the VigilVacCOVID study, we characterised the safety profile of both mRNA vaccines, and we observed that mRNA-1273 has greater reactogenicity than BNT162b2, in terms of a higher frequency of ARs and greater severity. These results were comparable to other real-world published studies. Besides, RNA-based vaccines were a new vaccine platform, and there were no existing data on some populations that had been underrepresented in pivotal clinical trials, such as immunosuppressed patients. In the VigilVacCOVID study, we compared the safety profile of HCPs and SOTRs, and we observed lower reactogenicity in the latter group. Other studies including immunosuppressed patients were published at the time, or subsequently, and they also reported a lower reactogenicity in this vaccinee group. In addition, we reported that age, sex, previous SARS-CoV-2 infection, and previous drug allergies were independent risk factors of reactogenicity, both in HCPs and SOTRs. These data were in line with other reports, although some risk factors, such as previous SARS-CoV-2 infection and previous drug allergies, had been poorly explored outside the VigilVacCOVID study. These results led us to formulate the hypothesis that there is a link between reactogenicity and elicited immunogenicity. This hypothesis was tested in the second part of the VigilVacCOVID study, where we demonstrated a positive association between reactogenicity and immunogenicity, particularly for certain subsets of systemic ARs, such as fever, malaise, and myalgia.

The causality assessment of ARs following immunisation primarily relies on four key criteria: consistency (supported by evidence from peer-reviewed literature), biological plausibility, temporality, and specificity. The causality assessment differs from that of conventional medicines due to the distinct immunological mechanisms underlying vaccines, compared with the mechanisms of action associated with therapeutic or diagnostic medicinal products. Although many COVID-19 vaccines are administered in two or more doses, there are limited opportunities to evaluate rechallenge, as reports often emerge after the second dose or between doses, typically with insufficient follow-up data regarding the effects of subsequent doses. In the context of newly developed vaccines, causality assessment is further complicated by the absence of sufficient evidence and the lack of data needed to properly assess biological plausibility. The emergence of data regarding the association between reactogenicity and immunogenicity supports the assessment of the biological plausibility of reported symptoms post-vaccination. In addition, one of the main limitations of vaccine immunosurveillance is the emergence of long-term ARs with a late time-to-onset. The current absence of biological markers specific to vaccination complicates the assessment of causality, especially when temporality is not assessable in long-term ARs. Therefore, further studies need to be performed to characterise the immunobiological basis of vaccination and reactogenicity. The kinetics of vaccine constructs, as well as elicited adaptive immunity (such as NK or dendritic cells), are an interesting and active area of research. Additional insight into these processes may help to elucidate the biological basis of long-term ARs reported after vaccination.

Of note, vaccines are generally considered immunobiological products (173). While pharmacovigilance of immune-mediated ARs, known as immunovigilance, has broadly focused on several types of immunotherapies, such as checkpoint inhibitors (411), vaccine safety has traditionally been kept aside. The experience from the previous pandemic has taught us that it is essential to establish and maintain well-organised multidisciplinary teams dedicated to pharmacovigilance, with an active focus on vaccine immunovigilance. At the local level, the Pharmacovigilance Program of the Hospital Clínic is an illustrative example of multidisciplinary collaboration. The close work between Clinical Pharmacology, Pharmacy, Preventive Medicine, Occupational Medicine, Immunology, Allergology, and Dermatology, as well as robust institutional support, enabled the implementation of a vaccine pharmacovigilance plan and the conduct of the VigilVacCOVID study.

Undoubtedly, the implementation of active surveillance systems requires an increase in resources and expertise. The Pharmacovigilance Program is a local example of the several international efforts that have been undertaken to implement and conduct these pharmacovigilance systems, in collaboration with a wide range of stakeholders. Ultimately, the COVID-19 pandemic and the vaccination campaign have highlighted the crucial lesson that a localised infection or epidemic can rapidly escalate and spread to all corners of the globe, and it is essential to be prepared in advance.

6 Conclusions

In summary, the work presented in this thesis has contributed to providing a deeper characterisation of the safety profile of mRNA vaccines. The implementation of the **VigilVacCOVID study** allowed the provision of additional evidence on the safety profile and immunogenicity of vaccination. This is a prospective comparative study where two vaccines (BNT162b2 and mRNA-1273) and two different populations (HCPs and SOTRs) vaccinated with mRNA-1273) were compared using the same structured questionnaire.

Overall, the findings from the **VigilVacCOVID study** support the following **conclusions**:

1. The implementation of the VigilVacCOVID study, conducted as an active pharmacovigilance project and designed as a cohort event-monitoring study, provided additional real-world evidence on the safety and immunogenicity of BNT162b2 and mRNA-1273. From this main conclusion, the following supporting intermediate conclusions can be drawn:

1.1 Local ARs, such as injection site pain and arm pain, and systemic ARs, such as fatigue, headache, and fever, were the most frequently reported solicited ARs for both vaccines. Most of these ARs were of mild severity.

1.2 The safety profile assessed in a real-world setting was mostly comparable with that reported in pivotal clinical trials in terms of overall reactogenicity, severity rate, and types of reported ARs.

1.3 Vaccination with mRNA-1273 showed greater reactogenicity and severity than BNT162b2, which is in line with previous studies. Both vaccines maintained an overall adequate tolerability profile, thereby supporting existing evidence.

1.4 Solid-organ transplant recipients have lower reactogenicity after vaccination with mRNA-1273, evidenced by a lower proportion of ARs and a milder severity of these ARs.

1.5 Age, sex, previous SARS-CoV-2 infection, and previous drug allergies were identified as risk factors for reactogenicity after vaccination.

1.6 Severe systemic ARs, such as fatigue, headache, myalgia, malaise and arthralgia, are more frequent after the second dose. No relevant differences are observed after the second dose for severe local ARs.

2. There is a significant association between the development of systemic ARs, especially the subset comprising fever, myalgia, and malaise, and elicited humoral immunogenicity.

2.1 There appears to be a slight trend, although not significant, suggesting a possible association between the development of some ARs—malaise, chills, arm pain, myalgia, and severe headache—and cellular immunogenicity. These results should be explored in-depth in future studies.

3. Vaccination with mRNA-1273 and BNT162b2 in HCPs showed similar immunogenicity rates, while reactogenicity seemed to be greater with mRNA-1273 than with BNT162b2, especially concerning severe AR rates. SOTRs vaccinated with mRNA-1273 exhibited decreased humoral immunogenicity and less reactogenicity compared with HCPs vaccinated with mRNA-1273. These results should be considered exploratory and should be further explored in future studies.

4. There is no association between immunogenicity-reactogenicity in terms of AR severity or vaccine doses.

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