Real-World And Genetic Evidence For The Management Of Opioid Epidemic And COVID-19 Pandemic: Population-Based Studies

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Trinity 2023

Acknowledgements

Heartfelt gratitude goes to my doctoral supervisors/mentors, Professor Daniel Prieto Alhambra, Dr Antonella Delmestri, Associate Professor Sara Khalid, Professor Gary Collins and Prof Chris Norbury, who have been an extraordinary blend of deep wisdom and enduring patience, guiding me through this exciting journey. My appreciation for other professionals is also immeasurable, particularly for Professor Sarah Parish. Without their guidance and support, I wouldn't have had this incredible opportunity to live and study at Oxford.

My immense thanks go to UK Biobank. Firstly, to the selfless volunteers who made this fantastic resource available. Also, to the project and its advocates who displayed incredible foresight in creating a tool for the global research community. I am sincerely thankful to the Jardine and Clarendon foundations for funding me over these past four years, and I hope this work reflects my deep appreciation. Additional thanks go to my NDORMS department, the Queens' college and International Society for Pharmacoepidemiology for funding my academic travels.

I've had the privilege of spending these years in Oxford alongside amazing peers: Mike Du, Yuchen Guo, Xintong Li and Leena Elhussein, and colleagues: Dr Annika Jödicke, Martí Català Sabaté, Ed Burn, Albert Prats-Uribe, Danielle Newby, Eng Hooi (Cheryl) Tan, Marta Pineda, Maria Sanchez Santos, Theresa Burkard. A nod to our shared laughter, unending support for each other, and enjoyable biscuits by the side of the magical "red box". A special shout-out to the many enlightening hours spent at The Butcher's Arms pub!

Being at the University of Oxford has given me a fantastic chance to learn from the brightest minds in the world. Also, living in Oxford city has exposed me to a myriad of diverse societies, which has significantly cultivated me a more comprehensive

understanding of global cultures. As I reflect, I see countless individuals who've shaped my journey. For those accidentally left out, I owe you a spicy Chinese-style feast as an apology!

Finally, I would like to express my deepest gratitude to my parents and my beloved wife (Chunxiao Li). She has been my confidant, my ally, and my comforter, sharing in my struggles, my triumphs, and all the moments in between in past years. Her companionship, love, and belief in my capabilities have played an instrumental role in the successful completion of this DPhil thesis.

Junqing Xie, May 24, 2023

Abstract

Background: The widespread use of opioids and the emergence of SARS-CoV-2 represent two pressing public health crises that require evidence-based decision-making. This thesis comprises six interconnected studies aimed at translating large, routinely-collected and Biobank data into reliable evidence to inform the management of the ongoing opioid epidemic and COVID-19 pandemic.

Methods: Four data sources were studied: primary care records from SIDIAP (Spain), US claims, and the UK Biobank. Exposures included opioids, ibuprofen, COVID-19 vaccines, human leucocyte antigen (HLA) genes, and SARS-CoV-2 infections. Cohort studies were used as the primary design, complemented by statistical techniques including regression, propensity scores, survival analyses, and negative and positive control outcomes, as well as Mendelian randomization. Study outcomes were adverse drug events, COVID-19 susceptibility and severity, and related complications.

Results: Incident opioid use increased in Catalonia from 2007 to 2019, with Tramadol being the most frequently used opioid in 2019. Compared to codeine, tramadol was associated with a higher risk of all-cause mortality, cardiovascular events, and fractures. No differential risk of COVID-19 was observed among users of ibuprofen versus other analgesics. When compared to two doses of ChAdOx1, vaccination with BNT162b2 was associated with lower risks of COVID-19 infection and hospitalization, respectively, during the study period when the Delta variant was dominant. Six independent HLA alleles significantly affected antibody response to COVID-19 vaccines, and the aggregated genetic score had a strong, collective, and causal influence on breakthrough COVID-19. COVID-19 infection was associated with an increased risk of venous thromboembolism (VTE) within 30 days, with highest risk in unvaccinated individuals. People with older

age, male sex, obesity, and inherited thrombophilia were also at a higher VTE risk post-COVID-19.

Conclusion: The integration of real-world and linked biobank data can be effectively leveraged using advanced analytical tools to generate timely and actionable evidence to tackle global health crises like the ongoing opioids epidemic and COVID-19 pandemic.

Statement of contribution to work

In the development of this thesis, I took on responsibility for the conceptualization, formulation, and crafting of the key research questions addressed. The study design and planning were undertaken with considerable guidance from my principal supervisor, Professor Daniel Prieto Alhambra, and contributions from my other supervisors: Dr. Antonella Delmestri, Associate Professor Sara Khalid, and Professor Gary Collins. I performed all statistical analyses, except for Chapter 5, where a co-author executed the computational process, and I conducted critical code reviews. I also adapted code lists for medication and disease phenotypes previously established within the Pharmaco- and Device Epidemiology group, as seen in Chapters 6 and 8. Apart from those modified by journal editors in published works, I originated all initial drafts and subsequent revisions of the tables and figures presented in this thesis. I took the lead in writing each chapter and interpreting research findings, with invaluable inputs and support provided by my supervisory team.

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List of publications and presentations from this thesis

Main articles

- **Xie J**, Strauss VY, Martinez-Laguna D, et al. Association of Tramadol vs Codeine Prescription Dispensation With Mortality and Other Adverse Clinical Outcomes. *JAMA*. 2021.
- **Xie J**, Reyes C, Prieto-Alhambra D. Association of Tramadol vs Codeine Prescriptions With Mortality and Other Adverse Clinical Outcomes.—Reply *JAMA*. 2022.
- **Xie J**, Strauss VY, Collins GS, Khalid S, Delmestri A, Turkiewicz A, Englund M, Tadrous M, Reyes C and Prieto-Alhambra D (2022) Trends of Dispensed Opioids in Catalonia, Spain, 2007–19: A Population-Based Cohort Study of Over 5 Million Individuals. *Front. Pharmacol*. 2022.
- **Xie J**, Prats-Uribe A, Feng Q, et al. Clinical and Genetic Risk Factors for Acute Incident Venous Thromboembolism in Ambulatory Patients With COVID-19. *JAMA Intern Med*. 2022.
- **Xie J**, Prieto-Alhambra D. Is There a Role for Thromboprophylaxis in Selected Outpatients With COVID-19?—Reply *JAMA Intern Med*. 2022.
- **Xie, J.**, Feng, S., Li, X. et al. Comparative effectiveness of the BNT162b2 and ChAdOx1 vaccines against Covid-19 in people over 50. *Nat Commun*. 2022.
- **Xie J**, Prats-Uribe A, Gordillo-Marañón M, Strauss VY, Gill D, Prieto-Alhambra D. Genetic risk and incident venous thromboembolism in middle-aged and older adults following COVID-19 vaccination. *J Thromb Haemost*. 2022.
- **Xie, J.**, Brash, J.T., Turkmen, C. et al. Risk of COVID-19 Diagnosis and Hospitalisation in Patients with Osteoarthritis or Back Pain Treated with Ibuprofen Compared to Other NSAIDs or Paracetamol: A Network Cohort Study. *Drugs*. 2023.

- **Xie, J**., HLA Allelic variations, COVID-19 Vaccine Antibody Response and Breakthrough Clinical Outcomes in Population (In Preparation)

Posters and oral presentations

- Temporal trends of opioid use among incident osteoarthritis patients in Catalonia, 2007-2016: a population-based cohort study (**oral**). *Annals of the Rheumatic Diseases 2020*.
- Temporal trends of opioid use in the management of fracture-related pain in Catalonia, 2007-2016: a population-based cohort study (**poster**). *World Congress on Osteoporosis, Osteoarthritis and Musculoskeletal Diseases 2020*.
- Temporal trends and changes in indication for opioid use in Catalonia, 2007-2017: a population-based study of 6 million individuals (**oral**). *37th ICPE 2020*.
- Treatment duration estimation based on claims vs primary care prescriptions data: an analysis of opioid use in the SIDIAP database (**poster**). *37th ICPE 2020*.
- The impact of refill gaps on the estimation of persistence with opioids use (poster). 37th ICPE 2020
- Secular Trends of Dispensed Opioid Prescriptions in Catalonia, Spain, 2007-19: A Population-based Cohort Study of over 5 Million Individuals (**oral**). *38th ICPE 2022*.
- Genetic Risk and Incident Venous Thromboembolism in Middle-aged and Older Adults Following COVID-19 Vaccination 2022 (**oral**). *38th ICPE 2022*.
- Comparative effectiveness of the BNT162b2 and ChAdOx1 vaccines against Covid-19 in people over 50 (**poster**). *UK Biobank scientific conference 2022*.
- Associations between HLA variations and serological response to COVID-19 vaccination (**oral**). *39th ICPE 2023*.
- The impact of HLA-DQB1*06 alleles on COVID-19 vaccine effectiveness (**oral**). *39th ICPE 2023*.

Additional articles

- **Xie J**, Yuliang Feng et al. Contribution of genetics and lifestyle to the risk of major cardiovascular and thromboembolic complications following COVID-19, *Nat Commun (In revision)*
- Yunhe Wang, **Xie J**[corr], et al. Long-term mental health outcomes after SARS-CoV-2 infection: prospective cohort study, *Nature Human Behaviour (In revision)*
- Hermosilla, E., Coma, E., **Xie, J**. et al. Comparative effectiveness and safety of homologous two-dose ChAdOx1 versus heterologous vaccination with ChAdOx1 and BNT162b2. *Nat Commun,* 2022.

Related awards

- ISPE 2022 Stanley A. Edlavitch Award, named after the Society's first Executive Director, is presented at each annual meeting to the student who submits the most highly rated abstract.
- Jardine Outstanding Leaders Award, awarded to outstanding leadership contribution in Policy Change in Global Vaccination Strategies.

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List of abbreviations

ASMD	Absolute Standardized Mean Difference
ATC	Anatomical Therapeutic Chemical
CI	Confidence Interval
CNCP	Chronic Non-Cancer Pain
COVAX	Covid-19 Vaccines Global Access
COVID-19	Coronavirus Disease-19
COX-2i	Cyclooxygenase-2 inhibitors
EHRs	Electronic Health Records
ER	Extended-Release
FDA	Food and Drug Administration
GP	General Practitioners
GRS/PRS	Genetic risk score/polygenic risk score
HES	Hospital Episode Statistics
HICs	High-Income Countries
HLA	human leukocyte antigen
HR	Hazard ratio
ICD	International Classification of Diseases
ICPE	International Conference on Pharmacoepidemiology & Therapeutic Risk Management
IPW	Inverse Probability Weighting
IR	Immediate-Release
ITT	Intention To Treat
LICs	Low-Income Countries
MDRR	Minimum Detectable Rate Ratio
MERS-COV	Middle East Respiratory Syndrome Coronavirus
MME	Morphine Milligram Equivalents
NSAID	Non-Steroidal Anti-Inflammatory Drug

OMOP-CDM	Observational Medical Outcomes Partnership Common Data Model
OT	On-Treatment
OA	Osteoarthritis
OT	On treatment
PHE	Public Health England
PHEIC	Public Health Emergency Of International Concern
PS	Propensity Score
RADARS	Researched Abuse, Diversion, And Addiction- Related Surveillance
RWD	Real world data
RWE	Real world evidence
SAIL	Secure Anonymised Information Linkage
SARS-Cov-2	severe acute respiratory syndrome coronavirus 2
SGSS	Second Generation Surveillance System
SIDIAP	The Information System for Research in Primary Care
UK	United Kingdom
US	United State
VOC	Variant of concern
VTE	Venous thromboembolism
WHO	World health organisation

Chapter 1

Introduction

1. Introduction

1.1.Opioid epidemic

The opioid crisis is a growing public health concern. In recent years, the use and abuse of opioids have reached staggering levels and resulted in a significant increase in opioid-related deaths, hospitalizations, and emergency department visits. This has been driven by a variety of factors, such as over-prescription of opioid painkillers, the availability of potent synthetic opioids, and the growth of the illicit opioid market. The opioid crisis has affected different population, including urban and rural communities, as well as different age groups and races. Complex neurobiological mechanisms for physical dependence and addiction have been involved, making ongoing opioid crisis a complex and multifaceted problem that requires a comprehensive solution.

In the following of this Introduction section, I will primarily describe the history of the opioid epidemic in the United States (US), including the incidence and prevalence of opioid use, with particular focus on its secular trends. Also, I will summarise the role of opioids in pain management, particularly for individuals experiencing chronic pain and the well-recognised adverse health consequences relating to opioid therapy.

1.1.1. Epidemiology of opioid use and consequences in the US

The US is widely recognized as being at the forefront and epicentre of the opioid epidemic, characterized by the widespread use and abuse of prescription and illicit opioids, which has had a profound impact on well-being of individuals and public health.

The epidemic began in the late 1990s, when US medical boards relaxed restrictions on laws governing opioid prescribing for chronic non-cancer pain.[1] It was then fuelled by aggressive pharmaceutical marketing tactics and changing medical standards in the diagnosis and treatment of chronic pain, together resulting in dramatic increases in

prescription opioids. For example, to promote the long-term use of opioids for chronic non-cancer pain, a pharmaceutical company by the name of Purdue Pharma funded more than 20,000 educational programmes on the topic.[2] In 1995, during the American Pain Society's annual meeting, the president unveiled the initiative "Pain is the Fifth Vital Sign." This campaign recommended more aggressive use of opioid for chronic non-cancer pain and urged medical personnel to assess pain with the "same ardour" as they do with vital signs. The Joint Commission, which accredits hospitals and other health care institutions, and the Veterans' Affairs health system soon adopted this "Pain is the Fifth Vital Sign" concept to promote the diagnosis and management of pain. Similarly, a consensus statement supporting the use of opioids for chronic non-cancer pain was released by the American Pain Society and the American Academy of Pain Medicine.[3] Despite the statement's warning against irresponsible prescribing, these organisations may have played a role in the subsequence sharp rise in medical opioid prescription.[4,5]

The initial wave

Sales and number of prescriptions

Between 1999 and 2010, sales of opioids for therapeutic use quadrupled in the US, with the amount of morphine equivalents per person rising from 96 mg in 1997 to 710 mg in 2010.[6,7] In particular, methadone, oxycodone, and fentanyl were the top three leading opioids that rose by 1293%, 866%, and 525% respectively, as depicted in **Table 1 1**.[8] As for number of prescriptions, over 256 million opioid prescriptions in 2009 were filled in the US, with immediate-release (IR) opioids accounting for 234 million prescriptions and extended-release (ER) opioids accounting for 22.9 million prescriptions. These numbers represent a significant increase from the 2007 figures of 223.9 million IR opioid prescriptions and 21.3 million ER opioid prescriptions. When compared to 2002, the data show a striking escalation, with ER opioid prescriptions increasing by 146% from 9.3

million to 22.9 million and IR opioid prescriptions increasing by 42% from 164.8 million to 234 million. The annual increase rates were 21% for ER opioids and 6% for IR opioids.

Table 1 1 Retail sales of opioids by grams from 1997 to 2007

Drug	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	% of Change from 1997
Methadone	518,737	692,675 (34%)	964,982 (39%)	1,428,840* (48%)	1,892,691 (32%)	2,649,559 (40%)	3,683,881 (39%)	4,730,157 (28%)	5,362,815 (13%)	6,621,687 (23%)	7,228,219 (9%)	1293%
Oxycodone	4,449,562	6,579,719 (48%)	9,717,600 (48%)	15,305,913 (58%)	19,927,286 (30%)	22,376,892 (12%)	26,655,152 (19%)	29,177,530 (9%)	30,628,973 (5%)	37,034,220 (21%)	42,977,043 (16%)	866%
Fentanyl Base	74,086	90,618 (22%)	107,141 (18%)	146,612* (37%)	186,083 (27%)	242,027 (30%)	317,200 (31%)	370,739 (17%)	387,928 (5%)	428,668 (11%)	463,340 (8%)	525%
Hydromorphone	241,078	260,009 (8%)	292,506 (12%)	346,574* (18%)	400,642 (16%)	473,362 (18%)	579,372 (22%)	655,395 (13%)	781,287 (19%)	901,663 (15%	1,011,028 (12%)	319%
Hydrocodone	8,669,311	10,389,503 (20%)	12,101,621 (16%)	14,118,637 (17%)	15,594,692 (10%)	18,822,619 (21%)	22,342,174 (19%)	24,081,900 (8%)	25,803,543 (7%)	29,856,368 (16%)	32,969,527 (10%)	280%
Morphine	5,922,872	6,408,322 (8%)	6,804,935 (6%)	7,807,511 (15%)	8,810,700 (13%)	10,264,264 (16%)	12,303,956 (20%)	14,319,243 (16%)	15,054,846 (5%)	17,507,148 (16%)	19,051,426 (9%)	222%
Codeine	25,071,410	26,018,054 (4%)	23,917,088 (-8%)	23,474,865* (-2%)	23,032,641 (-2%)	22,633,733 (-2%)	21,865,409 (-3%)	20,264,555 (-7%)	18,960,038 (-6%)	18,762,919 (-1%)	18,840,329 (0.4%)	-25%
Meperidine (Pethidine)	5,765,954	5,834,294 (1%)	5,539,592 (-5%)	5,494,898* (-1%)	5,450,204 (-1%)	5,412,389 (-1%)	5,239,932 (-3%)	4,856,644 (-7%)	4,272,520 (-12%)	4,160,033 (-3%)	3,936,179 (-5%)	-32%
Total	50,713,010	56,273,194 (11%)	59,445,465 (6%)	35,962,089.84 (15%)	75,294,939 (11%)	82,874,845 (10%)	92,987,076 (12%)	98,456,163 (6%)	101,251,950 (6%)	115,272,706 (14%)	126,477,091 (10%)	149%

Adapted from: Manchikanti L, et al. Therapeutic use, abuse, and nonmedical use of opioids: A ten-year perspective. Pain Physician 2010; 13:401-435 (32).

Characteristics of opioid users and prescribers

During a similar period, the percentage of opioid prescriptions varied by age, with a rise from 0.7% in the age group 0-9 years to over 50% among those aged 60 years and older.[9–11] Analysis of data from 2009 revealed that 11.7% of opioid prescriptions (9.3 million) were for individuals aged between 10 and 29 years, while 45.7% (36.4 million) were for those between 40 and 59 years old. Primary care physicians, including general practitioners, family physicians, and osteopathic physicians, were the principal prescribers of opioid medications, accounting for 28.8% (22.9 million) of the total number of prescriptions, followed by internists (14.6%, 11.6 million), dentists (8.0%, 6.4 million), and orthopaedic surgeons (7.7%, 6.1 million). Furthermore, a considerable proportion of patients had been previously prescribed opioid medications, with 56.4% (44.8 million) of all opioid prescriptions in 2009 filled by individuals who had received another opioid prescription within the previous month.[12]

Abuse and death

The escalation of opioid prescriptions was accompanied by a surge in opioid abuse, misuse, addiction, and fatalities among individuals with or without pain, and in patients who were prescribed opioids as well as those who were not. Between 1992 and 2002, in the US there was a staggering 90% increase in the number of individuals who admitted to abusing controlled prescription drugs.[8] Among those insured, the prevalence of opioid abuse was high and ranged between 6.7–8 per 10,000 persons.[13] In 2008, opioid pain relievers were implicated in 14,800 drug overdose deaths in the US, compared to 11,500 out of 27,500 fatal unintended drug overdose deaths in 2007, marking an increase of 3,300 fatalities in just one year.[14] It is in fact striking that in 2007opioid analgesic overdose deaths surpassed those resulting from heroin and cocaine combined.

The plateau

Numerous interventions have been implemented at local, regional, state, and federal levels since 2010 in response to the opioid epidemic, such as enacting legislation to establish prescription drug monitoring programmes and the revision of clinical recommendations for opioid medications. To evaluate the effects of these programmes, a milestone study published in 2015 examined trends in the diversion and abuse of prescription opioid analgesics. The study, based on the RADARS (Researched Abuse, Diversion, and Addiction- Related Surveillance) system in the US, revealed that despite significant increases in the rates of opioid diversion and abuse from 2002 to 2010, the rates levelled off or even declined from 2011 to 2013. For example, **Figure 1 1B** shows an increase in the calculated quarterly event rate for prescription opioids in the Drug Diversion Program, rising from approximately 1.5 per 100,000 population in 2002 to 2.9 in 2012, followed by a decline to 2.5 by the end of 2013. **Figure 1 1C** depicts a similar trend in the Poison Centre Program, with the quarterly abuse rate for opioid analgesics increasing from 0.20 per 100,000 population in 2003 to 0.56 in 2010 and then declining to 0.35 by the end of 2013. In the Opioid Treatment Program, the rate of prescription opioid abuse rose from 1.6 per 100,000 population in 2005 to 7.3 in 2010 and then decreased to 3.5 by the end of 2013, as illustrated in **Figure 1 1D**. Lastly, the proportion of prescription opioid abuse rose from 1.5 per 100,000 population in 2008 to 3.8 in 2011 and then declined to 2.8 by the end of 2013, as shown in **Figure 1 1E**. The only exception, however, was the College Survey Program where the rate of nonmedical use increased more than two-fold from 0.14 per 100,000 population in 2008 to 0.35 by the end of 2013 (**Figure 1 1F**). Consistent with these findings, several local and state intervention programmes have also reported decreases in the abuse and diversion of prescription opioids.[15,16]

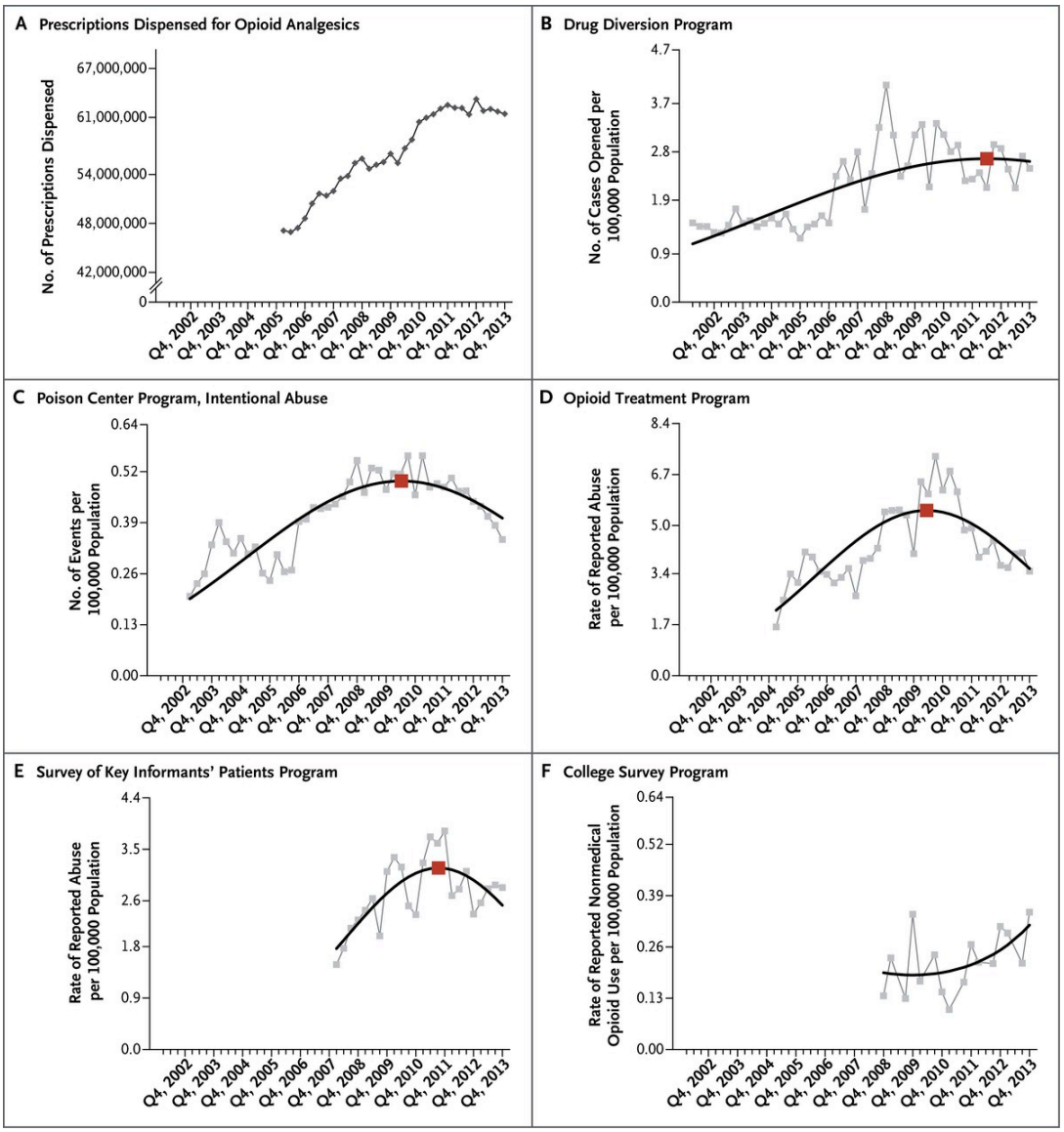


Figure 1 1 Prescriptions Dispensed for Opioid Analgesics and Rates of Abuse and Diversion, RADARS System, 2002–2013

Data are displayed according to calendar quarter. Panel A shows the number of prescriptions dispensed for the opioid analgesics. Panel B shows the rate of drug-diversion cases opened. Panel C shows the rate of cases of intentional abuse reported to participating poison centres. Panel D shows the rate of reported abuse by persons entering methadone programs. Panel E shows the rate of reported abuse by patients entering other substance-abuse treatment programs. Panel F shows the rate of reported nonmedical opioid use by college students. The red boxes in Panels B through E indicate the vertex of the quadratic curve. (There is no red box in Panel F because the quadratic terms were not significant).

Adapted from: Richard C. Dart, et al. Trends in Opioid Analgesic Abuse and Mortality in the United States. N Engl J Med 2015;372:241-8.

Recent trends in opioid use

A report from IQVIA based on five large US dispensation databases reveals a significant decline in the number of morphine milligram equivalents (MMEs) dispensed in the US in recent years, with a 51% decrease from 246 billion MMEs in 2011 to 120 billion MMEs in 2019 (**Figure 1 2**). The risks associated with prescription opioids, including addiction and abuse, increase with opioid exposure, particularly in patients receiving 90 or more MMEs per day. The most substantial reductions were observed in prescriptions for 90 or greater MME per day, with a 70% reduction since 2011, representing a shift in prescribing patterns away from high-dose opioid prescriptions (**Figure 1 2**). The least changed segment, <20 MME per day prescriptions, declined by 11% to 12 billion in 2019 (**Figure 1 2**).

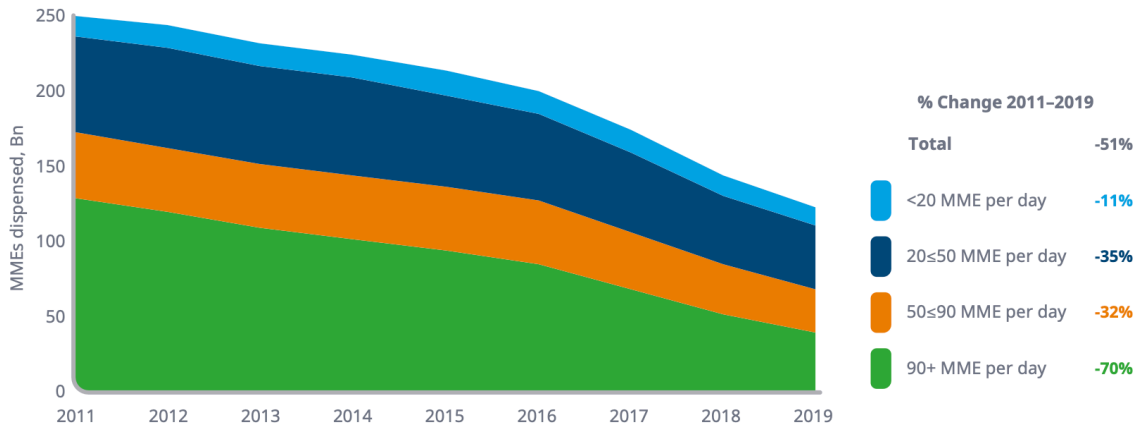


Figure 1 2 Prescription Opioid Use in the US Grouped by Morphine Milligram Equivalents per Day, 2011–2019.

In terms of specific pay types for opioid prescriptions, there has been a 17% decline in Medicare prescriptions since 2014. Commercial prescriptions, which comprise the largest share of prescription opioids, have declined by 51% (**Figure 1 3**). However, Medicare Part D prescriptions have increased by 2% since 2011 (**Figure 1 3**), with the

over 65 population requiring more procedures that result in opioid prescriptions, such as hip and knee surgeries.

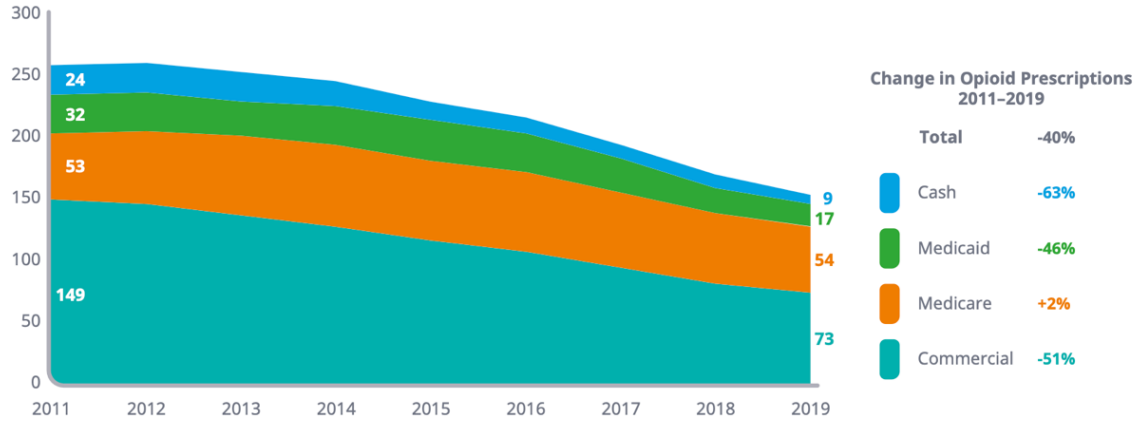


Figure 1 3 Number of Prescription Opioids Dispensed by Pay Type, Millions, 2011–2019.

Adapted from: Prescription Opioid Trends in the United States: Measuring and understanding progress in the opioid crisis. IQVIA Xponent, Mar 2020; IQVIA National Prescription Audit; IQVIA Institute, Nov 2020

However, findings from the National Survey on Drug Use and Health suggest that opioid misuse remained stable among adults and decreased among adolescents. Additionally, the survey indicated that in 2019 the rates of prescription pain reliever misuse, disorder, and initiation remained constant, while heroin misuse and disorder appeared to remain stable with a significant decline in heroin initiation. The Centres for Disease Control and Prevention data showed that in 2019 opioids were substantially co-involved with cocaine, amphetamine, and benzodiazepine overdoses. Moreover, there was a significant increase in the rates of emergency department-treated suspected nonfatal drug overdoses co-occurring with opioids and amphetamines in 2019 compared to 2018, and a near tripling of cocaine-involved overdoses.[17] Taken together, these findings suggest that the US may have entered a fourth wave characterized as a stimulant/opioid epidemic, reflecting the use of either or both substances, with an increased presence of co-occurring mental illness.[18]

1.1.2. Chronic non-cancer pain and safety of opioid treatment

A recent National Academies of Sciences report concluded that "The ongoing opioid crisis lies at the intersection of two substantial public health challenges— reducing the burden of suffering from pain and containing the rising toll of the harms that can result from the use of opioid medications".

The World Health Organization (WHO) considers opioids as essential medicines that are primarily used for cancer pain, palliative care, acute pain, and opioid dependence.[19] While short-term use of opioids has been shown to alleviate acute pain in trials,[20,21] the long-term effectiveness of opioids in the treatment of chronic non-cancer pain (CNCP) is still debatable. CNCP, as an emerging health concern, has negative consequences on patients, their families, and society as a whole. It features typically moderate to severe pain that lasts for at least six months and is caused by neuropathy, rheumatoid arthritis, lower back pain, osteoarthritis, fibromyalgia, and a variety of other chronic health conditions.[22] People with CNCP have difficulty sleeping, frequently feel fatigued and depressed, and struggle to form meaningful social relationships with their family and friends. According to 2016 National Health Interview Survey data, an estimated 20.4% (50.0 million) of U.S. adults had chronic pain and 8.0% of U.S. adults (19.6 million) had high-impact chronic pain. The findings of this report also indicated that CNCP rates was more common among specific demographic groups, including women, individuals who had previously been employed but were presently unemployed, residents living in or near poverty, and those residing in rural areas. Moreover, the age-adjusted prevalence of either chronic pain or high-impact chronic pain appeared significantly lower in adults with a minimum of a bachelor's degree, as compared to individuals with lower levels of educational attainment.[23]

In many developed countries, a large proportion of CNCP patients were prescribed opioid medications frequently to manage this condition despite the lack of well-established evidence supporting this practice.[24] For example, data from a nationally representative survey conducted in 2019 showed that approximately 22.1% of adults in the US with chronic pain had received opioid within the previous three months.[25] Similarly, a report from 2018 revealed that 12.7% of Canadians had used opioid pain relief medications during the preceding year.[26] Furthermore, a recent systematic review and meta-analysis[27] of patients with specific pain conditions show that opioid use ranged from 13.1% to 20.8% in cases of musculoskeletal pain, from 12% to 22% in cases of osteoarthritis, and from 8.4% to 22.4% in cases of fibromyalgia. It is noted that one study reported the highest prevalence of opioid use (81.2%) among elderly people.

Some opioids, such as morphine, hydromorphone, buprenorphine, oxycodone, fentanyl, and methadone, are typically classified as strong opioid and have increasingly been utilized in CNCP patients despite not being recommended.[28] For example, in Germany, 80% of prescribed opioid analgesics were indicated for patients with CNCP, and there has been a rise in the proportion of potent opioids.[29] Moreover, there has been a notable increase in prescriptions of potent opioids for opioid-naïve patients, such as fentanyl patches, which is not typically indicated for first-time opioid users.

Numerous studies have established a strong correlation between opioid medications and a range of side effects and adverse outcomes, including but not limited to drowsiness, constipation, nausea, vomiting, itching, sweating, and mood changes.[30] Additionally, other notable adverse effects such as hyperalgesia and endocrinological suppression have also been documented in association with opioid use.[31] It is estimated that 80% of individuals receiving opioid treatment will experience at least one of these side effects, which frequently lead to opioid rotation or treatment cessation.[32] Moreover, the risks of

respiratory depression, addiction, and drug misuse cause caution and apprehension among practitioners and patients alike.

1.2.The COVID-19 pandemic

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19) has led to a devastating global impact on demographics, resulting in over 6 million deaths worldwide, which represents the greatest threat to global health since the influenza pandemic of 1918.

In December of 2019, the SARS-CoV-2 was first reported in Wuhan, China. The WHO declared a Public Health Emergency of International Concern (PHEIC) on March 11th, 2020, due to the rapid global spread of a virus that later overwhelmed global healthcare systems (**Figure 1 4**). Even though significant progress has been made towards a better understanding of this novel virus, treatments for the disease' sequelae, and the implementation of massive vaccination programmes, the continued emergence of mutant variants remains a source of growing concern, leading to waves of outbreaks in many countries.

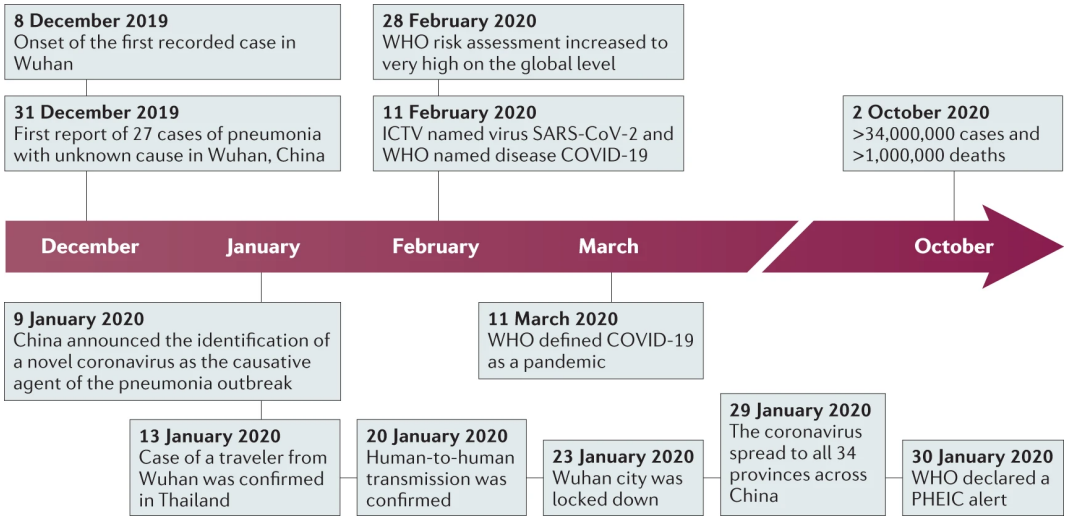


Figure 1 4 Timeline of the key events of the COVID-19 outbreak.

The first recorded cases were reported in December 2019 in Wuhan, China. Over the course of the following 10 months, more than 30 million cases have been confirmed worldwide. COVID-19, coronavirus disease 2019; ICTV, International Committee on Taxonomy of Viruses; PHEIC, public health emergency of international concern; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

Adapted from: Ben Hu, et al Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol 19, 141–154 (2021).

1.2.1. SARS-COV-2

Coronavirus virology

SARS-CoV-2 is a novel beta coronavirus classified under the same subgenus as the severe acute respiratory syndrome coronavirus (SARS-COV) and Middle East Respiratory Syndrome Coronavirus (MERS-COV), which were associated with SARS and MERS outbreaks, with mortality rates reaching up to 10% and 35%, respectively.[33] The animal origin of SARS-COV-2 remains unknown, although it is widely speculated to have emerged through zoonotic transmission. As with other RNA viruses, SARS-COV-2 is susceptible to genetic evolution and can develop mutations over time as it adapts to its human host, leading to the emergence of mutant variants that may differ in their characteristics from the ancestral strains.

During the current pandemic, multiple variants of SARS-COV-2 have emerged, and a few are designated as variants of concern (VOCs) due to their potential to enhance transmissibility or virulence, reduce neutralization by antibodies elicited by natural infection or vaccination, evade detection, or decrease the effectiveness of therapeutics or vaccines. According to the WHO epidemiological update, five VOCs of SARS-COV-2 have been identified since the onset of the pandemic.[34]

- Alpha (B.1.1.7): first variant of concern described in the UK in late December 2020
- Beta (B.1.351): first reported in South Africa in December 2020
- Gamma (P.1): first reported in Brazil in early January 2021
- Delta (B.1.617.2): first reported in India in December 2020
- Omicron (B.1.1.529): first reported in South Africa in November 2021

Epidemiology and risk factors

Since its declaration as a global pandemic by the WHO, SARS-CoV-2 has spread to 232 countries and resulted in more than 700 million confirmed cases and over 6 million reported deaths worldwide. For a graphical representation of the number of COVID-19 deaths over time by regions, please refer to **Figure 1 5**. According to a recent epidemiological update by the WHO, more than 200 countries have reported VOCs of SARS-CoV-2, among which the Omicron VOC has been identified as the most prevalent circulating VOC at present. Nonetheless, estimating the true burden of COVID-19 is a challenging task as only a fraction of cases is diagnosed and reported, which leads to a serious underestimation of the overall burden of this disease. Seroprevalence surveys conducted in the United States and Europe have suggested that, after accounting for potential false positives or negatives, the rate of prior exposure to SARS-CoV-2, as indicated by seropositivity, exceeds the incidence of reported cases by at least ten-fold or more.[35,36]

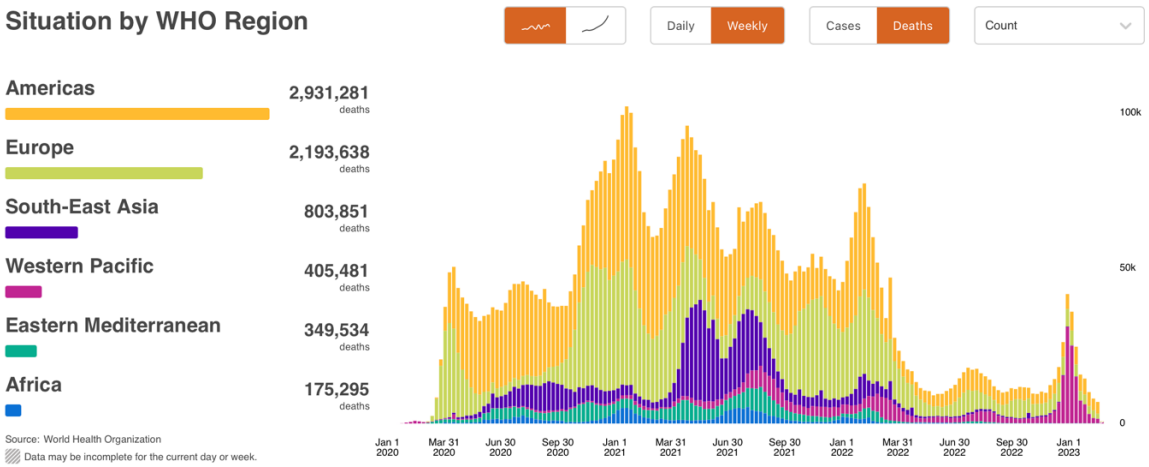


Figure 1 5 Number of COVID-19 death reported weekly by WHO Region, as of 1 January 2023

Adapted from: World Health Organization https://covid19.who.int.

Although individuals across all age groups face the risk of acquiring this infection and its consequential severe disease, older adults are at highest risk of seriously ill from COVID-19.[37] According to US data, over 81% of COVID-19-related deaths occurred in individuals aged 65 years and older. The COVID-19 fatality in this age group was almost 100 times higher when compared to those aged 18-29 years.[38] In the UK, a cross-sectional study indicated that individuals aged 40-64 years are at the greatest risk of contracting COVID-19 infection, followed by patients aged 75 years and above, and those aged 65-74 years.[39] It is notable that the risk of severe illness among older individuals is not only due to advanced age but also due to a higher prevalence of underlying medical comorbidities. Evidence from many nationwide cohort studies were consistent supporting that an individual's risk of severe illness from COVID-19 increases with the number of underlying medical comorbidities, including but not limited to obesity, cardiovascular disease, chronic kidney disease, diabetes, chronic lung disease, cancer, and solid organ or hematopoietic stem cell transplant patients[40,41].

In addition, data regarding gender discrepancies in COVID-19 showed that male patients had a greater susceptibility to COVID-19-related mortality, hospitalization, and clinical complications compared to their female counterparts.[42,43] As of May 20, 2021, the available sex-disaggregated data from Global Health 50/50 further substantiated this claim by revealing a higher infection fatality rate (IFR) in males relative to females in many countries such as Brazil, Yemen, Mexico, and Scotland.

Furthermore, the severity of infection and related mortality with COVID-19 also varies between different ethnic groups. A meta-analysis of 50 studies conducted by researchers in the United States and the United Kingdom revealed that individuals belonging to ethnic minority groups such as Black, Hispanic, and Asian populations experienced more adverse COVID-19 outcomes, which may stem from heightened risks to exposure to the

SARS-CoV-2 virus combined with a higher likelihood of developing severe forms of COVID-19 disease.[44] Thus, ethnicity constitutes a significant determinant or marker of COVID-19 burden, potentially intertwined with other social factors such as housing, socioeconomic status, employment, and general health status.[45]

Clinical manifestations and complications of COVID-19

The clinical presentation of COVID-19 is heterogeneous and ranges from an absence of symptoms to severe respiratory distress, septic shock, and multi-organ failure. Approximately 17.9% to 33.3% of infected individuals are asymptomatic, while the majority of symptomatic cases typically manifest with fever, cough, and dyspnea, and less commonly with sore throat, anosmia, dysgeusia, anorexia, nausea, malaise, myalgias, and diarrhoea.[46] Severity of illness is assessed based on a variety of clinical parameters, including laboratory and radiographic findings, hemodynamic stability, and organ function. The US National Institutes of Health have developed guidelines that classify COVID-19 into five categories based on disease severity, namely asymptomatic or presymptomatic infection, mild illness, moderate illness, severe illness, and critical illness.[47]

Despite the respiratory system being the primary target of COVID-19, it can be considered a systemic viral illness and multiple organ dysfunctions are associated with this illness. (**Figure 1 6**) Of noteworthy significance, newly emerged evidence highlights the occurrence of persistent symptoms in individuals who have recovered from COVID-19 infection, commonly termed "post-acute COVID-19 syndrome" or “Long Covid”.

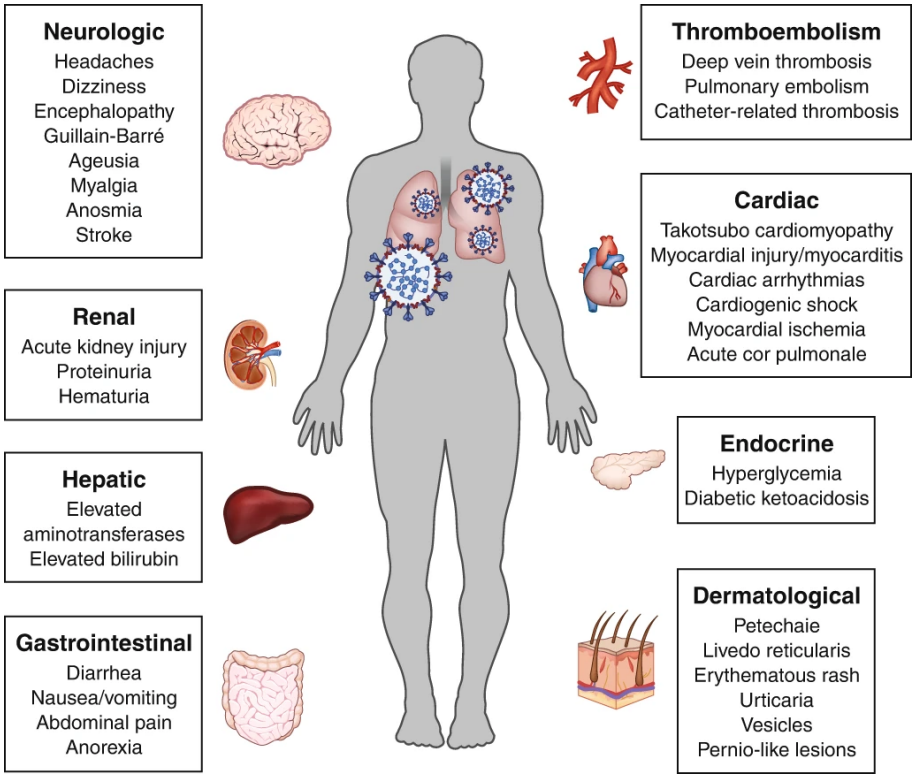


Figure 1 6 Extrapulmonary manifestations of COVID-19.

Adapted from: Gupta, A. et al. Extrapulmonary manifestations of COVID-19. Nat Med 26, 1017–1032 (2020)

1.2.2. COVID-19 vaccines

The rapid development and implementation of novel vaccines against SARS-CoV-2 is one of the most crucial steps to contain the pandemic of COVID-19. The number of doses delivered has reached 13.32 billion worldwide as of March 8, 2023, since the first COVID-19 vaccine was authorised for regular human use (beyond clinical trials) late in 2020, with 69.7% of the global population having received at least one dose. These vaccinations have undoubtedly saved millions of lives and are critical in mitigating the acute and long-term health burden imposed by SARS-CoV-2 and its ongoing variants, particularly in the absence of very effective therapies to date.

Vaccines and their efficacy against SARS-CoV-2

The mRNA vaccines developed by Pfizer-BioNTech and Moderna were the initial candidates in the COVID-19 vaccine race, and they received emergency use approval as the first vaccines distributed worldwide. Both vaccines are composed of nucleoside-modified mRNAs encapsulated in lipid nanoparticles, which encode the pre-fusion SARS-CoV-2 full-length S protein with proline substitutions and can trigger adaptive humoral and cellular immune responses.[48] Clinical trials indicate an overall vaccine efficacy of 94.1% and 94.6% for Pfizer-BioNTech and Moderna-mRNA-1273, respectively.[49]

Oxford-AstraZeneca and Johnson and Johnson/Janssen vaccines, constructed using adenoviral vectors that express the full-length SARS-CoV-2 spike protein, showed a lower vaccine efficacy than mRNA vaccines in placebo or active comparator RCTs. However, they still produced neutralizing antibodies in 91% and 100% of participants after prime and boost doses, respectively.[50] The diverse ethnicity of the study population and other factors such as new variants and variable places led to an overall vaccine efficacy ranging between 62% and 90% for the Oxford-AstraZeneca vaccine.[51]

In turn, Janssen vaccine (Ad26.COV2.S) is a recombinant, replication-deficient adenovirus-based vaccine encoding a full-length and stabilized spike protein. It induced humoral and cellular immune responses following a single dose. Neutralizing antibodies were detected in 90% and 100% of participants at days 29 and 57, respectively.[52] The overall efficacy of the Ad26.CoV2.S vaccine was 72% in the US, 66% in Latin America, and 57% in South Africa.[53]

Several other vaccine candidates are also currently in use to prevent COVID-19. Among these, inactivated vaccines are derived from viruses propagated in culture and chemically inactivated. Sinopharm and Sinovac are two examples of inactivated SARS-CoV-2 vaccines developed in China, both of which have been reported to be safe and relatively effective, with Sinopharm showing efficacy rates of 79% and 86%, while Sinovac has shown efficacy rates of 78%, 65%, and 91.25% depending on dosing and population. The recombinant protein-based vaccine is another notable vaccine platform and technique currently utilized in the context of COVID-19 pandemic. The Novavax vaccine (NVX-CoV2373) represents an example of a recombinant protein vaccine that employs a recombinant full-length, pre-fusion S protein with saponin-based Matrix-M adjuvant. In UK and South Africa phase 3 clinical trials, the vaccine recorded an overall efficacy of 89.3% and 60%, respectively. There are other vaccines that have been approved more recently in the EU, like the Sanofi Pasteur from France and the HIPRA PHH-1V from Spain.

The development of vaccines against COVID-19 continues to be a subject of significant interest. Data from the WHO COVID-19 vaccine tracker and landscape indicates that there are presently 180 new vaccines in various stages of clinical development.

Global vaccine rollout strategies and challenges

At the outset of vaccination efforts with limited vaccine access and supply, many countries opted to administer vaccines to prioritise high-risk populations, such as

healthcare workers and elderly individuals, for earlier vaccination. According to results from a prominent mathematical modelling study comparing five age-stratified vaccination prioritization schemes, mortality and years of life lost were minimized when vaccines were administered first to individuals over the age of 60 in most scenarios.[54] However, it also pointed out that a highly efficacious vaccine with transmission-blocking properties prioritized for adults between 20 and 49 years of age was more relevant to SARS-CoV-2 transmission.[55] Subsequently, in mid-2021, those countries with ample vaccine supplies shifted to mass vaccination campaigns targeting the adult population, and later, children. Subsequent booster doses were also introduced to sustain elevated levels of protection due to the declining efficacy of the vaccine and the emergence of new strains of concern.

However, the previous two years have seen substantial inequalities in the distribution of COVID-19 vaccines worldwide.[56] Prior to the global rollout of COVID-19 vaccines, a mechanism for the equitable allocation of vaccines had been established to address the issue of inequality, namely the COVID-19 Vaccines Global Access (COVAX) facility.[57] This initiative aimed to achieve a minimum of 20% vaccine coverage for eligible countries under the Advance Market Commitment (AMC) through the procurement of COVAX-secured vaccine doses by the end of 2021. To further enhance vaccine coverage, the World Health Organization subsequently set an ambitious global target of 70% coverage for all countries by mid-2022, with an interim goal of attaining 40% coverage by the end of 2021. Regrettably, these goals were not realized as planned. As of March 19, 2023, 3 in 4 people in high-income countries (HICs) had received at least one dose of a COVID-19 vaccine, as compared to only 1 in 3 people in low-income countries (LICs).[58]

Several obvious obstacles are limited vaccine supply to COVAX, which was further aggravated by certain countries obtaining a greater proportion of the global vaccine

supply, pharmaceutical companies failing to fulfil their contractual obligations to COVAX, and vaccines' short expiry windows. Besides, a four-dimensions framework (Development and production, Affordability, Allocation and Deployment) has been advanced to explicate the roots of vaccine inequities, involving the concentration of vaccine development and production in high-income nations, stockpiling of doses by these nations, high vaccine prices, and difficulties in deploying vaccines in resource-limited contexts.[59] Encouragingly, according to the latest Global COVID-19 Vaccination Strategy report by the World Health Organization, COVID-19 vaccine supplies worldwide are now abundant, with an annual manufacturing capacity of 11-16 billion doses and significant volumes accessible for LICs through contracts, donations via the COVAX, regional mechanisms, and bilateral approaches. External funding of over 3 billion USD has been allocated for these countries to bolster vaccine delivery, including through sizeable multi-partner technical support.[60] Also, at the local/national level, there was some evidence showing that fair vaccination campaigns have reduced inequity in the impact of the pandemic.[61]

1.3.The use of real-world and biobank data for evidence generation

In 2016, the US FDA provided an initial definition of real-world data (RWD) in the medical and healthcare field as the data "relating to patient health status and/or the delivery of health care routinely collected from a variety of sources".[62] This original definition has been substantially broadened, and the National Institute for Health and Care Excellence (NICE) in the United Kingdom currently characterizes RWD as any patient health or experience or care delivery data that is obtained outside the confines of a rigorously controlled clinical trial. One typical characteristic of RWD that is distinct from the clinical trial data is that interventions or exposures are not dictated by a study protocol, but rather determined by patients and healthcare providers. In the context of public health or social care, interventions may be influenced by individual behaviours, environmental exposures, or policy decisions.[63] However, it is true that certain interventional studies, such as pragmatic clinical trials, may also involve use of RWD to facilitate trial design, participant recruitment, or outcome data collection.

RWD generally originates from two primary sources, namely, routine databases and study designs that generate RWD.[64] A systematic review article shown that registries, electronic health records (EHRs), claims databases, administrative data, and patient-reported outcomes were the top five RWD sources frequently cited in literature documents.[65] A comprehensive list of various RWD types and sources in the field of medicine is presented in **Figure 1 7**.

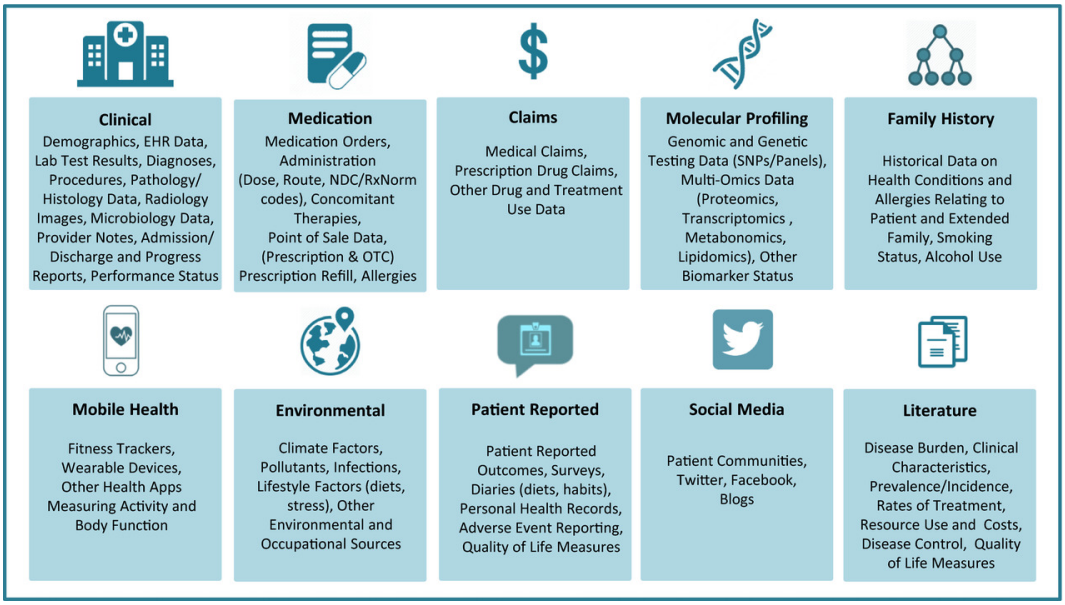


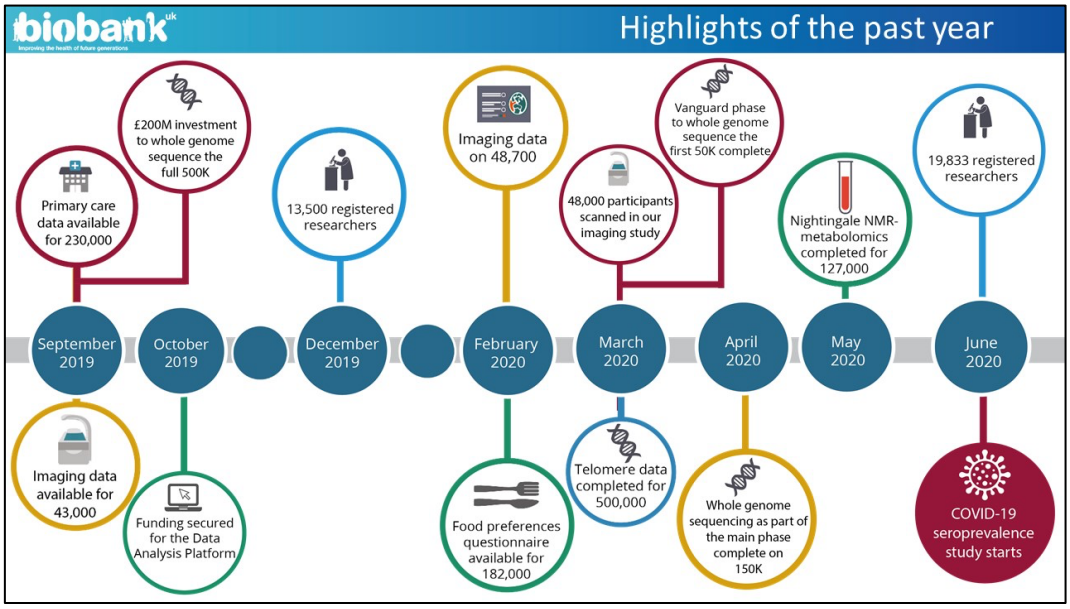
Figure 1 7 RWD Types and Sources

Adapted from: Swift B. et al. Innovation at the Intersection of Clinical Trials and Real-World Data Science to Advance Patient Care. Clin Transl Sci. 2018

In the medical field, "real-world evidence" (RWE) therefore refers to evidence obtained from the analysis of RWD, which typically implies clinical evidence concerning the utilization of medical products, such as treatment patterns or compliance, as well as the benefits and risks associated with such products in a defined population or subpopulations. The generation of RWE can involve a wide range of study designs and analytical methods, both quantitative and qualitative, depending on the research question or specific use case. Over the past few years, RWE has increasingly been utilized to support regulatory decision-making for medical products, particularly in rare disease and paediatric populations. For instance, a retrospective landscape study was conducted to analyse white and grey literature, as well as publicly available FDA reviews of medical products where RWE was submitted to support effectiveness. The study found that between 1954 and 2020, 26% of instances pertained to oncology, 18% to haematology, and 12% to neurology.[66]

By definition, “biobank” is a long-term storage and conservation facility for biological specimens. It collects, processes, stores, and distributes biospecimens and related data for use in medical research and clinical care.[67] Initially, biobanks were small-scale repositories established by universities to meet the specific research needs of individual projects. Over the past decades, the discipline of biobanking has evolved significantly over the past decades, with emerging institutional and government-supported biorepositories, commercial biobanks, disease-oriented biobanks, and population-based biobanks. Population-wide biobanks have been established majorly in developed countries, including Iceland, UK, USA, Estonia, Canada, Singapore, South Korea, Japan, and are rapidly emerging in developing countries such as China and Mexico. The UK Biobank, Estonia Biobank, and Danish National Biobank are among the preeminent population biobanks globally, which recruit participants and collect biological samples primarily from volunteers—without application of strict inclusion or exclusion criteria. These biobanks have progressively increased in size and complexity, both in terms of their sample collections and the associated data. For example, during the pandemic, biobanks served as an essential resource in the fight against COVID-19 and enabled researchers to investigate the aetiology of the virus, repurpose of drugs and evaluation of vaccines. These data sets linked to stored biospecimens now comprise detailed information about participants or patient phenotypes, including genetic, proteomic, and other 'omics' data. **Figure 1 8** shows an overview of ongoing data collection and linkage in UK Biobank between 2019 to 2020.

Figure 1 8 UK Biobank ongoing data collection and linkage.



Online source: https://twitter.com/uk_biobank/status/1275434774560038915.

As a result, modern biobanks have become complex infrastructures and powerful resources where clinicians, biologists, nurses, technicians, and bioethicists work together for advancing medical research and facilitating clinical care, with broad implications for the development of personalized medicine and the improvement of public health.[68]

1.4.Research rationale and aims

The opioid epidemic and the COVID-19 pandemic have both placed considerable strain on global healthcare systems. RWD has served as a critical resource for investigating these two health crises, offering timely, representative, and longitudinal information on diverse populations. Furthermore, the integration of large biobank data has significantly enhanced the scope and depth of these research by providing access to biological samples, genomic data, and multi-omics information.

However, big data itself does not equate to knowledge. There is an urgent demand for understanding how to translate such voluminous and exponentially increasing information into evidence regarding to the two public crises. This includes insights into how opioids are utilized in non-US countries, their safety for managing chronic pain, whether older on-market drugs could still be indicated during the pandemic, the real-world effectiveness of various vaccines, and the clinical and genetic risk factors for COVID-19.

My overarching DPhil program aims to generate trustworthy and actionable evidence to fill the aforementioned knowledge gaps through leveraging both RWD and biobank data, in conjunction with various epidemiological principles and modern statistical techniques. It comprises six specific objectives, formatted according to the PICO (population/intervention/comparison/outcome) model where relevant:

- To characterize trends and patterns of opioid use from 2007 to 2019 in general adults in Catalonia.
- To compare the risk of seven adverse opioid events and death in new adult opioid users following the initiation of Tramadol vs Codeine.
- To assess the risk of COVID-19 disease and its severity among prevalent ibuprofen users with osteoarthritis or back pain.

- To study the comparative effectiveness of BNT162b2 versus ChAdOx1 vaccines against COVID-19 and hospitalization in people over 50.
- To investigate the effects of critical immunogenetics (human leukocyte antigens) on vaccine effectiveness and breakthrough COVID-19 in the vaccinated population.
- To quantify the excess risk of venous thromboembolism in patients with COVID-19 compared to those who are COVID-19 naive, and to identify relevant clinical and genetic determinants.

1.5.Outline of the thesis

Chapter 1 provides a comprehensive background to the two public health crises under investigation: the opioid epidemic and the COVID-19 pandemic. This chapter introduces the concepts of RWD and Biobank Data as critical resources for studying these complex issues. Furthermore, it elucidates the rationale and overarching aims of the research, as well as outlines the key research objectives to be explored in Chapters 3 through 8.

Chapter 2 presents a brief overview of the data sources utilized in this DPhil thesis, including the UK Biobank, The Information System for Research in Primary Care (SIDIAP), and two US claims databases, namely Open Claims and PharMetrics Plus. Additionally, this chapter outlines the design of the study cohorts, highlighting the custom modifications used for pharmacoepidemiological study. Lastly, the chapter delves into the analytical methods implemented to address potential biases inherent in different observational data and research questions.

Chapter 3 examines the secular trends in opioid dispensation and characterizes the patterns of various opioid types prescribed for diverse painful conditions. While numerous opioid utilization studies exist, the majority focuses on the United States, Canada, Australia, and northern European countries. Data concerning opioid usage in southern European countries remains limited, with a lack of information on subpopulations and standardized measurements. Moreover, no previous studies have analysed opioid indications and prescribing patterns in this context. The research presented in this chapter is based on the SIDIAP database and study opioid use from January 1, 2007, to December 31, 2019, among approximately five million adults, representing over 75% of the population in Catalonia, Spain. The work from this chapter has been published in **Front. Pharmacol**.

Chapter 4 evaluates the comparative risk of Tramadol versus Codeine concerning eight pre-specified side effects previously associated with opioid use. A review of opioid utilization studies conducted in 2019-2020 reveals that tramadol was the most prescribed opioid in the United Kingdom, Netherlands, and Spain, consistent with my findings from Chapter 3. Although tramadol has been widely perceived as a relatively safe opioid by many patients and clinical societies, including the American Academy of Orthopaedic Surgeons, there remains a paucity of evidence from clinical trials and observational studies to substantiate this assertion. In this chapter, I establish a propensity score-matched cohort comprising 184,480 Tramadol users and an equal number of Codeine users. I then compare the incidence of all-cause mortality, cardiovascular events, fractures, falls, delirium, constipation, sleep disorders, and opioid abuse/dependence within a one-year period, providing valuable insights into the safety profile of these two medications. The work from this chapter has been published in **JAMA**.
Chapter 5 investigates the association between Ibuprofen and risk of incident COVID-19 diagnosis and hospitalisation. During the early stages of the COVID-19 pandemic in 2020, concerns arose about the potential risks of non-steroidal anti-inflammatory drug (NSAID) use, particularly ibuprofen, in patients with the virus. This was primarily due to anecdotal evidence from a French case-report study, prompting some health organizations and experts to recommend alternatives like paracetamol. Despite a few subsequent observational studies suggesting that Ibuprofen do not increase the risk of severe complications or death in COVID-19 patients, methodological limitations, particularly indication bias, persist in these investigations. The lack of reliable causal evidence and the presence of non-comparable participant groups in such studies highlight the need for more rigorous research to better understand the relationship between ibuprofen usage and COVID-19 outcomes. In this chapter, I design a prevalent user-active comparator

cohort study and curate over 10 million patients with osteoarthritis or back pain based on two US claim databases mapped to Common Data Model. I compare Ibuprofen users with other non-selective NSAID, Cyclooxygenase or Paracetamol users in relation to developing a COVID-19 diagnosis or COVID-19 hospitalisation, with application of a series of advanced statistical methods to control confounding. The work from this chapter has been published in **Drugs**.

Chapter 6 compares the effectiveness of the BNT162b2 and ChAdOx1 vaccines against Covid-19. In July 2021, the Medicines and Healthcare products Regulatory Agency in the UK has approved four COVID-19 vaccines: Pfizer-BioNTech BNT162b2, Moderna's mRNA-1273, Oxford-AstraZeneca's ChAdOx1, and Janssen's Ad26.CoV2.S. While phase 3 trials show high clinical efficacy for each vaccine, direct comparisons of their effect were challenging, due to disparities in study designs, varying populations recruited across distinct regions and timeframes, diverse primary endpoint definitions, and heterogeneous statistical analysis methodologies. With rapid rollout of the Covid-19 vaccination programme worldwide, evidence on their comparative performance has become more crucial to inform policy decisions on optimizing vaccine implementation strategies. In this chapter, I use data from population-based UK Biobank cohort and create balanced groups of BNT162b2 vaccinees and ChAdOx1 vaccinees using a method of propensity score-based inverse probability of treatment weighting. I include 70,097 and 98,551 people received the first BNT162b2 and ChAdOx1 Covid-19 vaccines, respectively, and 67,813 and 89,030 accordingly for people receiving the second dose. I then evaluate risk of COVID-19 infection and hospitalization over time from the vaccination to 30/09/2021. The work from this chapter has been published in **Nat Communications**.

Chapter 7 studies Human leukocyte antigens (HLA) genetic impact on antibody response to COVID-19 vaccines and subsequent clinical COVID-19 outcomes. The rapid development, approval, and distribution of COVID-19 vaccines have been unparalleled, with over 13 billion doses administered globally. However, the universal "one-size-fits-all" approach to vaccination has led to considerable heterogeneity in vaccine effectiveness among different subgroups, leaving a significant portion of fully vaccinated individuals still susceptible to the virus. While some acquired risk factors have been identified, the role of host genetic determinants in vaccine effectiveness remains largely unexplored. HLA are a group of genes receiving particular interest in due to their established role in modulating chronic infections, autoimmune diseases, cancers, as well as their potential on vaccine-induced immune responses. In this chapter, I replicate previous findings regarding the association between HLA-DQB1:06 alleles and seroconversion rates and identify novel HLA alleles influencing antibody response following COVID-19 vaccination in a cross-sectional study of 185,519 vaccinees from the UK Biobank. In addition, by incorporating electronic health records of 368,098 vaccinated individuals, I prospectively evaluate the impact of 39 HLA variations on the risk of COVID-19 breakthrough infections and hospitalizations, both individually and collectively, using traditional epidemiological approaches, polygenic risk score and Mendelian randomization methodologies. The work from this chapter has been submitted to **Nature**.

Chapter 8 quantifies short-term venous thromboembolism (VTE) risk associated with SARS-CoV-2 infection in ambulatory settings and investigates the related clinical and genetic risk factors. Numerous epidemiological studies have highlighted the high risk of VTE in patients with severe COVID-19, leading to the implementation of anticoagulation therapies during hospital admission. However, limited interventions have been established for VTE prevention in ambulatory patients with SARS-CoV-2 infection,

partially due to conflicting findings on the infection's association with VTE occurrence and mixed evidence on the benefits of oral anticoagulation. As milder COVID-19 cases treated in ambulatory settings continue to rise globally due to vaccine rollouts, relaxed public health restrictions, and the highly transmissible Omicron variant, there is an increasing need to refine prophylaxis approaches, including dates and dosing regimens, for outpatient settings to address this critical aspect of COVID-19 management. In this chapter, I analyse data from 26,210 UK Biobank participants with SARS-CoV-2 infection between March 1, 2020, and September 30, 2021, from which I identify 18,818 milder COVID-19 cases. Using the propensity score method, I match these infected individuals 5:1 with uninfected counterparts, controlling for various covariates. I assess the 30-day risk of VTE following infection and evaluate the association of COVID-19-assoicated VTE with conventional risk factors including age, sex, ethnicity, socioeconomic status, obesity, vaccination status, and inherited thrombophilia. The work from this chapter has been published in **JAMA Intern Med**.

Chapter 9 summarise the principal findings and relevant methodological considerations in this thesis, discusses the strengths, limitations, and implications of the conducted studies. Additionally, this chapter provides recommendations for future research in this filed.

Chapter 2

Methods

2. Methods

Data from routinely collected electronic health records (EHR), claims-based databases, or a research-oriented prospective cohort were used for the analyses in this thesis. The current chapter provides a brief description of each data source, including SIDIAP, U.S. claims, and UK Biobank. Key elements of epidemiological study design and analytic techniques of relevant across the studies are also summarized in this chapter. Further information regarding bespoke settings, cohort specifications, exposure and outcome definitions, confounders, and variations in statistical analysis specific to each study are presented in subsequent chapters.

2.1.Data sources

The main considerations for including these three data sources in my thesis are data availability and granularity for answering each specific research question. The frequency of data updates and ethical approval within the expected timeline for each study are also important factors in my decision.

2.1.1. SIDIAP

The Information System for Research in Primary Care is a research-oriented electronic medical records database, which includes routinely collected data from primary care practices provided by Catalonia's largest healthcare provider, Catalan Health Institute (Institut Catala` de la Salut, ICS). Currently, SIDIAP contains information from 274 primary health centres and covers 5.8 million distinctive people (~ 75% of the population living in Catalonia).[69] The SIDIAP database contains comprehensive data fields, such as demographics, clinical diagnoses, and treatment histories. The quality of these data in SIDIAP is generally considered high. However, it remains important to validate the

accuracy of diagnostic codes and other clinical variables within the specific research context if additional information is available and linkable.

Source population and settings

Catalonia, an autonomous community in north-eastern Spain, has a health system similar to the UK's National Health Service, where primary care provides free treatment for registered residents and is the first point for public to access non-emergency health-related services. Typically, a primary care centre in Catalonia consists of a team of health providers, including general practitioners, dentists, paediatricians, nurses, social workers. Other support services such as sexual and reproductive health care are also incorporated.[70] The population characteristics included in SIDIAP has been described elsewhere.[71] As of 30 June 2021, the sex and age distribution of the SIDIAP participants was similar to that of the general Catalonia population, with 50.7% female and a median age of 44 years old. Also, the large majority of people live in urban areas (88.4%) and 75.3% resides in the Barcelona region.

Main data collected

Data in SIDIAP have been updated and expanded on a regular basis every six months. It contained information on individual level socio-demographic characteristics, such as date of birth, sex, residential area (rural or urban), and nationality. General metrics relating to socio-economic status were curated through combining individual and ecological indicators (e.g., MEDEA [Mortalidad en a´reas pequenas Espanolas y Desigualdades Socioecono´micas y Ambientales], a standard deprivation index used at the census tract level).[71] More accurate personal data such as income level (<18,000 €, 18,000 to 100,000€, >100,000€ per year) and type of occupation (active or retired) were

also available through the pharmaceutical co-payment information. Two important lifestyle variables, smoking and alcohol drinking statues, were collected.

Disease diagnoses for registered individuals were captured and coded by healthcare professionals using the Tenth Revision of International Classification of Diseases codification system (ICD-10). Both date of beginning and end associated with a diagnosis can be extracted if recorded by a GP. Data on therapeutic interventions, measurements and laboratory test results were captured. For example, vaccinations (types and doses), common scales (e.g., cognitive, pain, mental health, etc.), routine examinations (e.g., weight, height, blood pressure, heart rate etc.) and as well as serology and biochemistry parameters were integrated into the SIDIAP.

One of the notable advantages of SIDIAP for conducting pharmacoepidemiological studies is the comprehensive information contained regarding both prescriptions and dispensations of medications. Drugs financed by the Spanish National Health System and prescribed by ICS healthcare professionals were recorded in the prescriptions dataset. Those then filled in community pharmacies were also documented in the dispensations dataset. The standard Anatomical Therapeutic Chemical (ATC) Classification System was used to code each prescription and dispensation. What's more, medication details including dosage, drug units per day, defined daily dose (DDD) recommended by the World Health Organization, the strength, the number of units per package and the administration route were available.

Linked data

In addition to information collected from primary care practices, secondary care data are readily available to research on a project-by-project basis, which covers emergency and hospitalization-based diagnoses, procedures, and medications. Moreover, several disease

specific registries such as cancer and dementia have been linked individually using anonymized personal identifier. It is noted that environmental related variables (air pollution, noise, green spaces and built environment) can also be studied through linkage to relevant databases at the census tract level.[72]

2.1.2. US claims

IQVIA, formerly known as Quintiles and IMS Health, Inc., is an American multinational company serving the combined industries of health information technology and clinical research.[73] Two prescription claims databases used in this thesis were provided by IQVIA: the open-source longitudinal pharmacy and medical claims [IQVIA LRx/Dx], and the adjudicated claims [IQVIA PharMetrics Plus]. The data quality of both databases is generally considered reliable and has been widely utilized for research purposes in US. However, these databases may be limited by potential coding errors, missing data, and the absence of certain types of clinical information.

IQVIA PharMetrics Plus

The IQVIA PharMetrics Plus database, which is derived from payers, is a comprehensive source of adjudicated medical and pharmacy claims for patients in the United States.[74,75] The database contains information on over 150 million individuals from 2006 onward, with approximately 40 million active patients in 2017. These data are representative of the commercially insured US population, covering 90% of US hospitals, 80% of all US doctors' offices, and 85% of Fortune 100 companies.[76]

In addition to standard fields such as inpatient and outpatient diagnoses and procedures, retail and mail-order prescription records, PharMetrics Plus provides detailed information on pharmacy and medical benefits (including copayments and deductibles), inpatient stays (including admission type, source and discharge status), and provider

details (including specialty and provider ID). Given the broad coverage of the data, individuals in the PharMetrics Plus database are comparable to the national commercially insured population in terms of age and gender for individuals aged 65 and under.

The data are longitudinal, with more than 30 million individuals having three or more years of continuous enrolment with both medical and pharmacy coverage. Overall, the PharMetrics Plus database is a valuable resource for researchers seeking to investigate healthcare utilization and outcomes among a large and diverse population of commercially insured individuals in the US.

IQVIA LRx/Dx

LRx and Dx databases are major sources of healthcare data in the United States, with LRx receiving nearly 4 billion electronically transmitted prescription claims annually.[77,78] These claims cover up to 92% of retail channels, 62% of traditional and specialty mail orders, and 76% of claims for long-term care. Dx collects approximately 1 billion professional fee claims per year, representing over 870,000 practitioners per month. The LRx database contains prescription claims, payer information (e.g., commercial, public, cash), patient demographics including sex, age, and geographic information, and drug product information. The Dx database includes diagnoses, medical procedures, prescribed medications, health care encounters (primarily outpatient based), and patient demographic and payer/pay type information.

LRx and Dx databases obtain data from various sources, such as pharmacies, payers, software providers, transactional clearing houses, and healthcare practitioners, making them payer agnostic and representing a broader range of encounters and filling behaviours compared to payer-based databases. Additionally, IQVIA databases offer the advantage of a short data lag, with all claims being nearly complete within 1-2 months.

For the purpose of obtaining information on patients' race variable, consumer attribute data from the Experian Marketing Services Consumer View database were linked with LRx and Dx data. This national marketing database includes demographic, lifestyle (e.g., purchasing habits, interests, leisure), and financial attributes.

2.1.3. UK Biobank

UK Biobank (UKBB) is one of the world largest population-based prospective cohorts. It recruited over 500,000 participants from England (89%), Scotland (7%) and Wales (4%) aged 40-69 years old between 2006 and 2010, covering a variety of different settings, socioeconomic and ethnic heterogeneity and urban–rural mix (**Figure 2 1**).[79] The UKBB team adopts rigorous data collection methods, including standardized questionnaires and medical tests, to ensure high data accuracy. It is generally considered a high-quality resource for research in epidemiology and population health. However, the follow-up data through linkage to external data sources may suffer from some quality issues similar to those found in most electronic health record databases.

Overall study design and recruitment

The UKBB was initiated to investigate the impact of a wider variety of exposures on health and different diseases outcomes. It has been collecting extensive phenotypic and genotypic details and facilitating innovative and collaborative science by maximising access to the resource, with a long-term aim of improving the prevention, diagnosis, treatment, and management of diseases of middle and later life. The initial study proposal of UKBB is open to access and provides greater depth of descriptions given in present thesis.[80] Ethics approval for the UK Biobank was granted by the North West Multi-Centre Research Ethics Committee as a Research Tissue Bank (RTB) approval, which is renewed on a 5-yearly cycle.[81] This approval guarantee that all UKBB based studies undertaken in my thesis do not require separate ethical clearance and can operate under the UKBB

application number 65,397. The informed written consent has been obtained for all participants in UKBB for their participation and long-term follow-up.

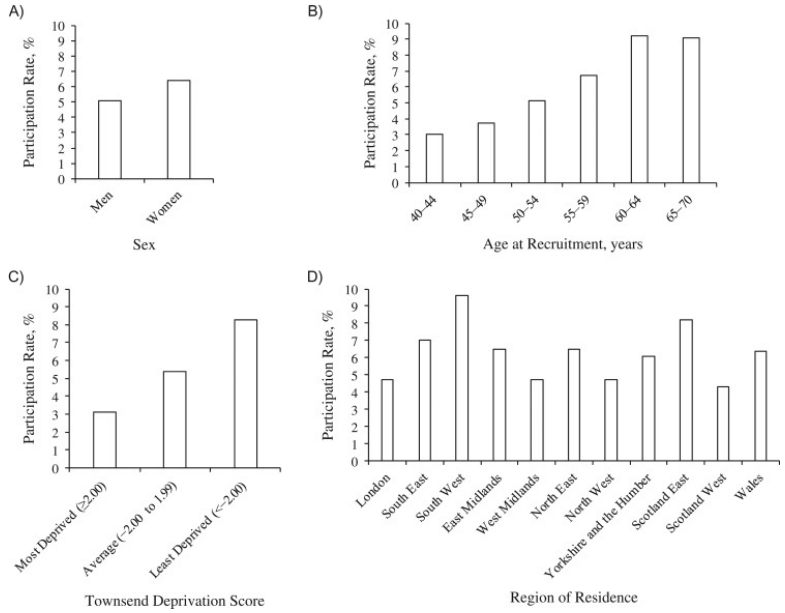


Figure 2 1 Rate of participation in the UK Biobank

According to sex (A), age at recruitment (B), Townsend deprivation score (C), and region of residence (D), 2006–2010.

Adapted from: Anna Fry, et al Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. Am J Epidemiol. 2017 Nov 1;186(9)

Baseline measurements

At one of 22 assessment centres, each participant was first invited to sign an electronic consent. A touch-screen questionnaire and a computer-assisted interview was then undertaken to collect extensive baseline self-reported information, followed by detailed measurements and a collection of biological samples of blood, urine, and saliva. Briefly, these self-reported data covered aspects of individual's sociodemographics, lifestyles, psychosocial factors, health and medical history, and cognitive function and family background such as illnesses of father/mother/siblings. As for objective measures, they included physical measures (e.g., blood pressure, and heart rate), anthropometrics (e.g., weight body mass index and whole-body bioimpedance measures). Samples collected during baseline assessment were appropriately stored in the aliquots for long-term

frozen storage and were preserved in two geographically different places to protect loss of resources against any unforeseen accidents.

Socio-demographics

The participant's sociodemographic characteristics were used in UKBB-based studies (more details are given in each relevant chapter), due to the well-established evidence of their impact on risks of a wide range of exposure levels and diseases outcomes. Specifically, individuals' ethnicity, employment, education, and household information were collected with variable levels of data granularity using designed questionnaire. For example, seven primary categories of ethnicity were available for participants to select (White, Mixed, Asian or Asian British, Black or Black British, Chinese, Other ethnic group, Do not know, Prefer not to answer).

Lifestyle factors

In regard to assessment of lifestyle, UKBB collected lifestyle-related variables for all individuals at the baseline visit, including physical activity, diet, sleep, smoking, alcohol, and electronic device use, using questions adapted from mostly sourced general population surveys (such as the 2001 Census[82] and the Health Survey for England[83]). Information on physical activity was collected using adapted questions from the validated short International Physical Activity Questionnaire.[8485] Diet were measured based on food frequency questionnaire, which includes self-reported weekly intake of a range of common food and drink items, covering meat, fish, fruit and vegetable etc.. Sleep-related variables were mainly comprised of two aspects: sleep duration and insomnia symptoms. Comprehensive questions on participants' smoking habits were designed, covering smoking duration, frequency, and age started smoking. Most information on alcohol consumption was collected in a similar way as smoking behaviour, but

additionally included beverage specificity.[80] Information on electronic device use were measured with questions such as “In a typical day, how many hours do you spend watching TV?” and ‘In a typical day, how many hours do you spend using a computer? (not including time using a computer at work)”.These questions has been widely used in previous studies as a lifestyle proxy for screen or sedentary time.

Follow-up

Individuals provided their consent for long-term follow-up after undergoing the baseline assessment. Currently, UKBB has adopted both passive data linkage and active data collection strategies to capture health-related outcomes. For the former approach, data already available for full cohorts included secondary care (hospital admissions), national cancer registries, and national death registries, which were updated regularly. Specifically, the hospital inpatient admissions data was provided by the Data Access Request Service and managed by the National Health Service. This dataset is generally referred to as Hospital Episode Statistics Admitted Patient Care. At the time of writing this thesis, the latest records for these datasets are current up to October 2022. In addition, primary care data (general practitioners, GP) were also made available for ~230,000 participants up to 2016 or 2017 depending on the data supplier. To facilitate researchers identifying and phenotyping outcomes more reliably, UKBB have developed bespoke algorithms and generated information on first diagnosis of a small number of health conditions incorporating self-reported and linked health data.[86] Given that not all health-related data can be easily and validly captured by the passive procedure, active data collection was also initiated among a sub-cohort to supplement information on exposures that may vary over time or health outcomes that are difficult to obtain in routine electronic health records. For example, data relating to participants’ cognitive function (a series of cognitive tests), mental health (lifetime mental health events),

gastrointestinal health (gastrointestinal symptoms) and pain (causes of pain, severity and duration of chronic pain) has been specifically collected using web-based questionnaires after the baseline assessment.[87] It is noted that because follow-up is chiefly conducted through linkages to routinely available electronic datasets, it is the main approach applied in the current study.

COVID-19 specific data

To facilitate rapid COVID-19 research, UKBB has been linked to Public Health England (PHE) and Secure Anonymised Information Linkage, where COVID-19 results (positive/negative) were documented for all UKBB participants based on the reverse transcription polymerase chain reaction (PCR) testing. This linkage to the external database could be a reliable source for identifying SARS-CoV-2 infection status at scale, as it covers all PCR results reported during the pandemic in community, outpatient, and inpatient settings. Moreover, PCR-based tests are generally more accurate than lateral flow rapid tests, with much lower rates of both false positives and negatives. To guarantee the completeness and validity of this new linkages, a pilot study was firstly conducted on small number of participants to determine the feasibility of linking PHE's Second Generation Surveillance System (SGSS) to UKBB. A rapid dynamic linkage system was then implemented, which allows a near to real time data on SARS-CoV-2 test results for all UKB participants.[88] Besides those key variables such as negative or positive results for SARS-CoV-2 and date of specimen, necessary auxiliary information was also available, including specimen type as recorded on the laboratory request form (e.g., nasal, nose and throat, sputum) and name of laboratory that processed the sample. Moreover, an inpatient indictor was constructed using a bespoke algorithm developed by PHE's SGSS to infer whether an individual was an inpatient when the sample was taken. [89]

2.2.Cohort designs

2.2.1. Rationale

The majority of observational studies in this thesis used a cohort design, analysed prospectively for UK Biobank-based studies and retrospectively for others. This choice was made due to the distinct advantages of the cohort design over the case-control design. First of all, it is less susceptible to recall bias which typically hinders a reliable inference of causality when studying the effectiveness or adverse reaction of a drug. Also, it allows investigating multiple endpoints so that a broader picture of both the risks and benefits associated with a specific exposure can be revealed at the same time. Moreover, the selection of different control groups to reduce the risk of indication bias is a common practice in pharmacoepidemiologic studies, which is more logically straightforward by doing in a prospective way. Lastly, studying the combined effects of lifestyle, environment, genotype, and other exposures appears to be more feasible within a cohort.

Admittedly, the cohort design is an umbrella term to describe study characteristics and involves identifying the study groups based on the exposure. The cohort specifications typically vary depending on the research questions and extent to which causal effect can be inferred. Here, I summarise the key features of all primary cohorts included in the present thesis and will provide more details in each separate study chapter wherever relevant.

2.2.2. General cohort

All participants registered in the SIDIAP database, aged 18 years and older, constitute the source cohort. I followed up with eligible participants from the first day of each annual year to the last day of the same year. Users, either incident or prevalent, were included as

the numerator cohorts, depending on which epidemiological metrics—such as incidence or prevalence—were calculated in **Chapter 3**.

2.2.3. Incident drug-user/vaccine-recipient cohort

The incident or new user design has been regarded as the standard one for contemporary pharmacoepidemiology study evaluating the causal relevance between drug exposure and occurrence of health outcomes.[90] It has conceptually advantages in mitigating biases, such as indication bias and healthy adherer bias. In **Chapter 4**, I created cohorts of new opioid users and anchored the index date (start of follow-up) as the first treatment episode/prescription/dispensation based on clinically informed washout period during the observational period. Also, in **Chapter 6 and 7**, I included participants receiving first and second doses of novel COVID-19 vaccines, which falls into the concept of incident cohort.

2.2.4. Prevalent drug-user cohort

In contrast to applying incident drug user cohort, I designed a prevalent drug user cohort for a study of COVID-19 susceptibility risk regarding the use of older analgesics where the implementation of incident cohort is not feasible or suboptimal.[91] The primary reason for the choice of prevalent user design is because all studied analgesics, such as Ibuprofen and Paracetamol, were widely used drugs before the outbreak of COVID-19. Identifying a group of people without historical use of these drugs would exclude a large number of subjects and likely lead to severe selection bias. Besides, my research is more prone to address a clinical question in relation to chronic or long-term drug exposure to analgesics rather than intermediate or immediate exposure. Taken these into consideration, in **Chapter 5**, I constructed cohorts by including a mix of both incident and prevalent users.

2.2.5. Active comparator and naïve control cohort

Besides the use of new user design, setting one or more active control groups is another recommended approach to reduce confounding bias in pharmacoepidemiologic studies or broadly non-experimental studies of adverse drug effects. In a historical publication in 1987, Kramer and colleagues[92] stated that "it is important to compare that risk with that of some other real therapeutic option for patients with the same clinical indication. Just as in a clinical trial investigating treatment efficacy, any epidemiologic study of treatment risks should compare two or more viable treatment alternatives." and "it is the comparison among available treatments that is essential in clinical and regulatory decision making". Later on in a 1990 paper, Gerstman and colleagues commented[93] "the fact that pharmaceutical drugs are usually used to treat underlying illnesses suggests that disease rates in exposed individuals will often reflect the condition for which drugs were prescribed in the first place. This phenomenon, often referred to as "confounding by indication", implies that it may be preferable to study pharmaceutical drug-associated risks in relation to the indication for which drugs were originally prescribed". Although those previous suggestions of comparing drugs risks in their proper clinical context were more built upon the worthiness of the risk being measured, it may also aid in controlling for the selection and detection biases, as shown in more recent methodological papers.[90,94] In the current thesis, I adhered to this active comparator principal in all studies of drug or vaccine's effects, including **Chapter 4, 5, and 6**.

2.2.6. First COVID-19 infection cohort

Individuals can have multiple COVID-19 infections in their lifetime or over long observational period under study, a scenario similar to exposure to certain drugs. In terms of inferring COVID-19 sequala either during acute or ongoing stages as study in **Chapter 8**, following people from confirmed first infection onward benefits from several

methodological strengths. Firstly, it provides a clear definition for curating exposure cohort and enables the determination of index date in an intuitive way. Secondly, it is less likely to be impacted by reverse causality bias, where an outcome of interest is the cause of an exposure such as immunodeficiency disorders leading to COVID-19 reinfection but not the reverse. Lastly, it can minimise the risk of misclassifying exposures and controls in observational studies. In real-world settings, COVID-19 testing efforts, including capacity, coverage, and policy coercivity, varied dramatically over time. This generally results in a COVID-19 cohort that likely includes more symptomatic cases and individuals more willing to seek health services.

2.3.Analytic approaches

Details of the descriptive analysis, such as the calculation of annual incidence or cohort characteristics, are provided in the statistical section of each study chapter. Estimating causal effects of pharmaceutical interventions on outcomes is one of the primary goals of pharmacoepidemiologic studies. However, due to the non-randomized nature of the treatment or exposure, systematic differences of baseline characteristics likely remain between comparison groups even after applying stringent design components, such as the active comparator new user design described above, which leads to observed differences in outcomes not entirely attributable to the treatment. Accordingly, appropriate statistical methods must be considered in order to reduce potential biases.

2.3.1. Propensity scores

Propensity score has been increasingly used to examine the safety and efficacy of drugs or vaccines in parallel with availability of big health data.[95–97] In theory, it represents the probability of a patient being assigned to an intervention given on a set of measured baseline covariates, and can guarantee the same distribution of those measured baseline covariates between groups if a true propensity score is obtained.[98] Because of this property, conditioning on the propensity score enable the mimic of some RCT's characteristics: balance of covariates at baseline between the different treatment arms. However, unlike randomisation that removes both measured and unmeasured subject characteristics, unmeasured covariates may still exist after conditioning on the propensity score. Currently, four primary applications of propensity score have dominated the medical literature to estimate treatment effects, including matching, inverse probability weighting (IPW), stratification and covariate adjustment. In the present thesis, I used the propensity score matching (**Chapter 4**, **5**, **8**), IPW (**Chapter 6**) as the primary analyses for each study.

2.3.2. Regression model

Regression model such as the Cox proportional hazards model is another statistical approach to deal with confounding bias when analysing observational data, and is the most commonly used method in conventional epidemiology before the propensity score was introduced.[99,100] Similar to most statistical techniques, the validity of "adjusted" or "corrected" estimate of effects from multivariable regression depends on the extent to which the underlying assumptions about the model are met. Compared to propensity score-based approach for adjusting for baseline difference, this direct covariate adjustment method has been primarily criticized for the potential of overfitting, especially when number of patients or outcome events are small, which may lead to either biased estimates and/or loss of precision. Historically, a rule of thumb for sample size insufficient for regression adjustments is to have less than 10 events per covariate included in the model.[100] However, because all of my studies had adequate sample and was not limited by this rule, I decided to implement this approach in **Chapter 7**, **8** where more than one exposures of interest were studied simultaneously.

2.3.3. Negative controls

Following the philosophy of experimental studies where biologists routinely designed one or multiple "negative control" laboratory tests to check whether a prior known non-effect can be verified with the current research framework, an analogous negative control experiment using observational data was proposed and has been increasingly accepted in the field of epidemiology. After the adoption of typical statistical techniques to address confounding by measured variables, the negative control analysis can be performed as a supplement to detect the potential residual biases resulting either from confounding, mismeasurement, selection or analytic error, especially when the casual nature of observed association is of particular interest for the research. Initially, Marc and

colleagues[101] suggested that ideally a negative control outcome should be selected as a condition without a hypothesized underlying causal mechanism linked to the exposure but is very likely to involve the same sources of bias as the original studied outcome. In practice, however, reliable pre-specification of such outcome that met all correct assumptions is challenging and relies heavily on the investigator's subjective existing knowledge. Instead, a more data-driven approach has been recently developed to select negative outcomes. In brief, it comprises a series of semiautomated processes including extracting information from literature, product labels, and spontaneous reporting and using a logistic regression model to fit a list of negative and positive controls as the reference, as detailed elsewhere. In the present thesis, I used both hypothesis-generated (**Chapter 6, 8**) and data-driven (**Chapter 5**) tactics to strengthen the causal interpretation of results from epidemiologic studies.

2.3.4. Mendelian randomisation

Mendelian randomisation is a fast-developing methodological approach in epidemiology that uses genetic variants as instrumental variable (IV) for modifiable risk factors influencing population health.[102] By definition, an IV is a variable that is used in econometrics and statistics to identify and estimate causal relationships when controlled experiments are not feasible. This technique is gaining popularity due to its potential to overcome the primary limitation of observational studies: uncontrolled confounding. Therefore, the evidence resulting from it sits at the interface of experimental and observational studies (**Figure 2 2**). However, as with all epidemiological methodologies, the conclusions drawn from Mendelian randomization studies rely on specific assumptions. Three fundamental assumptions underlying this method are: first, IVs present an association with the risk factor/exposure under investigation (the relevance assumption); second, they lack a shared causal relationship with the outcome (the

independence assumption); and third, their influence on the outcome occurs solely through the risk factor/exposure (the exclusion restriction assumption). I used Mendelian randomization to triangulate findings in **Chapter 7**.

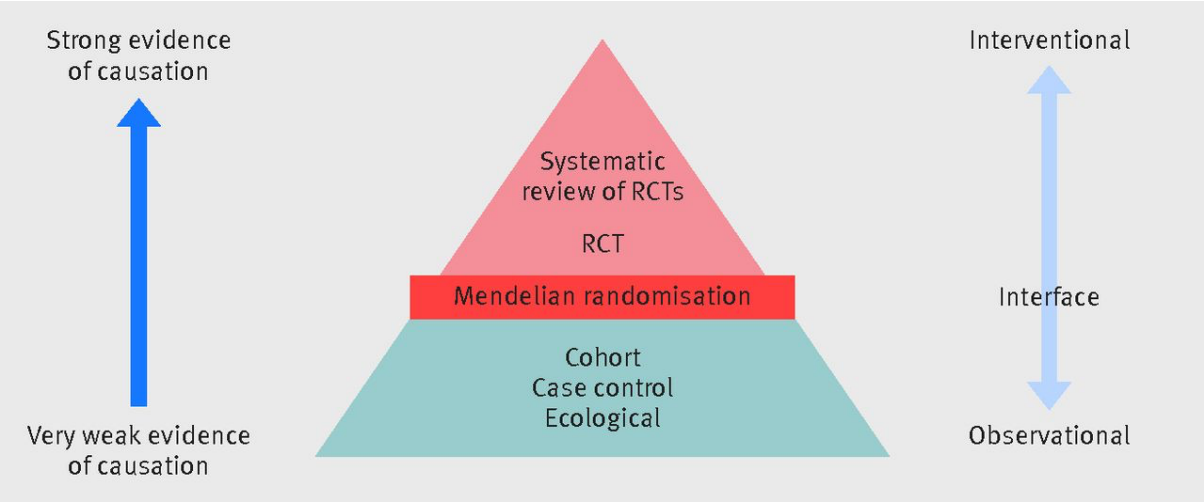


Figure 2 2 A hierarchy of observational and experimental data

Adapted from: Neil M Davies, et al. Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ 2018; 362.

Chapter 3

Opioid utilisation in Catalonia

3. Opioid utilisation in Catalonia

3.1.Aim

To characterize trends and patterns of opioid use from 2007 to 2019 in general adults in Catalonia.

3.2.Introduction

The risks associated with opioid use are increasingly recognised. According to the 2019 World Drug Report, 35 million people worldwide suffered from substance use disorders, and two-thirds of the deaths associated with substance use disorders were caused by opioids. The US is the country that appears to be the most affected, with 4 percent of its population using opioids and an increase in its age-adjusted rate of drug overdose deaths between 1999 and 2018. [103] In Europe, opioid consumption is lower than in the United States. However, the rapid increase in opioid prescriptions (e.g., a 34 percent increase in opioid prescriptions in England between 1998 and 2016 and a twofold increase in the number of opioid users in the Netherlands between 2008 and 2017) portends a potential upcoming public health crisis in prescribing opioid. [104,105]

Due to methodological differences and types of opioids analysed, it is difficult to make direct comparisons between European reports on opioid consumption. [106,107] Despite the fact that two previous studies reported an increase in the use of opioids in Spain over the past decade, they were limited by a lack of data on critical subpopulations and doses of opioids measured in MME.[108,109] In addition, none of them analysed the opioid indications and prescribing patterns. Therefore, the purpose of this study is to comprehensively characterise the trend of opioid use in Catalonia (number of users, dispensations, and MME) (Spain).

3.3.Methods

3.3.1. Data sources

I used the data from the SIDIAP, which has been described in the Method Chapter.

3.3.2. Study design

All participants registered in the SIDIAP database aged 18 years or older from January 1, 2007, to December 31, 2019, were included for the current analyses.

3.3.3. Opioids classification

I quantified opioids use based on pharmacies dispensations rather than on general practitioners (GP) prescriptions to better reflect the actual opioid consumption. I identified all types of opioids and their combinations by 7-digit ATC codes. I grouped opioids by ingredient: codeine [R05DA04, N02AA59, N02AJ06, N02AJ07, N02AJ08], tramadol [N02AX02, N02AJ13, N02AJ14], oxycodone [N02AA05, N02AA55], tapentadol [N02AX06], fentanyl [N02AB03], and morphine [N02AA01]. All additional opioids were combined in the category of other opioids, including dihydrocodeine [N02AA08], hydromorphone [N02AA03], dextropropoxyphene [N02AC04], buprenorphine [N02AE01], pethidine [N02AB02], and pentazocine [N02AD01].

For each dispensation of opioids, I extracted the total dose, unit, and dispensation date and converted the original opioids dose to oral MME based on prespecified conversion factors (**sTable 3 1**).

3.3.4. Statistical analyses

I calculated the following summary measures to assess yearly opioid use (over 12 months from 1st Jan to 31st Dec): (1) number of opioid users per 1000 individuals, (2) number of opioid dispensations per 1000 individuals, and (3) oral MME per 1000 individuals. Accordingly, the numerators were calculated as the number of unique individuals who

had received at least one opioid dispensation, number of any opioid dispensations, and total oral MME of any opioid dispensations during the index year. The denominator is the number of eligible individuals (defined as those registered in the database up to the first day of the index year) and is the same for the three measures. I calculated each measure in the overall population (total opioids and each opioid subtype) and subgroups stratified by age (five-year age groups from 18 up to 80), sex (male or female), living area (rural or urban), and socioeconomic status (MEDEA) which was divided into fifths: the first representing the least deprived (U1), and the last (U5) the most deprived. To adjust for demographic changes over time, an age direct standardization was performed using the 2007 population as the reference with 5-year age bands. I quantified the secular trend between 2007 and 2019 with percentage change (PC) using the formula: (value in 2019 - value in 2007)/ (value in 2007) * 100 and calculated its 95% confidence intervals based on Poisson regressions with robust standard errors.[110]

I estimated the relative proportions of nine pain-related conditions (cancer, back pain, neck pain, osteoarthritis, fibromyalgia, cough, fracture, falls, surgery) as potential clinical indications occurring in the year before the opioid dispensation. Specifically, in each year, I defined the first opioid dispensation as the index date and categorized people as incident or prevalent users depending on whether they were dispensed the same opioid one year before the index date. Users can have multiple indications if relevant diagnoses were identified on or 1-year before the index date, but each indication was only counted once for each person. These conditions were prespecified a priori through operational definitions using ICD-10 codes (**sTable 3 2**). All analyses were conducted with R 4.04.

3.4.Results

3.4.1. Overall trends

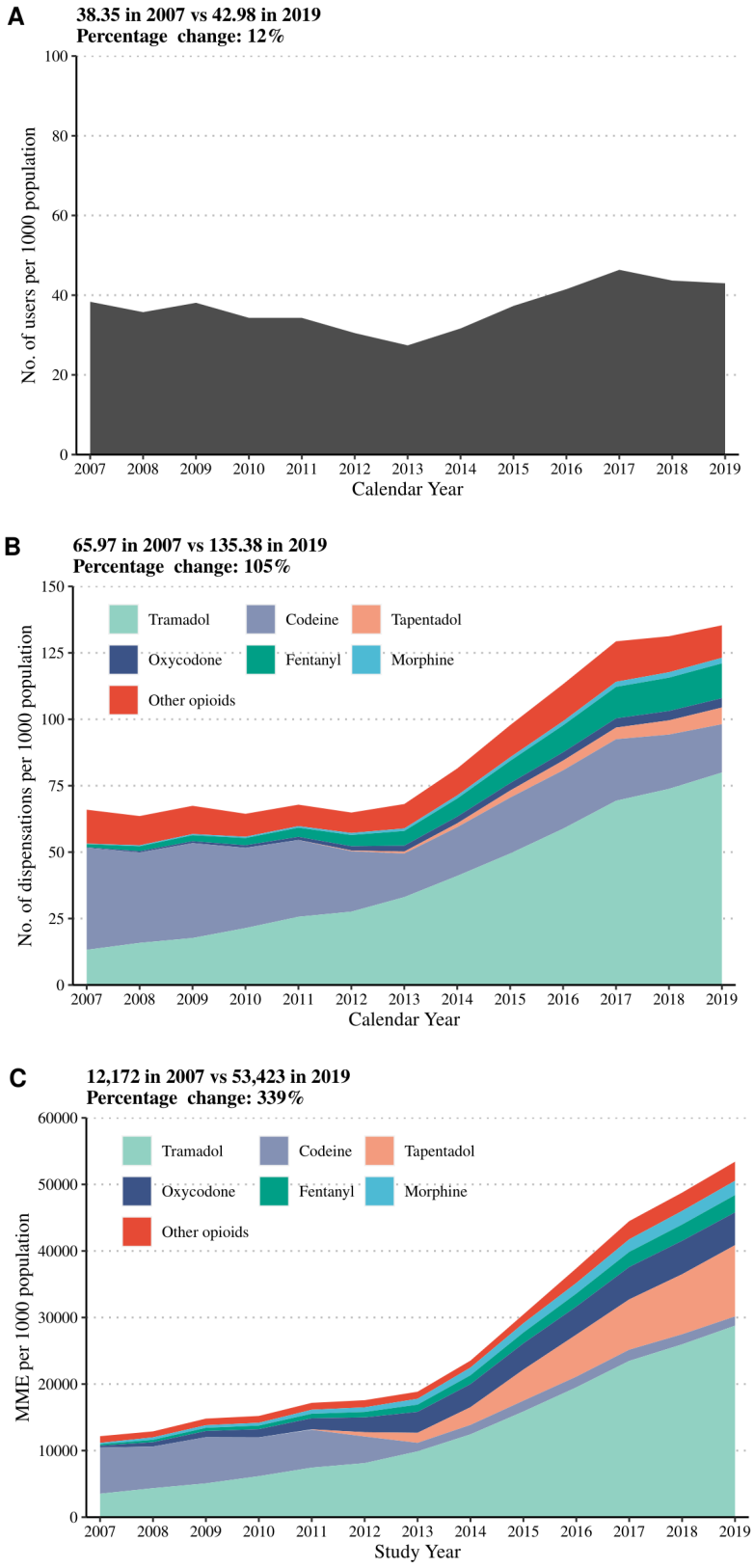
The size and composition of the population covered by SIDIAP remained stable between 2007 to 2019 (**Table 3 1**). There were 4,656,197 participants in 2007 vs 4,798,114 in 2019. The mean age increased from 47.3 (SD: 18.4) in 2007 to 50.4 (SD:18.6) in 2019. The proportion of males and females were similar over the study period, with 510 females per 1000 people and 512 females per 1000 people in 2007 and 2019, respectively.

Table 3 1 Basic demographic information of entire registered residents in SIDIAP by year

Study year	Registered residents	Age	Sex		Living area			Socioeconomic status					
	Number	Mean (SD)	Female (%)	Male (%)	Urban (%)	Rural (%)	Missingness (%)	U1 (%)	U2 (%)	U3 (%)	U4 (%)	U5 (%)	Missingness (%)
2007	4656197	47.3	2376117	2280080	3765841	876934	13422	662894	645092	648310	641566	617919	1440416 (30.9)
2008	4791568	47.4	2440103	2351465	3884031	893012	14525	681906	662310	665906	661993	643374	1476079 (30.8)
2009	4903562	47.6	2491639	2411923	3985097	905678	12787	698032	676642	681163	679506	666174	1502045 (30.6)
2010	4963931	47.8	2520563	2443368	4040398	911924	11609	708803	686299	689124	689437	678314	1511954 (30.5)
2011	4966548	48.2	2522892	2443656	4044544	910754	11250	709464	688071	691783	694016	682616	1500598 (30.2)
2012	4966393	48.6	2523389	2443004	4046763	908734	10896	714658	691936	693974	694908	685222	1485695 (29.9)
2013	4875535	49.0	2481589	2393946	3970599	896281	8655	706661	689637	691132	690674	679395	1418036 (29.1)
2014	4855506	49.3	2473821	2381685	3953824	893162	8520	710235	692605	691730	687668	677613	1395655 (28.7)
2015	4790736	49.6	2443679	2347057	3902606	879670	8460	704882	690489	688442	682102	669193	1355628 (28.3)
2016	4772900	49.9	2437454	2335446	3888962	875391	8547	709429	694429	690761	683864	670648	1323769 (27.7)
2017	4775950	50.1	2442100	2333850	3893455	873785	8710	715084	700415	695413	688785	676535	1299718 (27.2)
2018	4786105	50.3	2449613	2336492	3906884	871314	7907	723349	708867	702914	696312	686786	1267877 (26.5)
2019	4798114	50.4	2456520	2341594	3922138	868051	7925	731733	717815	711406	705876	699897	1231387 (25.7)

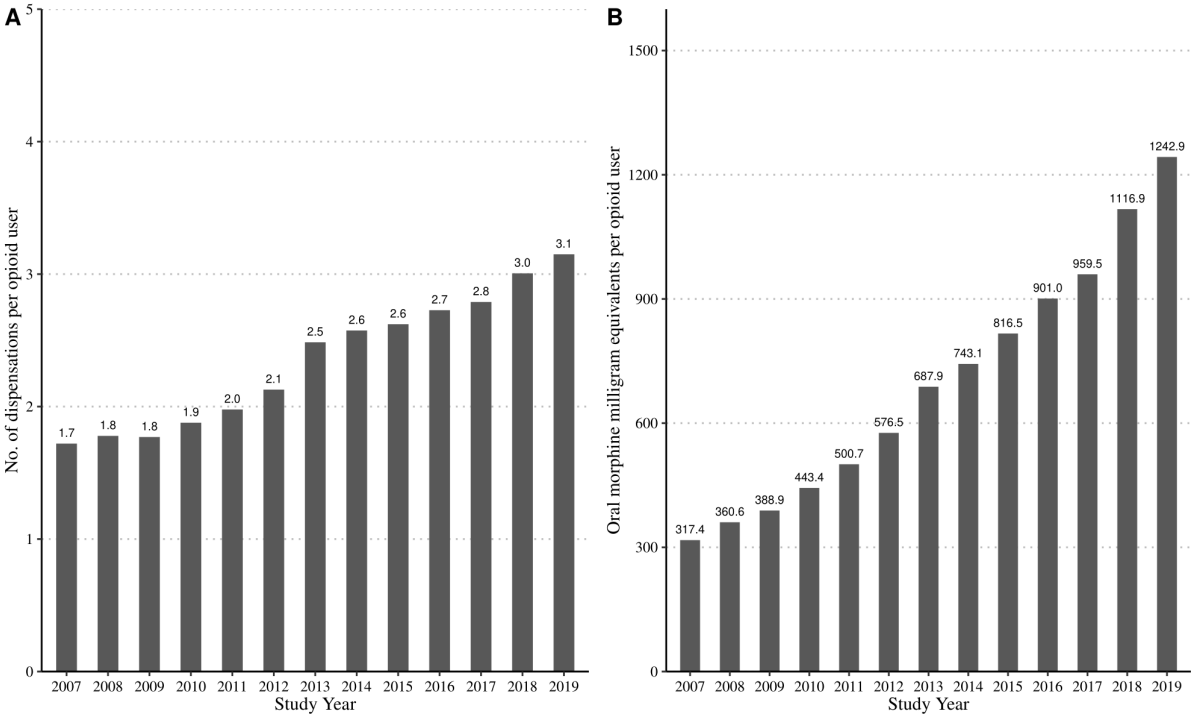
In the Error! Reference source not found. and **sTable 3 3,** the number of people with opioid dispensations increased by 12% (from 38.4 per 1000 in 2007 to 43.0 per 1000 individuals in 2019, P <0.01) during the study period. After counting for multiple dispensations per person during the year and correcting for opioid strength, there was a 105% increase in the number of opioid dispensations (from 66.0 per 1000 to 135.4 per 1000 individuals, P <0.01) and a 339% increase in MME (from 12,172 to 53,423 mg per 1000 individuals, P <0.01). After age-standardization, the increasing trend of opioid use was mitigated to 3%, 82%, and 282% accordingly.

Figure 3 1 Number of opioid users (A), number of opioid dispensations (B), and oral MME (C) per 1000 population from 2007 to 2019 in the overall population



Of note, the opioid dispensations and MME per opioid user increased from 1.7 to 3.1 and 309 to 1350 respectively between 2007 and 2019 (**Table 3 2**).

Figure 3 2 Number of opioid dispensations (A) and oral morphine milligram equivalents (B) per opioid user from 2007 to 2019



The number of opioid users per 1000 individuals and the number of opioid dispensations per 1000 individuals remained constant until 2013, after which, it increased sharply until the end of the study period. The increase from 2013 was driven mainly by the use of tramadol (**sTable 3 3**). In 2007, codeine was the most dispensed opioid (31.5 users and 38.4 dispensations per 1000 individuals), followed by tramadol (7.5 users and 13.2 dispensations per 1000 population). In comparison, in 2019, tramadol users increased to 26.2 per 1000 individuals and dispensations to 80.0 per 1000 individuals, making up 61% and 59% of all opioid users and dispensations.

When considering oral MME, opioid use seemed to continuously increase, primarily due to a rise in tramadol use from MME 3,527 per 1000 individuals in 2007 to MME 28,776 per 1000 individuals in 2019, making up 50% of total oral MME.

3.4.2. Subgroups

Between 2007 and 2019, there was an age-dependent shift in opioid use, with a slight decrease among younger adults but a dramatic increase among the elderly. For example, opioid users aged 18-24 years old decreased from 36.4 in 2007 to 10.5 in 2019 per 1000 individuals (PC -71.1%); but increased from 27.5 in 2007 to 72.0 in 2019 per 1000 individuals (PC 162.2%) in ≥ 80-year-old (**Table 3 2**).

Table 3 2 The percentage changes of opioid use between 2007 and 2019 among subgroups by age, sex, living area and socioeconomic status

	Number of users per			**Number of**			**Oral MME per 1000**		
	2007	2019	PC (%)	2007	2019	PC	2007	2019	PC
Age									
[18,25)	36.41	10.53	-71	54.52	18.14	-67	7677	1715	-78
[25,30)	31.86	15.36	-52	47.71	27.12	-43	7020	3463	-51
[30,35)	32.29	18.67	-42	48.80	35.61	-27	7622	7289	-4
[35,40)	34.40	24.70	-28	52.40	51.79	-1	8633	14842	72
[40,45)	35.85	31.29	-13	56.12	72.72	30	9717	25609	164
[45,50)	38.50	37.62	-2	63.46	95.83	51	12135	37985	213
[50,55)	42.09	47.07	12	71.94	133.81	86	13790	58490	324
[55,60)	46.77	55.43	19	82.07	172.66	110	15358	75822	394
[60,65)	50.18	62.77	25	92.15	203.39	121	18201	85175	368
[65,70)	50.75	67.16	32	100.17	220.48	120	20724	88325	326
[70,75)	48.04	73.18	52	100.44	259.59	158	21550	104054	383
[75,80)	41.34	75.83	83	88.90	293.16	230	19083	120296	530
>= 80	27.46	71.99	162	63.16	331.01	424	14558	135364	830
Sex									
Female	42.04	50.28	20	74.19	166.45	124	13977	66744	378
Male	34.49	35.33	2	57.41	102.78	79	10291	39448	283
Living area									
Rural	42.28	44.55	5	73.45	142.70	94	13914	58170	318
Urban	37.48	42.67	14	64.32	133.85	108	11783	52400	345
Missingness	24.51	26.75	9	40.60	87.82	116	7493	39424	426
Socioeconomic									
U1	29.34	30.03	2	50.62	96.05	90	9190	37674	310
U2	38.24	39.90	4	65.12	126.10	94	11793	48418	311
U3	41.52	44.86	8	71.17	140.43	97	12760	53726	321
U4	42.83	49.89	16	72.45	152.67	111	13108	59032	350
U5	42.01	51.15	22	70.73	157.32	122	12623	62818	398
Missingness	37.54	42.79	14	66.16	138.85	110	12840	56968	344

Also, 2019 than 2007 had consistently higher rates of opioid users for other subgroups, with PC of 19.6% 13.9% and 21.8% in female, urban residents, and the most deprived subgroup respectively. More pronounced trends were observed across all studied subgroups in terms of opioid dispensations and MME

3.4.3. Pain-related diagnoses among opioid users

Over the 13 years, the relative proportions of the nine pain-related conditions analysed remained stable for the main weak opioid subtypes (tramadol and codeine) as well as for tapentadol and the category of other opioids (dihydrocodeine, hydromorphone, dextropropoxyphene, buprenorphine, pethidine and pentazocine) (**sFigure 3 1** and **sFigure 3 2**). Back and neck pain represented more than 50% of the indications of weak opioids (tramadol and codeine) and of tapentadol which is an opioid classified as strong.[111] Strong opioids such as fentanyl, morphine and oxycodone were more frequently indicated for cancer, however, there is an increasing trend of use of these strong opioids to treat non-cancer medical conditions; For example, the non-cancer indications for morphine only comprised 29% of any pain-related medical conditions in 2007, but this figure gradually increased to 60% in 2019.

3.5.Discussion

Based on a representative cohort of adults covering over 80% of the population in Catalonia, I observed that the use of opioids was relatively stable from 2007 to 2013 but increased sharply after that until 2019. These increases dominated particularly in older adults and were even more pronounced when accounting for opioid strengths. Regarding the opioid indications, this report shows increasing use of strong opioids (such as morphine, fentanyl, and oxycodone) to treat non-cancer medical conditions.

3.5.1. Possible factors contributing to the rise

Several factors might underline the recent rising trends of opioid use in Catalonia amid the global calling for early and active treatment of pains.

First, the ageing of the population with the consequent increase in chronic painful medical conditions, such as osteoarthritis, could have contributed to the overall increase in the opioid dispensations observed as effective medications for pain management in the elderly is highly needed. Also, poor modern lifestyle habits and the expanded occupational risk factors could have also contributed to the high proportion of back and neck pains observed in my study, potentially leading to increased use of both weak and strong opioids.[112] Safety concerns regarding the potential cardiovascular risks for the use of non-steroidal anti-inflammatory drugs (NSAIDs) might have facilitated clinicians to prescribe opioids as an alternative therapy in this population[4]. Two meta-analyses published in 2011 and 2013 found that almost all NSAIDs were associated with a higher risk of cardiovascular events such as myocardial infarction, stroke, or cardiovascular death [113]. Even though uncertainty remains, it is still recommended that cardiovascular risk be considered when prescribing NSAIDs[114,115].

Second, the observed growth of the opioid dispensations has been even more pronounced since 2013, which coincides with changes in the regulatory framework to facilitate opioid

prescribing (integrating these opioids to the electronic prescription and extending its renewal from quarterly to annually).[116] Although more research is needed to quantify the impact of this reform on the clinical practice of opioid prescription in Spain, changes in policy regulations have been previously identified in other countries as responsible for the increase in opioid prescriptions.[117]

In this study, I found that tramadol was the only type of opioid that experienced a consistent increase during the entire period from 2007 to 2019, which is in line with previous national reports that showed a 10-fold increase in five years from 1993 to 1998[118]. Since first licensed in Spain, tramadol had been marketed as an "atypical" opioid with fewer side effects and addiction potential compared to other opioids, which could explain the increase in dispensations observed. Tramadol and codeine are both classified as "weak" opioids and could be prescribed for similar indications, although my recent research showed a noticeable difference in their safety profile.[119] In this study, I found that the number of users and dispensations of these two opioids varied substantially, with a stable decrease of codeine use after 2013. This difference can be again explained by changes in governmental policies in 2012[116] where many products containing codeine were no longer funded by the Spanish social security, leading patients to seek another similar pain killer such as tramadol.

3.5.2. Opioid pandemic

The increased rate of opioid dispensations in Catalonia is similar to reported in other regions of Spain[108,109]. A recent cross-sectional study, carried out in a mid-eastern region of Spain, analysed the opioid prescription and MME from 2010 to 2018.[109] During this period, they found that the MME of tramadol increased 2 fold, fentanyl more than tripled, and tapentadol was the second in terms of MME in 2018. My results go in the same line, with a much more pronounced increase in MME for tramadol (increasing more than 7

fold from 2007 to 2019), fentanyl (increasing more than 10 fold from 2007 to 2019), and tapentadol (increasing more than 160 fold from very low levels after its commercialization in 2011).

As in this study, other European reports have also observed an overall increasing trend in opioid use in the last decade[105–107,120]. Methodological differences of these studies render comparison difficult, and their results also vary on the type of opioid that drives the increase. For the Netherlands[105], this increase is at the expense of oxycodone (with a 4-fold increase from 2007 to 2017). In the UK, buprenorphine and fentanyl are the ones responsible for the increase between 2008 and 2018[120]. Fentanyl is the opioid most consumed in western, northern EU countries and Spain between 2014 and 2016.[107] Results on the use of tramadol and codeine are also differing; while tramadol use declined in the Netherlands especially from 2013 to 2017,[105] in France tramadol and codeine increased a 62 and 42% from 2006 to 2015[106] respectively. My study accounted for all the opioids commercialized in Spain including tramadol and codeine which are commonly used as the second step in the WHO analgesic ladder.[111] Results show that tramadol was responsible for the increase both in the number of dispensations and total MME.

Although the opioids use in Catalonia is still far from what is reported in the US,[121] the increasing trend of the opioid dispensations and MME reported in this study, which is similar to what has been published in other regions of Spain, foresees a future health care problem in this country. The opioid epidemic is a multifaceted crisis that requires the coordinated action of both governmental regulations and clinicians. The sharper increase of the opioid dispensations reflected in my data from 2013 onwards, which coincides with a loosening of the opioid prescription's regulation suggests that prevention strategies should start here. More stringent opioid regulations have been already

implemented in the US, and recent reports attribute it to the decreasing trend in the opioid prescriptions seen in recent years.[122]

3.5.3. Strengths and limitations

The main strength of this study is the use of a representative cohort of more than 4.5 million patients in Catalonia and the use of real-world data of primary health care centres with detailed drug dispensation information, including ATC code, dose, unit, and duration.

Several limitations warrant consideration in this study. First, my data provide no information on opioid-related complications, overdose, illicit opioid use, or mortality. Evidence on both opioid dispensations and side effects is crucial to weigh opioid risks and benefits to public health. While an increase in opioid dispensations may reflect more significant attention to effective pain management or patients' improved expectations of pain relief, the increase in total MME might reflect the burden of opioid exposure in my population.

Second, the retrospective review of pharmacy dispensing data has limitations; I were unable to assess direct drug compliance and therefore all drug dispensed was assumed to be used. However, I expect that the non-compliance rate is stable over the study period and would have little impact on my estimate of trends. Furthermore, compared to most prior population-level-based studies that used prescribing databases to surrogate opioid consumption, my study using dispensed prescriptions is unlikely to be subject to overestimation of opioid use due to unfilled prescriptions.

Third, the opioid dispensations were not automatically linked to a specific medical condition and although an operational definition was defined a priori to assess the indications, the risk of misclassification still exists. Such information is of great value for distinguishing opioids used for acute pain, cancer pains, or chronic non-cancer pain and understanding the driving force behind the increase.

Fourth, I only accounted for dispensations prescribed in the community pharmacies and were not able to cover those extended at the hospital level, with the consequent risk of underestimating the real opioid consumption. However, these would mostly affect acute treatments as chronic treatments are regulated by primary care clinicians and therefore would be captured at some point in my database.

In conclusion, my findings show a considerable increase in opioid dispensations and MME among the elderly population over 2007-2019 and a shift in the opioid indications towards using strong potent opioids to treat non-cancer pain. Authorities should implement more rigorous opioid stewardship, particularly among older people, to prevent further escalation in Catalonia.

3.6.Supplements

sTable 3 1 Conversion factors of Morphine Milligram Equivalents for each studied opioid

ATC codes	Opioid	Conversion Factor
N02AJ13	Tramadol	0.1
N02AX02	Tramadol	0.1
N02AJ14	Tramadol	0.1
R05DA04	Codeine	0.15
N02AJ06	Codeine	0.15
N02AA59	Codeine	0.15
N02AJ08	Codeine	0.15
N02AJ07	Codeine	0.15
N02AJ06	Codeine	0.15
N02AX06	Tapentadol	0.4
N02AA05	Oxycodone	1.5
N02AA55	Oxycodone	1.5
N02AB03	Fentanyl	7.2
N02AA01	Morphine	1
N02AE01	Other opioids (Buprenorphine)	7
N02AA03	Other opioids (Hydromorphone)	4
N02AC04	Other opioids (Dextropropoxyphene)	0.23
N02AB02	Other opioids (Pethidine)	0
N02AD01	Other opioids (Pentazocine)	0.37
N02AA08	Other opioids (Dihydrocodeine)	0.25

sTable 3 2 ICD-10 codes for the identification of nine pain-related conditions

Osteoarthritis	M18.9, M17.9, M47.9, M18.10, M15.9, M47.899, M19.90, M19.049, M15.0, M19.039, M16.9, M18.0, M15.1, M17.10, M15.2, M15.4, M17.0, M16.0, M16.10, M15.8, M15.3, M17.30, M16.30, M16.50, M16.6, M18.50, M16.7, M17.4, M17.2, M16.2, M18.2, M16.4, M18.30, M19.071, M17.5, M19.019, M19.271, M19.079, M19.011, M19.072, M19.041, M19.172, M19.111, M19.029, M19.012, M18.4, M19.119, M19.219, M19.272, M19.279, M19.171, M19.042, M19.112, M19.141, M19.149, M19.031, M19.021, M19.179, M19.0, M19.032, M19.242, M19.2, M19.241, M19.022
Fibromyalgia	M79.7
Back pain	M54.5, M54.9, M54.89, M51.26, M54.40, M54.2, M43.10, M51.9, M54.30, M54.10, M43.00, M54.6, M53.1, M53.3, M43.8X9, M43.9, M51.24, M43.20, M53.9, M51.34, M51.17, M51.86, M51.16, M43.3, M53.0, M51.06, M51.47, M51.05, M51.25, M53.80, M51.36, M43.5X9, M51.46, M51.35, M51.84, M51, M51.37, M51.2, M43.4, M51.27, M51.15, M51.45, M51.87 M51.85,M51.1,M51.0
Neck/shoulder pain	M43.6, M54.2, M25.50, M50*, M53.0, M53.1,
Cancer	C00*-D49*
Bone fractures	S62.90XA, T14.8, T07, S92.309A, S52.90XA, S22.009A, S22.39XA, S32.10XA, S72.90XA, S62.309A, S42.009A, S62.109A, S42.309A, S82.209A, S82.009A, S72.009A, S82.409A, S92.009A, S42.409A, S32.009A, S82.90XA, S22.20XA, M80.80XA, S82.899A, S62.009A, S32.2XXA, S32.9XXA, S42.209A, S82.66XA, S42.109A, S82.56XA, S62.209A, S82.109A, S72.109A, S32.309A, S92.109A, S22.49XA, S82.309A, S72.26XA, S32.409A, S32.509A, S72.409A, S72.309A, S22.5XXA, S72.8X9A
Cough	R05

Falls	W19.XXXA, W00.0XXA, W01.0XXA, W06.XXXA, W10.9XXA, W08.XXXA, W10.1, W03.XXXA, W00.9XXA, W18.39XA, W01.0, W16.42XA, W12.XXXA, W07.XXXA, W15.XXXA, W05.0XXA, V00.891A,W14.XXXA, W11.XXXA, W17.89XA, W18.30, W13.9XXA, W09.8XXA, W18.01XS, W04.XXXA, W13.3XXS, W13.3XXD, W13.3XXA, W10.8, W10.1XXS, W13.3, W01.198D, W10.8XXS, W18.31XS, W18.30XA, W18.09, W17.81, W06.XXXD, W18.30XD, W13.2XXA, W01.198A, W01.10XS, W18.30XS, W18.39, W00.0XXS, W10.8XXD, W06, W10.8XXA, W01.190A
Major surgeries (Joint replacement, limb amputation)	Z96.6, Y83.5

*all subcodes included

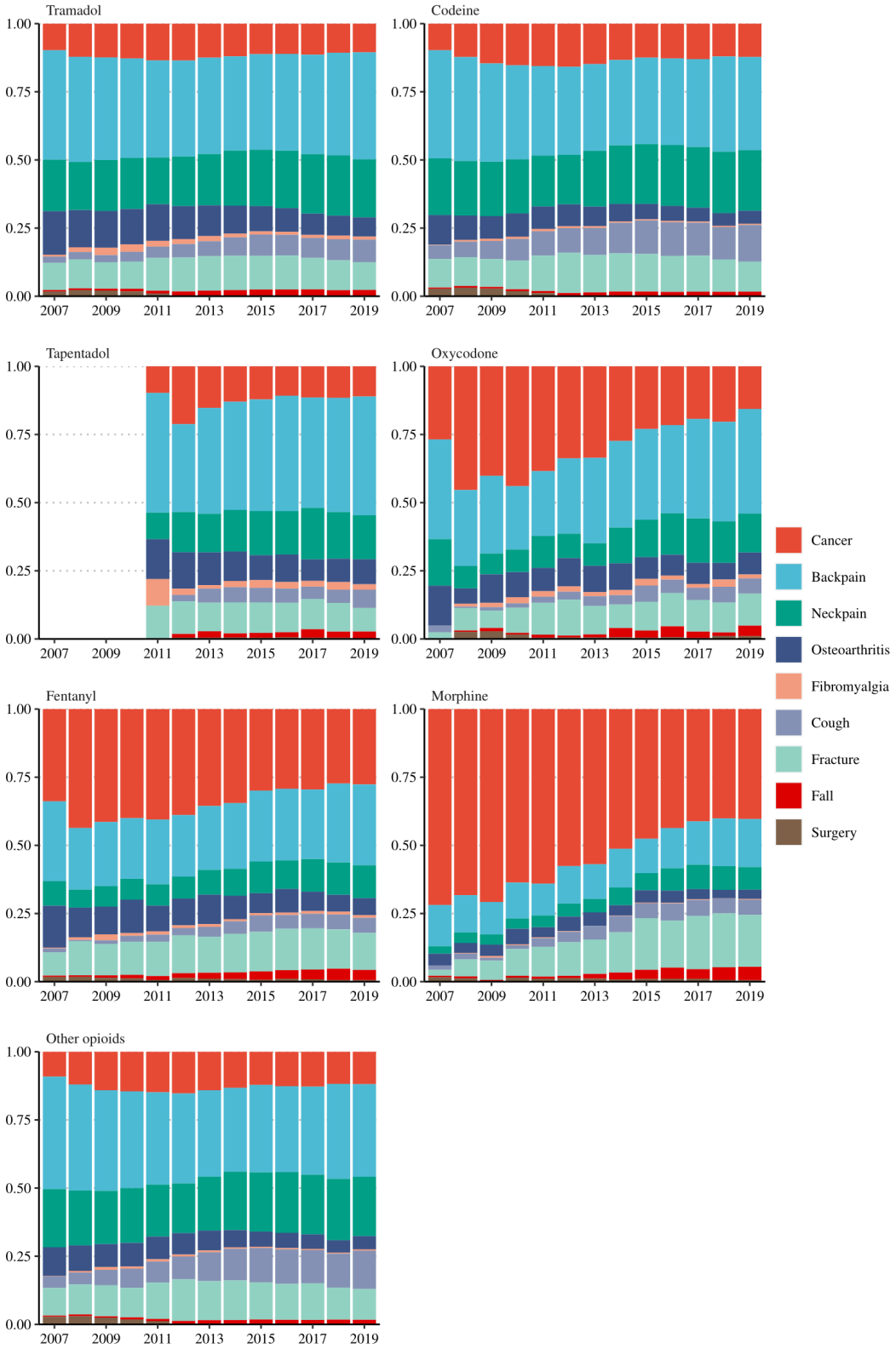
sTable 3 3 Use of opioids and their subtypes, measured by different metrics from 2007 to 2019

Study year	Opioids	Opioids (AS)	Tramadol	Codeine	Tapentadol	Oxycodone	Fentanyl	Morphine	Others
Number of users per 1000 population									
2007	38.35	38.35	7.47 (0.19)	31.52 (0.82)	NA	0.04 (0)	0.41 (0.01)	0.09 (0)	11 (0.29)
2008	35.74	35.74	8.36 (0.23)	27.83 (0.78)	NA	0.12 (0)	0.55 (0.02)	0.15 (0)	9.38 (0.26)
2009	38.09	38.06	9.06 (0.24)	29.47 (0.77)	NA	0.15 (0)	0.64 (0.02)	0.19 (0)	9.07 (0.24)
2010	34.31	34.16	10.12 (0.29)	24.57 (0.72)	NA	0.17 (0)	0.69 (0.02)	0.21 (0.01)	7.1 (0.21)
2011	34.31	33.98	11.06 (0.32)	23.5 (0.68)	0.02 (0)	0.23 (0.01)	0.82 (0.02)	0.24 (0.01)	6.57 (0.19)
2012	30.49	29.86	11.55 (0.38)	18.85 (0.62)	0.09 (0)	0.32 (0.01)	0.93 (0.03)	0.3 (0.01)	6.3 (0.21)
2013	27.40	26.53	13.2 (0.48)	13.62 (0.5)	0.21 (0.01)	0.41 (0.01)	1.14 (0.04)	0.34 (0.01)	7.8 (0.28)
2014	31.66	30.42	15.78 (0.5)	15.14 (0.48)	0.42 (0.01)	0.47 (0.01)	1.38 (0.04)	0.41 (0.01)	8.73 (0.28)
2015	37.31	35.53	18.66 (0.5)	17.67 (0.47)	0.74 (0.02)	0.52 (0.01)	1.67 (0.04)	0.49 (0.01)	10.57 (0.28)
2016	41.51	39.29	21.71 (0.52)	18.52 (0.45)	0.93 (0.02)	0.59 (0.01)	1.95 (0.05)	0.56 (0.01)	12.05 (0.29)
2017	46.37	43.44	25.42 (0.55)	19.56 (0.42)	1.08 (0.02)	0.64 (0.01)	2.19 (0.05)	0.64 (0.01)	13.03 (0.28)
2018	43.67	40.36	25.04 (0.57)	17.06 (0.39)	1.26 (0.03)	0.6 (0.01)	2.23 (0.05)	0.62 (0.01)	11.45 (0.26)
2019	42.98	39.33	26.23 (0.61)	14.96 (0.35)	1.41 (0.03)	0.57 (0.01)	2.28 (0.05)	0.62 (0.01)	10.13 (0.24)
Percentage change	12	3	251	-53	6950	1325	456	589	-8
Number of dispensations per 1000 population									
2007	65.97	65.97	13.22 (0.2)	38.41 (0.58)	NA	0.18 (0)	1.21 (0.02)	0.18 (0)	12.77 (0.19)
2008	63.57	63.58	15.84 (0.25)	34.05 (0.54)	NA	0.48 (0.01)	1.84 (0.03)	0.36 (0.01)	11 (0.17)
2009	67.42	67.31	17.67 (0.26)	35.75 (0.53)	NA	0.69 (0.01)	2.26 (0.03)	0.44 (0.01)	10.63 (0.16)
2010	64.43	63.91	21.43 (0.33)	30.22 (0.47)	NA	0.93 (0.01)	2.73 (0.04)	0.49 (0.01)	8.64 (0.13)
2011	67.86	66.64	25.67 (0.38)	28.86 (0.43)	0.03 (0)	1.18 (0.02)	3.44 (0.05)	0.59 (0.01)	8.07 (0.12)
2012	64.89	62.81	27.59 (0.43)	22.75 (0.35)	0.29 (0)	1.6 (0.02)	4.22 (0.07)	0.76 (0.01)	7.69 (0.12)
2013	68.1	64.88	33.07 (0.49)	16.41 (0.24)	0.73 (0.01)	2.24 (0.03)	5.52 (0.08)	0.94 (0.01)	9.2 (0.14)
2014	81.51	76.75	41.06 (0.5)	18.31 (0.22)	1.46 (0.02)	2.53 (0.03)	6.82 (0.08)	1.16 (0.01)	10.15 (0.12)
2015	97.81	91.01	49.48 (0.51)	21.04 (0.22)	2.66 (0.03)	2.81 (0.03)	8.37 (0.09)	1.34 (0.01)	12.11 (0.12)
2016	113.22	104.31	58.82 (0.52)	21.98 (0.19)	3.66 (0.03)	3.11 (0.03)	10.15 (0.09)	1.56 (0.01)	13.95 (0.12)
2017	129.34	117.57	69.34 (0.54)	23.16 (0.18)	4.39 (0.03)	3.48 (0.03)	11.85 (0.09)	1.9 (0.01)	15.24 (0.12)
2018	131.27	117.68	73.81 (0.56)	20.44 (0.16)	5.35 (0.04)	3.53 (0.03)	12.55 (0.1)	2.03 (0.02)	13.57 (0.1)

2019	135.38	120.26	79.95 (0.59)	18.2 (0.13)	6.28 (0.05)	3.5 (0.03)	13.13 (0.1)	2.1 (0.02)	12.22 (0.09)
Percentage change	105	82	505	-53	20833	1844	985	1067	-4
Oral morphine milligram equivalents per 1000 population									
2007	12172	12172	3527 (0.29)	6937 (0.57)	NA	290 (0.02)	220 (0.02)	191 (0.02)	1006 (0.08)
2008	12888	12886	4357 (0.34)	6264 (0.49)	NA	646 (0.05)	363 (0.03)	370 (0.03)	889 (0.07)
2009	14814	14755	5081 (0.34)	6944 (0.47)	NA	922 (0.06)	479 (0.03)	395 (0.03)	993 (0.07)
2010	15211	15026	6166 (0.41)	5836 (0.38)	NA	1221 (0.08)	542 (0.04)	421 (0.03)	1026 (0.07)
2011	17178	16766	7447 (0.43)	5684 (0.33)	64 (0)	1677 (0.1)	676 (0.04)	601 (0.03)	1029 (0.06)
2012	17576	16866	8125 (0.46)	3991 (0.23)	651 (0.04)	2227 (0.13)	823 (0.05)	691 (0.04)	1070 (0.06)
2013	18852	17677	9891 (0.52)	1280 (0.07)	1505 (0.08)	3150 (0.17)	1083 (0.06)	886 (0.05)	1056 (0.06)
2014	23526	21715	12446 (0.53)	1421 (0.06)	2657 (0.11)	3476 (0.15)	1326 (0.06)	1158 (0.05)	1044 (0.04)
2015	30461	27757	15880 (0.52)	1622 (0.05)	4673 (0.15)	3946 (0.13)	1624 (0.05)	1382 (0.05)	1333 (0.04)
2016	37404	33572	19505 (0.52)	1603 (0.04)	6316 (0.17)	4219 (0.11)	1961 (0.05)	1588 (0.04)	2213 (0.06)
2017	44488	39391	23481 (0.53)	1681 (0.04)	7557 (0.17)	4870 (0.11)	2268 (0.05)	1890 (0.04)	2741 (0.06)
2018	48773	42747	25970 (0.53)	1510 (0.03)	9028 (0.19)	5023 (0.1)	2448 (0.05)	2035 (0.04)	2758 (0.06)
2019	53423	46486	28776 (0.54)	1390 (0.03)	10712 (0.2)	4926 (0.09)	2602 (0.05)	2149 (0.04)	2870 (0.05)
Percentage change	339	282	716	-80	16638	1599	1083	1025	185

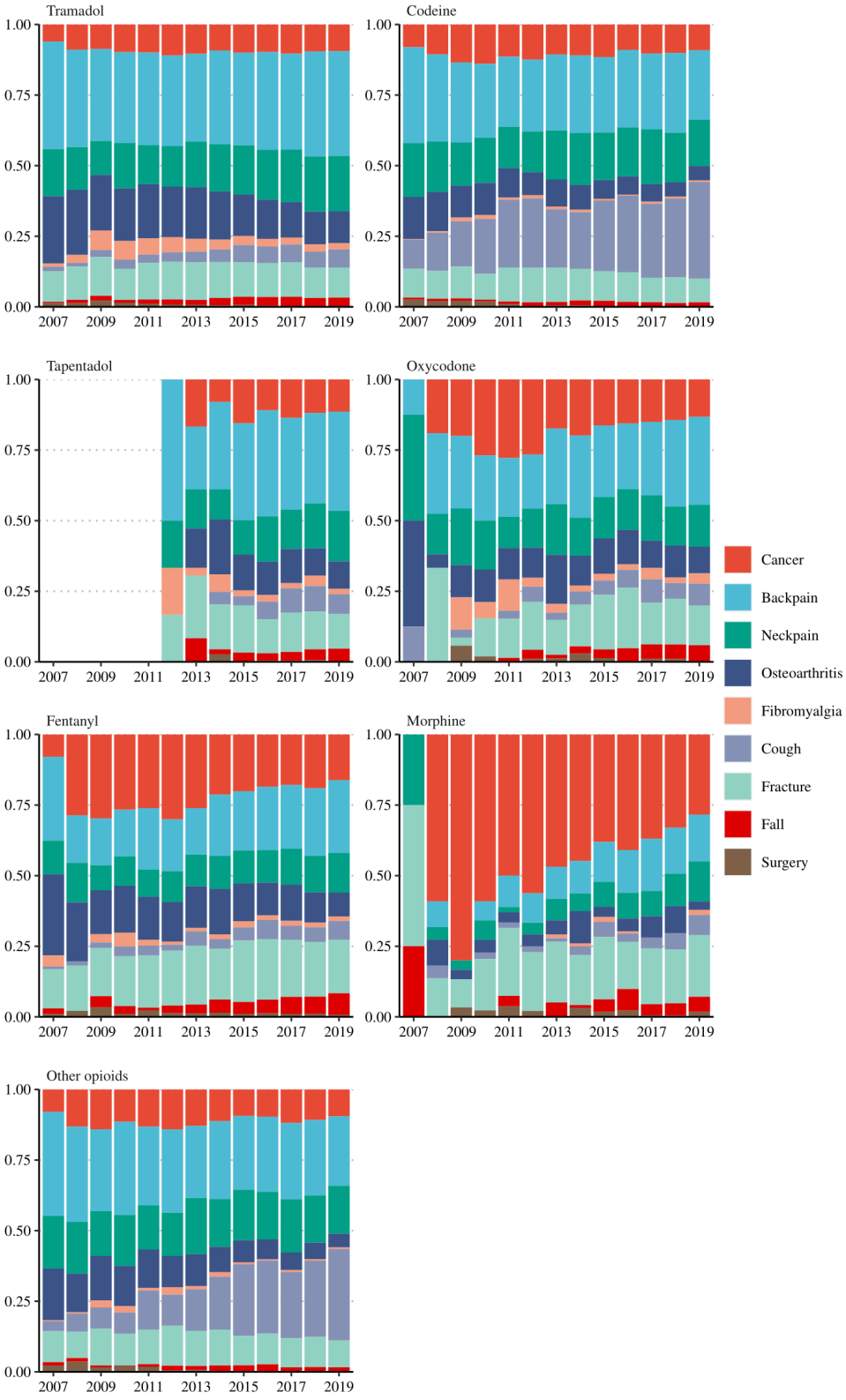
AS: Age-standardised. The numbers in the bracket are the proportions of each opioid type. The sum of proportions in each year for the opioid user metrics may be greater than 1. The percentage change for tapentadol was calculated between 2011 and 2019. Other opioids included dihydrocodeine [N02AA08], hydromorphone [N02AA03], dextropropoxyphene [N02AC04], buprenorphine [N02AE01], pethidine [N02AB02], and pentazocine [N02AD01]. NA: not available for the opioid type in Catalonia.

sFigure 3 1 The proportion of nine pain-related diagnoses among incident opioid users, stratified by opioid subtypes



Other opioids included dihydrocodeine [N02AA08], hydromorphone [N02AA03], dextropropoxyphene [N02AC04], buprenorphine [N02AE01], pethidine [N02AB02], and pentazocine [N02AD01]

sFigure 3 2 The proportion of nine pain-related diagnoses among prevalent opioid users, stratified by opioid subtypes



Chapter 4

Comparative safety between tramadol and codeine

4. Comparative safety between tramadol and codeine

4.1.Aim

To compare the risk of seven adverse opioid events and death in new adult opioid users following the initiation of Tramadol vs Codeine.

4.2.Background

Opioids are used in the pharmaceutical treatment of moderate-to-severe cancer pain and are commonly prescribed for chronic non-cancer pain when alternative therapies no longer provide sufficient relief. [24,111] Tramadol was considered to be a relatively safe opioid[123–125] and in 2013 was strongly recommended by the American Academy of Orthopaedic Surgeons for patients with symptomatic knee osteoarthritis. [126] Data from opioid utilization studies conducted in 2019-2020, found that tramadol was the most prescribed opioid in the United Kingdom[104], Netherlands[105], and Spain[109]. Regarding the United States, the age-adjusted rate of drug overdose deaths involving synthetic opioids (mostly fentanyl but also tramadol) increased from 1.0 per 100,000 in 2013 to 11.4 in 2019. [127]

Tramadol's common non-life-threatening adverse effects (e.g., headache, itching, and nausea) are well-established. However, evidence regarding serious adverse events compared to other opioids is still scarce.[128–130] An observational study found that tramadol initiation was associated with increased risk of all-cause mortality in patients with osteoarthritis. [131]

Given the widespread use of tramadol to manage a broad spectrum of painful conditions, its safety profile should be comparatively assessed, and its beneficial effects weighed against potential harms. This population-based cohort study compared risks of all-cause mortality, cardiovascular events, fractures, constipation, delirium, falls, opioid abuse/dependence, and sleep disorders between patients who received prescription dispensations for tramadol versus codeine. It also investigated whether the relative risk was consistent across different pain indications in adults.

4.3.Methods

4.3.1. Data sources

I used the data between January 1, 2007, and December 31, 2017 from the Information System for Research in Primary Care (SIDIAP).

4.3.2. Study design and cohort definition

I conducted a retrospective cohort study to compare the risks associated among patients with new prescription dispensations of tramadol versus codeine. "New dispensation" was defined by applying a fixed 12-month look-back period in which the patient had continuous data coverage but did not have any opioid dispensation. If a patient had multiple new dispensation episodes, only the first one was used. All patients who were dispensed tramadol and/or codeine and categorized in the tramadol or codeine cohort according to the first redeemed drug. Patients with both drugs dispensed on the same day were excluded. Cohorts were constructed by including patients who were aged 18 years or older, had at least one year of database enrolment before the first drug dispensation (index date), and had not been dispensed any opioids in the 12 months before or on entering the study. Those with a history of burn injuries, traffic crashes, major surgeries (amputation and joint replacement surgery), or any of the studied outcomes on or before the index date were excluded.

4.3.3. Baseline characteristics

The study population was characterized at the index date considering the following potential confounders: sociodemographic factors, medical conditions, Charlson comorbidity index, concomitant medication use, and healthcare utilization. Socioeconomic deprivation was measured with the MEDEA (Mortalidad en áreas pequeñas Españolas y Desigualdades Socioeconómicas y Ambientales) index that

describes socioeconomic and environmental inequalities among 175 small areas of Spain. Healthcare utilization was quantified by frequency of general practice visits and hospital admissions in the past 12 months.

The following health conditions expected to be linked to the opioid prescription dispensation or to be associated with any of the study outcomes were identified. These included common opioid indications, musculoskeletal diseases, chronic obstructive pulmonary disease, fibromyalgia, dyspnea, peripheral vascular disease, angina, transient ischemic attack, osteoporosis, diarrhea, rheumatoid arthritis, malabsorption disorders, pulmonary edema, diabetes, chronic liver disease, chronic kidney disease, Alzheimer's disease, and Parkinson's disease.

4.3.4. Controlling for confounding

Potential confounding by indication was reduced by using propensity score matching[132]. For each inclusion year, propensity scores for tramadol versus codeine dispensation were calculated using a logistic regression model that included all baseline characteristics. Patients with a new tramadol dispensation were matched 1:1 to those with a new codeine dispensation using caliper matching within a caliper of 0.2 times the standard error of the logit propensity score and an exact matching of inclusion year. Individuals who were not matched with another patient were excluded.

4.3.5. Study outcomes

The study outcomes were all-cause mortality, cardiovascular events (stroke, cardiac arrhythmia, myocardial infarction, and heart failure), fracture (hip, vertebral, and others), falls, delirium, constipation, sleep disorders, and opioid abuse/dependence within one year.

4.3.6. Statistical analyses

Baseline characteristics of the tramadol and codeine cohort participants before and after matching were compared. Missing values for living area and socioeconomic deprivation were imputed with one dataset using binary and polytomous logistic regression models, respectively. Covariate balance was assessed using the absolute standardized mean difference (ASMD). Acceptable matching was indicated by an ASMD under 0.1[133].

For each individual, person-years of follow-up for each outcome from the index date to the first of database disenrollment, 1 year after indexing, end of the study period (December 31, 2017), or outcome occurrence was calculated. One-year cumulative incidences were computed using the Kaplan-Meier method and survival curves were plotted. Hazard ratios (HRs) were estimated using cause-specific Cox proportional hazard regression accounting for competing risk of death[134]. Proportional hazard assumption was tested for exposure with respect to each study outcome using the Kolmogorov supremum test and report the HR at 3, 6, and 9 months if the assumption was violated[135]. Absolute rate differences (ARDs) were calculated as $I_0 \times (HR - 1)$, where I_0 is the incidence in the control group (codeine). To ensure that the overall confidence level for multiple tests is 0.95, Bonferroni-corrected confidence intervals (CIs) in the main analyses are reported, which for eight primary outcomes is [1 – (0.05/8)]*100 = 99.375%. All estimates from subgroup or sensitivity analyses are provided with regular 95% CIs.

4.3.7. Prespecified subgroup and sensitivity analyses

All subsequent analyses were only conducted for outcomes that had an incidence rate higher than 10.0 per 1,000 person-years or had a statistically significant difference in the main analysis. Prespecified subgroup analyses on the matched cohort with further adjustment of propensity scores in the Cox model, categorizing by age (18-39, 40-59, and

≥60), sex (female and male), common pain indications (cancer, cough, neurologic diseases, back pain, neck/shoulder pain, and osteoarthritis), Charlson comorbidity index (0, 1-2, and ≥3), and concomitant psychotropic and NSAID prescription dispensation (yes and no) were performed. The likelihood ratio test was used to examine significant multiplicative interaction effects in the Cox model.

Eight sensitivity analyses were conducted. First, a multivariable Cox-proportional hazard model to estimate the HRs of study outcomes in the entire cohort[136]. Second, a "stratified Cox model" to control for matching structure in the matched cohort. Third, a complete-case analysis, excluding participants with missing baseline characteristic values. Fourth, to better identify new tramadol and codeine dispensations, the look-back period was extended from 1 to 3 years. Fifth, to increase drug specificity, the analysis was limited to tramadol single-formulation, excluding combinations with another non-narcotic chemical. Sixth, tramadol may have been prescribed to relieve pain caused by an undiagnosed disease under study, such as fractures. A 3-month lag window, excluding patients with outcomes that occurred within three months after the index date was introduced[137]. Seventh, a Fine-Gray subdistribution hazard, an alternative approach to account for the competing risk of death was used[134]. Eighth, a comparison of the risks during the "on-treatment" period that began with the initial prescription date and ended 30 days after the last fill would have run out was carried out. A gap of 90 days or more without a fill terminated the on-treatment period. All analyses were conducted using R software (version 3.6.2). *P* values were 2-sided and < 0.05 was considered significant.

4.4.Results

4.4.1. Study population and baseline characteristics

Of the 1,093,064 patients with a tramadol or codeine dispensation between January 1, 2007 and December 31, 2017, 326,921 were first dispensed tramadol, 762,492 were first dispensed codeine, and 3,651 were first dispensed both drugs concomitantly and were excluded. A total of 839,489 tramadol/codeine initiators were identified by excluding 86,660 patients who were dispensed tramadol and 163,264 patients who were dispensed codeine and did not meet eligibility criteria. After propensity score matching, the matched cohort included 368,960 participants: 184,480 and 184,480 in the tramadol and codeine cohort. **sFigure 4 1** shows the participant selection process. **Table 4 1** reports the participants' baseline characteristics before and after propensity score matching. After matching, the two cohorts of new users were comparable for all observed features, with no ASMD exceeding 0.1 and most not exceeding 0.01. Patients in the tramadol and codeine cohort had a mean (SD) age of 52.7 (16.2) and 53.5 (16.1) years old, respectively. The prevalence of cancer was 3.2% and 3.3%, respectively. Their most common diagnoses were back pain (47.5% vs 48.5%), neck/shoulder pain (28.6% vs 29.5%), and osteoarthritis (15.3% vs 15.5%). The drugs most frequently dispensed were ibuprofen (34.4% vs 34.3%) and paracetamol (37.1% vs 36.8%). The proportion of residential area and socioeconomic deprivation index data missing across the entire dataset were 0.04% and 27.04%, respectively.

Table 4 1 Baseline characteristics of new tramadol and codeine users before and after propensity-score matching

	Before matching			After matching		
	Tramadol	**Codeine**	**ASMD**	**Tramadol**	**Codeine**	**ASMD**
Number of participants	240261	599228		184480	184480	
Calendar year, N (%)						
2007	22545 (9.4)	108010 (18.0)	0.25	21631 (11.7)	21631 (11.7)	<0.01
2008	22887 (9.5)	92706 (15.5)	0.18	21446 (11.6)	21446 (11.6)	<0.01
2009	21339 (8.9)	87784 (14.6)	0.18	19810 (10.7)	19810 (10.7)	<0.01
2010	21345 (8.9)	61408 (10.2)	0.04	18645 (10.1)	18645 (10.1)	<0.01
2011	20423 (8.5)	60337 (10.1)	0.05	17414 (9.4)	17414 (9.4)	<0.01
2012	18659 (7.8)	42382 (7.1)	0.02	14940 (8.1)	14940 (8.1)	<0.01
2013	19250 (8.0)	26778 (4.5)	0.14	12477 (6.8)	12477 (6.8)	<0.01
2014	21073 (8.8)	28438 (4.7)	0.16	13289 (7.2)	13289 (7.2)	<0.01
2015	22550 (9.4)	31725 (5.3)	0.15	14648 (7.9)	14648 (7.9)	<0.01
2016	24070 (10.0)	30047 (5.0)	0.19	14515 (7.9)	14515 (7.9)	<0.01
2017	26120 (10.9)	29613 (4.9)	0.22	15665 (8.5)	15665 (8.5)	<0.01
Sociodemographics						
Age, year, mean (SD)	54.1 (16.2)	45.3 (16.3)	0.54	52.7 (16.2)	53.5 (16.1)	0.04
Sex, N (%)						
Male	102280 (42.6)	271274 (45.3)	0.05	78703 (42.7)	78713 (42.7)	<0.01
Female	137981 (57.4)	327954 (54.7)	0.05	105777 (57.3)	105767 (57.3)	<0.01
Socioeconomic deprivation, N (%)						
U1 (least deprived)	40231 (16.7)	95500 (15.9)	0.02	30942 (16.8)	31080 (16.8)	<0.01
U2	47841 (19.9)	118451 (19.8)	<0.01	36695 (19.9)	36645 (19.9)	<0.01
U3	50517 (21.0)	128510 (21.4)	0.01	38944 (21.1)	38943 (21.1)	<0.01
U4	52901 (22.0)	131550 (22.0)	<0.01	40425 (21.9)	40497 (22.0)	<0.01
U5 (most deprived)	48771 (20.3)	125217 (20.9)	0.01	37474 (20.3)	37315 (20.2)	<0.01
Residence = rural, N (%)	44060 (18.3)	115718 (19.3)	0.02	33562 (18.2)	33649 (18.2)	<0.01
Common opioids indications, N (%)						
Back pain	129471 (53.9)	184539 (30.8)	0.48	87606 (47.5)	89710 (48.6)	0.02

Neck/shoulder pain	79040 (32.9)	111010 (18.5)	0.33	52851 (28.6)	54489 (29.5)	0.02
Osteoarthritis	45476 (18.9)	40877 (6.8)	0.36	28206 (15.3)	28433 (15.4)	<0.01
Cough	14472 (6.0)	54295 (9.1)	0.11	11602 (6.3)	11189 (6.1)	<0.01
Migraine	10712 (4.5)	22071 (3.7)	0.03	7895 (4.3)	8084 (4.4)	<0.01
Cancer	10168 (4.2)	8515 (1.4)	0.17	5963 (3.2)	6115 (3.3)	<0.01
Other comorbidities, N (%)						
Diabetes	26232 (10.9)	37168 (6.2)	0.16	17958 (9.7)	18599 (10.1)	0.01
Other musculoskeletal disorders	13309 (5.5)	15861 (2.6)	0.14	8496 (4.6)	8677 (4.7)	<0.01
Chronic obstructive pulmonary disease	6811 (2.8)	8771 (1.5)	0.09	4511 (2.4)	4646 (2.5)	<0.01
Chronic kidney disease	6306 (2.6)	4710 (0.8)	0.14	3468 (1.9)	3497 (1.9)	<0.01
Fibromyalgia	4461 (1.9)	1631 (0.3)	0.15	1984 (1.1)	1555 (0.8)	0.02
Dyspnea	3863 (1.6)	5030 (0.8)	0.07	2407 (1.3)	2500 (1.4)	<0.01
Peripheral vascular disease	2597 (1.1)	2018 (0.3)	0.08	1420 (0.8)	1420 (0.8)	<0.01
Angina	2249 (0.9)	2692 (0.4)	0.05	1515 (0.8)	1554 (0.8)	<0.01
Transient ischemic attack	1242 (0.5)	1290 (0.2)	0.05	754 (0.4)	806 (0.4)	<0.01
Osteoporosis	845 (0.4)	488 (0.1)	0.05	440 (0.2)	395 (0.2)	<0.01
Parkinson's disease	892 (0.4)	759 (0.1)	0.04	534 (0.3)	539 (0.3)	<0.01
Alzheimer's disease	953 (0.4)	1104 (0.2)	0.03	702 (0.4)	725 (0.4)	<0.01
Chronic liver disease	680 (0.3)	710 (0.1)	0.03	424 (0.2)	428 (0.2)	<0.01
Diarrhea	747 (0.3)	1720 (0.3)	<0.01	554 (0.3)	540 (0.3)	<0.01
Rheumatoid arthritis	445 (0.2)	372 (0.1)	0.03	227 (0.1)	236 (0.1)	<0.01
Malabsorption disorders	465 (0.2)	969 (0.2)	<0.01	333 (0.2)	336 (0.2)	<0.01
Pulmonary edema	64 (0.0)	67 (0.0)	0.01	32 (0.0)	37 (0.0)	<0.01
Charlson comorbidity index[a], N (%)						
0	176076 (73.3)	502922 (83.9)	0.26	140794 (76.3)	139713 (75.7)	0.01
1-2	55479 (23.1)	89439 (14.9)	0.20	38885 (21.1)	39961 (21.7)	0.01
≥3	8706 (3.6)	6867 (1.1)	0.16	4801 (2.6)	4806 (2.6)	<0.01
Concomitant medication, N (%),						
Psychotropics						
Benzodiazepines	71881 (29.9)	99437 (16.6)	0.31	48709 (26.4)	49543 (26.9)	0.01
Anticonvulsant	32153 (13.4)	18590 (3.1)	0.38	16321 (8.8)	14400 (7.8)	0.03

SSRIs	26972 (11.2)	48296 (8.1)	0.10	20252 (11.0)	20461 (11.1)	<0.01
Hypnotics	15147 (6.3)	20045 (3.3)	0.13	10653 (5.8)	10618 (5.8)	<0.01
NSAIDs						
Ibuprofen	80916 (33.7)	207684 (34.7)	0.02	63472 (34.4)	63381 (34.4)	<0.01
Diclofenac	54571 (22.7)	56862 (9.5)	0.36	36215 (19.6)	35353 (19.2)	0.01
Other NSAIDs[b]	46642 (19.4)	34885 (5.8)	0.41	27197 (14.7)	25653 (13.9)	0.02
Naproxen	29280 (12.2)	29194 (4.9)	0.26	16630 (9.0)	17042 (9.2)	<0.01
Celecoxib	5832 (2.4)	2569 (0.4)	0.16	2903 (1.6)	2320 (1.3)	0.02
Other analgesics						
Paracetamol	97358 (40.5)	191456 (32.0)	0.17	68365 (37.1)	68032 (36.9)	<0.01
Metamizole	53545 (22.3)	38815 (6.5)	0.46	29202 (15.8)	27668 (15.0)	0.02
Health care utilization, mean (SD),						
General practitioner visits	7.1 (5.3)	5.3 (4.4)	0.35	6.6 (5.1)	6.6 (5.0)	<0.01
Hospital admissions	3.3 (5.6)	2.0 (3.8)	0.27	2.9 (4.9)	2.9 (5.1)	<0.01

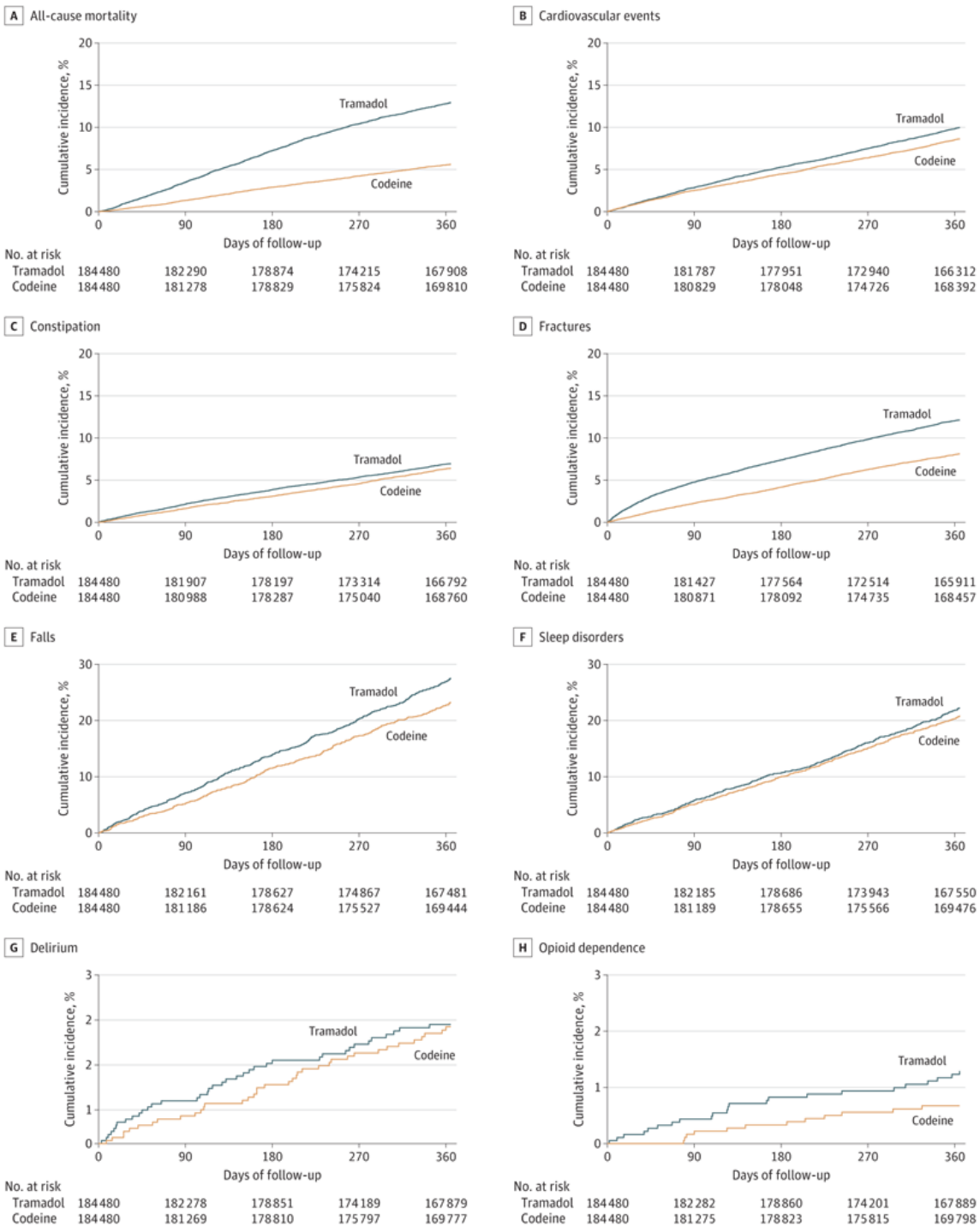
Abbreviation: ASMD, absolute standardized mean difference. COPD, chronic obstructive pulmonary disease. SSRIs, selective serotonin reuptake inhibitors. NSAIDs, non-steroidal anti-inflammatory drugs.

[a] Charlson comorbidity index was calculated using ICD-10 coding algorithm based on the R *"comorbidity"* package[138].

[b] All other NSAIDs included.

Figure 4 1 shows Kaplan-Meier plots in the matched cohorts. During 1-year follow-up, participants with a prescription dispensation of tramadol had a significantly higher risk of all-cause mortality, cardiovascular disease, and fractures, with 1-year cumulative incidence of 12.86 (95% CI, 12.34 to 13.38), 9.97 (95% CI, 9.50 to 10.43) and 12.07 (95% CI, 11.56 to 12.57) per 1,000 participants respectively in the tramadol cohort, compared with 5.59 (95% CI, 5.25 to 5.94), 8.62 (95% CI, 8.19 to 9.05) and 8.08 (95% CI, 7.67 to 8.50) per 1,000 participants in the codeine cohort.

Figure 4 1 Kaplan-Meier Cumulative Incidence Plots for All Study Outcomes Within 1 Year in the Matched Cohort



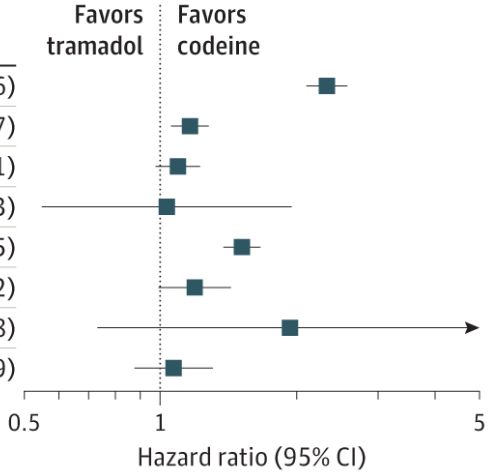
Most patients in each group were observed for the whole year. There were no primary or secondary outcomes; all study outcomes were prespecified and treated equally. Cumulative incidence (95% CI) per 1000 participants, tramadol vs codeine: A, 12.86 (12.34-13.38) vs 5.59 (5.25-5.94). B, 9.97 (9.50-10.43) vs 8.62 (8.19-9.05). C, 6.92 (6.54-7.31) vs 6.39 (6.02-6.76). D, 12.07 (11.56-12.57) vs 8.08 (7.67-8.50). E, 2.75 (2.50-2.99) vs 2.32 (2.09-2.54). F, 2.22 (2.00-2.44) vs 2.08 (1.87-2.29). G, 0.21 (0.14-0.2) vs 0.20% (0.14-0.27). H, 0.12 (0.07-0.18) vs 0.06 (0.02-0.10).

4.4.2. Association of drug dispensation with adverse outcomes

Compared with codeine, tramadol prescription dispensation was significantly associated with a higher risk of mortality (13.00 vs 5.61 per 1,000 person-years; HR, 2.31 [95% CI, 2.08 to 2.56]; ARD, 7.37 [95% CI, 6.09 to 8.78] per 1,000 person-years), cardiovascular events (10.03 vs 8.67 per 1,000 person-years; HR, 1.15 [95% CI, 1.05 to 1.27]; ARD, 1.36 [95% CI, 0.45 to 2.36] per 1,000 person-years), and fractures (12.26 vs 8.13 per 1,000 person-years; HR, 1.50 [95% CI, 1.37 to 1.65]; ARD, 4.10 [95% CI, 3.02 to 5.29] per 1,000 person-years). For tramadol compared with codeine prescription dispensation, there was no significant difference in the risk of constipation (6.98 vs 6.41 per 1,000 person-years; HR, 1.08 [95% CI, 0.97 to 1.21]; ARD, 0.56 [95% CI, -0.17 to 1.39] per 1,000 person-years), delirium (0.21 vs 0.20 per 1,000 person-years; HR, 1.02 [95% CI, 0.54 to 1.93]; ARD, 0.00 [95% CI, -0.06 to 0.18] per 1,000 person-years), falls (2.75 vs 2.32 per 1,000 person-years; HR, 1.18 [95% CI, 0.98 to 1.42]; ARD, 0.43 [95% CI, -0.02 to 0.98] per 1,000 person-years), opioid abuse/dependence (0.12 vs 0.06 per 1,000 person-years; HR, 1.91 [95% CI, 0.72 to 5.08]; ARD, 0.05 [95% CI, -0.01 to 0.24] per 1,000 person-years), and sleep disorders (2.22 vs 2.08 per 1,000 person-years; HR, 1.06 [95% CI, 0.87 to 1.29]; ARD, 0.13 [95% CI, -0.26 to 0.62] per 1,000 person-years) (**Figure 4 2**). As the proportional hazard assumption was violated for the mortality ($p < 0.001$) and fractures ($p < 0.001$) outcomes, the follow-up period was shortened to ≤ 3, 6, and 9 months.

Figure 4 2 Event counts, incidence rates, absolute rate differences, and adjusted hazard ratios for 8 study outcomes within 1 year in the matched cohort

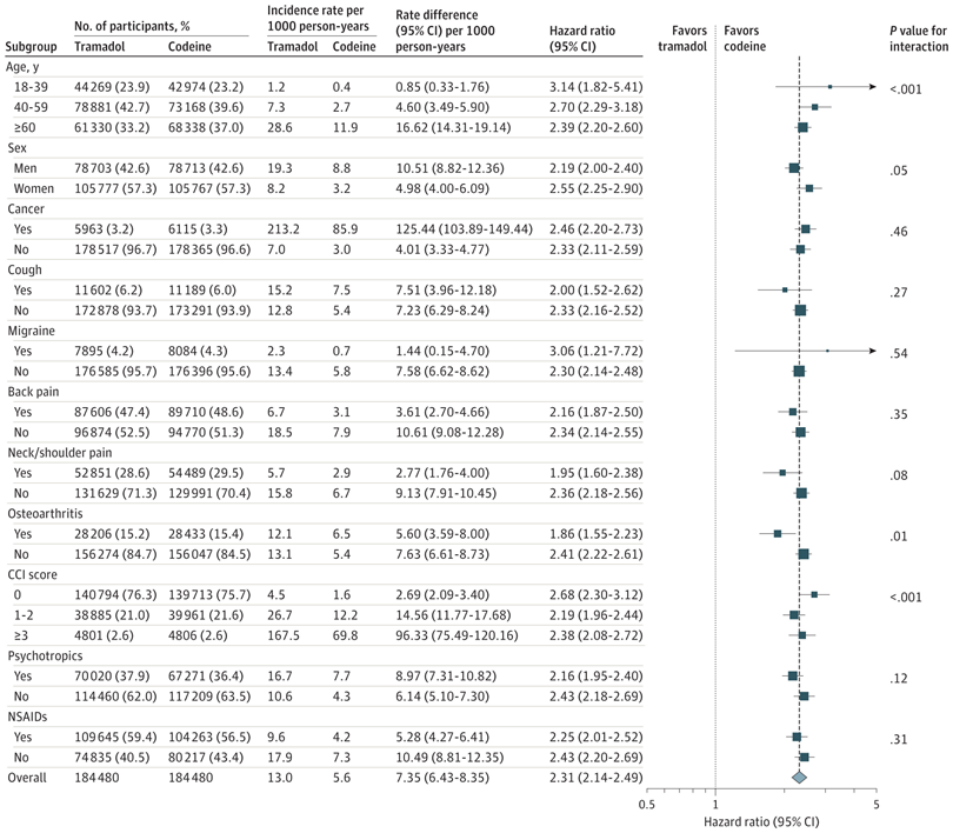
	Tramadol (n = 184 480)		Codeine (n = 184 480)			
Outcome	No. of Events	Incidence rate per 1000 person-years	No. of Events	Incidence rate per 1000 person-years	Rate difference (95% CI) per 1000 person-years	Hazard ratio (95% CI)
All-cause mortality	2307	13.00	999	5.61	7.37 (6.09 to 8.78)	2.31 (2.08 to 2.56)
Cardiovascular events	1772	10.03	1536	8.67	1.36 (0.45 to 2.36)	1.15 (1.05 to 1.27)
Constipation	1234	6.98	1137	6.41	0.56 (-0.17 to 1.39)	1.08 (0.97 to 1.21)
Delirium	38	0.21	37	0.20	0.00 (-0.09 to 0.18)	1.02 (0.54 to 1.93)
Fractures	2160	12.26	1442	8.13	4.10 (3.02 to 5.29)	1.50 (1.37 to 1.65)
Falls	488	2.75	413	2.32	0.43 (-0.02 to 0.98)	1.18 (0.98 to 1.42)
Opioid abuse/dependence	23	0.12	12	0.06	0.05 (-0.01 to 0.24)	1.91 (0.72 to 5.08)
Sleep disorders	394	2.22	371	2.08	0.13 (-0.26 to 0.62)	1.06 (0.87 to 1.29)



4.4.3. Subgroup and sensitivity analyses

The increased mortality risk associated with tramadol was significantly greater among younger as compared with older adults (HR, 3.14 [95% CI, 1.82 to 5.41] vs HR, 2.39 [95% CI, 2.20 to 2.60]; p for interaction < 0.001). Female participants had a significantly greater risk of cardiovascular events than male participants (HR, 1.32 [95% CI, 1.19 to 1.46] vs 1.03 [95% CI, 0.93 to 1.13], *p* for interaction < 0.001). Participants with the most prevalent comorbidities compared with the least prevalent comorbidities had a significantly higher fracture risk (HR, 2.20 [95% CI, 1.57 to 3.09] vs 1.47 [95% CI, 1.35 to 1.59], *p* for interaction = 0.004). Subgroups' ARDs varied depending on their baseline risk for each outcome (**Figure 4 3**). The sensitivity analyses consistently indicated that patients in the tramadol cohort experienced significantly higher risks of mortality, cardiovascular events, and fractures than patients in the codeine cohort.

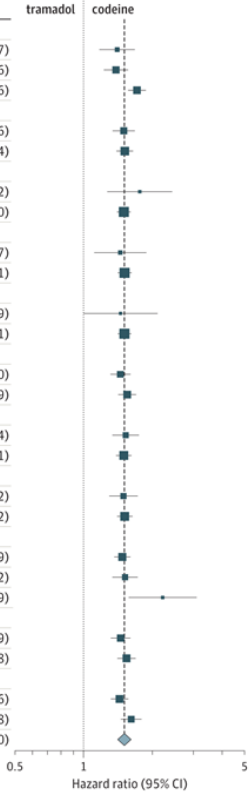
Figure 4 3 Subgroup-specific associations of tramadol vs codeine with all-cause mortality, cardiovascular events, and fractures.



Subgroup	No. of participants, %		Incidence rate per 1000 person-years		Rate difference (95% CI) per 1000 person-years	Hazard ratio (95% CI)	*P* value for interaction
	Tramadol	Codeine	Tramadol	Codeine			
Age, y							
18-39	44 269 (23.9)	42 974 (23.2)	1.2	0.4	0.85 (0.33-1.76)	3.14 (1.82-5.41)	<.001
40-59	78 881 (42.7)	73 168 (39.6)	7.3	2.7	4.60 (3.49-5.90)	2.70 (2.29-3.18)	
≥60	61 330 (33.2)	68 338 (37.0)	28.6	11.9	16.62 (14.31-19.14)	2.39 (2.20-2.60)	
Sex							
Men	78 703 (42.6)	78 713 (42.6)	19.3	8.8	10.51 (8.82-12.36)	2.19 (2.00-2.40)	.05
Women	105 777 (57.3)	105 767 (57.3)	8.2	3.2	4.98 (4.00-6.09)	2.55 (2.25-2.90)	
Cancer							
Yes	5963 (3.2)	6115 (3.3)	213.2	85.9	125.44 (103.89-149.44)	2.46 (2.20-2.73)	.46
No	178 517 (96.7)	178 365 (96.6)	7.0	3.0	4.01 (3.33-4.77)	2.33 (2.11-2.59)	
Cough							
Yes	11 602 (6.2)	11 189 (6.0)	15.2	7.5	7.51 (3.96-12.18)	2.00 (1.52-2.62)	.27
No	172 878 (93.7)	173 291 (93.9)	12.8	5.4	7.23 (6.29-8.24)	2.33 (2.16-2.52)	
Migraine							
Yes	7895 (4.2)	8084 (4.3)	2.3	0.7	1.44 (0.15-4.70)	3.06 (1.21-7.72)	.54
No	176 585 (95.7)	176 396 (95.6)	13.4	5.8	7.58 (6.62-8.62)	2.30 (2.14-2.48)	
Back pain							
Yes	87 606 (47.4)	89 710 (48.6)	6.7	3.1	3.61 (2.70-4.66)	2.16 (1.87-2.50)	.35
No	96 874 (52.5)	94 770 (51.3)	18.5	7.9	10.61 (9.08-12.28)	2.34 (2.14-2.55)	
Neck/shoulder pain							
Yes	52 851 (28.6)	54 489 (29.5)	5.7	2.9	2.77 (1.76-4.00)	1.95 (1.60-2.38)	.08
No	131 629 (71.3)	129 991 (70.4)	15.8	6.7	9.13 (7.91-10.45)	2.36 (2.18-2.56)	
Osteoarthritis							
Yes	28 206 (15.2)	28 433 (15.4)	12.1	6.5	5.60 (3.59-8.00)	1.86 (1.55-2.23)	.01
No	156 274 (84.7)	156 047 (84.5)	13.1	5.4	7.63 (6.61-8.73)	2.41 (2.22-2.61)	
CCI score							
0	140 794 (76.3)	139 713 (75.7)	4.5	1.6	2.69 (2.09-3.40)	2.68 (2.30-3.12)	<.001
1-2	38 885 (21.0)	39 961 (21.6)	26.7	12.2	14.56 (11.77-17.68)	2.19 (1.96-2.44)	
≥3	4801 (2.6)	4806 (2.6)	167.5	69.8	96.33 (75.49-120.16)	2.38 (2.08-2.72)	
Psychotropics							
Yes	70 020 (37.9)	67 271 (36.4)	16.7	7.7	8.97 (7.31-10.82)	2.16 (1.95-2.40)	.12
No	114 460 (62.0)	117 209 (63.5)	10.6	4.3	6.14 (5.10-7.30)	2.43 (2.18-2.69)	
NSAIDs							
Yes	109 645 (59.4)	104 263 (56.5)	9.6	4.2	5.28 (4.27-6.41)	2.25 (2.01-2.52)	.31
No	74 835 (40.5)	80 217 (43.4)	17.9	7.3	10.49 (8.81-12.35)	2.43 (2.20-2.69)	
Overall	184 480	184 480	13.0	5.6	7.35 (6.43-8.35)	2.31 (2.14-2.49)	

Subgroup	No. of participants, %		Incidence rate per 1000 person-years		Rate difference (95% CI) per 1000 person-years	Hazard ratio (95% CI)	*P* value for interaction
	Tramadol	Codeine	Tramadol	Codeine			
Age, y							
18-39	44 269 (23.9)	42 974 (23.2)	0.8	0.5	0.22 (-0.06 to 0.72)	1.45 (0.86 to 2.44)	.14
40-59	78 881 (42.7)	73 168 (39.6)	4.0	3.0	1.01 (0.37 to 1.79)	1.33 (1.12 to 1.59)	
≥60	61 330 (33.2)	68 338 (37.0)	24.5	19.8	4.65 (2.88 to 6.56)	1.23 (1.14 to 1.33)	
Sex							
Men	78 703 (42.6)	78 713 (42.6)	12.0	11.6	0.34 (-0.71 to 1.51)	1.03 (0.93 to 1.13)	<.001
Women	105 777 (57.3)	105 767 (57.3)	8.5	6.4	2.09 (1.27 to 2.99)	1.32 (1.19 to 1.46)	
Cancer							
Yes	5963 (3.2)	6115 (3.3)	20.1	16.6	3.39 (-1.51 to 9.91)	1.20 (0.90 to 1.59)	.77
No	178 517 (96.7)	178 365 (96.6)	9.7	8.4	1.32 (0.66 to 2.03)	1.15 (1.07 to 1.24)	
Cough							
Yes	11 602 (6.2)	11 189 (6.0)	12.5	10.5	1.97 (-0.82 to 5.58)	1.18 (0.92 to 1.53)	.83
No	172 878 (93.7)	173 291 (93.9)	9.8	8.5	1.31 (0.64 to 2.03)	1.15 (1.07 to 1.23)	
Migraine							
Yes	7895 (4.2)	8084 (4.3)	3.8	3.3	0.46 (-1.08 to 3.08)	1.14 (0.67 to 1.93)	.95
No	176 585 (95.7)	176 396 (95.6)	10.3	8.9	1.39 (0.71 to 2.13)	1.15 (1.08 to 1.23)	
Back pain							
Yes	87 606 (47.4)	89 710 (48.6)	6.9	6.0	0.93 (0.16 to 1.81)	1.15 (1.02 to 1.30)	.93
No	96 874 (52.5)	94 770 (51.3)	12.7	11.1	1.65 (0.63 to 2.76)	1.14 (1.05 to 1.24)	
Neck/shoulder pain							
Yes	52 851 (28.6)	54 489 (29.5)	7.5	6.2	1.31 (0.27 to 2.52)	1.21 (1.04 to 1.40)	.47
No	131 629 (71.3)	129 991 (70.4)	11.0	9.6	1.34 (0.53 to 2.21)	1.13 (1.05 to 1.23)	
Osteoarthritis							
Yes	28 206 (15.2)	28 433 (15.4)	18.6	15.1	3.49 (1.23 to 6.07)	1.23 (1.08 to 1.40)	.27
No	156 274 (84.7)	156 047 (84.5)	8.4	7.4	0.96 (0.32 to 1.66)	1.13 (1.04 to 1.22)	
CCI score							
0	140 794 (76.3)	139 713 (75.7)	7.6	6.4	1.18 (0.52 to 1.89)	1.18 (1.08 to 1.29)	<.001
1-2	38 885 (21.0)	39 961 (21.6)	16.2	14.7	1.52 (−0.24 to 3.51)	1.10 (0.98 to 1.23)	
≥3	4801 (2.6)	4806 (2.6)	35.3	26.2	8.83 (1.21 to 18.55)	1.33 (1.04 to 1.70)	
Psychotropics							
Yes	70 020 (37.9)	67 271 (36.4)	12.0	10.0	2.07 (0.89 to 3.38)	1.20 (1.08 to 1.33)	.23
No	114 460 (62.0)	117 209 (63.5)	8.7	7.8	0.86 (0.10 to 1.69)	1.11 (1.01 to 1.21)	
NSAIDs							
Yes	109 645 (59.4)	104 263 (56.5)	8.2	7.0	1.13 (0.36 to 1.98)	1.16 (1.05 to 1.28)	.78
No	74 835 (40.5)	80 217 (43.4)	12.7	10.7	1.96 (0.82 to 3.22)	1.18 (1.07 to 1.30)	
Overall	184 480	184 480	10.0	8.6	1.35 (0.69 to 2.05)	1.15 (1.08 to 1.23)	

Subgroup	No. of participants, %		Incidence rate per 1000 person-years		Rate difference (95% CI) per 1000 person-years	Hazard ratio (95% CI)	*P* value for interaction
	Tramadol	Codeine	Tramadol	Codeine			
Age, y							
18-39	44 269 (23.9)	42 974 (23.2)	6.9	4.9	1.95 (0.83-3.29)	1.39 (1.17-1.67)	<.001
40-59	78 881 (42.7)	73 168 (39.6)	8.5	6.1	2.35 (1.38-3.44)	1.38 (1.22-1.56)	
≥60	61 330 (33.2)	68 338 (37.0)	20.9	12.2	8.62 (6.84-10.56)	1.70 (1.56-1.86)	
Sex							
Men	78 703 (42.6)	78 713 (42.6)	10.4	6.9	3.41 (2.33-4.62)	1.49 (1.33-1.66)	.88
Women	105 777 (57.3)	105 767 (57.3)	13.6	9.0	4.59 (3.50-5.78)	1.51 (1.38-1.64)	
Cancer							
Yes	5963 (3.2)	6115 (3.3)	18.9	10.6	8.00 (2.85-15.13)	1.75 (1.26-2.42)	.33
No	178 517 (96.7)	178 365 (96.6)	12.0	8.0	3.96 (3.18-4.81)	1.49 (1.39-1.60)	
Cough							
Yes	11 602 (6.2)	11 189 (6.0)	13.4	9.2	4.09 (1.05-8.04)	1.44 (1.11-1.87)	.74
No	172 878 (93.7)	173 291 (93.9)	12.1	8.0	4.07 (3.26-4.93)	1.50 (1.40-1.61)	
Migraine							
Yes	7895 (4.2)	8084 (4.3)	9.0	6.2	2.78 (0.00-6.79)	1.44 (1.00-2.09)	.83
No	176 585 (95.7)	176 396 (95.6)	12.4	8.2	4.15 (3.34-5.02)	1.50 (1.40-1.61)	
Back pain							
Yes	87 606 (47.4)	89 710 (48.6)	11.0	7.6	3.40 (2.34-4.56)	1.44 (1.30-1.60)	.32
No	96 874 (52.5)	94 770 (51.3)	13.3	8.6	4.71 (3.57-5.95)	1.54 (1.41-1.69)	
Neck/shoulder pain							
Yes	52 851 (28.6)	54 489 (29.5)	10.8	7.1	3.72 (2.38-5.26)	1.52 (1.33-1.74)	.81
No	131 629 (71.3)	129 991 (70.4)	12.8	8.5	4.21 (3.27-5.23)	1.49 (1.38-1.61)	
Osteoarthritis							
Yes	28 206 (15.2)	28 433 (15.4)	17.1	11.4	5.60 (3.33-8.21)	1.49 (1.29-1.72)	.89
No	156 274 (84.7)	156 047 (84.5)	11.3	7.5	3.82 (2.99-4.70)	1.50 (1.39-1.62)	
CCI score							
0	140 794 (76.3)	139 713 (75.7)	10.8	7.3	3.46 (2.62-4.36)	1.47 (1.35-1.59)	.004
1-2	38 885 (21.0)	39 961 (21.6)	16.1	10.6	5.46 (3.55-7.63)	1.51 (1.33-1.72)	
≥3	4801 (2.6)	4806 (2.6)	25.0	11.2	13.51 (6.39-23.50)	2.20 (1.57-3.09)	
Psychotropics							
Yes	70 020 (37.9)	67 271 (36.4)	15.0	10.3	4.62 (3.23-6.15)	1.44 (1.31-1.59)	.38
No	114 460 (62.0)	117 209 (63.5)	10.5	6.8	3.65 (2.74-4.65)	1.53 (1.40-1.68)	
NSAIDs							
Yes	109 645 (59.4)	104 263 (56.5)	11.9	8.3	3.60 (2.61-4.69)	1.43 (1.31-1.56)	.09
No	74 835 (40.5)	80 217 (43.4)	12.7	7.8	4.76 (3.53-6.13)	1.61 (1.45-1.78)	
Overall	184 480	184 480	12.2	8.1	4.09 (3.30-4.93)	1.50 (1.40-1.60)	



4.5.Discussion

In this retrospective population-based cohort study, the prescription dispensation of tramadol was significantly associated with greater risks of mortality, cardiovascular events, and fractures than the prescription dispensation of codeine. No significant difference was found in the risks of constipation, delirium, falls, opioid abuse/dependence, or sleep disorders in the year after treatment initiation. The results remained consistent and robust across subgroup and sensitivity analyses.

Musich et al.[139] recently reported 59%, 41%, and 21% increased risk of cardiovascular hospitalizations, falls/fractures, and mortality, respectively, among patients with osteoarthritis ≥65 years old starting tramadol, compared with non-users. However, this comparison has limited clinical relevance, as individuals prescribed an opioid are likely to be unhealthier than those who are not. Two recent studies using the UK's Health Improvement Network database found patients with osteoarthritis who used tramadol had a greater risk of hip fracture (HR, 1.28 [95% CI, 1.13 to 1.46])[140] and myocardial infarction (HR, 1.23 [95% CI, 0.95 to 1.60])[141] than those who used codeine. Comparing tramadol and codeine is clinically relevant, as they are both "weak" opioids (lower morphine equivalent dose) used for the second step of the WHO pain ladder, such as moderate non-cancer pain.

This study expanded on previous studies by comparing tramadol with codeine for various indications in all adult age-groups, and found that, compared with codeine, tramadol prescription dispensation was significantly associated with a higher risk of mortality, cardiovascular events, and fractures in all age groups, independent of previous comorbid conditions.

Two studies have shown results that differ from the results of the present study. Solomon et al. compared five commonly prescribed opioids in the United States in 31,375 Medicare

patients[142]. They found that codeine users had higher incidence rates of mortality, cardiovascular events, and fractures (70, 170, and 270 per 1,000 person-years, respectively) than tramadol users (40, 110 and 70 per 1,000 person-years, respectively) during 180 days of follow-up. However, this study may have been subject to selection bias, as codeine users also had greater risks of these outcomes than users of oxycodone, propoxyphene, and hydrocodone, which contradicts what might be expected from physicians' experience and the morphine milligram equivalents of the five drugs.

Zeng et al.[131] conducted a head-to-head comparison of mortality risk between tramadol, codeine, and several NSAIDs and found that tramadol initiation was associated with an increased risk of mortality compared with NSAIDs, but not codeine (HR, 0.94 [95% CI, 0.83 to 1.05]). However, they used a UK-based sample of 16,922 older patients with osteoarthritis (mean age 71 years), who differed substantially from the general population in terms of comorbidities and polypharmacy. This sample was possibly subject to depletion of susceptibility bias, in which early increased risk of acute adverse events is attenuated after a long drug exposure. The study relied on general practice prescriptions, which are more susceptible to exposure misclassification than drug dispensation records because non-adherence to opioid medications is common[143]. The participants were also unlikely to truly be new users, as weak opioids used to be available over-the-counter in the UK[144].

4.5.1. Limitations

This study has several limitations. First, although tramadol and codeine have been widely indicated for managing moderate-to-severe pain, confounding by indication could have affected the study findings. For example, codeine is often prescribed to control coughs and a higher prevalence of cough among patients with codeine prescription dispensation (9.1%) has been observed, than among patients with tramadol prescription dispensation

(6.0%) before matching. However, the results remained almost unchanged when restricting the analysis to patients with or without a prior cough diagnosis, suggesting this residual indication bias is unlikely to have had a great effect. Also, tramadol may be expected to be prescribed for patients with severe illness due to a perception that it has a relatively safe profile, which could explain part of the observed associations. Second, despite that the propensity score matching balanced the observed baseline characteristics between the two groups and that the subgroup analyses produced results that were highly consistent with the main analysis, unmeasured confounders may have nonetheless biased the estimates. Third, the magnitude of associations between the packages of tramadol dispensation and the adverse outcomes should be interpreted cautiously. Dispensed packages of tramadol is a crude proxy for assessing dose and could instead be a reflection of anticipated pain levels, which might explain the observed association.

Fourth, this study did not include the cause of death. Given the difficulty of disentangling how much of the mortality risk was specifically related to the actual dispensation of tramadol, this estimate may be falsely elevated. Fifth, some of the study outcomes, especially delirium and sleep disorders, may have been underestimated in routine clinical practice. Also, the tramadol or codeine prescription dispensation does not necessarily indicate exposure to either drug as the compliance is largely unknown. However, any underestimation and nonadherence are likely to have been the same in both groups, either not affecting the between-group comparison or distorting the associations toward the null.

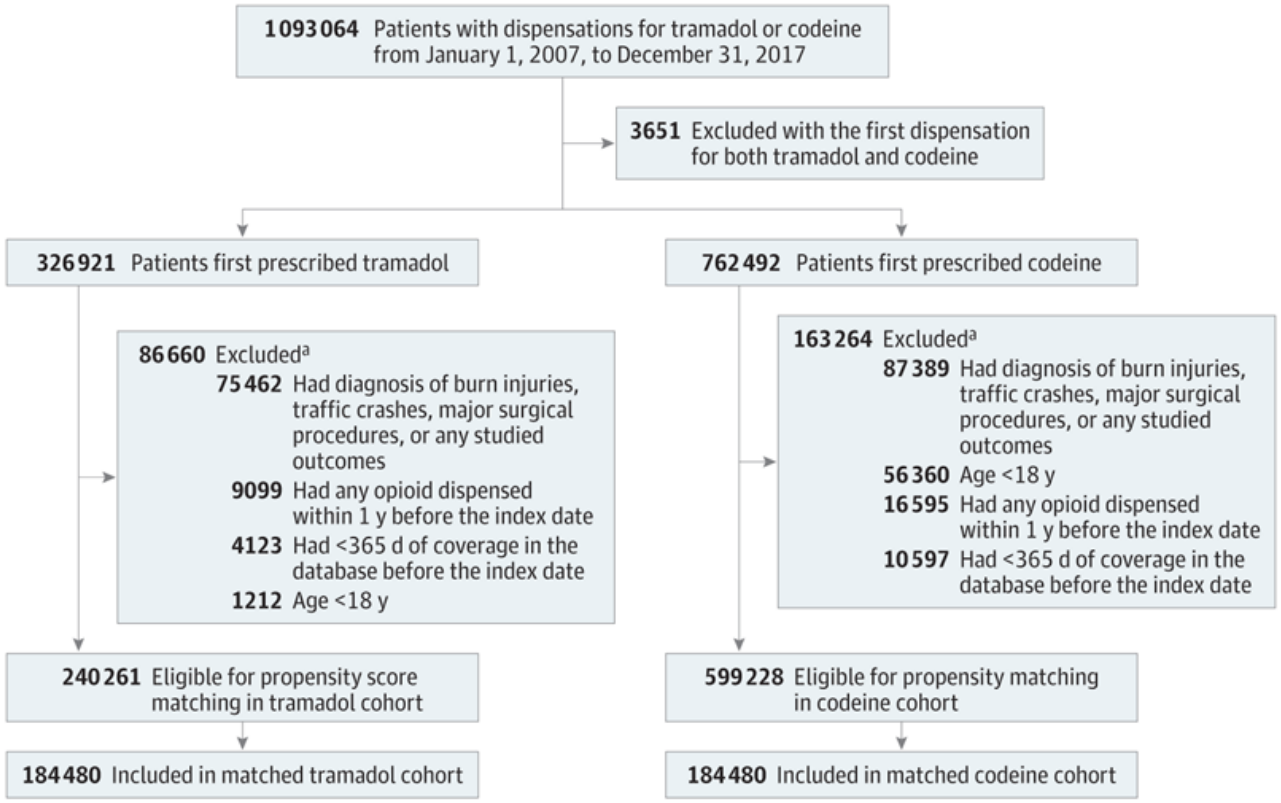
4.5.2. Conclusions

In this population-based cohort study, a new prescription dispensation of tramadol, compared with codeine, was significantly associated with higher risk of subsequent all-

cause mortality, cardiovascular events, and fractures, but there was no significant difference in the risk of constipation, delirium, falls, sleep disorders, and opioid abuse/dependence. The findings should be interpreted cautiously given the potential for residual confounding.

4.6. Supplements

sFigure 4 1 Selection process of participants for the study



Chapter 5

Risks of COVID-19 associated with ibuprofen use vs other NSAIDs

5. Risks of COVID-19 associated with ibuprofen use

5.1.Aim

To assess the risk of COVID-19 disease and its severity among prevalent ibuprofen users with osteoarthritis or back pain.

5.2.Introduction

In the early stages of the COVID-19 pandemic in 2020, a doctor in France reported four young patients with COVID-19 developing severe symptoms after using ibuprofen, a typical type of non-steroidal anti-inflammatory drug (NSAID)[145]. The French Health Minister and some UK experts immediately endorsed this case study, suggesting that patients with SARS-CoV-2 infection avoid using ibuprofen[146,147]. This triggered a significant public health concern about prescribing NSAIDs in the COVID-19 naïve population, and presented a clinical dilemma for those who require NSAIDs for relieving symptoms caused by a spectrum of diseases[148]. On the one hand, there was speculation that regular use of NSAIDs might increase the expression of the angiotensin-converting enzyme 2 receptor (ACE2) through which SARS-CoV-2 enters into host cells[149,150]. On the other hand, studies found that uncontrolled inflammation due to diseases such as active arthritis was associated with increased COVID-19 infection risk[151]. Given the absence of reliable casual evidence linking NSAID usage with COVID-19 outcomes, health regulatory agencies and clinical societies stated that NSAIDs therapy should not be discontinued[152,153]. However, as a precautionary approach, they recommended that alternative analgesics, such as paracetamol, might be preferable for managing the symptoms of COVID-19[154].

Some observational studies have since emerged showing that NSAID use does not increase the risk of severe complications or death in patients with COVID-19, yet only two studies have examined susceptibility to COVID-19 for general NSAIDs users in outpatient settings[155,156]. Despite informative, these studies are subject to methodological limitations, particularly the indication bias, by comparing NSAIDs users with either non-users or with users of opioid-containing drugs. Numerous empirical studies have shown that inappropriate design of reference groups can result in entirely non-comparable

participants, which is unlikely to be addressed by traditional statistical approaches based on limited confounder adjustment.

Given the conundrum that persists for the public and medical professionals regarding the safety of ibuprofen in the context of the ongoing COVID-19 pandemic, I attempted to assess the causality between ibuprofen use and COVID-19 susceptibility and severity in the COVID-19 naive population by leveraging two large US claims databases, head-to-head comparisons, and state-of-the-art statistical techniques to account for measured and unmeasured confounding. Specifically, I designed a prevalent-user active comparators cohort study, comparing ibuprofen users against either other non-selective NSAIDs (ns-NSAIDs), COX-2i or paracetamol users in relation to developing COVID-19 diagnosis or COVID-19 hospitalization, and restricted to a pool of patients with osteoarthritis (OA) or back pain given that they are commonly prescribed for those analgesics. In addition, I explored the potential heterogeneity of the associations during different calendar periods considering there was a significant change in prescribing patterns for these drugs due to the pandemic.

5.3.Methods

5.3.1. Study Design

I conducted a prevalent-user active comparators cohort study using two US administrative claims databases that had previously been mapped to the Observational Medical Outcomes Partnership (OMOP) Common Data Model (CDM v5).[157] Specifically, I used the IQVIA US Open Claims and IQVIA PharMetrics Plus databases, which contain pre-adjudicated and adjudicated health insurance data, respectively. Additional information on these data sources is detailed in Error! Reference source not found.. All data partners had previous institutional review board approval or exemption for their participation.

5.3.2. Cohort eligibility and enrolment period

I included participants registered in either data source who were aged 18 years or older, with a history of osteoarthritis or back pain, and received at least one eligible prescription of any study drugs between November 1 2019 and January 31 2020 (the pre-pandemic observational period), or between February 1 2020 and October 31 2020 (the pandemic period). I used the last prescription of any study drugs as the index date in the pre-pandemic period to reduce exposure misclassification due to medication switching between drugs. However, during the pandemic period, I chose the first prescription to reduce reverse causality (people taking these same drugs to treat fever and other COVID-19 related symptoms). Patients with less than 180 days observable data, previous exposure to the comparator drug within the 180 days, or with a diagnosis of COVID-19 on or before the index date were excluded.

5.3.3. Exposures and follow-up

I classified patients into the target (ibuprofen) or the active comparator groups (ns-NSAIDs, or COX-2 inhibitors[COX-2i], paracetamol,) according to the prescription

received on the index date. I excluded those who had both target and comparator drugs prescribed concomitantly on the index date. Participants were followed from index date to the earliest of a study outcome, death, loss or deregistration from the database, date of last data collection (last possible drug prescription start in Open Claims: August 2021, PharMetrics Plus: March 2021), record of comparator drug, or end of index treatment (on-treatment [OT] analysis). I additionally performed an intention to treat (ITT) sensitivity analysis, wherein patients were followed for 6 months following index date.

5.3.4. Outcomes

I assessed two outcomes for COVID-19 susceptibility, 1) COVID-19 diagnosis, and 2) COVID-19 hospitalization (hospital admission with a COVID-19 diagnosis during or up to three weeks before admission). The COVID-19 status for both outcomes was identified by SNOMED COVID-19 diagnostic codes. The phenotyping process of these outcomes based on claims data has been previously described and validated.[158,159]

5.3.5. Statistical Analyses

As prior knowledge was limited to estimate minimal sample size, I, instead, provided a minimum detectable rate ratio (MDRR) that presents achieving a 5% type-I error rate and 80% statistical power for each target–comparator–outcome combination by using all patients who met the eligibility criteria from the specific data source and study window.[160]

I used large-scale propensity score (PS) matching to balance target and comparison cohorts and control for measured and potential unmeasured confounding. For instance, when comparing ibuprofen with paracetamol, I first derived a PS for each individual by building a regularized logistic regression model that includes the binary treatment assignment as the dependent variable and a large set of predefined baseline patient

demographics, previous conditions, drug exposures, procedures, and health service use behaviours as the explanatory variables.[161] Of note, I excluded baseline features that occurred in fewer than 0.1% of patients within the target and comparator cohorts before fitting the PS model but evaluated their balance between groups after PS matching. I then created 1:1 PS-matched patients' cohorts and replicated the process to assemble 12 pairs of PS-matched cohorts (1 target * 3 comparators * 2 databases * 2 study windows).

I quantified the relative risk of outcome between the target and comparator treatments by hazard ratios (HRs) derived from the Cox proportional hazards model. To account for potential residual confounding, I included up to 217 negative control outcomes (NCOs) for each comparison, i.e., outcomes for which the null hypothesis was believed to be true. NCOs were identified through a data-rich algorithm[162,163] and reviewed by clinicians. I used the empirical null distributions to calibrate HR estimates if more than 5% of negative experiments were rejected.

I reported study diagnostics, including preference score distributions (a transformation of propensity score that adjusts for prevalence differences between populations) to evaluate the empirical equipoise and population generalizability, absolute standardised mean differences (ASMD) of patient characteristics to evaluate the cohort balance before and after propensity score matching, negative control calibration plots to assess the likelihood of residual bias, Kaplan-Meier plots to examine the proportional hazard assumption of COX model. For each model, I defined the HR estimate as invalidated if any ASMD of baseline covariates after PS matching was greater than 0.1 or the Cox model failed due to violation of the proportionality assumption. Using a fixed-effect model, I aggregated HRs and their 95% CI estimates (without correcting for multiple testing) across the data sources.

I conducted this study using the open-source OHDSI CohortMethod R package with large-scale analytics achieved through the Cyclops R package.

5.4.Results

5.4.1. Population

Study cohorts were created from a pool of patients with osteoarthritis or backpain, and were designed to enable comparisons between ibuprofen users (target cohort) and either ns-NSAID users, COX-2i users, or paracetamol users (comparator cohorts). The number of patients eligible for each cohort varied by data source and enrolment window (**Table 5 1**).

Table 5 1 Population size

Comparisons	Database	Target cohort	Comparator cohort	All patients
Pre-pandemic enrolment (November 2019 - January 2020)				
Ibuprofen vs COX-2i	PharMetrics Plus	118,841	60,347	179,188
Ibuprofen vs COX-2i	Open Claims	930,231	393,788	1,324,019
Ibuprofen vs COX-2i	Combined	1,049,072	454,135	1,503,207
Ibuprofen vs ns-NSAIDs	PharMetrics Plus	82,742	508,085	590,827
Ibuprofen vs ns-NSAIDs	Open Claims	640,574	2,708,452	3,349,026
Ibuprofen vs ns-NSAIDs	Combined	723,316	3,216,537	3,939,853
Ibuprofen vs Paracetamol	PharMetrics Plus	87,305	338,249	425,554
Ibuprofen vs Paracetamol	Open Claims	611,173	2,756,871	3,368,044
Ibuprofen vs Paracetamol	Combined	698,478	3,095,120	3,793,598
Pandemic enrolment (February 2020 - October 2020)				
Ibuprofen vs COX-2i	PharMetrics Plus	211,088	98,905	309,993
Ibuprofen vs COX-2i	Open Claims	1,574,159	649,594	2,223,753
Ibuprofen vs COX-2i	Combined	1,785,247	748,499	2,533,746
Ibuprofen vs ns-NSAIDs	PharMetrics Plus	150,744	869,877	1,020,621
Ibuprofen vs ns-NSAIDs	Open Claims	1,105,008	4,391,839	5,496,847
Ibuprofen vs ns-NSAIDs	Combined	1,255,752	5,261,716	6,517,468
Ibuprofen vs Paracetamol	PharMetrics Plus	163,801	536,024	699,825
Ibuprofen vs Paracetamol	Open Claims	1,102,235	4,173,434	5,275,669
Ibuprofen vs Paracetamol	Combined	1,266,036	4,709,458	5,975,494

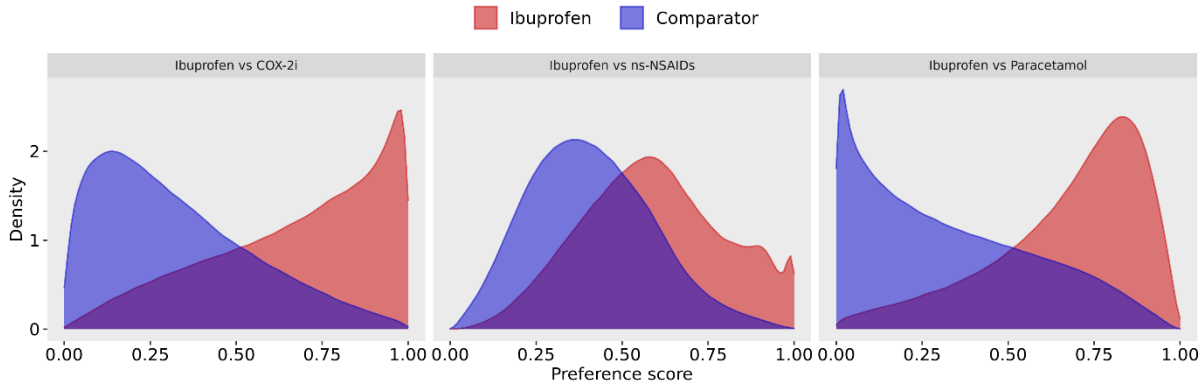
In the pre-pandemic enrolment window (November 2019 – January 2020), 1,503,207 patients were eligible for the ibuprofen vs ns-NSAIDs user cohorts, 3,939,853 patients were eligible for ibuprofen vs COX-2i user cohorts, and 3,793,598 patients were eligible for ibuprofen vs paracetamol user cohorts. In the pandemic enrolment window (February 2020 – October 2020), the corresponding figures were 6,876,630 patients,

2,370,693 patients and 5,551,200 patients, respectively. Cohorts from the Open Claims database had more patients than equivalent cohorts in the PharMetrics Plus database, consistent with Open Claims being the larger database.

5.4.2. Patient characteristics and PS matching

The number of baseline patient characteristics used to construct a PS model ranged from 3,174 to 3,789 covariates for the ibuprofen vs ns-NSAIDs user cohorts, 3,491 to 4,090 covariates for the ibuprofen vs COX-2i user cohorts, and 3,639 to 4,227 for the ibuprofen vs paracetamol user cohorts, with variation arising between data sources and study windows. Substantial overlap in PS-distribution between unmatched ibuprofen and ns-NSAID user cohorts indicated minimal violation of the positivity assumption for causal inference[164] (**Figure 5 1**). Indeed, unmatched ibuprofen and ns-NSAID user cohorts were similar with respect to age, gender, and prevalence of clinical conditions (**Table 5 2**).

Figure 5 1 Preference score distribution in ibuprofen versus comparator cohorts



Illustrative preference score distributions for Ibuprofen versus comparator user cohorts from the February-October 2020 enrolment window, Open Claims database. Preference scores are a transformation of propensity scores (PS), and a PS is the probability a patient received the target drug over the comparator drug, given the patient's baseline covariates.

In contrast, a more polarized PS-distribution existed between ibuprofen user cohorts and COX-2i or paracetamol user cohorts, suggesting that these patients had less comparable

baseline characteristics before PS-matching. COX-2i users tended to be older than ibuprofen users, with a higher prevalence of musculoskeletal-related procedures and a lower prevalence of emergency room visits (**Table 5 3**). Paracetamol users appeared generally less healthy than ibuprofen users, as indicated by higher mean clinical index scores **(Table 5 4**). Nevertheless, following PS-matching, all measured covariates were adequately balanced between analysis cohort pairs (ASMD < 0.1 for all covariates).

Table 5 2 Covariate balance before and after PS-matching Ibuprofen-nsNSAID cohorts

	Before PS-matching			After PS-matching		
	Ibuprofen (%)	ns-NSAID (%)	ASMD	Ibuprofen (%)	ns-NSAID (%)	ASMD
Demographics						
Female	63.3	62.7	0.01	62.1	62.8	-0.01
Age 18 - 19	1.3	0.5	0.09	1.1	0.9	0.01
Age 20 - 24	3.7	1.5	0.14	2.8	2.6	0.01
Age 25 - 29	4.9	2.1	0.15	3.7	3.5	0.01
Age 30 - 34	6.2	3.1	0.15	5.1	4.9	0.01
Age 35 - 39	7	4.1	0.12	6.4	6.5	0
Age 40 - 44	7.6	5.3	0.09	7.5	7.6	0
Age 45 - 49	8.7	6.9	0.07	8.9	8.8	0
Age 50 - 54	10.7	9.5	0.04	11.2	11.1	0
Age 55 - 59	12.7	12.3	0.01	13.4	13.8	-0.01
Age 60 - 64	12.2	13.4	-0.03	13	13.4	-0.01
Age 65 - 69	9.9	13.1	-0.1	10.6	10.5	0
Age 70 - 74	6.9	11.4	-0.16	7.5	7.6	0
Age 75 - 79	4.1	7.8	-0.16	4.4	4.5	0
Age 80 - 84	4.1	9.1	-0.2	4.4	4.4	0
Conditions						
Amenorrhea, any time prior	6.1	3.4	0.13	5	5.2	-0.01
Hypertension, any time prior	52.7	62.3	-0.2	54.8	55.6	-0.02
Hip pain, any time prior	17	22	-0.13	17.6	18.1	-0.01
Hyperlipidemia, any time prior	40.5	49.8	-0.19	42.4	42.9	-0.01
Nicotine dependence, any time	17.8	13.8	0.11	16.8	17.8	-0.03
Cataracts, any time prior	13	20.1	-0.19	13.9	14.1	0
Osteoarthritis, any time prior	20.1	28	-0.19	21.3	22.1	-0.02
Hip OA, any time prior	7.9	12.1	-0.14	8.5	8.6	-0.01
Knee OA, any time prior	27	38.9	-0.26	28.9	29.8	-0.02
Osteoporosis, any time prior	7.9	12.3	-0.15	8.4	8.5	0
Lumbar spine stenosis, any time	15.7	20.3	-0.12	16.6	17.2	-0.01
Low back pain, 6 months prior	43.1	35.8	0.15	41.7	41.8	0

Hip OA, 6 months prior	3.6	6.1	-0.11	3.9	4	0
Knee OA, 6 months prior	12.9	20.3	-0.2	13.9	14.3	-0.01
Knee OA, 1 month prior	5.5	10.5	-0.18	5.9	6.2	-0.01
Cerebrovascular disease	2.1	3	-0.06	2.2	2.3	-0.01
Chronic obstructive lung disease	12.5	14.3	-0.05	13.1	13.6	-0.02
Diabetes mellitus	0.7	0.7	0	0.7	0.8	-0.01
Ischemic heart disease	0.2	0.3	-0.01	0.2	0.2	0
Obesity	25.8	25.3	0.01	25.6	26.3	-0.01
Malignant neoplastic disease	0.2	0.2	-0.01	0.2	0.2	0
Procedures and visit						
Knee arthroscopy, any time	3	5.6	-0.13	3.2	3.5	-0.01
DXA scan, any time prior	13	20.3	-0.2	14	14.2	-0.01
ECG, any time prior	37.6	44.3	-0.14	38.9	39.2	-0.01
Knee X-ray, any time prior	15	20.4	-0.14	15.6	16.5	-0.02
Manual/ physiotherapy , any	37	44.4	-0.15	38.2	38.6	-0.01
Total knee replacement, any	0.6	1.4	-0.08	0.7	0.8	-0.01
Arthrocentesis, 6 months prior	5.3	8.8	-0.14	5.8	5.9	0
Emergency visit, 6 months prior	14.5	12.3	0.06	12	12.5	-0.02
Clinical Index Scores [1]						
CHADS2VASc	2.06	2.54	-0.29	2.12	2.15	-0.02
Diabetes Comorbidity Severity	1.52	1.95	-0.18	1.58	1.63	-0.02
Charlson index - Romano	2.1	2.55	-0.16	2.17	2.23	-0.02

[1]Data for these covariates are given as a mean score for the cohort, not % of the cohort. Select characteristics before and after PS-matching, showing the (weighted) percentage of subjects with the characteristics in the Ibuprofen versus comparator cohorts, as well as the absolute standardized mean difference (ASMD). Data shown from the OpenClaims, OT, February-October 2020 analysis.

Table 5 3 Covariate balance before and after PS-matching Ibuprofen-COX-2i cohorts

	Before PS-matching			After PS-matching		
	Ibuprofen (%)	COX-2i (%)	ASMD	Ibuprofen (%)	COX-2i (%)	ASMD
Demographics						
Female	64.5	63.6	0.02	63.8	63.1	0.02
Age 18 - 19	1.1	0.1	0.12	0.2	0.2	0
Age 20 - 24	3.3	0.4	0.21	0.5	0.6	-0.01
Age 25 - 29	4.5	0.7	0.24	0.8	0.9	-0.01
Age 30 - 34	5.9	1.2	0.26	1.5	1.6	-0.01
Age 35 - 39	6.9	2	0.24	2.5	2.7	-0.01
Age 40 - 44	7.9	3.2	0.2	4.1	4.1	0
Age 45 - 49	9.1	5	0.16	6.1	6.3	-0.01
Age 50 - 54	11.3	8.1	0.11	9.7	9.7	0
Age 55 - 59	13.3	11.9	0.04	13.9	13.4	0.01
Age 60 - 64	12.4	14.7	-0.07	15.8	15.6	0.01
Age 65 - 69	9.8	16.3	-0.19	15.5	15.5	0
Age 70 - 74	6.7	14.9	-0.26	12.7	12.7	0
Age 75 - 79	3.9	10.4	-0.25	8.1	8.3	-0.01
Age 80 - 84	3.9	11	-0.27	8.5	8.4	0
Conditions						
Amenorrhea, any time prior	6.3	2.2	0.2	2.7	2.6	0
Hypertension, any time prior	55	66.4	-0.23	65.5	64	0.03
Hip pain, any time prior	18.6	27.2	-0.21	24.7	23.9	0.02
Hyperlipidemia, any time prior	41.9	54.5	-0.25	52.5	51.6	0.02
Nicotine dependance, any time prior	19.3	9.8	0.27	12	11.5	0.01
Cataracts, any time prior	13.5	24.2	-0.28	21.3	20.9	0.01
Osteoarthritis, any time prior	21.8	35	-0.3	32.3	31.7	0.01
Hip OA, any time prior	8.4	19.4	-0.32	15	14.8	0
Knee OA, any time prior	29.1	51.1	-0.46	45.7	44.6	0.02
Osteoporosis, any time prior	8.2	15.2	-0.22	13.7	13.2	0.01
Lumbar spine stenosis, any time prior	16.5	26.4	-0.24	24.6	23.8	0.02
Low back pain, 6 months prior	43.5	27.3	0.34	30.7	31	-0.01
Hip OA, 6 months prior	3.8	11.6	-0.29	7.7	8	-0.01
Knee OA, 6 months prior	14.3	28.6	-0.36	24	23.9	0
Knee OA, 1 month prior	5.9	16.3	-0.34	11.5	11.7	0
Cerebrovascular disease	2.2	3	-0.05	3	2.8	0.01
Chronic obstructive lung disease	13.4	13.8	-0.01	15.5	14.2	0.04
Diabetes mellitus	0.8	0.6	0.02	0.7	0.7	0
Ischemic heart disease	0.2	0.3	-0.01	0.3	0.2	0.01
Obesity	27.7	25	0.06	26.1	25.2	0.02
Malignant neoplastic disease	0.2	0.3	-0.01	0.3	0.3	0
Procedures and visit						
Knee arthroscopy, any time prior	3.1	11.1	-0.32	6.8	6.9	0
DXA scan, any time prior	13.5	25.4	-0.3	22.6	21.8	0.02
ECG, any time prior	39	49.2	-0.21	47.5	46.1	0.03
Knee X-ray, any time prior	16.5	26	-0.23	23	22.1	0.02

Manual therapy/ physiotherapy, any	38.6	53.3	-0.3	49.5	48.6	0.02
Total knee replacement, any time	0.6	4.6	-0.25	1.8	1.9	-0.01
Arthrocentesis, 6 months prior	6.1	11.8	-0.2	10.5	10.2	0.01
Emergency visit, 6 months prior	17.6	6.2	0.36	7.5	7.1	0.02
Clinical Index Scores [1]						
CHADS2VASc	2.12	2.74	-0.38	2.62	2.57	0.03
Diabetes Comorbidity Severity Index	1.62	1.95	-0.14	1.95	1.87	0.04
Charlson index - Romano adaptation	2.22	2.57	-0.13	2.6	2.48	0.04

[1]Data for these covariates are given as a mean score for the cohort, not % of the cohort. Select characteristics before and after PS-matching, showing the (weighted) percentage of subjects with the characteristics in the Ibuprofen versus comparator cohorts, as well as the absolute standardized mean difference (ASMD). Data shown from the OpenClaims, OT, February-October 2020 analysis.

Table 5 4 Covariate balance before and after PS-matching Ibuprofen-Paracetamol cohorts

	Before PS-matching			After PS-matching		
	Ibuprofen (%)	Paracetamol (%)	ASMD	Ibuprofen (%)	Paracetamol (%)	ASMD
Demographics						
Female	64	60.6	0.07	62.3	62.8	-0.01
Age 18 - 19	1.4	0.3	0.11	0.8	0.7	0.01
Age 20 - 24	3.8	1.1	0.18	2.3	2.1	0.01
Age 25 - 29	5	1.6	0.19	3.4	3.2	0.01
Age 30 - 34	6.4	2.5	0.19	4.9	4.8	0
Age 35 - 39	7.1	3.5	0.16	6.1	6.2	0
Age 40 - 44	7.8	4.6	0.13	7.3	7.5	0
Age 45 - 49	8.8	6.1	0.1	8.8	8.8	0
Age 50 - 54	10.8	8.6	0.07	11.2	11.2	0
Age 55 - 59	12.5	11.8	0.02	13.5	13.6	0
Age 60 - 64	11.8	13.5	-0.05	13.2	13.4	-0.01
Age 65 - 69	9.6	13.7	-0.13	10.9	11.1	0
Age 70 - 74	6.9	12.3	-0.19	8	7.9	0
Age 75 - 79	4.1	8.9	-0.2	4.8	4.8	0
Age 80 - 84	4	11.5	-0.28	4.8	4.8	0
Conditions						
Amenorrhea, any time prior	6.2	3	0.15	4.9	4.9	0
Hypertension, any time prior	51.4	68.3	-0.35	55.6	56.4	-0.02
Hip pain, any time prior	16.5	24.2	-0.19	17.6	18.3	-0.02
Hyperlipidemia, any time prior	39.9	53.7	-0.28	42.9	43.9	-0.02
Nicotine dependence, any time	16.6	16.3	0.01	16.2	16.9	-0.02
Cataracts, any time prior	13.2	21.5	-0.22	14.6	15.3	-0.02
Osteoarthritis, any time prior	19.9	29.1	-0.22	21.8	23	-0.03
Hip OA, any time prior	7.6	13.5	-0.19	8.6	8.8	-0.01
Knee OA, any time prior	27.3	39	-0.25	29.9	30.7	-0.02
Osteoporosis, any time prior	7.9	13.5	-0.18	8.9	9.1	-0.01
Lumbar spine stenosis, any time	13.5	26.9	-0.34	15.4	16.3	-0.02
Low back pain, 6 months prior	43	36.6	0.13	41.3	41.3	0
Hip OA, 6 months prior	3.6	6.5	-0.13	4.1	4.1	0
Knee OA, 6 months prior	13.9	18	-0.11	15.1	15.1	0
Knee OA, 1 month prior	5.9	9.3	-0.13	6.5	6.4	0
Cerebrovascular disease	1.9	4.1	-0.13	2.1	2.3	-0.01
Chronic obstructive lung disease	10.9	19.9	-0.25	12.4	13	-0.02
Diabetes mellitus	0.7	0.9	-0.03	0.7	0.8	-0.01
Ischemic heart disease	0.2	0.3	-0.03	0.2	0.2	0
Obesity	25.9	26.1	0	25.6	26.1	-0.01
Malignant neoplastic disease	0.2	0.3	-0.03	0.2	0.2	0
Procedures and visits						
Knee arthroscopy, any time prior	2.6	6.7	-0.2	3	3.2	-0.01
DXA scan, any time prior	13.3	20.5	-0.19	14.7	15.2	-0.01

ECG, any time prior	37.6	46.7	-0.18	39.1	40.1	-0.02
Knee X-ray, any time prior	14.9	21.7	-0.18	15.8	16.5	-0.02
Manual/ physiotherapy, any time	36.8	45.7	-0.18	38.1	39.1	-0.02
Total knee replacement, any time	0.4	2.1	-0.16	0.4	0.5	-0.01
Arthrocentesis, 6 months prior	5.8	8.2	-0.09	6.4	6.6	-0.01
Emergency visit, 6 months prior	15.4	13.5	0.05	12.8	13.4	-0.02
Clinical Index Scores [1]						
CHADS2VASc	2.02	2.85	-0.48	2.15	2.19	-0.03
Diabetes Comorbidity Severity	1.43	2.49	-0.41	1.57	1.65	-0.03
Charlson index - Romano	2	3.19	-0.41	2.14	2.27	-0.05

[1]Data for these covariates are given as a mean score for the cohort, not % of the cohort. Select characteristics before and after PS-matching, showing the (weighted) percentage of subjects with the characteristics in the Ibuprofen versus comparator cohorts, as well as the absolute standardized mean difference (ASMD). Data shown from the OpenClaims, OT, February-October 2020 analysis.

5.4.3. Incidence rates of COVID-19 outcomes

OT incidence of COVID-19 outcomes in PS-matched cohorts are reported in **Table 5 5**. In the Open Claims database, the OT incidence rates of COVID-19 diagnosis were 37.6 vs 40.0 (ibuprofen vs ns-NSAIDs), 38.6 vs 38.0 (ibuprofen vs COX-2i), and 39.3 vs 43.0 (ibuprofen vs paracetamol) per 1,000 person-years for patients enrolled during the pandemic window. Within the same database and study window, the incidence rates of COVID-19 hospitalization were 10.1 vs 9.9 (ibuprofen vs ns-NSAIDs), 11.2 vs 9.9 (ibuprofen vs COX-2i), and 10.3 vs 11.6 (ibuprofen vs paracetamol) per 1,000 person-years. In general, the incidence rates of COVID-19 diagnosis were higher in PharMetrics Plus, but the incidence rates for COVID-19 hospitalization were similar between the two databases. Furthermore, the incidence rates of both COVID-19 outcomes were higher for cohorts enrolled during the pandemic observation period.

Table 5 5 Incidence of COVID-19 outcomes in PS-matched Ibuprofen and comparator cohorts

Comparisons	Database	Persons[1]	Cases		Follow up (years)		Incidence (%)		Incidence (per 1k person years)	
			Ibuprofen	Comparator	Ibuprofen	Comparator	Ibuprofen	Comparator	Ibuprofen	Comparator
COVID-19 diagnosis (Nov 2019- Jan 2020)										
Ibuprofen vs COX-2i	PharMetrics Plus	39,572	156	480	6,931	14,539	0.39	1.21	22.5	33.0
Ibuprofen vs COX-2i	OpenClaims	272,097	1,407	3,432	62,849	138,466	0.52	1.26	22.4	24.8
Ibuprofen vs ns-NSAIDs	PharMetrics Plus	69,723	174	449	9,038	16,081	0.25	0.64	19.3	27.9
Ibuprofen vs ns-NSAIDs	OpenClaims	563,839	1,885	4,100	98,946	168,055	0.33	0.73	19.1	24.4
Ibuprofen vs Paracetamol	PharMetrics Plus	54,074	119	414	6,830	10,454	0.22	0.77	17.4	39.6
Ibuprofen vs Paracetamol	OpenClaims	437,928	1,334	4,523	71,973	141,905	0.30	1.03	18.5	31.9
COVID-19 diagnosis (Feb 2020 - Oct 2020)										
Ibuprofen vs COX-2i	PharMetrics Plus	67,190	532	1,247	10,894	21,898	0.79	1.86	48.8	57.0
Ibuprofen vs COX-2i	OpenClaims	457,280	3,551	7,370	92,004	193,931	0.78	1.61	38.6	38.0
Ibuprofen vs ns-NSAIDs	PharMetrics Plus	120,954	689	1,334	14,424	24,796	0.57	1.10	47.8	53.8
Ibuprofen vs ns-NSAIDs	OpenClaims	943,006	5,382	9,682	143,309	241,999	0.57	1.03	37.6	40.0
Ibuprofen vs Paracetamol	PharMetrics Plus	101,635	575	834	11,617	15,820	0.57	0.82	49.5	52.7
Ibuprofen vs Paracetamol	OpenClaims	776,963	4,325	8,106	110,110	188,659	0.56	1.04	39.3	43.0
Hospitalized with COVID-19 (Nov 2019- Jan 2020)										
Ibuprofen vs COX-2i	PharMetrics Plus	39,572	29	89	6,963	14,660	0.07	0.22	4.2	6.1
Ibuprofen vs COX-2i	OpenClaims	272,097	446	961	63,317	139,848	0.16	0.35	7.0	6.9
Ibuprofen vs ns-NSAIDs	PharMetrics Plus	69,723	24	91	9,073	16,183	0.03	0.13	2.7	5.6
Ibuprofen vs ns-NSAIDs	OpenClaims	563,839	561	1,171	99,526	169,551	0.10	0.21	5.6	6.9
Ibuprofen vs Paracetamol	PharMetrics Plus	54,074	18	82	6,854	10,565	0.03	0.15	2.6	7.8
Ibuprofen vs Paracetamol	OpenClaims	437,928	386	1,274	72,392	143,803	0.09	0.29	5.3	8.9
Hospitalized with COVID-19 (Feb 2020 - Oct 2020)										
Ibuprofen vs COX-2i	PharMetrics Plus	67,190	112	211	10,979	22,153	0.17	0.31	10.2	9.5
Ibuprofen vs COX-2i	OpenClaims	457,280	1,044	1,948	92,847	196,320	0.23	0.43	11.2	9.9
Ibuprofen vs ns-NSAIDs	PharMetrics Plus	120,954	128	262	14,506	25,020	0.11	0.22	8.8	10.5
Ibuprofen vs ns-NSAIDs	OpenClaims	943,006	1,456	2,432	144,396	244,609	0.15	0.26	10.1	9.9
Ibuprofen vs Paracetamol	PharMetrics Plus	101,635	120	185	11,687	16,004	0.12	0.18	10.3	11.6
Ibuprofen vs Paracetamol	OpenClaims	776,963	1,143	2,228	110,941	191,379	0.15	0.29	10.3	11.6

[1]Ibuprofen and comparator cohorts are matched 1:1 on PS and are therefore the same size. OT Incidence rates of COVID-19 diagnosis or hospitalization with COVID-19 in ibuprofen vs comparator (comp) user cohorts. For each pairwise comparison, I report the PS-matched cohort size, follow-up years, the number of events and incidences.

5.4.4. Empirical calibration and hazard ratios

The proportional hazards assumption of Cox regression was held for all comparisons, except for one paracetamol comparison. Prior to empirical calibration, all comparisons had detectable systematic bias, defined here as > 5% significant NCOs in a comparison. After empirical calibration, most comparisons produced <5% significant NCOs, and all comparison produced < 8% NCOs.

In the OT-analyses, meta-analysis of calibrated HRs revealed no significant differential risk of COVID-19 diagnosis or hospitalization with COVID-19 in users of ibuprofen versus users of COX-2i, ns-NSAIDs or paracetamol (**Table 5 6, Figure 5 2**, comparisons that violated the Cox proportional hazards assumption were excluded). For example, the aggregated HRs for COVID-19 diagnosis in the pre-pandemic cohorts were 1.00 (95% CI 0.83 – 1.22) for ibuprofen vs ns-NSAID users, 1.06 (95% CI 0.84 – 1.35) for ibuprofen vs COX-2i users, and 0.97 (95% CI 0.71 – 1.33) for ibuprofen vs paracetamol users. Further, the aggregated HRs for hospitalization with COVID-19 in the pandemic enrolment window were 1.23 (95% CI 0.99 – 1.52), 1.26 (95% CI 0.96-1.65), and 1.01 (95% CI 0.65 – 1.58) accordingly. Although a single significant HR was observed in the Open Claims database when comparing ibuprofen vs ns-NSAID users for risk of hospitalization with COVID-19 (pandemic period). no similarly significant HRs were observed in PharMetrics Plus, nor in either database during the pre-pandemic period.

The results of the ITT6 sensitivity analyses were largely consistent with the OT analysis, bar two comparisons. Specifically, a significantly increased risk of COVID-19 diagnosis was observed in an ibuprofen - paracetamol comparison (pre-pandemic enrolment, meta-analysis HR 1.27, 95% CI 1.06 – 1.52), and a significantly increased risk of hospitalization with COVID-19 was observed in an ibuprofen - COX-2i comparison (pandemic enrolment window, HR 1.25, 95% CI 1.07 – 1.46). Neither result was

replicated in the alternative study period, nor in the OT analysis. Notably, the median length of drug use in these cohorts was substantially less than 6 months, with a large difference between median ibuprofen and COX-2i usage.

Figure 5 2 OT calibrated hazard ratios estimates for COVID-19 outcomes in users of ibuprofen versus comparator analgesics

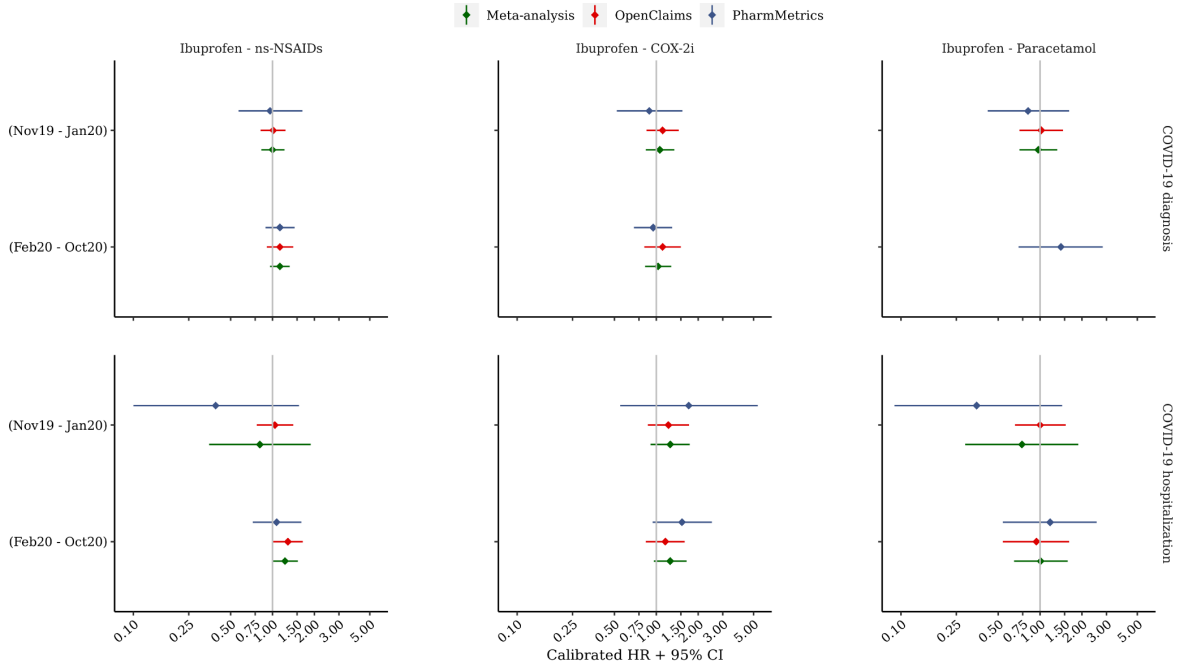


Table 5 6 OT calibrated hazard ratios for COVID-19 outcomes in users of ibuprofen versus comparator analgesics

Comparisons	Database	COVID-19 diagnosis HR (95% CI)		Hospitalization with COVID-19 HR (95% CI)	
		Nov 2019 - Jan 2020	Feb 2020 - Oct 2020	Nov 2019 - Jan 2020	Feb 2020 - Oct 2020
		On treatment			
Ibuprofen - ns-NSAIDs	Open Claims	1.01 (0.82 - 1.24)	1.13 (0.91 - 1.41)	1.04 (0.77 - 1.41)	1.29 (1.01 - 1.65)
Ibuprofen - ns-NSAIDs	PharMetrics Plus	0.96 (0.57 - 1.64)	1.13 (0.89 - 1.44)	0.39 (0.10 - 1.55)	1.07 (0.72 - 1.61)
Ibuprofen - ns-NSAIDs	Meta-analysis	1.00 (0.83 - 1.22)	1.13 (0.96 - 1.33)	0.81 (0.35 - 1.88)	1.23 (0.99 - 1.52)
Ibuprofen - COX-2i	Open Claims	1.11 (0.85 - 1.45)	1.11 (0.82 - 1.50)	1.22 (0.87 - 1.72)	1.16 (0.84 - 1.60)
Ibuprofen - COX-2i	PharMetrics Plus	0.89 (0.52 - 1.54)	0.95 (0.69 - 1.30)	1.71 (0.55 - 5.36)	1.53 (0.94 - 2.51)
Ibuprofen - COX-2i	Meta-analysis	1.06 (0.84 - 1.35)	1.03 (0.83 - 1.28)	1.26 (0.91 - 1.74)	1.26 (0.96 - 1.65)
Ibuprofen - Paracetamol	Open Claims	1.02 (0.71 - 1.46)	NA	1.00 (0.66 - 1.53)	0.94 (0.54 - 1.62)
Ibuprofen - Paracetamol	PharMetrics Plus	0.82 (0.42 - 1.62)	1.41 (0.70 - 2.82)	0.35 (0.09 - 1.44)	1.18 (0.54 - 2.55)
Ibuprofen - Paracetamol	Meta-analysis	0.97 (0.71 - 1.33)	NA	0.74 (0.29 - 1.88)	1.01 (0.65 - 1.58)

NA: not available due to the failure of negative control diagnosis.

5.5.Discussion

In this cohort study, including 6,707,247 and 10,154,597distinct patients with osteoarthritis or back pain during the two observation periods, I found no differential risks of incident COVID-19 diagnosis or COVID-19 hospitalization among ibuprofen users compared with other ns-NSAIDs, COX-2i or paracetamol. My findings support regulatory recommendations that NSAIDs, including ibuprofen, should be prescribed as indicated in the same way as before the COVID-19 pandemic, especially for those who rely on ibuprofen or NSAIDs to manage chronic arthritis or musculoskeletal pain symptoms.

A few laboratory-based studies have proposed possible biological mechanisms linking ibuprofen or NSAIDs exposure and COVID-19 complications. For example, in vitro experiments found that NSAIDs could disrupt the resolution of the inflammatory process by inhibiting prostanoid biosynthesis, which theoretically might weaken immune responses against pathogens.[165,166] In diabetic rats, ibuprofen has the potential to upregulate ACE2, to which the SARS-CoV-2 virus binds before entering host cells[167]. However, it remains unknown whether these results can be generalised to humans.

Based on patients hospitalized with COVID-19, several studies have consistently shown that the previous use of NSAIDs did not exacerbate the severity of COVID-19 or dying from the disease[168–171]. Nevertheless, data are limited on the susceptibility to COVID-19 associated with NSAIDs indicated for other health conditions. Among a few studies, AY et al. found no differential risk of COVID-19 related deaths among NSAIDs users (536,423 users from the general community and 175,495 users from rheumatoid arthritis/osteoarthritis population) compared to non-users.[156] This study exploited clinical knowledge with an informed directed acyclic graph approach to control potential confounding bias. However, the quantitative bias analysis in this study showed that even

a moderate unmeasured confounder could fully explain the observed associations, which is likely to occur given the known systematic difference between NSAIDs users and non-users driven by the drug indications. Another community-based cohort study, in contrast, used an active comparator design by including 13,202 users of NSAIDs and 12,457 users of co-codamol or co-dydramol[155]. Although the observed association with COVID-19 infection was statistical no-significance [HR, 0.79 (95% CI 0.57–1.11)], it is questionable that opioid-containing drugs acted as a meaningful active comparator given that it is likely prescribed for patients with moderate or severe acute pain with different susceptibility to COVID-19.

My study extended existing knowledge and produced more reliable causal findings in the following ways. First, for the first time, I specifically studied the effect of ibuprofen, the type of drug under the spotlight among the NSAIDs class, compared with other alike analgesics on COVID-19 related outcomes. It is of special importance for clinicians and patients to balance the risks associated with ibuprofen over its alternative medications, because of the very large consumption of these drugs in primary care and over-the-counter settings. Second, previous studies that used general practitioners' prescription records as the source of drug exposure were likely subject to considerable medication non-compliance.[172] Instead, I based my analyses on claim data, and, therefore, the concern of non-compliance between the drug prescription and drug dispensation can be vastly dismissed. Third, COVID-19 is a novel infectious disease, and current knowledge of its risk factors is relatively limited. The conventional approach of pre-specifying potential confounders based on clinical experience might be insufficient and defective, especially during the pandemic's lockdown periods when doctors' routine drug prescribing practices have been seriously disrupted. Even though I applied a large-scale propensity

score approach to balance thousands of baseline covariates that maximally rules out the confounding, evidence from NCOs existed for residual confounding in my study, highlighting the importance of empirically calibrating estimates in observational healthcare database studies.

However, several limitations warrant attention in my study. First, ibuprofen, other ns-NSAIDs and paracetamol were commonly used through the over-the-counter self-medication[173], which inevitably misclassified groups of exposures and controls. Although the misclassification between the target and comparator drugs could be no-differential, it would likely distort associations toward the null. Second, due to the limited availability of testing resources at the earlier pandemic stage and the fact that many infected patients are asymptomatic, under-diagnosis of people with COVID-19 infection was possible. To address this limitation inherent in identifying asymptomatic COVID-19 cases, I defined a hospital admission-based COVID-19 outcome because the claim data can capture most COVID-19 hospitalization cases. Also, as the COVID-19 test capacity had been gradually improved in the US community, the underestimation of COVID-19 should be primarily mitigated in the second observational window up to October 31, 2020. Third, when a data driven model was applied in the context the prevalent-user design, there was always a potential risk of inclusion of mediators in the covariates adjustment. In this specific case, all study analgesic medications were commonly prescribed for symptoms relief, the pathology of underlying diseases that causes painful conditions was unlikely to be modified by these drugs. Therefore, the likelihood of over-adjustment seemed minimal. Fourth, indication bias could not be ruled out, even though the target and comparison drugs had a significant degree of overlap for their indications. Fifth, I cannot differentiate short-term or long-term users as information on indications is not readily available. By

restricting to OA or back pain patients, I expected most participants to be regular users of NSAIDs. Sixth, a proportion of people initiating any study drugs to relieving COVID-19 symptoms might be falsely included. However, this is unlikely to occur during the first enrolment window before the pandemic, showing consistent results.

In summary, my findings reassured that using ibuprofen in the community, compared to other ns-NSAIDs, COX-2i or paracetamol, was not associated with an increased risk of susceptibility to or severity of COVID-19.

Chapter 6

Comparative effectiveness of the BNT162b2 and ChAdOx1 vaccines

6. Comparative effectiveness of the BNT162b2 and ChAdOx1 vaccines

6.1.Aim

To study the comparative effectiveness of BNT162b2 versus ChAdOx1 vaccines against COVID-19 and hospitalization in people over 50.

6.2.Introduction

To date, four vaccines against the Coronavirus disease 2019 (Covid-19) have been approved for use in the UK by the Medicines and Healthcare products Regulatory Agency: the Pfizer-BioNTech BNT162b2, Moderna's mRNA-1273, Oxford-AstraZeneca's ChAdOx1, and Janssen's Ad26.CoV2.S. Although phase 3 trials suggested that all four have high clinical efficacy, mRNA vaccines presented numerically greater efficacy than adenoviral-based ones: BNT162b2 reported 95% efficacy against symptomatic covid-19,[174] mRNA-1273 94.1% efficacy,[175] whilst ChAdOx1 had 70.4%,[51] and Ad26.CoV2.S had 66.9% efficacy.[53] However, notable differences in study designs made it difficult to compare vaccine efficacy based on these trials, including different populations recruited in different regions and at different times, diverse primary endpoint definitions, and heterogeneous statistical analysis methods.

Specific for the two more widely utilized vaccines, BNT162b2 and ChAdOx1, several observational studies have recently evaluated their effectiveness in multiple real-world settings from Israel,[176] the UK,[177] and Spain,[7] amongst others. Although useful, none of these studies conducted head-to-head comparisons of vaccine effectiveness. With the ongoing pandemic and rapid rollout of the Covid-19 vaccination programme all over the world, evidence on their comparative performance has become more crucial to inform policy decisions on optimizing vaccine implementation strategies, not only in countries where greater coverage of the prime dose is urgently required or in countries where boost doses and sequential immunization are being considered. Therefore, this study aimed to estimate the comparative effectiveness of BNT162b2 vs ChAdOx1 vaccines against Covid-19 infection and hospitalization in a large and population-based prospective cohort of people aged 50 years or older.

6.3.Methods

6.3.1. Study population and design

In this study, I analysed data from UK Biobank participants from England, as vaccination data for Scotland and Wales were not available. Although vaccination started in early December 2020 in the UK, I restricted my analyses to periods when both vaccines were rolled out to maximise comparability in individual- and population-level characteristics, including indication for vaccination, ongoing public health restrictions and predominant virus variants. With this in mind, I curated one-dose and two-dose Covid-19 vaccine cohorts covering from Jan 11, 2021 to Feb 28, 2021, and from March 22, 2021 to May 9, 2021 respectively. Participants with primary care records collected using the TTP software, which did not contain a specific vaccine type, were excluded.

6.3.2. Data sources

Several external data sources have been linked to UK Biobank to enable Covid-19 research, including primary care electronic health records, hospital admissions data from Hospital Episode Statistics (HES), and Covid-19 tests from Public Health England (PHE).[88]

6.3.3. Vaccination status

Vaccination status was obtained from GP prescription records, including the date of receipt of each dose and the vaccine brand. I used dm+d codes (Dictionary of medicines and devices used across the UK's National Health Service) to identify BNT162b2 [39115611000001103] and ChAdOx1 [39114911000001105] Covid-19 vaccination.

6.3.4. Study outcomes

In the UK, people at any age with any of these three Covid-19 symptoms (a high temperature, a new and continuous cough, or loss or change in the sense of smell or taste)

are recommended to take a free Polymerase-chain-reaction (PCR) test by ordering a self-swab home PCR test kit or booking an appointment at a walk-in or drive-through test site. People could also access this service if they were at high risk of infection, e.g., close contact with a case. All these tests were recorded by PHE and linked to UK Biobank, providing information on test dates and results. In this study, I defined infection as having a positive PCR test for SARS-CoV-2; and Covid-19 hospitalization (infection requiring hospital admission) based on the UKBB derived algorithm.

6.3.5. Covariates

I assessed multiple characteristics potentially associated with Covid-19 risk and/or vaccination, therefore considered as confounders. Study covariates included socio-demographics (age, sex, ethnicity), socio-economic status (index of multiple deprivations, education levels), physical measurements (body mass index), healthcare resource use (prescribed medications and the number of hospital admissions three years before the vaccination date), and comorbidities. The calendar week of receipt of the Covid-19 vaccine was also included as it affected the probability of receiving different vaccines and infection risk (through changes in community transmission level).

6.3.6. Statistical analyses

The outcome risk assessment window for the one-dose cohort went from receiving the first dose to the earliest of outcome occurrence, receiving the second dose, or 14 weeks after the vaccination. For the two-dose cohort, follow-up was from receiving the second dose to outcome occurrence or end of the study (18/10/2021).

I used the propensity score-based inverse probability of treatment weighting (IPW) to minimise confounding.[98] The specification of propensity score modelling was described in **Supplementary Methods**. I generated Kaplan-Meier plots to depict the cumulative

incidence of outcome over time in each cohort. I applied Cox proportional hazards regression with robust variance estimators to derive average hazard ratio (HR) and calculated incidence rates using weighted counts and follow-up time. I assessed the proportionality of hazards in the Cox models by visually inspecting scaled Schoenfeld residuals.

I conducted the negative control experiment to assess potential residual (unobserved) confounding. Three clinical outcomes (limb pain, fracture, and peptic ulcer events) were pre-specified and should not be associated with vaccination status (BNT162b2 vs ChAdOx1) if potential confounders have been adequately controlled for.

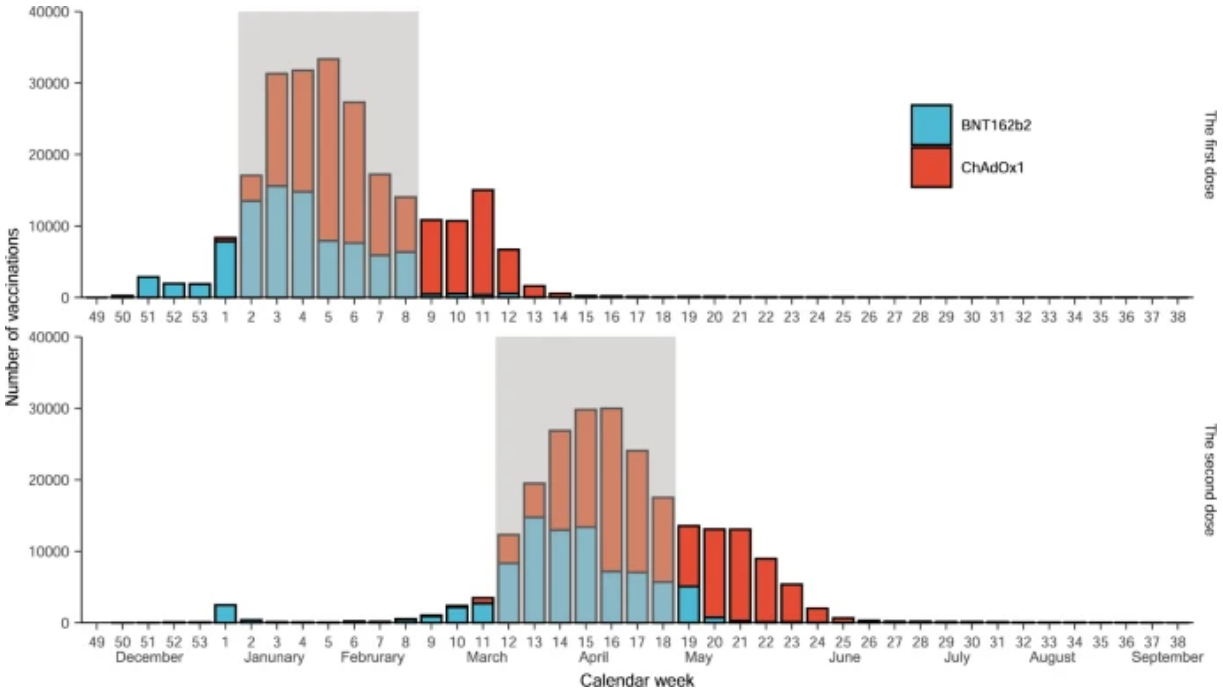
Finally, I performed a sensitivity analysis using propensity score 1:1 matching without replacement. Specifically, I set a caliper of width equal to 0.2 of the standard deviation of the logit of the propensity score.[178] I reported the standard 95% confidence intervals and used R version 4.0.4 for all analyses.

6.4.Results

6.4.1. Data linkage and study cohorts

During the one-dose enrolment period, 70,097 and 98,551 people received the first BNT162b2 and ChAdOx1 Covid-19 vaccines, respectively. These figures were 67,813 and 89,030 accordingly for people receiving the second dose during the two-dose enrolment period. Vaccine uptake over calendar time in my study population is depicted in **Figure 6 1**.

Figure 6 1 The number of people receiving either BNT162b2 or ChAdOx1 Covid-19 vaccine in the study cohort from Dec 01, 2020, to Sep 21, 2021.



6.4.2. Baseline characteristics

In the one-dose vaccine cohorts, people receiving BNT162b2 were slightly older (mean (sd) age: 71.35 (7.21) years) than those receiving ChAdOx1 (mean (sd) age: 71.06 (6.02) years). Sex (44.5% vs 44.1% male) and ethnicity (91.2% vs 92.6% White) were comparable between the two groups. Little difference was seen in the prevalence of

medicines or comorbidities. The main differences between cohorts were vaccination dates and socio-economic factors such as income. Similar patterns of baseline characteristic differences were also seen in the two-dose comparison cohorts (**Table 6 1** Incidence rate and hazard ratio of COIVD-19 infection and hospitalisation following one and two doses of vaccines.

	BNT162b2			ChAdOx1				
Covid-19 outcomes	**Total PYs at**	**Cases**	**Incidence, per**	**Total PYs at**	**Cases**	**Incidence, per**	**Unadjusted HR**	**Adjusted HR**
One dose								
Infection	14630	200	13.70	20714	261	12.60	1.08 (0.90	0.72 (0.58
Hospitalis	14654	45	3.07	20746	45	2.17	1.41 (0.95	0.87 (0.53
Two doses								
Infection	34991	1361	38.90	44084	2497	56.60	0.72 (0.57	0.70 (0.65
Hospitalis	35176	122	3.47	44421	202	4.55	0.66 (0.62	0.71 (0.55

PYs: person-years, HR: hazard ratio

Figure 6 2). All covariates were balanced after inverse probability weighting (IPW) with an absolute standardised mean difference < 0.1, including the date (calendar week) of vaccination.

6.4.3. Incidence rate and hazard ratio

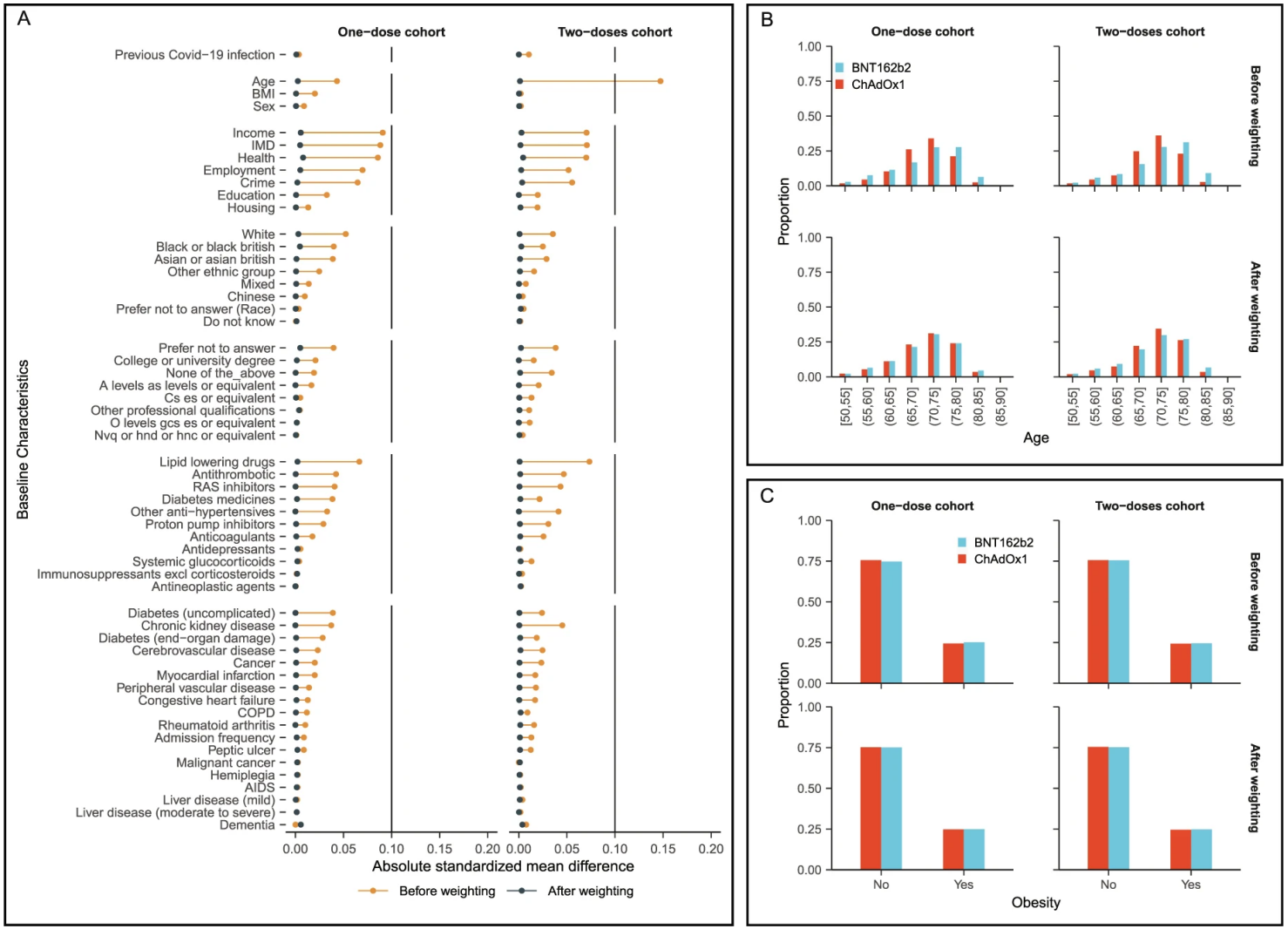
Over the 14,630 and 20,714 person-years of follow-up for the one dose BNT162b2 and ChAdOx1 recipient, 200 and 261 people tested positive for SARS-CoV-2, equivalent to incidence rates (IR) of 13.7 and 12.6/1,000 person-years, respectively, and an unadjusted hazard ratio (HR) of 1.08 (95% 0.90 - 1.30). After IPW, the HR changed to 0.72 (95% 0.58 - 0.88), favouring BNT162b2 in the overall population. In contrast, the incidences of Covid-19 hospitalisation were similar among the one dose BNT162b2 (IR: 3.07 per 1,000 person-years) and ChAdOx1 (IR: 2.17 per 1,000 person-years) cohorts, with no noticeable differences between both vaccine groups: adjusted/weighted HR 0.87 (95% 0.53 - 1.41). After the second dose, 1,361/34,991 person-years (IR: 38.9 per 1,000 person-years) and 2,497/44,084 person-years (IR: 56.6 per 1,000 person-years) were identified positive for SARS-CoV-2 among BNT162b2 and ChAdOx1 recipients respectively. Unadjusted (0.72, 95% 0.57 - 0.91) and adjusted HR (0.70, 95% 0.65 - 0.75) were almost identical, favouring BNT162b2. The rates of Covid-19 hospitalisation remained low in both cohorts, but higher amongst ChAdOx1 (IR: 4.55 per 1,000 person-years) compared to BNT162b2 recipients (IR: 3.47 per 1,000 person-years), with adjusted HR of 0.71 (95% 0.55 - 0.90) favouring BNT162b2.

Table 6 1 Incidence rate and hazard ratio of COIVD-19 infection and hospitalisation following one and two doses of vaccines.

Covid-19 outcomes	BNT162b2			ChAdOx1			Unadjusted HR	Adjusted HR
	Total PYs at risk	Cases	Incidence, per 1000	Total PYs at risk	Cases	Incidence, per 1000		
One dose								
Infection	14630	200	13.70	20714	261	12.60	1.08 (0.90 - 1.30)	0.72 (0.58 - 0.88)
Hospitalisation	14654	45	3.07	20746	45	2.17	1.41 (0.95 - 2.09)	0.87 (0.53 - 1.41)
Two doses								
Infection	34991	1361	38.90	44084	2497	56.60	0.72 (0.57 - 0.91)	0.70 (0.65 - 0.75)
Hospitalisation	35176	122	3.47	44421	202	4.55	0.66 (0.62 - 0.71)	0.71 (0.55 - 0.90)

PYs: person-years, HR: hazard ratio

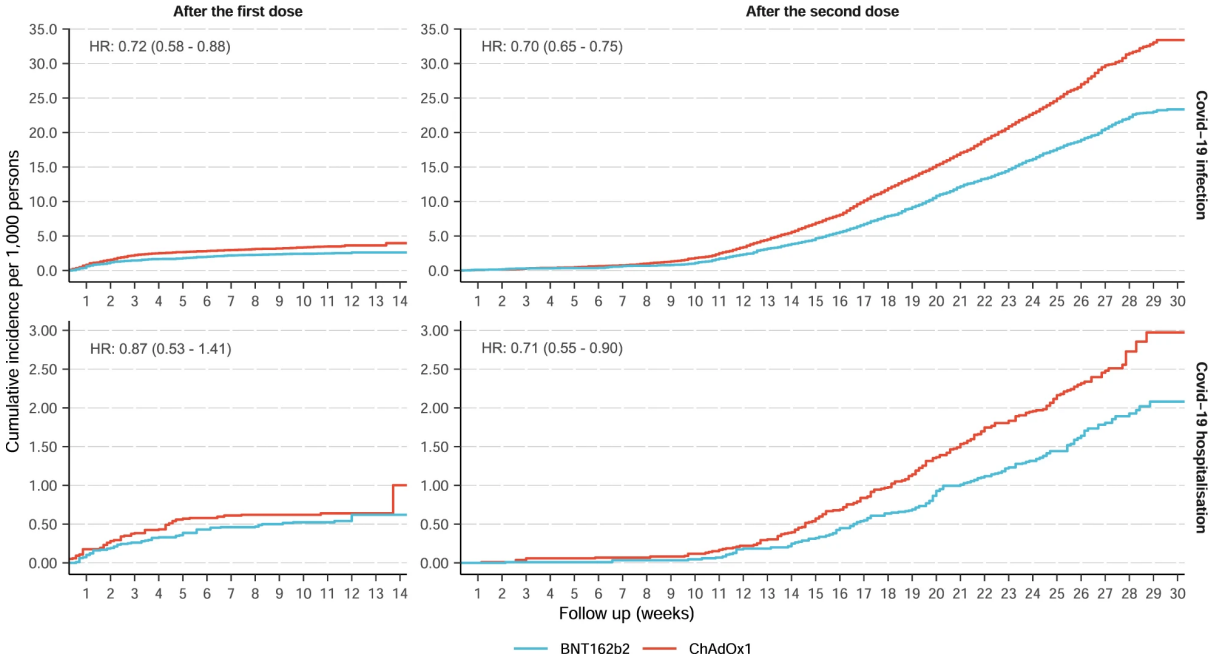
Figure 6 2 Balance of baseline covariates for the one dose and two doses cohorts, before and after weighting.



6.4.4. Kaplan–Meier curve of Covid-19 infection and hospitalisation

Kaplan–Meier curves stratified by vaccine depicted similar trends between Covid-19 infection and hospitalisation. The cumulative incidence after the first dose increased rapidly in the early follow-up but flattened later until 14 weeks after vaccination, corresponding to the calendar period from January to March 2021. Conversely, the trend of cumulative incidence was reversed for the two-dose cohorts, with a substantial increase starting 12 weeks after the second dose (**Figure 6 3**), corresponding to the calendar period from June to October 2021.

Figure 6 3 Kaplan-Meier cumulative incidence curves of Covid-19 infection and hospitalisation after one and two doses of vaccines.

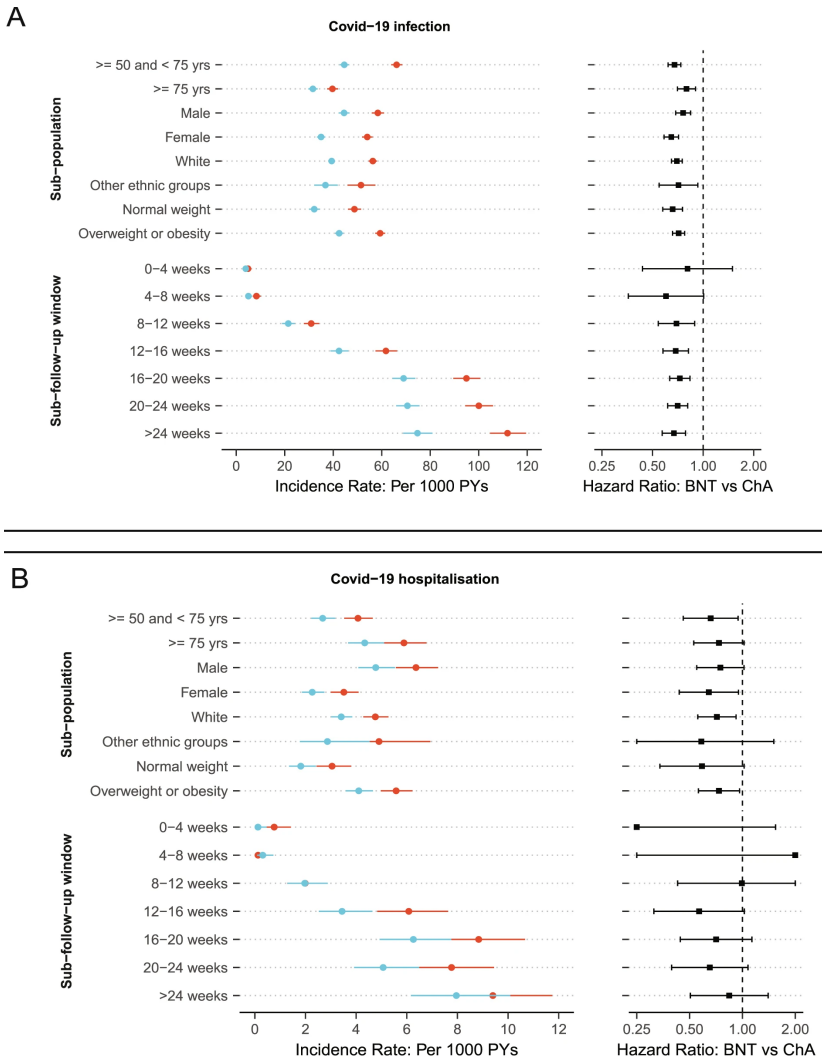


6.4.5. Comparative effectiveness across sub-populations and over-time

The risk of Covid-19 infection was consistently lower in people receiving two doses of BNT162b2 compared to those receiving two doses of ChAdOx1 across all strata. HRs ranged from 0.65 (95% 0.59 - 0.71) for females to 0.80 (95% 0.70 - 0.90) for oldest-old adults. Notably, incidence rates of Covid-19 infection increased substantially during the

study period, from 3.93 and 4.87 per 1,000 person-years at the 0–4-week window to 74.74 and 111.87 per 1,000 person-years at the >24-week window in BNT162b2 and ChAdOx1 cohorts, respectively. However, as reflected by HRs, the comparative risks were stable for at least six months (**Figure 6 4 A**). Although a similar pattern was observed for Covid-19 hospitalisation, power was limited for the time-split analysis, in particular for the 0–4-week window (HR: 0.16, 95% 0.02 - 1.54) and 4–8-week window (HR: 2.48, 95% 0.16 - 39.66), due to the extremely rare events observed in the first few weeks after the second vaccination (**Figure 6 4 B**).

Figure 6 4 Comparative effectiveness of two doses BNT162b2 vs ChAdOx1 vaccine on Covid-19 infection and hospitalisation across key subgroups and over time.



6.4.6. Negative control and sensitivity analyses

Adjusted hazard ratios for proposed negative clinical outcomes according to vaccine received among the one dose cohort were 1.01 (0.95 to 1.17) for limb pain, 0.93 (0.82 to 1.05) for fracture, and 0.88 (0.51 to 1.51) for peptic ulcer. The main results were consistent in the propensity score-matched cohorts with slightly wider confidence intervals.

6.5.Discussion

Among adults aged 50 years and older, I found that people receiving two doses of BNT162b2 vaccine had 30% lower risk of both Covid-19 diagnosis and related hospital admission, compared with those vaccinated with ChAdOx1. A similar difference was also observed among the one dose recipients, yet, only for Covid-19 diagnosis. Notably, the two-dose comparative effectiveness was comparable across several high-risk groups, such as oldest-old,[37] male,[179] ethnic minority[180] and those with overweight or obesity,[181] and persisted over time for over six months, verifying the hypothesis from a few recent preprint studies.[182,183]

To date, only two Phase 2 randomized controlled trial has directly compared the efficacy of mRNA with adenovirus-based Covid-19 vaccines by using immunogenic endpoints. Liu et al.[184] found that the BNT162B2-BNT162B2 appeared more immunogenic than the ChAdOx1-ChAdOx1 schedule, with higher levels of antibodies at 28 days after the first and second dose. A similar finding was reported in the Borobia et al. study comparing the heterologous BNT162B2-ChAdOx1 and the homogenous ChAdOx1-ChAdOx1 vaccine regimens.[185,186] In line with this evidence, my study showed a lower risk of Covid-19 outcomes in people vaccinated with BNT162b2 overall and across subgroups.

However, a recent preprint study by Hulme et al.[187] showed no difference among recipients of these two vaccines regarding the risk of SARS-CoV-2 positive test, COVID-19 related accident & emergency attendance and hospital admission. Several fundamental factors might explain the disagreement with my results. First, Hulme's study only included health and social care workers who are more likely to be exposed to SARS-CoV-2 than my cohort: a community-based "healthy volunteer" population,[188] which was corroborated by the higher infection rate in their study (58 per 1,000 person-years) compared to my study (~13 per 1,000 person-years) during the first few weeks following the first dose. Second, both my and Hulme's studies found that, on average, rollout of BNT162B2 happened earlier than ChAdOx1. Given the variation in community transmission over time in England, insufficient control for vaccination date could result in biased estimates of their comparative effect. Informed by this, I observed a substantial change of hazard ratio after the complete alignment of the first vaccination date using the weighting approach when the background transmission varied substantially (**sFigure 6 1**, Exposure anchor period). In contrast, adjusting/weighting the second vaccination date had little impact on the estimate, likely due to the relatively low and stable virus circulation at that period. Third, Hulme's risk assessment period started from the first dose (January to February 2021) and ended on 13 June 2021, reflecting a mixed effect of one and two doses of vaccines.

The leading challenges in estimating vaccine effectiveness with observational data lies in confounding by indication and potentially differential testing rates between exposed vaccinated and unvaccinated populations.[189,190] However, my study minimized the impact of such differences by comparing two vaccines and restricting the analysis to a period when both vaccines were available and had a similar national delivery. UK data

are ideal for comparative effectiveness research into Covid-19 vaccines, as both BNT162b2 and ChAdOx1 were rolled out simultaneously for the target population (adults ≥ 50 years) included in my analyses.[191] [192,193] .

However, a few limitations remain. Information on some participants' characteristics such as socio-economic status was collected ten years ago and may have changed since then. However, given that all people at the cohort recruitment were already middle-aged or older adults (40-69 years old), I expected any changes in those features are likely to be minor or unrelated to the choice of vaccine types. Admittedly, misclassification of covariates could bias the genuine association towards the null and lead to underestimating the comparative vaccine effectiveness. Also, my data are limited to participants aged 50 or above. However, this is precisely the most vulnerable population in the COVID-19 pandemic.

My study has several unique strengths. First, the granularity of UK Biobank data and comprehensive linkage to external data sources allowed us to measure and control for an extensive array of confounders, including demographics, socio-economic deprivation, comorbidity, and medication usage. My negative control outcome analyses provided reassurance that no significant residual confounding remained after adjusting the mentioned covariates using IPW methods. Secondly, the proposed study outcomes (Covid-19 and related hospitalization) were identified through linkage to official national databases of tests and hospital inpatient data, minimising the risk of misclassification. Finally, the sample size of my cohort triplicated that of the largest phase III trials, enabling us to detect differences in hospital admission rates, which seemed underpowered in randomized trials.

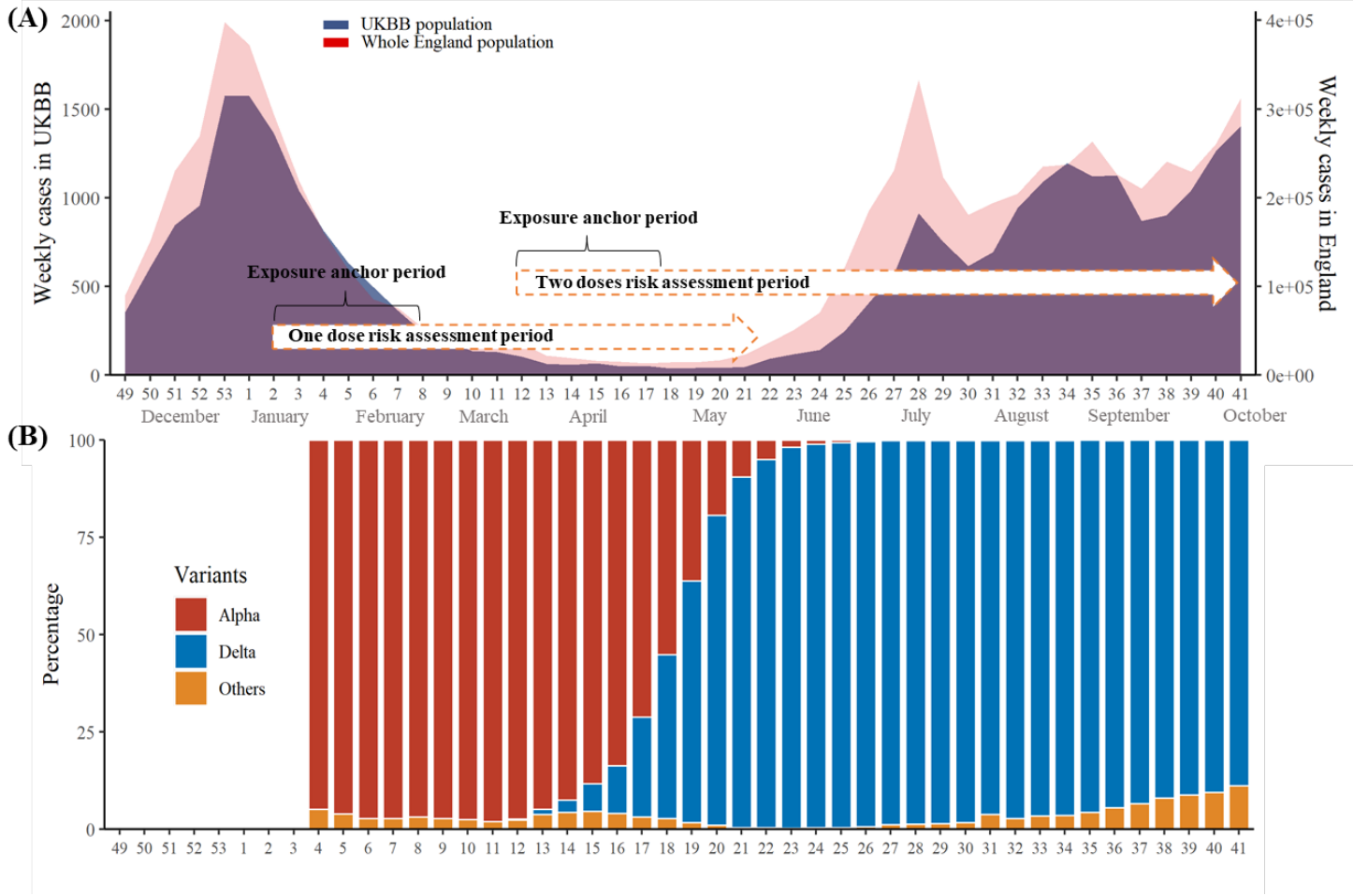
My findings support evidence from pivotal trials suggesting that BNT162b2 provides additional protection against Covid-19 and hospitalisation than ChAdOx1 vaccination. For the first time, I found that this comparative effectiveness endured over six months when the Delta variant was predominant, and community transmission kept increasing in the UK. These findings highlight the importance of continuous monitoring of the effectiveness of different vaccines against emerging SARS-CoV-2 variants to inform future booster campaigns and vaccine combinations strategies.

6.6.Supplements

sMethods: Propensity score modelling and inverse probability of treatment weighting

I calculated propensity scores for a vaccination with BNT162b2 against ChAdOx1 using logistic regression. The variables included in the model were previous Covid-19 infection status (binary), age on the vaccination date (continuous linear), sex (binary), ethnicity (categorial, collected at the UK Biobank recruitment), multiple socio-economic deprivation scores (Townsend deprivation index, income score, employment score, health score, education score, housing score, and crime score; continuous linear, collected at the UK Biobank recruitment), education levels (categorial, collected at the UK Biobank recruitment), body mass index (continuous linear, collected at the UK Biobank recruitment), a list of pre-specified medications (binary, obtained from primary care prescription records), the number of hospital admissions (continuous linear, obtained from HES), and comorbidities (binary, obtained from primary care diagnosis records). The weights for each participant were then computed based on the Rosenbaum formula: Wi= Zi/ei+ ((1-Zi))/((1-ei)).27 To reduce unstable estimate due to extreme weights in the tails of the propensity score distribution, I used asymmetric trimming to exclude people whose propensity score was below the 1th percentile of the propensity score of the BNT162b2cohort and above the 99th percentile of the propensity score of the ChAdOx1 cohort.28

sFigure 6 1 The transmission dynamics of SARS-CoV-2 (A) and its dominant variants (B) during the study period.



Notes: The data for weekly Covid-19 cases and SARS-CoV-2 variants of concern in England were from the Public Health England (https://coronavirus.data.gov.uk/).

Chapter 7

Real-world impact of HLA genes on COVID-19 vaccines response and effectiveness

7. Real-world impact of HLA genes on COVID-19 vaccines antibody responses and effectiveness

7.1.Aim

To investigate the effects of critical immunogenetics (human leukocyte antigens) on vaccine effectiveness and breakthrough COVID-19 in the vaccinated population.

7.2.Background

The COVID-19 pandemic has led to an unprecedented development, approval, and rollout of vaccines in the history of vaccinology. As of June 8, 2023, over 13 billion doses have been delivered worldwide, with over 70% of the global population receiving at least one dose.[194] The "one-size-fits-all" vaccination strategy[195,196] has shown remarkable variability in impact across different subpopulations.4–6 For COVID-19 vaccine, this heterogeneity has led to a considerable number of fully vaccinated individuals remaining susceptible to COVID-19, requiring further booster doses.

Historically, immunogenetic variations, particularly the human leukocyte antigens (HLA) genes, have been recognized as significant influencers of adaptive immune responses to various vaccines, including hepatitis B, measles, and influenza.7–9 However, the role of HLA in the context of COVID-19 vaccines, a novel exogenous antigen, remains largely elusive.10 A recent genome-wide association study (GWAS)11 focusing on post-vaccination antibodies against COVID-19 unveiled a number of genome-wide significant single nucleotide polymorphisms (SNPs) and pinpointed HLA-DQB1:06, DQB1:06:02 subtype in particular, as the potential causal alleles. Because of the complex structure in the major histocompatibility complex (MHC) region, including intense gene density, high polymorphism, and long linkage disequilibrium, the statistically fine-mapping of GWAS output proximally near this region is subject to critical methodological challenges and limitations.12,13 As a result, the predicted DQB1:06 alleles likely represent a fraction of HLA allelic variations that influence heterogenous antibody response to COVID-19 vaccines, offering a glimpse into the larger, more comprehensive picture of its genetic basis. Furthermore, in the same study, HLA-DQB1:06 alleles were found to confer over 30% lower risk of breakthrough infection among a cohort of trials participants. The

extent to which this association can be generalised to a broader general population is however unclear.14

This population-based research consisted of three interconnected analyses to fill existing knowledge gaps. Firstly, I aimed to replicate prior findings on the effect of HLA-DQB1:06 alleles on enhancing vaccine-induced antibody responses and reducing COVID-19 breakthrough infection risk. Secondly, I aimed to discover and validate novel genetic associations between HLA alleles and antibody response, and to investigate their clinical significance. Finally, I used Mendelian randomisation analyses to estimate the portion of effectiveness against SARS-CoV-2 infection and against severe COVID-19 attributable to vaccine-induced antibodies.

7.3.Methods

7.3.1. Study design and participants

The UK Biobank (UKBB) is a longitudinal population-based cohort of over 500,000 individuals aged 40 to 69 years at the time of recruitment between 2006 and 2010, recruited from England, Scotland, and Wales of the United Kingdom. Its study design and participant characteristics have been previously described in detail elsewhere.25

On enrolment, participants responded to socio-demographic, lifestyle, and health-related questionnaires, undertook physical assessments, and provided electronically signed consent. Biological samples of blood, urine, and saliva were collected and stored to facilitate the use of diverse assays, such as genotyping data, as used in this investigation. Participants granted permission to follow up their health outcomes over an extended period through linkage to electronic health records, including data related to the COVID-19 pandemic.

Nested SARS-CoV-2 coronavirus antibody seroprevalence study in UKBB

Alive UKBB participants were re-invited for a SARS-CoV-2 coronavirus antibody study from February 2021 to July 2021, when the UK's vaccination program was being rapidly implemented. At the design stage of the self-test antibody epidemiological study, potential participants of both sexes and all age groups who met the predetermined inclusion and exclusion criteria were eligible for recruitment. These individuals were invited to participate through an email containing a brief overview of the study, links to the information sheet, an instructional video, a list of frequently asked questions, and the online consent form. A concerted effort was made to ensure that as many people as possible participated in this study. For instance, interested individuals were instructed to confirm their contact information and consent to receive a lateral flow self-test kit at their residence. Non-participants were also encouraged to confirm their non-

participation via the same website. Upon consenting, participants received an acknowledgement email confirming their participation and providing details on when to expect their antibody testing kit. Participants were notified by email one to three days before kit shipment, and their addresses were securely transferred to a third-party mailing house and shipping company. A reminder email was sent to participants who had not returned a test result one week after their kit was dispatched.

Participant recruitment was done in two phases. The first phase targeted individuals who had previously attended a UK Biobank imaging assessment centre, with approximately ~34,713, ~22,390, and ~21,405 participants sequentially invited. The second phase invited the remaining ~371,985 participants who were not eligible for inclusion in phase 1.

Nested COVID-19 infection seroprevalence study in UKBB

As the lateral flow test (LFT) device used in the SARS-CoV-2 antibody seroprevalence study could not distinguish between antibodies produced in response to infection and those generated by vaccination, a follow-on COVID-19 infection seroprevalence study was conducted. Individuals who had previously participated in the self-test antibody study (phase 1 or phase 2) and had reported a "positive" antibody test result were re-invited to provide a capillary blood sample for laboratory analysis of specific antibodies (i.e., nucleocapsid) that are solely produced in response to COVID-19 infection.

Secondary data linkage

Multiple electronic health records databases have been linked at the individual level to enable following up on the health and disease status of UKBB participants. The pertinent databases used in this study included primary care records (prescriptions and diagnoses), hospital inpatient admissions (diagnoses), cause-specific death registrations, and national infectious diseases surveillance data (COVID-19 test results).26 My research

team and other investigators have comprehensively scrutinized the data linkage methodology for COVID-19 research, assuring its quality and validity.27,28

7.3.2. Analytic cohort curation

I generated a cross-sectional (CS) and prospective (PS) cohort for current analyses. The cross-sectional cohort was composed of participants from the SARS-CoV-2 antibody and infection study, which was stratified according to number of vaccine doses received at the time of testing antibody: the CS-1-dose cohort, consisting of individuals who received one dose of the COVID-19 vaccine, and the CS-2-dose cohort, consisting of those who received two doses. Participants were excluded from the CS-1-dose cohort if they received the first vaccine dose on the day of or more than 84 days (12 weeks) prior to the antibody test. Similarly, participants were excluded from the CS-2-dose cohort if they received the second vaccine dose on the day of or more than 84 days (12 weeks) before the antibody test. Individuals with evidence of prior infection, as confirmed by the presence of nucleocapsid antibody, were excluded from both cohorts. To ensure the robustness and reliability of the identified genetic associations, I partitioned participants into discovery (90%) and validation (10%) subsets in a non-random manner. The discovery subset performed genotype calling using the UK Biobank Axiom array, while the validation subset was processed using the UK BiLEVE Axiom array. This methodological strategy can maximize the independence across both subsets.

The prospective cohort (PS) included all participants who had a record of COVID-19 vaccination in their linked primary care data between December 1, 2020, and September 30, 2021. The follow-up for the PS cohort began on the date of receipt of the first vaccine dose. Participants with a previous positive PCR result for COVID-19 before vaccination were excluded from the cohort.

7.3.3. Genotyping and HLA imputation

Genotyping and initial quality control of the genetic dataset for UKBB participants have previously been documented.24 Briefly, genotyping of UK Biobank participants was undertaken in 2 phases using two custom-built genome-wide arrays that share 95% of over 820,000 SNP marker content. SNP genotyping was used to impute classical HLA types with four-digit resolution at the MHC class I and the class II regions. The HLA imputation was conducted using a modified HLA*IMP:02 model, which was designed to operate on a multi-population reference panel.29

This imputation model performed reasonably well in the entire UK Biobank sample, with a 4-digit accuracy of HLA alleles ranging from 93.9% for HLA-DRB1 gene to 99.5 for HLA-DPA1 gene among European populations with a posterior probability call threshold of 0.7 (94% of the UKBB individuals self-reported as White). The utility of the HLA imputation was also confirmed in a previous study by replicating signals of known associations between HLA alleles and 11 self-reported immune-mediated diseases.30

I grouped people with any of six specific HLA alleles (HLA-DQB1*06:01, HLA-DQB1*06:02, HLA-DQB1*06:03, HLA-DQB1*06:04, HLA-DQB1*06:05, and HLA-DQB1*06:09) as the carrier of HLA-DQB1*06 alleles subtype. Finally, I examined 203 alleles at the four-digit resolution with frequency >= 1/1000 across 11 HLA genes, including HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DRB3, HLA-DRB4, HLA-DRB5, HLA-DQA1, HLA-DQB1, HLA-DPA1, and HLA-DPB1.

7.3.4. Ascertainment of antibody positive and COVID-19 outcomes

The presence of detectable antibodies against SARS-COV-2 was defined as antibody positive. The LFT testing assays shown a good clinical performance in detecting IgG antibodies, with a sensitivity of 98.4% and 98.0% and a specificity of 99.8% and 99.5% for the Fortress Fast COVID-19 Device and AbC-19TM Rapid Test, respectively.

Breakthrough infection was defined through a combination of data sources, including a positive result on PCR testing, hospital admission with a COVID-19 related diagnosis (as indicated by the ICD-10 codes U07.1 and U07.2), or a death certificate in which COVID-19 was listed as the cause of death (using the same ICD-10 code). Severe cases were defined as those for whom breakthrough infection required hospitalization or resulted in death.

7.3.5. Statistical approach

Characterisation of vaccinated cohorts

I evaluated characteristics of HLA-DQB1*06 carriers and HLA-DQB1*06 non-carriers at baseline, which was when the antibody test was performed for the cross-sectional cohort and when prospective cohort participants received their first dose of COVID-19 vaccine. Factors that were potentially confounders for the genetic analysis were specified based on prior knowledge, including age, sex, vaccine types (ChAdOx1 and BNT162b2), and time since the latest vaccine dose by week.

Genome-wide association analysis

The GWAS of antibody response following COVID-19 vaccination was performed using REGENIE, controlling for population stratification, relatedness and case-control imbalance, and adjusted for baseline age (at the date of antibody testing), sex, genetic batch, and first ten genetic principal components (PCs). I retain only high-quality variants satisfying the following criteria (1) missing call rates ≤ 1%; (2) minor allele frequency ≥1%; (3) minor allele count ≥ 20; and (4) Hardy-Weinberg equilibrium ≥1*10-15. To further minimize the influence of population stratification, my analysis was restricted to participants to those of the Caucasian ethnic group.

HLA gene-based association analysis

I examined the proportion of antibody positivity among different HLA genotype groups, stratified by time. To quantify the association between HLA alleles and antibody response

in the CS cohort, I used a multivariable logistic regression model with each allele of interest as the independent variable (number of copy: 0, 1, 2) and the presence of antibodies (positive or negative) as the dependent variable. The model for the primary analysis was adjusted for age, sex, ethnicity, genotyping arrays, and the first ten genetic PCs derived from the entire UK Biobank population. The significance of HLA alleles in the logistic regression model was tested using the Wald test while accounting for multiple comparisons with FDR correction. Associations between HLA alleles and vaccine antibody response were estimated separately in the CS-1-dose and CS-2-dose cohorts. Only alleles with FDR-correct P-value below 0.05 in both discovery and validation dataset were considered as significant and subsequently analyzed.

To investigate the associations between HLA alleles affecting antibody response and the risk of breakthrough infection and severe COVID-19 in real-world settings, I conducted a survival analysis in the PS cohort using the Cox proportional hazard model. Potential confounders were adjusted including age, sex, ethnicity, genotyping arrays, and 10 PCs. In my primary analysis, I started follow-up from the first vaccination date until death, outcome of interest or the end of the study (30th November 2021 before the Omicron outbreak in the UK), whichever occur first.

Genetic score

I constructed genetic score to measure the combined effect of all HLA allelic variations on the antibody positivity phenotype. The GS was calculated by aggregating the product of the effect size for an allele and the quantity of allele copies across all HLA genes. Effect size estimates for each allele were coefficients (Beta) derived from the logistic regression model and subsequently modified using three approaches: (GS1) forcing the effect size estimate to zero for all alleles with an FDR-correct P-value below 0.05, (GS2) forcing the effect size estimate to zero for all alleles with an uncorrected P-value below 0.05, and

(GS3) directly applying the original estimate regardless of the statistical significance. This process of shrinking some coefficients to zero is equivalent to exclude corresponding alleles from the GS calculation.

Drug/vaccine-target Mendelian randomisation analysis

I used a Mendelian randomization analysis to study the causal effect between the exposure of antibody positivity and COVID-19 outcomes. I selected HLA alleles that were statistically significant with antibody response and used them as genetic instrumental variable. The effect size of the instrumental variables with exposures (antibody positivity following the initial vaccine dose and the subsequent dose) and with outcomes (incident breakthrough outcomes) were obtained from my analyses of CS and PS cohorts, respectively. In the primary analysis, I only used independent alleles as instrumental variables. For the sensitivity analysis, all significant alleles were used regardless of their intercorrelation. The analysis was performed using the R packages TwoSampleMR.

7.4.Results

Figure 7 1 summarizes the impact of eligibility criteria for participants of each analysis. Out of the 194,371 individuals who enrolled in the SARS-CoV-2 antibody and infection seroprevalence study, 181,812 who self-reported having received either one or two doses of COVID-19 vaccines at the time of antibody testing and had no previous COVID-19 infection as confirmed by the presence of antibodies against SARS-CoV-2 nucleocapsid antigen were included for the cross-sectional (CS) cohort. Baseline characteristics of the overall, discovery, and validation CS cohorts are detailed in **Table 7 1**. Participants were stratified according to whether they had received one dose (CS-1-dose) or two doses of COVID-19 vaccine (CS-2-dose), and further analyses were conducted accordingly. Following a median interval of 52 days (interquartile range: 36 to 64 days) after the first dose of vaccination, 28.4% of recipients tested positive for anti-spike SARS-CoV-2 antibodies. This proportion escalated to 65.5% among individuals who had the antibody test a median of 20 days (interquartile range: 8 to 33 days) after administration of the second vaccine dose.

Figure 7 1 Study flowchart

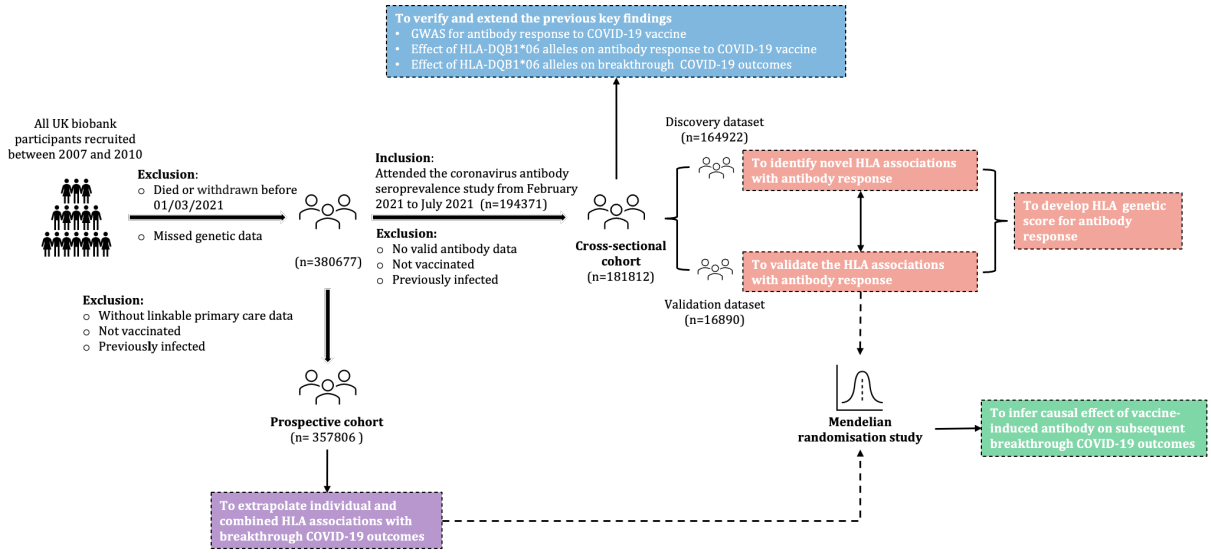


Table 7 1 Baseline characteristics of eligible participants with the antibody test in the cross-sectional cohort

	One-dose vaccine recipients			Two-dose vaccine recipients		
	All	**Discovery dataset**	**Validation dataset**	**All**	**Discovery dataset**	**Validation dataset**
Number	107175	97097	10078	67825	61013	6812
Age, mean (SD)	67.27 (7.59)	67.23 (7.60)	67.61 (7.53)	71.22 (6.56)	71.21 (6.57)	71.29 (6.50)
Age category, n (%)						
50-59 years	42872 (40.0)	38671 (39.8)	4201 (41.7)	45931 (67.7)	41302 (67.7)	4629 (68.0)
60-69 years	19970 (18.6)	18223 (18.8)	1747 (17.3)	5085 (7.5)	4582 (7.5)	503 (7.4)
>=70 years	44333 (41.4)	40203 (41.4)	4130 (41.0)	16809 (24.8)	15129 (24.8)	1680 (24.7)
Sex, n (%)						
Female	59761 (55.8)	54584 (56.2)	5177 (51.4)	39417 (58.1)	35733 (58.6)	3684 (54.1)
Male	47414 (44.2)	42513 (43.8)	4901 (48.6)	28408 (41.9)	25280 (41.4)	3128 (45.9)
Ethnicity						
White	90511 (84.5)	81825 (84.3)	8686 (86.2)	56814 (83.8)	50933 (83.5)	5881 (86.3)
Others	16664 (15.5)	15272 (15.7)	1392 (13.8)	11011 (16.2)	10080 (16.5)	931 (13.7)
Weeks since the latest vaccination, n (%)						
1 week	1677 (1.6)	1513 (1.6)	164 (1.6)	13222 (19.5)	11941 (19.6)	1281 (18.8)
2 weeks	2957 (2.8)	2683 (2.8)	274 (2.7)	12911 (19.0)	11624 (19.1)	1287 (18.9)
3 weeks	4609 (4.3)	4173 (4.3)	436 (4.3)	10715 (15.8)	9675 (15.9)	1040 (15.3)
4 weeks	7053 (6.6)	6408 (6.6)	645 (6.4)	8939 (13.2)	7963 (13.1)	976 (14.3)
5 weeks	9188 (8.6)	8361 (8.6)	827 (8.2)	7018 (10.3)	6295 (10.3)	723 (10.6)
6 weeks	10489 (9.8)	9558 (9.8)	931 (9.2)	4989 (7.4)	4483 (7.3)	506 (7.4)
7 weeks	12350 (11.5)	11171 (11.5)	1179 (11.7)	3426 (5.1)	3068 (5.0)	358 (5.3)
8 weeks	14840 (13.8)	13424 (13.8)	1416 (14.1)	2438 (3.6)	2211 (3.6)	227 (3.3)
9 weeks	15613 (14.6)	14088 (14.5)	1525 (15.1)	1718 (2.5)	1543 (2.5)	175 (2.6)
10 weeks	14655 (13.7)	13263 (13.7)	1392 (13.8)	1275 (1.9)	1153 (1.9)	122 (1.8)
11 weeks	11319 (10.6)	10272 (10.6)	1047 (10.4)	733 (1.1)	650 (1.1)	83 (1.2)

12 weeks	2425 (2.3)	2183 (2.2)	242 (2.4)	441 (0.7)	407 (0.7)	34 (0.5)
Vaccine types, n (%)[1]						
received, n (%)[1]						
ChAdOx1	39948 (37.3)	36366 (37.5)	3582 (35.5)	18578 (27.4)	16721 (27.4)	1857 (27.3)
BNT162b2	15188 (14.2)	13690 (14.1)	1498 (14.9)	17587 (25.9)	15886 (26.0)	1701 (25.0)
Unknown	52039 (48.6)	47041 (48.4)	4998 (49.6)	31660 (46.7)	28406 (46.6)	3254 (47.8)

[1] Calculated based on a subgroup of participants who had complete linkage to primary care records to obtain information on COVID-19 vaccine types

In the prospective cohort (PS) of 357,806 individuals with recorded COVID-19 vaccination in the linked primary care database, the mean age was 69.3 years (SD: 8.0) with 55.3% being female (baseline demographics are shown in **sTable 7 1**). During the one-year follow-up period post-first dose vaccination, a total of 17,068 individuals experienced a breakthrough infection as confirmed by polymerase chain reaction test, and 1,353 were hospitalized with or died from COVID-19.

7.4.1. Verification and extension of previous key findings

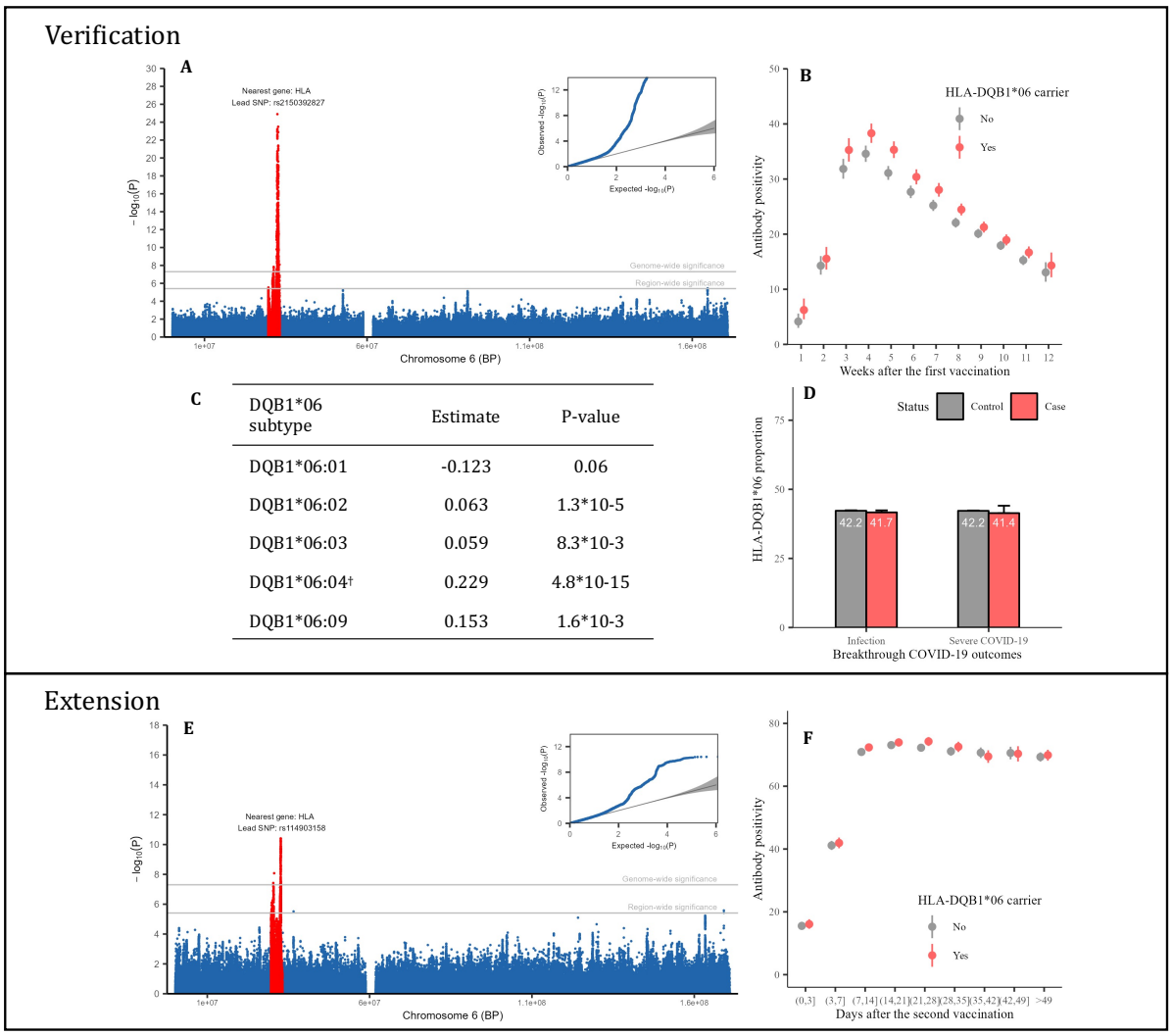
In the GWAS of the first-dose antibody positivity, a number of genome-wide significant associations were observed. The lead SNP, rs2150392827 (Position: 6:32451297;OR, 0.865; P, $1.27*10^{-25}$), was located in the chromosome 6 MHC region with HLA genes to be nearest one (Error! Reference source not found.**a**). Similar GWAS signals were found for the second-dose antibody positivity, with the lead SNP rs11490315 (Position: 6:32663047; OR, 0.863; P, $3.89*10^{-11}$), also nearest to HLA genes (**Figure 7 2 e**).

In the candidate HLA-gene based analysis, 42.1% of people in the overall CS cohort carried at least one HLA-DQB1:06 alleles, a proportion similar to the previous report of 44.1% in trial's participants. The distribution of temporal duration between the date of vaccination and the date of antibody testing was similar between carriers and non-carriers of the HLA-DQB1:06 alleles, which verified my assumption that genetic

variations were independent of this critical determinant for the antibody positivity **(sTable 7 2**). On average, the antibody response post-first dose was positive in 29.5% of HLA-DQB1:06 alleles carriers, compared to 27.4% in non-carriers. This difference of antibody positivity remained consistent over time and was observed up to eight weeks after the initial vaccination (**Figure 7 2 b**). A higher but attenuated rate of antibody positivity was observed post-second dose in people carrying the DQB1*06 alleles (66.1%) than those without carrying the DQB1*06 alleles (65.2%) (**Figure 7 2 f**).

After adjusting for potential confounding factors, a significant positive association was found between HLA-DQB1*06 alleles and the antibody response in the CS-1-dose cohort (odds ratio OR 1.13, 95% CI 1.10 to 1.17) and in the CS-2-dose cohort (OR 1.04, 95% CI 1.00 to 1.07). When considering the five specific subtypes of DQB1:06 alleles (**Figure 7 2 b**), the DQB1:06:04 subtype showed a markedly stronger association with vaccine-induced antibodies, compared to the DQB1:06:02 subtype, which was previously hypothesized to be primarily responsible for binding to the SARS-CoV-2 spike protein more effectively. Furthermore, the frequency of DQB1:06 alleles shown no association with COVID-19 outcomes after vaccination (therefore breakthrough COVID-19) (**Figure 7 2 d**), with adjusted OR corresponding to 0.98 (95% CI 0.94 to 1.02) for infection, and 1.00 (95% CI of 0.88 to 1.13) for severity, defined as hospitalisation with or death from COVID-19.

Figure 7 2 Verification and extension of previous key findings on HLA-DQB1*06 alleles



DQB1*06 subtype	Estimate	P-value
DQB1*06:01	-0.123	0.06
DQB1*06:02	0.063	1.3*10-5
DQB1*06:03	0.059	8.3*10-3
DQB1*06:04†	0.229	4.8*10-15
DQB1*06:09	0.153	1.6*10-3

The verification and extension results are presented in panels a-d and e-f, respectively. (**A**) Manhattan plot of GWAS in chromosome 6 on antibody response to first-dose COVID-19 vaccine. SNPs within the MHC region are labelled in red colour, with the nearest gene to the lead SNP presented. (**B**) The proportion of individuals with a positive antibody response stratified by their HLA-DQB1*06 status in the CS-1 cohort. Individuals with one or two DQB1*06 alleles are classified as carriers, whereas those with zero DQB1*06 alleles are as non-carriers. The maximum observation period is to eight weeks following the first vaccination. (**C**) The association of five specific HLA-DQB1*06 subtypes with the antibody response. The estimate presents the coefficient of each allele in the logistic regression model. The p-value is presented raw and does not account for multiple test corrections. † indicates the strongest association among the five DQB1*06 alleles subtype (**D**) The proportion of individuals carrying HLA-DQB1*06 alleles, comparing COVID-19 cases with controls (those not infected). Specific numerical data is labelled with white text within each bar. (**E**) Manhattan plot of GWAS in chromosome 6 on antibody response to second-dose COVID-19 vaccine. SNPs within the MHC region are labelled in red colour, with the nearest gene to the lead SNP presented. (**F**) The proportion of individuals with a positive antibody response stratified by their HLA-DQB1*06 status in the CS-2 cohort. Individuals with one or two DQB1*06 alleles are classified as carriers, whereas those with zero DQB1*06 alleles are as non-carriers. The maximum observation period is to eight weeks following the second vaccination. The x-axis is grouped slightly different from the panel B due to the different time distribution.

7.4.2. Discovery and validation of novel HLA allele associations with antibody response

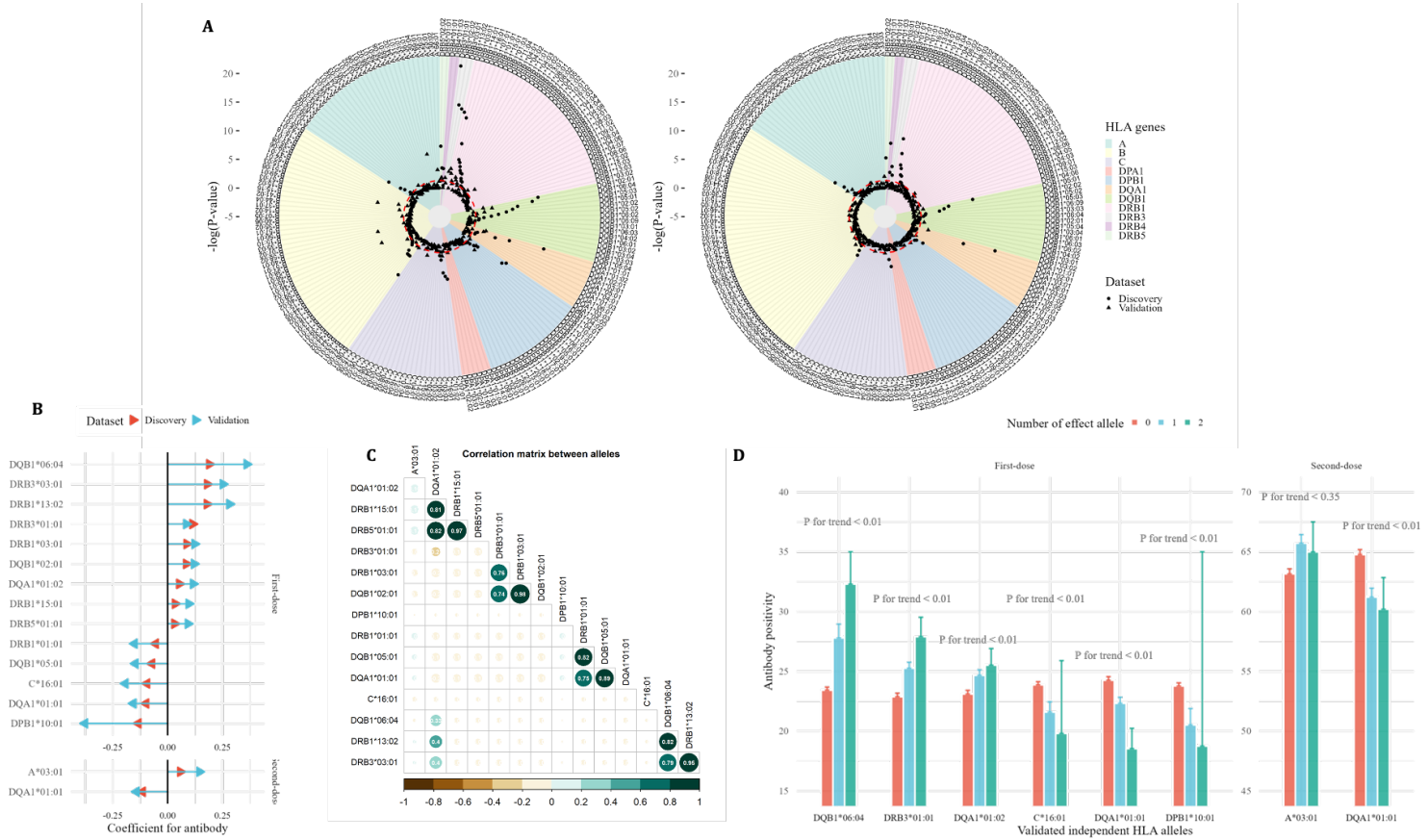
I then proceeded to identify novel HLA associations with antibody response by conducting a gene-based analysis across 213 classic alleles in 11 HLA genes. In general, the associations were more pronounced in relation to the first-dose (**Figure 7 3 a** left) than the second-dose (**Figure 7 3 a** right) antibody response.

In total, 15 alleles of 2 class I and 6 class II HLA genes (A, C, DQA1, DQB1, DRB1, DRB3, DRB5, DPB1) retained statistical significance in both the discovery and validation dataset after FDR-correction (**sTable 7 3**). Among them, 14 HLA alleles were associated with first-dose antibody response, 2 with second-dose antibody response, and 1 with both (**Figure 7 3 b)**. 9 HLA alleles were linked to a higher antibody positivity (enhancer), while 6 alleles linked to lower antibody positivity (suppressor).

The HLA-DQB1*06:04 allele was the most potent enhancer of antibody response (β = 0.213 in discovery and 0.384 in validation) following first-dose vaccination. However, no effect was detected for this allele after the second dose. In contrast, DQA1*01:01 consistently functioned as an antibody suppressor either following the first (β = -0.122 in discovery and -0.180 in validation) or second vaccination (β = -0.137 in discovery and -0.165 in validation). Several haplotypes or clusters of HLA alleles were identified among all validated alleles (**Figure 7 3 c**): Cluster 1: DQA1*01:02; DRB5*01:01; DRB1*15:01, Cluster 2: DRB3*01:01; DRB1*03:01; DQB1*02:01, Cluster 3: DRB1*01:01; DQB1*05:01; DQA1*01:01, Cluster 4: DQB1*06:04; DRB3*03:01; DRB1*13:02.

After removing highly correlated alleles from each cluster, 7 independent HLA alleles remained significantly associated with antibody response (DQB1*06:04, DQA1*01:02, DRB3*01:01, C*16:01, DPB1*10:01, A*03:01, DQA1*01:01). All alleles, except for A*03:01, appeared to have an additive effect on antibody response (**Figure 7 3 d**).

Figure 7 3 Association of 203 HLA alleles with COVID-19 vaccine antibody response



(**A**) Circular Manhattan plot of HLA-gene based genetic analysis for the first-dose antibody response (left panel) and second-dose antibody response (right panel) in the discovery dataset. The red dashed circle delineating the y-axis indicates a P-value of 0.05. (**B**) Association strength and direction of HLA alleles that significant in both the discovery and validation datasets following the False Discovery Rate correction of total number of alleles tested. The upper portion of the panel represents the results for the first-dose antibody response, while the lower portion represents the second-dose antibody response. (**C**) Pairwise correlation plot between all significant HLA alleles. (**D**) Antibody positivity rate among individuals with varying numbers of risk alleles (0, 1, 2) at each locus. The left portion of the panel represents the results for the first-dose antibody response, while the right portion for the second-dose antibody response.

I constructed genetic scores (GS) of antibody response to assess its combined impact by aggregating all HLA allelic variations with different statistical thresholding. The distribution of each GS is provided in **sFigure 7 1**. The results showed a significant association of the GS with antibody response. More specifically, for GS1, an adjusted OR of 1.14 (95% CI: 1.13 to 1.16) per SD increase was found for antibody positivity following first-dose vaccination, and an OR of 1.10 (95% CI: 1.08 to 1.11) for 2-dose antibody positivity. For GS2, the corresponding ORs were 1.16 (95% CI: 1.14 to 1.18) and 1.12 (95% CI: 1.10 to 1.14) respectively. GS3 presented the strongest association (1.17 [95% CI: 1.15 to 1.18] for the first dose, and 1.14 [95% CI: 1.12 to 1.15] for the second dose) and was selected for subsequent analyses (**sFigure 7 2**).

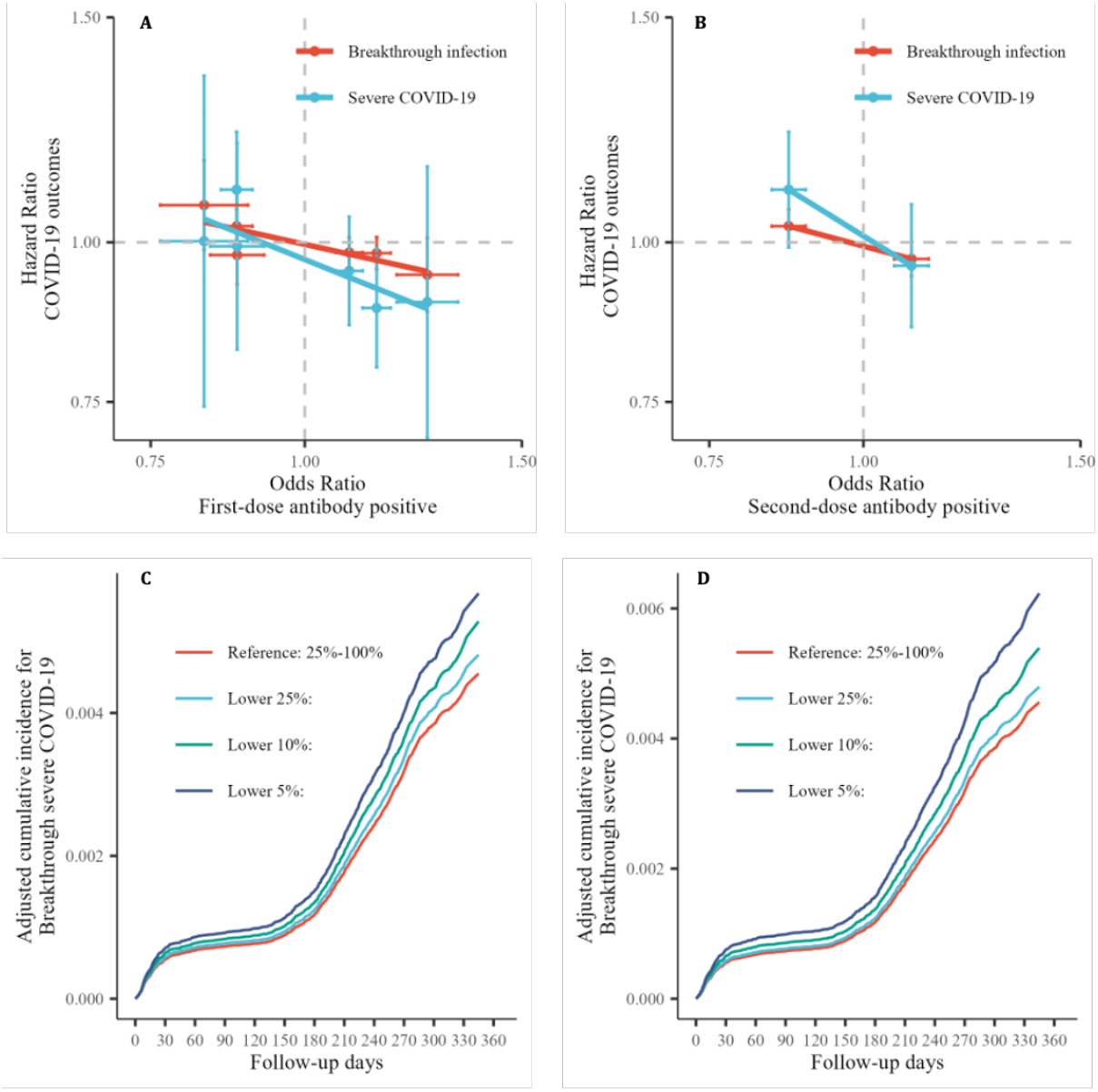
7.4.3. Extrapolation of HLA effects on COVID-19 breakthrough outcomes

Among the 7 validated HLA alleles with associations with either first-dose or second-dose antibody response, none of them was individually associated with clinical outcomes of breakthrough COVID-19 (**sTable 7 4**). However, a clear dose-dependent correlation between antibody response and COVID-19 outcome predisposed by each HLA allele was observed (**Figure 7 4**). Individuals who carried an allele that enhanced antibody response had a numerically (but not significant) lower risk of breakthrough COVID-19 outcomes, with HLA-DQB1:06:04 exhibiting most strongest effects on both breakthrough infection (Hazard ratio HR 0.94 95% 0.88 to 1.01) and severe COVID-19 (HR 0.90 95% 0.70 to 1.15). In general, the severe COVID-19 was more correlated with antibody response conferred by each HLA allele than the breakthrough infection outcome, as suggested by a steeper slope.

In comparison with the GS of first-dose antibody response, GS of second-dose antibody response showed a stronger association with breakthrough COVID-19 outcomes, including infection (HR per GS unit increase 0.94, 95% CI 0.90 to 0.98) and hospitalization

or death (HR 0.87, 0.76 to 0.99). Furthermore, I identified 5% of participants who had a 26% (HR 1.26 95% CI 1.01 to 1.58) or a 34% (HR 1.34 95% CI 1.08 to 1.67) increased risk of severe COVID-19 based on first-dose or second-dose GS respectively (**Figure 7 4**).

Figure 7 4 Extrapolation of individual and combined HLA effects



(**A**) Correlation between the effect of six independent HLA alleles on first-dose antibody response and their subsequent effects on breakthrough COVID-19 outcomes. (**B**) Correlation between the effect of two independent HLA alleles on second-dose antibody response and their subsequent effects on breakthrough COVID-19 outcomes. Age-sex adjusted cumulative incidence curve of severe COVID-19 stratified by the first-dose (**C**) and second-dose (**D**) genetic scores.

Finally, I estimated the causal effect of vaccine-induced antibody positivity on breakthrough COVID-19 outcomes. Based on the 7 validated independent alleles used as instrumental variables in Mendelian randomisation analyses, I estimated an effectiveness of antibody positivity ranging from 16.84% (95% CI 6.39% to 26.12%) to 21.68% (5.99% to 34.76%) in preventing breakthrough infection, and 46.44% (95% CI -1.49% to 71.73%) to 49.94% (95% CI 15.52% to 70.34%) in preventing severe COVID-19 (**Table 7 2**). Sensitivity analyses produced consistent results (**sTable 7 5**).

Table 7 2 Effect of vaccine-induced antibody on breakthrough COVID-19 outcomes

Exposure	**Breakthrough outcomes**	**Number of HLA alleles**	**Relative Risk**	**Effectiveness (%)**
First-dose antibody	Infection	6	0.83 (0.74 to 0.94)	16.84 (6.39 to 26.12)
	Severe COVID-19	6	0.55 (0.36 to 0.84)	49.94 (15.52 to 70.34)
Second-dose antibody	Infection	2	0.78 (0.65 to 0.94)	21.68 (5.99 to 34.76)
	severe COVID-19	2	0.54 (0.28 to 1.01)	46.44 (-1.49 to 71.73)

7.5.Discussion

The present study is the largest and most comprehensive to investigate the genetic determinants of antibody response to COVID-19 vaccines and their impact on subsequent clinical outcomes, with a particular focus on the HLA genes. Firstly, I confirmed earlier findings that the HLA-DBQ1*06 allele family enhances the antibody response to initial COVID-19 vaccination. However, I refuted the notion that it can meaningfully impact clinical outcomes of breakthrough COVID-19 in isolation. Additionally, I found that the specific HLA-DBQ1*06:04 allele is likely the driving subtype, as opposed to the previously hypothesized HLA-DBQ1*06:02.

Secondly, I identified and validated six new HLA alleles that independently influence antibody response after COVID-19 vaccines. The cumulative effect of multiple HLA variants, represented in the form of a genetic score, was associated with both the susceptibility to, and the severity of breakthrough COVID-19.

Thirdly, I estimated that vaccine-induced antibody response alone confers approximately 20% protection against SARS-CoV-2 infection, and to a much greater extent (around 50%) against severe COVID-19 outcomes.

Findings in context

Few population-based studies have been undertaken to understand the relationship between HLA alleles and antibody responses to COVID-19 vaccines. The majority of these have been constrained by limited sample size, typically including dozens of individuals recruited from convenient populations, such as healthcare workers or clinical trial participants. The first significant HLA allele was reported by a Spanish study of 87 healthcare workers,[15] showing a positive association between HLA-DRB1*07:01 and antibody levels 30 days post-receipt of the second dose of the mRNA-1273 vaccine. This finding was partially supported by my data, with an elevated antibody positivity post-

second dose vaccination amongst HLA-DRB107:01 carriers. However, this association did not meet statistical significance. Other studies involving healthcare workers from Japan (100 participants),[16] Italy (56 participants),[17] and the UK (251 participants)[18] have all failed to detect any significant associations between HLA alleles and anti-spike antibody response or seroprevalence following vaccination, except for the last UK study supporting DRB1*15:01 linked to stronger spike T-cell responses. In my study, DRB1*15:01 is one of the validated alleles associated with increased antibody rate, but it was highly correlated with DQA1*01:02 and DRB5*01:01 alleles.

In addition to vaccine antibody response, a GWAS among 17,440 vaccinated participants reported a robust association between HLA-A03:01 and vaccine-related side effects, such as chills and fever.[19] In my study, the HLA-A03:01 allele was also associated with an enhanced antibody response. This observation may reflect the epidemiological evidence of a correlation between reactogenicity and immunogenicity following COVID-19 vaccination[20] and suggests a potential genetic colocalization of stronger antibody responses and higher risk side effects via the HLA-A03:01 allele. However, further mechanistic research is warranted to confirm this hypothesis.

To date, there is a paucity of data on the impact of HLA variations on the risk of post-vaccination breakthrough COVID-19 outcomes. One previous study based on pivotal trial data found a substantial reduction (over 30%) in breakthrough infection among HLA-DQB1:06 allele carriers.[11] However, I were unable to replicate this finding in my larger and more extensive community-based population-based cohort. This divergence could be attributed to immunosenescence,[21,22] considering the relatively younger (mean age of 37 years) and healthier population in the prior study, and the design, as trial participants exclusively received the ChAdOx1 vaccine.

There is a clinical agreement that vaccination is efficacious and offers superior protection against severe COVID-19 than infection. This consensus is based on evidence gathered from initial vaccine trials as well as routinely collected health data. My research represents the first application of genetic methodologies to estimate vaccine effectiveness. My findings can further improve confidence in COVID-19 vaccines and tackle vaccine hesitancy. Moreover, my estimations provide quantitative evidence of the proportion of protection attributable to antibody-mediated immunity following vaccination, informing the additional protection conferred by cellular immunity. Notably, a recent meta-analysis of 15 studies modelled that detectable neutralizing antibodies may provide approximately 20% protection against infection and 50% protection against severe COVID-19.[23] This figure was surprisingly aligned with my estimation.

Implications

While current vaccines have shown substantial effectiveness, cases of non-responsiveness and breakthrough infections continue to persist, even following booster doses. My study has produced a comprehensive map of HLA associations with vaccine-induced antibody responses. These most significant alleles should be prioritized in mechanistic research to elucidate their functional implications, thereby informing the development of novel vaccines. Compared to previous genetic risk scores that predicted COVID-19 outcomes by aggregating numerous SNPs from COVID-19 GWAS,[24] my newly developed HLA genetic score is biologically understandable. This GS could be implemented through a tailored HLA-based genotyping array.

Limitations

Although my gene allele-based investigation was better positioned to identify functional HLA variants that have potentially been overlooked in previous SNP-based GWAS studies, experimental data are still needed to support the associations found. Antibody response

is a dynamic and intricate process. The genetic profile for binary anti-S antibody seropositivity may not fully encapsulate other immune attributes such as peak antibody titre, durability, and specificity. Also, it is imperative to explore these associations in individuals who have received booster doses in future research. I used routine linked public health data collection rather than active surveillance to capture COVID-19 events. The resulting potential outcome misclassification may lead to underestimation of genetic effects on asymptomatic breakthrough infection, but less likely for severe cases.

Using an extensive dataset incorporating host genetics, immunological biomarkers, vaccination statuses, and disease outcomes, alongside advanced methodologies and multidisciplinary expertise within my research team, I successfully identified six previously unreported HLA alleles that regulate the COVID-19 vaccine antibody response. Furthermore, I shown that variations within the HLA strongly, collectively, and causally impact critical COVID-19 outcomes within a vaccinated population. These novel can inform vaccine developments by substantially advancing understanding of genetic mechanisms of vaccine immunogenicity and clinical effectiveness, which my open new paths for personalised vaccination in the foreseeable future.

7.6.Supplements

sTable 7 1 Baseline characteristics of participants at the time of receiving the first COVID-19 vaccine dose in the prospective cohort

	All participants
Number of recipients	357,806
Age, mean (SD)	69.28 (7.99)
Sex, n (%)	
Female	197824 (55.3)
Male	159982 (44.7)
Ethnicity	
White	328557 (91.8)
Other ethnic groups	29249 (8.2)
Vaccine types, n (%)[1]	
ChAdOx1	135237 (37.8)
BNT162b2	83202 (23.3)
Unknown	139367 (39.0)

sTable 7 2 Baseline characteristics of HLA DQB1*06 carriers and non-carriers in the cross-sectional cohort

	One-dose vaccine recipients			Two-dose vaccine recipients		
	DQB1*06 carriers	**DQB1*06 non-carriers**	**SMD**	**DQB1*06 carriers**	**DQB1*06 non-carriers**	**SMD**
Number	61984	45191		39317	28508	
Age, mean (SD)	67.26 (7.59)	67.28 (7.59)	0.002	71.19 (6.56)	71.26 (6.56)	0.011
Age category, n (%)						
50-59 years	24865 (40.1)	18007 (39.8)	0.007	26592 (67.6)	19339 (67.8)	0.009
60-69 years	11567 (18.7)	8403 (18.6)		2985 (7.6)	2100 (7.4)	
>=70 years	25552 (41.2)	18781 (41.6)		9740 (24.8)	7069 (24.8)	
Sex, n (%)						
Female	34590 (55.8)	25171 (55.7)	0.002	22879 (58.2)	16538 (58.0)	0.004
Male	27394 (44.2)	20020 (44.3)		16438 (41.8)	11970 (42.0)	
Ethnicity						
White	52372 (84.5)	38139 (84.4)	0.003	33007 (84.0)	23807 (83.5)	0.012
Others	9612 (15.5)	7052 (15.6)		6310 (16.0)	4701 (16.5)	
Weeks since the latest vaccination, n (%)						
1 week	988 (1.6)	689 (1.5)	0.021	7609 (19.4)	5613 (19.7)	0.024
2 weeks	1709 (2.8)	1248 (2.8)		7448 (18.9)	5463 (19.2)	
3 weeks	2613 (4.2)	1996 (4.4)		6205 (15.8)	4510 (15.8)	
4 weeks	4051 (6.5)	3002 (6.6)		5198 (13.2)	3741 (13.1)	
5 weeks	5286 (8.5)	3902 (8.6)		4074 (10.4)	2944 (10.3)	
6 weeks	6040 (9.7)	4449 (9.8)		2899 (7.4)	2090 (7.3)	
7 weeks	7192 (11.6)	5158 (11.4)		2030 (5.2)	1396 (4.9)	
8 weeks	8611 (13.9)	6229 (13.8)		1447 (3.7)	991 (3.5)	
9 weeks	8984 (14.5)	6629 (14.7)		1006 (2.6)	712 (2.5)	
10 weeks	8552 (13.8)	6103 (13.5)		729 (1.9)	546 (1.9)	
11 weeks	6511 (10.5)	4808 (10.6)		433 (1.1)	300 (1.1)	

12 weeks	1447 (2.3)	978 (2.2)		239 (0.6)	202 (0.7)	
Vaccine types, n (%)[1]						
ChAdOx1	23039 (37.2)	16909 (37.4)	0.012	10824 (27.5)	7754 (27.2)	0.022
BNT162b2	8893 (14.3)	6295 (13.9)		10316 (26.2)	7271 (25.5)	
Unknown	30052 (48.5)	21987 (48.7)		18177 (46.2)	13483 (47.3)	

SMD, standardised mean difference

[1] Calculated based on a subgroup of participants who had complete linkage to primary care records to obtain information on COVID-19 vaccine types.

sTable 7 3 Significant HLA alleles for COVID-19 vaccine antibody response in both discovery and validation datasets

Significant alleles[1]	Discovery dataset			Validation dataset			Direction of effects
	Beta	**P-value**	**FDR-corrected P-value**	**Beta**	**P-value**	**FDR-corrected P-value**	
Antibody response after the first-dose vaccination							
DQB1*06:04	0.213	4.2*10^-12	1.1*10^-10	0.384	5.5*10^-05	2.6*10^-03	Positive
DRB1*13:02	0.203	1.9*10^-14	7.5*10^-13	0.305	2.5*10^-04	5.2*10^-03	Positive
DRB3*03:01	0.204	6.4*10^-15	4.3*10^-13	0.275	9.5*10^-04	7.4*10^-03	Positive
DRB1*03:01	0.110	1.4*10^-13	4.7*10^-12	0.146	1.6*10^-03	9.4*10^-03	Positive
DQB1*02:01	0.108	3.2*10^-13	9.1*10^-12	0.144	1.9*10^-03	9.7*10^-03	Positive
DQA1*01:02	0.077	1.3*10^-08	2.1*10^-07	0.139	7.8*10^-04	7.3*10^-03	Positive
DRB1*15:01	0.058	1.6*10^-04	1.1*10^-03	0.119	1.1*10^-02	4.6*10^-02	Positive
DRB5*01:01	0.055	3.1*10^-04	1.9*10^-03	0.116	1.4*10^-02	4.7*10^-02	Positive
DRB3*01:01	0.137	2.8*10^-22	5.6*10^-20	0.109	1.4*10^-02	4.7*10^-02	Positive
DQB1*05:01	-0.095	1.6*10^-08	2.3*10^-07	-0.170	1.4*10^-03	9.3*10^-03	Negative
DRB1*01:01	-0.078	3.8*10^-05	2.9*10^-04	-0.175	4.0*10^-03	1.9*10^-02	Negative
DQA1*01:01	-0.122	9.3*10^-15	4.7*10^-13	-0.180	3.3*10^-04	5.2*10^-03	Negative
C*16:01	-0.117	1.4*10^-05	1.1*10^-04	-0.216	1.2*10^-02	4.7*10^-02	Negative
DPB1*10:01	-0.157	3.1*10^-04	1.9*10^-03	-0.519	6.4*10^-04	7.3*10^-03	Negative
Antibody response after the second-dose vaccination							
A*03:01	0.081	3.0*10^-06	7.7*10^-05	0.168	1.2*10^-03	1.5*10^-02	Positive
DQA1*01:01	-0.137	7.6*10^-16	1.5*10^-13	-0.165	1.2*10^-03	1.5*10^-02	Negative

[1] The alleles is ordered by the magnitude of associations as measured by the beta value of the logistic regression model.

sTable 7 4 Hazard ratios for the association between individual HLA alleles, composite HLA genetic score, and breakthrough COVID-19 outcomes

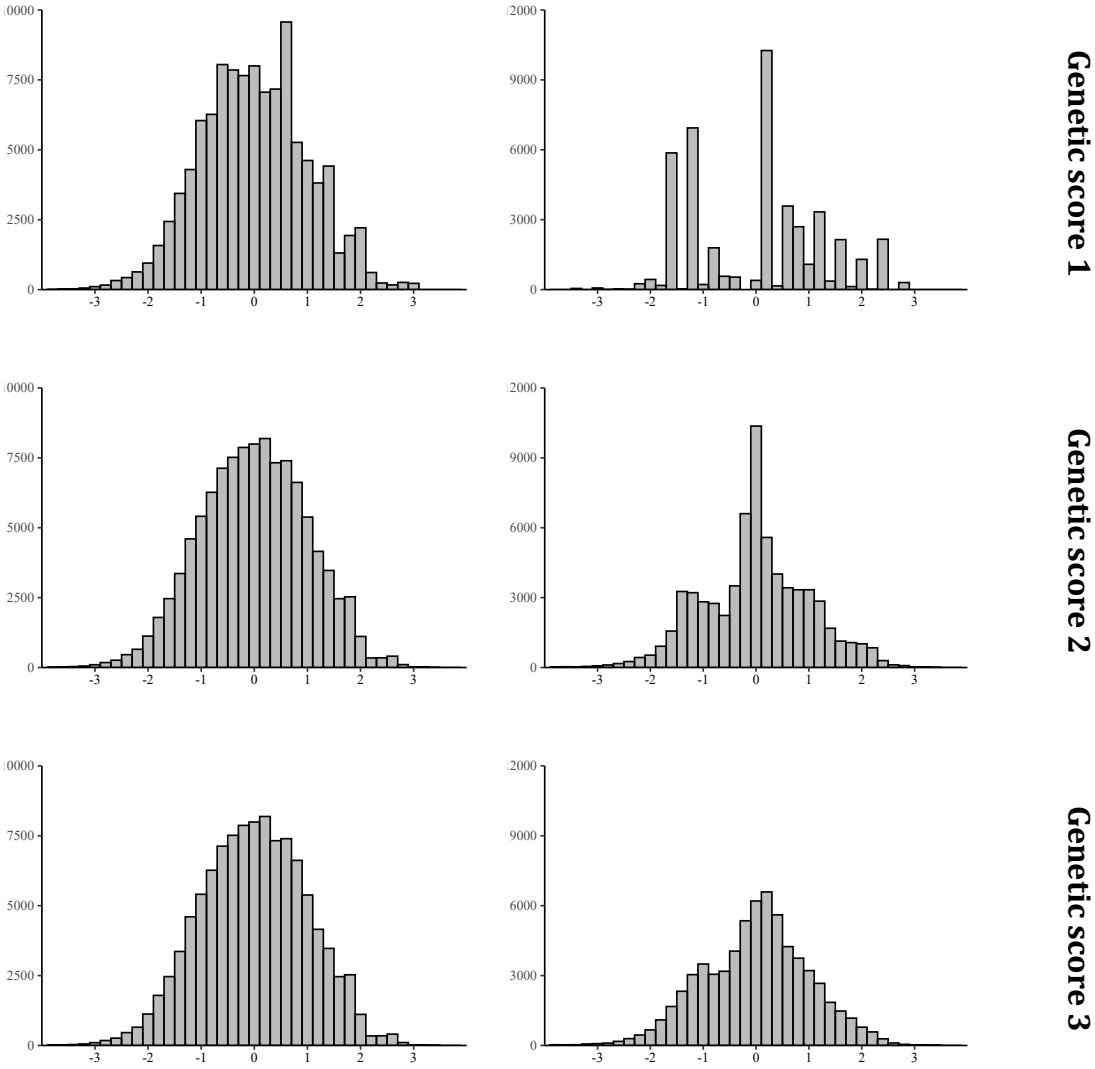
	Breakthrough infection	Breakthrough severe COVID-19
7 validated HLA alleles		
A*03:01	0.97 (0.94 to 1.00)	0.96 (0.86 to 1.07)
C*16:01	0.98 (0.93 to 1.03)	0.99 (0.82 to 1.20)
DPB1*10:01	1.07 (0.99 to 1.16)	1.00 (0.74 to 1.35)
DQA1*01:01	1.03 (1.00 to 1.06)	1.10 (0.99 to 1.22)
DQA1*01:02	0.98 (0.96 to 1.01)	0.95 (0.86 to 1.05)
DQB1*06:04	0.94 (0.88 to 1.01)	0.90 (0.70 to 1.15)
DRB3*01:01	0.98 (0.95 to 1.01)	0.91 (0.80 to 1.00)
Continuous first-dose GS	1.02 (0.99 to 1.05)	0.89 (0.82 to 0.98)*
First-dose GS subgroups		
Lower 25% vs Higher 25%-100%	NC	1.06 (0.94 to 1.20)
Lower 10% vs Higher 25%-100%	NC	1.16 (0.97 to 1.37)*
Lower 5% vs Higher 25%-100%	NC	1.26 (1.01 to 1.58) *
Continuous second-dose GS	0.94 (0.90 to 0.98)*	0.87 (0.76 to 0.99)*
Second-dose GS subgroups		
Lower 25% vs Higher 25%-100%	1.04 (1.01 to 1.08)*	1.05 (0.93 to 1.19)
Lower 10% vs Higher 25%-100%	1.05 (1.00 to 1.11)*	1.16 (0.98 to 1.38)*
Lower 5% vs Higher 25%-100%	1.05 (0.98 to 1.12)	1.34 (1.08 to 1.67)*

GS, genetic score. The asterisk denotes statistical significance at P<0.05 without correction. NC, Not calculated due to the lack of statistical significance for the continuous form of genetic score.

sTable 7 5 Sensitivity Mendelian randomisation estimation

Exposure	**Methods**	**Number of HLA alleles**	**Breakthrough infection (effectiveness)**	**Breakthrough severe COVID-19 (effectiveness)**
First-dose antibody	MR Egger	24	11.19 (-5.39 to 25.16)	52.82 (14.14 to 74.08)
	Weighted median	24	14.69 (5.95 to 22.62)	46.90 (25.50 to 62.15)
	Inverse-variance weighted	24	14.51 (8.38 to 20.23)	41.13 (24.64 to 54.00)
	Simple mode	24	17.73 (4.69 to 28.98)	46.10 (11.97 to 67.00)
	Weighted mode	24	15.20 (3.64 to 25.38)	47.39 (20.86 to 65.03)
Second-dose antibody	MR Egger	4	-2.06 (-139.77 to 56.56)	64.88 (-622.50 to 98.29)
	Weighted median	4	20.08 (5.35 to 32.52)	49.51 (10.11 to 71.64)
	Inverse-variance weighted	4	21.34 (9.29 to 31.78)	46.56 (12.09 to 67.51)
	Simple mode	4	20.46 (0.81 to 36.22)	48.91 (-12.29 to 76.75)
	Weighted mode	4	19.58 (2.14 to 33.92)	50.07 (2.28 to 74.49)

sFigure 7 1 Distribution of different HLA genetic scores



sFigure 7 2 Association of different HLA genetic scores with antibody response

Category	First-dose antibody			Second-dose antibody		
	GS 1	**GS 2**	**GS 3**	**GS 1**	**GS 2**	**GS 3**
Continuous*	1.14 (1.13 to 1.16)	1.16 (1.14 to 1.18)	1.17 (1.15 to 1.18)	1.10 (1.08 to 1.11)	1.12 (1.10 to 1.14)	1.14 (1.12 to 1.15)
Quintile 1	1 ref	1 ref	1 ref	1 ref	1 ref	1 ref
Quintile 2	1.19 (1.14 to 1.25)	1.17 (1.12 to 1.23)	1.16 (1.11 to 1.22)	1.11 (1.06 to 1.17)	1.13 (1.07 to 1.18)	1.09 (1.04 to 1.14)
Quintile 3	1.26 (1.21 to 1.32)	1.28 (1.22 to 1.34)	1.30 (1.24 to 1.36)	1.14 (1.09 to 1.20)	1.18 (1.12 to 1.24)	1.21 (1.15 to 1.27)
Quintile 4	1.37 (1.30 to 1.43)	1.37 (1.31 to 1.44)	1.38 (1.32 to 1.45)	1.29 (1.23 to 1.36)	1.26 (1.20 to 1.32)	1.26 (1.20 to 1.32)
Quintile 5	1.47 (1.41 to 1.54)	1.52 (1.45 to 1.59)	1.54 (1.47 to 1.61)	1.28 (1.22 to 1.34)	1.38 (1.32 to 1.46)	1.39 (1.32 to 1.46)

Chapter 8

COVID-19 infection and risk of venous thromboembolism

8. COVID-19 infection and risk of venous thromboembolism

8.1.Aim

To quantify the excess risk of venous thromboembolism in patients with COVID-19 compared to those who are COVID-19 naive, and to identify relevant clinical and genetic determinants.

8.2.Introduction

Numerous hospital-based studies and case series have found a high risk of venous thromboembolism (VTE) in severe patients with COVID-19. A recent meta-analysis reported a pooled VTE rate of 14.7% and 23.2% among those admitted to the hospital and intensive care units.[197] Additionally, emerging randomized controlled trials[198–200] have shown the benefit-risk of anticoagulation for patients with COVID-19 at different stages of the disease, and clinical societies have recommended initiating routine antithrombotic therapy during hospital admission.[201]

In contrast, fewer clinical interventions have been implemented to prevent VTE amongst ambulatory patients with SARS-CoV-2 infection, partially due to conflicting findings on the association between the infection and VTE occurrence. For example, previous cohort studies showing no elevated risk[202] but self-controlled case series studies consistently showing a substantial increase of acute risk.[203–205] Also, mixed evidence of benefit from oral anticoagulation complicated clinical decisions for ambulatory COVID-19.[206] Given the ongoing global rollout of vaccines, relaxation of public health restrictions, and widespread highly transmissible omicron variant, the absolute number of milder COVID-19 cases managed in ambulatory settings continues to rise worldwide.[207,208] These collectively suggest that prophylaxis, including time and dosing regimens, requires further refinement, particularly in the outpatient setting.[198–200,209,210] Moreover, a lack of insight into the impact of clinical, socioeconomic, and genetic risk factors for infection-related VTE persists.[211]

This study aimed to (1) quantify the magnitude of acute VTE risk attributable to SARS-CoV-2 infection identified in ambulatory settings; and (2) investigate the clinical and genetic determinants of VTE risk after SARS-CoV-2 infection.

8.3.Methods

I included UK Biobank (UKBB) participants from England who were alive on 01 March 2020.

8.3.1. Data sources and study cohorts

I obtained data from UK Biobank comprising multiple linked sources, including baseline surveys conducted between 2006-2010, individual genetic data, primary care electronic medical records, hospital inpatient data from Hospital Episode Statistics, diagnostic COVID-19 test data from the Public Health England's Second Generation Surveillance System (PHE-SGSS)[88] and death records from the national death registry (Office of National Statistics).

I curated an infected cohort by enrolling individuals with positive polymerase-chain-reaction SARS-CoV-2 results confirmed between 1 March 2020 and 30 September 2021. Participants who were never tested or only had negative test results were classified into the non-infected cohort. The index date was the date of the first positive specimen sample for the infected cohort. A random date that follows the same calendar period distribution of index date as the infected was assigned to the non-infected. Participants with historical VTE or who used oral anticoagulants and antiplatelet drugs one year before index dates were excluded. Additionally, I excluded those in the infected cohort who were already hospitalised at the time of testing positive for COVID-19. Any information after the index date was not used for the cohort exclusion.

8.3.2. Inherited thrombophilia

Information on genotyping and imputation procedures in UK Biobank has been detailed in previous studies.[212] Briefly, genome-wide single nucleotide polymorphisms (SNPs) were genotyped using two closely related purpose-designed arrays (the UK BiLEVE

Axiom array and UK Biobank Axiom array). I defined inherited thrombophilia carriers as having any of two risk SNP variants in Factor V Leiden (rs6025) or Prothrombin G20210A (rs1799963). I also defined a positive genetic control exposure by calculating a 297-SNPs polygenic risk score (PRS) for VTE that did not include these two variants.[213]

8.3.3. Covariates

I prespecified a list of covariates for adjustment based on clinicians' knowledge, including demographics (age, sex, self-reported race and ethnicity grouped into White or Other Racial Categories that consisted of Asian or Asian British, Black or Black British, Chinese, and unspecific/unknown ethnicity, given that approximately 90% of the UKBB participants were white), socio-economic status measured by the Index of Multiple Deprivation (a continuous summary deprivation measurement used in England containing seven aspects in crime, education, employment, health, housing, income and living environment),[214] body mass index (BMI), medications for chronic illness prescribed within one year before the index date, and trauma-related diagnosis and all comorbidities included in the Charlson Comorbidity Index.[215] The orthopaedic surge, number of hospital admissions in the past year (proxy of healthcare utilization), and vaccination status (not or partially vaccinated vs fully vaccinated) were also studied.

8.3.4. Outcomes

Incident VTE, comprising either deep vein thrombosis or pulmonary embolism, was identified using ICD-10 codes based on hospital records (**sTable 8 2**). Eligible participants were followed up for up to 30 days after the index date, given that VTE occurring after 30 days was much less likely due to a direct consequence of SARS-CoV-2 infection.

8.3.5. Statistical analyses

I used propensity score (PS) matching to minimize confounding in studying the association between SARS-CoV-2 infection and VTE. I fitted multivariable logistic regression models to estimate PS as the probability of infection based on all predefined covariates. I then matched infected with non-infected individuals with a ratio of 1:5 based on PS values with a caliper width of up to 0.2 standard deviations of the logit of the PS, with exact matching on index dates.[95,98] I assessed covariate balance between the cohorts before and after matching using absolute standardized mean differences (SMD) and specified SMD>0.1 as relevant imbalances.[133] Cause-specific Cox survival models were applied to estimate the hazard ratio (HR) for VTE according to exposure, in which death was considered a competing risk.[216] The overall HR and that in subgroup by prior vaccination status were calculated. The multiplicative interaction effect between the infection and vaccination status was tested statistically on the probability scale.

To study clinical determinants, I fitted multivariable Cox models of 30-day VTE in the ambulatory COVID-19 cohort, including age, sex, ethnicity, socio-economic status, obesity (BMI less than vs equal or more than 30 kg/m2), vaccination status, cancer, fall, fracture, and the number of comorbidities. For the analysis of inherited thrombophilia' effect on post-COVID-19 VTE, I adjusted Cox models for age, sex and genetic ancestry (quantified by the first three principal components), assuming that genetic variants were independent of all other baseline characteristics.

I performed a sensitivity analysis repeating the modelling of clinical determinants with VTE in the uninfected cohort, where no association is expected between vaccination and COVID-19-unrelated VTE. I also introduced a positive exposure control (PRS for VTE) and a negative outcome (arterial thromboembolism [ATE]) experiment to detect residual

confounding and potential unresolved bias for the exposure of inherited thrombophilia.[217]

All statistical tests were 2-sided, where a P = 0.05 or a 95% CI that did not contain unity were considered statistically significant for the primary analyses. All analyses and data visualizations were conducted using R statistical software. Genetic data management and quality controls were performed using Plink 1.9.[218]

8.4.Results

8.4.1. Baseline characteristics

Out of 407,311 UKBB participants, 26,210 were infected with SARS-CoV-2 between 1 March 2020 and 30 September 2021. After applying exclusion criteria, 21,724/ 26,210 (83.0%) infected and 317,943/ 380,398 (83.5%) non-infected were eligible for analyses (**sFigure 8 1**). For all infections, 2,877 (13.2 %) and 18,847 (86.8 %) tested in hospital and outpatient settings, respectively. Only the latter were included for subsequent analyses.

Baseline characteristics by infection status and incident VTE outcome are summarized in **Table 8 1** and **sTable 8 1**. Before matching, ambulatory COVID-19 participants were younger than the uninfected (mean age [sd] 64.32 [8.03] vs 67.96 [8.03]), more likely male (43.8% vs 41.5%), from non-White ethnicity (12.0 % vs 6.4%), and more socioeconomically deprived and obese. After 1:5 PS matching, 18,818 patients with COVID-19 were matched to 93,179 uninfected counterparts (98.6% of COVID-19 with five matches). Of note, all covariates became balanced (e.g., mean age 64.31 years and 44.0% male in COVID-19 vs mean age 64.33 years and 43.8% male in controls).

Table 8 1 Baseline characteristics of participants stratified by the SARS-CoV-2 infection status before and after matching.

	Un-matched Cohorts			Matched cohorts		
	Uninfected	**Infected**	**SMD[a]**	**Uninfected**	**Infected**	**SMD[a]**
Number	317943	18847		93179	18818	
Age, mean (sd)	67.96 (8.03)	64.32 (8.03)	0.453	64.31 (7.92)	64.33 (8.03)	0.002
Sex, no. (%)						
Male	132046 (41.5)	8247 (43.8)	0.045	41002 (44.0)	8238 (43.8)	0.005
Female	185897 (58.5)	10600 (56.2)	0.045	52177 (56.0)	10580 (56.2)	0.005
Race and ethnicity, no. (%)						
White	297595 (93.6)	16590 (88.0)	0.194	83261 (89.4)	16588 (88.1)	0.038
Multiracial/unknown	20348 (6.4)	2257 (12.0)	0.194	9918 (10.6)	2230 (11.9)	0.038
Index of multiple deprivation, mean (sd)	17.03 (13.51)	19.87 (14.68)	0.201	19.41 (14.92)	19.83 (14.65)	0.028
Body mass index, mean (sd)	27.04 (4.61)	27.65 (4.83)	0.13	27.58 (4.99)	27.64 (4.81)	0.014
Vaccination status (%)						
Not or partially vaccinated	195574 (61.5)	11156 (59.2)	0.047	55183 (59.2)	11135 (59.2)	0.001
Fully vaccinated	122369 (38.5)	7691 (40.8)	0.047	37996 (40.8)	7683 (40.8)	0.001
Recent medications (%)						
Lipid lowering drugs	87164 (27.4)	4634 (24.6)	0.064	22507 (24.2)	4627 (24.6)	0.01
RAS inhibitors	57832 (18.2)	3296 (17.5)	0.018	16035 (17.2)	3292 (17.5)	0.008
Other anti-hypertensives	25961 (8.2)	1367 (7.3)	0.034	6750 (7.2)	1363 (7.2)	0.001
Proton pump inhibitors	78080 (24.6)	5263 (27.9)	0.077	25371 (27.2)	5246 (27.9)	0.015
Diabetes medicines	16108 (5.1)	1112 (5.9)	0.037	5238 (5.6)	1106 (5.9)	0.011
Antidepressants	43310 (13.6)	3188 (16.9)	0.092	15483 (16.6)	3178 (16.9)	0.007
Systemic glucocorticoids	14237 (4.5)	972 (5.2)	0.032	4623 (5.0)	969 (5.1)	0.009
Immunosuppressants	3541 (1.1)	203 (1.1)	0.004	938 (1.0)	203 (1.1)	0.007
Antineoplastic agents	178 (0.1)	14 (0.1)	0.007	66 (0.1)	14 (0.1)	0.001
Recent orthopaedic surgery (%)	6018 (1.9)	371 (2.0)	0.006	1844 (2.0)	371 (2.0)	0.001
Recent hospital admissions, mean (sd)	0.29 (1.48)	0.31 (1.95)	0.014	0.30 (1.61)	0.31 (1.95)	0.011
Coexisting conditions (%)						
Fracture	57539 (18.1)	3415 (18.1)	0.001	16850 (18.1)	3414 (18.1)	0.002

Fall	24063 (7.6)	1381 (7.3)	0.009	6721 (7.2)	1374 (7.3)	0.003
Cancer	31271 (9.8)	1476 (7.8)	0.071	7175 (7.7)	1474 (7.8)	0.005
Malignant cancer	1119 (0.4)	63 (0.3)	0.003	297 (0.3)	63 (0.3)	0.003
Diabetes (uncomplicated)	23250 (7.3)	1496 (7.9)	0.024	7203 (7.7)	1490 (7.9)	0.007
Diabetes (end-organ damage)	6714 (2.1)	415 (2.2)	0.006	2004 (2.2)	415 (2.2)	0.004
Congestive heart failure	1666 (0.5)	89 (0.5)	0.007	445 (0.5)	89 (0.5)	0.001
Myocardial infarction	658 (0.2)	34 (0.2)	0.006	161 (0.2)	34 (0.2)	0.002
Cerebrovascular disease	2500 (0.8)	138 (0.7)	0.006	655 (0.7)	138 (0.7)	0.004
Peripheral vascular disease	1379 (0.4)	49 (0.3)	0.03	213 (0.2)	49 (0.3)	0.006
Liver disease (mild)	1506 (0.5)	81 (0.4)	0.007	401 (0.4)	80 (0.4)	0.001
Liver disease (moderate to severe)	607 (0.2)	46 (0.2)	0.011	231 (0.2)	46 (0.2)	0.001
COPD	49834 (15.7)	3309 (17.6)	0.051	16182 (17.4)	3302 (17.5)	0.005
Chronic kidney disease	12847 (4.0)	691 (3.7)	0.019	3367 (3.6)	690 (3.7)	0.003
Peptic ulcer	6256 (2.0)	419 (2.2)	0.018	2039 (2.2)	414 (2.2)	0.001
Rheumatoid arthritis	8363 (2.6)	511 (2.7)	0.005	2412 (2.6)	510 (2.7)	0.008
Dementia	1902 (0.6)	205 (1.1)	0.054	998 (1.1)	202 (1.1)	0.001
Hemiplegia	242 (0.1)	15 (0.1)	0.001	83 (0.1)	15 (0.1)	0.003
AIDS	309 (0.1)	17 (0.1)	0.002	76 (0.1)	17 (0.1)	0.003

SMD: Standardised mean difference, COPD: Chronic obstructive pulmonary disease, AIDS: Acquired immune deficiency syndrome. The look-back window of covariates for recent medications, orthopaedic surgery, and hospital admissions was past one year before the index date, and for coexisting conditions was any time before the index date. Age, index of multiple deprivation, body mass index and recent hospital admissions were included as the continuous and other covariates as binary in logistic regression model for the generation of propensity score.

[a] Value ≤0.10 is considered good balance.

Table 8 2 Associations between ambulatory COVID-19 and venous thromboembolism overall and in subgroups by vaccination status

	Uninfected			Infected				
	No. of People	**No. of Events**	**Incidence per 1000 PYs**	**No. of People**	**No. of Events**	**Incidence per 1000 PYs**	**Hazard ratio (95% CI)**	***P*-interaction**
Overall	93179	17	2.37	18818	73	50.99	21.42 (12.63 to 36.31)	
Subgroups								0.02
Not or partially vaccinated[a]	55183	12	2.67	11135	67	74.96	27.94 (15.11 to 51.65)	
Fully vaccinated	37996	5	1.87	7683	6	11.15	5.95 (1.82 to 19.51)	

[a] 92.2% of people were not vaccinated and 7.8% were partially vaccinated.

8.4.2. Association of SARS-CoV-2 infection with incident VTE

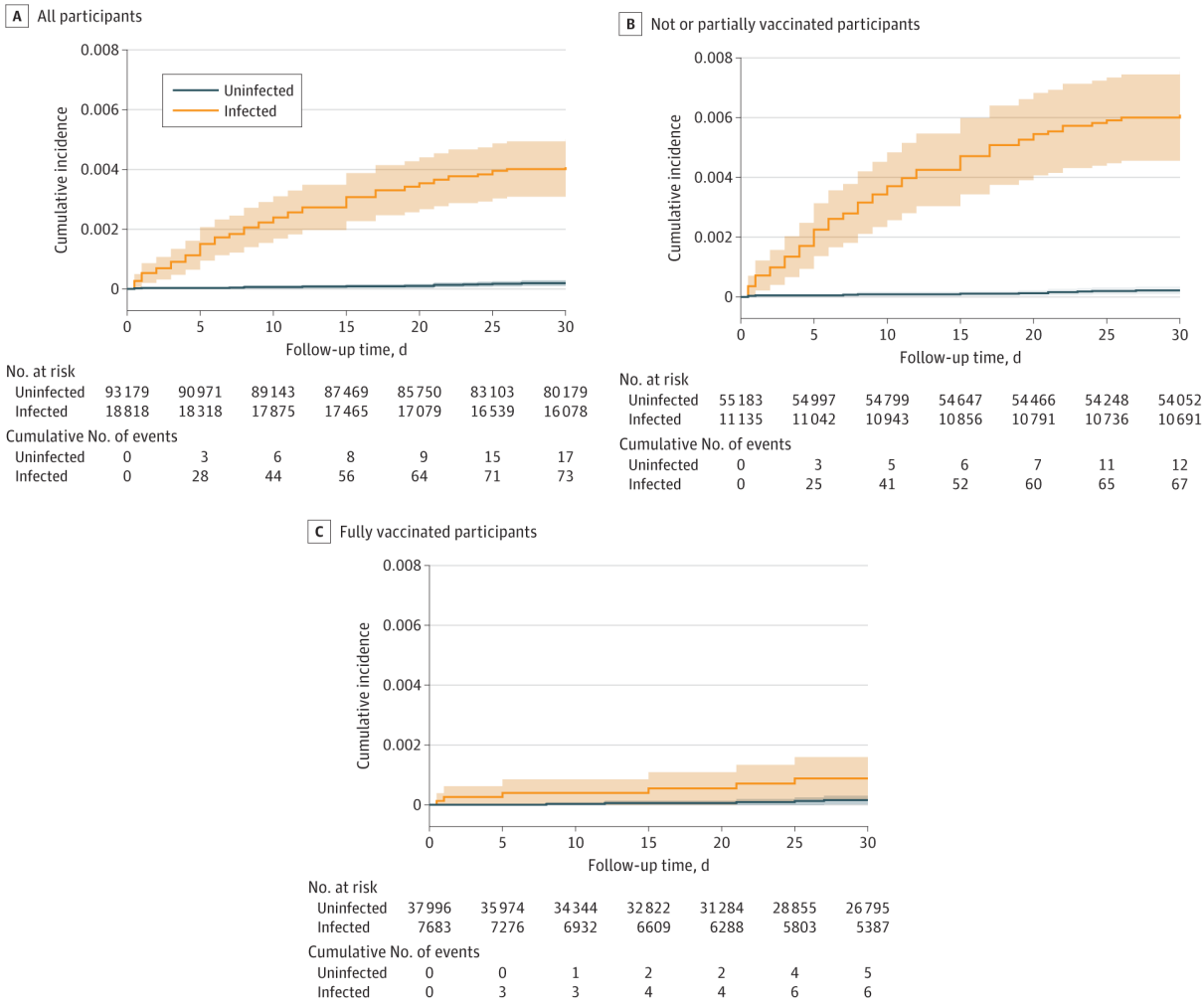
Figure 8 1 depicts the cumulative incidence of VTE according to infection status, showing an early separation of the matched cohorts, with continued divergence over time. A total of 73 and 17 VTE events were seen within 30 days amongst the ambulatory COVID-19 cases and matched uninfected, corresponding to incidence rates of 50.99 and 2.37 per 1,000 person-years, respectively. Survival analyses (**Table 8 2**) suggested that the infection was associated with a substantial increase in VTE risk: HR: 21.42, 95% CI 12.63 to 36.31. The observed risk was more pronounced in the unvaccinated patients (HR: 27.94, 95% CI 15.11 to 51.65) and significantly mitigated in the fully vaccinated (HR: 5.95, 95% CI 1.82 to 19.51, P-interaction: 0.02).

8.4.3. Clinical determinants of post-COVID-19 VTE

The associations between socio-demographic and clinical factors (including vaccination status) and the risk of post-COVID-19 VTE are shown in **Table 8 2**. Older participants had a higher risk, with an approximate doubling of risk per each 10-year increase in age (adjusted HR: 1.87, 95% CI 1.50 to 2.33). Men were at higher risk than women (adjusted HR: 1.69, 95% CI 1.30 to 2.19), and people with obesity at a higher risk than non-obese (adjusted HR: 1.83, 95% 1.28 to 2.61). These associations were similarly seen for COVID-19-unrelated VTE in both direction and magnitude.

Additionally, no or partial vaccination was associated with evident higher risk of COVID-19-related VTE (adjusted HR: 5.50, 95% 3.00 to 10.08). A sensitivity analysis supported no association between vaccination status and COVID-19-unrelated VTE amongst the uninfected participants, with HR equal to 1.07 (95% 0.80 to 1.42).

Figure 8 1 Cumulative Incidence Curves of Venous Thromboembolism Within 30 Days Overall and in Subgroups by Vaccination Status



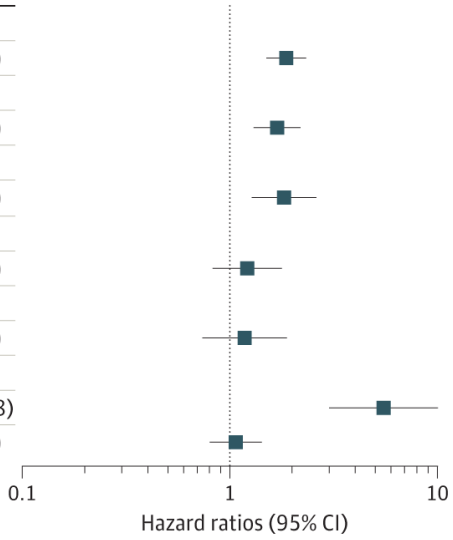
8.4.4. Inherited thrombophilia and post COVID-19 VTE

Among 21,055 infected participants with complete genetic data, 1,287 (6.11%) had inherited thrombophilia, with 909 (4.32%) and 392 (1.86%) carrying risk variant/s of Factor V Leiden and Prothrombin G20210A, respectively (**Table 8 3**). The frequency of these genetic variations in the infected cohort was like that in the overall UKBB cohort and consistent with reports from previous literature. No differences in any of the measured covariates (except for ethnicity), including socio-demographics, medications, or comorbidities, were observed when comparing those with vs without inherited thrombophilia.

Participants with inherited thrombophilia had a higher risk of VTE following SARS-CoV-2 infection than those without (adjusted HR: 2.05, 95% CI 1.15 to 3.66). For each risk variant, the adjusted HR was 2.17 (95% CI 1.13 to 4.15) for Factor V Leiden carriers and 1.52 (95% CI 0.48 to 4.79) for Prothrombin G20210A carriers. Also, individuals with higher PRS values had greater VTE risk: adjusted HR per 1-SD increase of PRS 1.33, 95% 1.11 to 1.59. As expected, no associations were observed between inherited thrombophilia and the negative control outcome ATE, with adjusted HR ranging from 0.91 (95% CI 0.29 to 2.84) to 0.99 (95% CI 0.49 to 2.01).

Figure 8 2 Hazard Ratio of Clinical Risk Factors for Venous Thromboembolism (VTE) Among Patients With COVID-19

Variable	Hazard ratios (95% CI)
Age (per 10-y increase)	
Infection-related VTE	1.87 (1.50-2.33)
Sex (male vs female)	
Infection-related VTE	1.69 (1.30-2.19)
Obesity (BMI ≥30 vs <30)	
Infection-related VTE	1.83 (1.28-2.61)
Socioeconomic status (higher 50% IMD vs lower 50%)	
Infection-related VTE	1.21 (0.83-1.78)
Ethnicity (other ethnic vs White)	
Infection-related VTE	1.18 (0.74-1.88)
Vaccination status (not or partial vs full)	
Infection-related VTE	5.50 (3.00-10.08)
Other VTE	1.07 (0.80-1.42)



Covariates included in the single multivariable Cox regression model were age, sex, race and ethnicity, socioeconomic status, obesity, vaccination status, cancer, fall, fracture, and number of comorbidities. The estimate of hazard ratios for other VTE was calculated among the uninfected group, which was predefined as a negative control outcome analysis. BMI indicates body mass index (calculated as weight in kilograms divided by height in meters squared); IMD, index of multiple deprivation.

Table 8 3 Effects of inherited thrombophilia on venous and arterial thromboembolism amongst the patients with COVID-19.

	Primary outcome			Negative outcome		
	Venous thromboembolism			Arterial thromboembolism		
Exposure	**Unadjusted HR**	**Adjusted HR**	***P* value**	**Unadjusted HR**	**Adjusted HR**	***P***
Inherited thrombophilia[a]	1.82 (1.02 to 3.23)	2.05 (1.15 to 3.66)	0.01	0.94 (0.51 to 1.73)	0.98 (0.53 to 1.80)	0.95
Factor V Leiden	1.97 (1.03 to 3.76)	2.17 (1.13 to 4.15)	0.02	0.97 (0.48 to 1.98)	0.99 (0.49 to 2.01)	0.97
Prothrombin G20210A[b]	1.31 (0.42 to 4.11)	1.52 (0.48 to 4.79)	0.45	0.84 (0.27 to 2.62)	0.91 (0.29 to 2.84)	0.86
Positive control						
Continuous PRS[c]	1.29 (1.08 to 1.54)	1.34 (1.11 to 1.60)	<0.001*	NC	NC	NC
Categorical PRS[d]	1.39 (0.73 to 2.66)	1.54 (0.80 to 2.95)	0.20	NC	NC	NC

HR: Hazard ratio, PRS: polygenic risk score, NC: Not calculated.

[a] Any risk mutations in Factor V Leiden and Prothrombin G20210A. [b] Minimum detectable incidence rate ratio associated with the exposure of prothrombin G20210A is 3.58 under the current sample size. [c] Per 1-SD increase of PRS, [d] Top 6% of PRS vs the lower.

To set an informative, positive exposure arm, I classified people into low and high polygenic risk groups according to their PRS value with a cut-off point at the 94% percentile. This threshold was chosen based on the proportion of inherited thrombophilia in the general UK Biobank population (~6%).

8.5.Discussion

In this community-based cohort of UKBB participants, including 26,210 with PCR-confirmed ambulatory COVID-19 cases and 380,398 matched contemporary non-infected controls, I found that SARS-CoV-2 infection was associated with an increased VTE risk within 30 days of a positive test (HR: 21.42). However, this risk was largely attenuated in the fully vaccinated participants who then suffered a breakthrough infection (HR: 5.95). Known clinical determinants of VTE, including older age, male sex, and obesity, applied to post-COVID-19 VTE. Also, compared to the full vaccination, none or partial vaccination was associated with an increased risk of post-COVID-19 VTE. As expected, vaccination did not impact VTE risk in the uninfected peers. Finally, Factor V Leiden thrombophilia carriers had an additional double risk of post-COVID-19 VTE compared to non-carriers, equivalent to the excess risk attributable to an increase of 10 years of age.

My finding of a substantially higher incidence of VTE in ambulatory COVID-19 disagreed with a previous meta-analysis of seven heterogeneous small COVID-19 cohorts, which had suggested that mild COVID-19 was not a risk factor for VTE, with a combined overall relative risk equal to 1.18 (95% CI 0.79 to 1.77).[202] However, my data are in much line with more extensive self-controlled case series studies[203–205] that better accounted for within-person confounding and consistently showed orders of magnitude increases in VTE risk after the infection (e.g., over 46-fold and 7-fold higher risk at 7-14 day for pulmonary embolism and deep vein thrombosis respectively)[203]. Most recently, a large network cohort study in five European countries estimated that 90-day incidence of VTE ranged from 0.2% to 0.8% among any patients with COVID-19 and up to 4.5% for those hospitalised.[219]

Public interest and concerns have been placed predominantly on vaccine-related rare thromboembolic events [220], which has led to vaccine hesitancy and restrictions on its

use.[221] My study found that the vaccination can offset SARS-CoV-2-induced VTE risk even if people get a breakthrough infection. This evident benefit should not be ignored in the ongoing global vaccination campaigns.

For the first time, I showed that inherited thrombophilia conferred a double risk of COVID-19-related VTE, echoing previous clinical findings of elevated factor V activity in patients with severe COVID-19.[222–224] More relevantly, a relatively high proportion of congenital thrombophilia was detected in a small pilot study of 87 COVID-19 cases, but the minimal sample size precluded further robust inference.[225]

I analysed rich linked data combining extensive community SARS-CoV-2 testing, well-recorded vaccination status, ambulatory and hospital-based clinical outcomes, as well as large-scale genotyping data readily available for UKBB participants. The results of this analysis have many noteworthy implications. First, VTE risk management needs re-evaluation for milder ambulatory COVID-19. With emerging evidence and guidelines focusing on VTE prophylaxis for hospitalized patients with COVID-19, further work is necessary to mitigate the risk in the community. The recent ACTIV-4B study[226] shown no benefit of use of aspirin or apixaban for VTE prevention among the younger outpatients (median age, 54 years [IQR,46-59]), largely due to a very low thrombotic event rate. My study of older participants (mean age, 64 years) might inform ongoing[227] or new trials targeting the more elderly population, particularly those with multiple risk factors identified in this study. Second, although the aetiology of post-Covid VTE is complex and multifaceted, my findings elucidated the role of Factor V and possibly Prothrombin proteins as contributing factors. Third, although genetic testing of inherited thrombophilia for VTE prevention has been previously discussed in many clinical scenarios,[228,229] this newly identified effect on COVID-19-related VTE, comparable to a 10-year ageing effect, supports the potential value of targeted genetic screening for

thrombophilia in the infected older adults. Finally, my data support the significant impact of vaccination on minimizing the risk of COVID-19 VTE.

Limitations

This study also has some limitations. Residual confounding cannot be ruled out in this observational study, although robust statistical approaches for causal inference were applied, including PS matching and knowledge-driven negative control analyses. Differential outcome ascertainment might also contribute to the elevated risk to some extent, given that SARS-CoV-2 infection since the pandemic was strongly believed to be a risk factor for VTE, clinicians were probably more intentionally looking for VTE in patients with COVID-19 than others requiring hospital care. Although participants with COVID-19 were from non-hospital settings, they were tested likely due to the presence of typical symptoms of COVID-19. The extent to which purely asymptomatic infection is associated with VTE risk warrants further investigation. Also, the VTE in my study appeared to be clinically relevant events that trigger ICD coding. However, the diagnoses themselves did not necessarily reflect VTE status and severity, e.g., asymptomatic, incidental or symptomatic, which requires bespoke screening for VTE in patients with COVID-19. My study was performed amongst the antithrombotic use naïve population, and before the monoclonal antibody infusion or antivirals were approved for use, whether these treatments can mitigate ambulatory COVID-19-related VTE risk remain unclear. Moreover, the estimates from my analyses were an average and mixed acute (30-day) effect of several SARS-CoV-2 strains from the original wild type to Delta,[230] which should be cautiously extrapolated to ongoing or post-acute periods and novel variants, such as omicron. Finally, participants recruited in UKBB were not fully representative of the general population,[188,231] and the data included in this study had very few participants

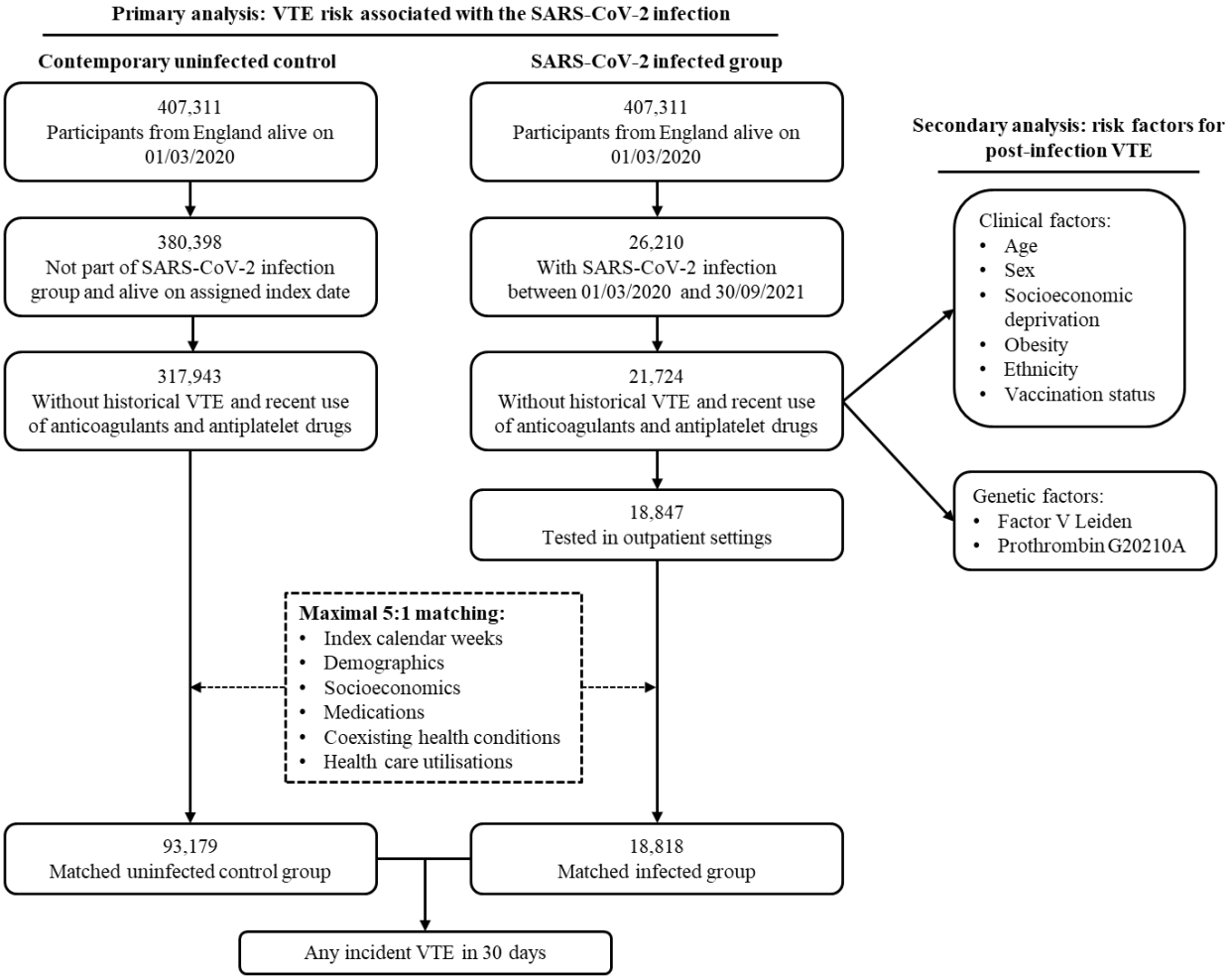
who identify as Asian or Asian British, Black or Black British, or Chinese, which may limit the generalizability of the findings.

Conclusions

In this population-based cohort study of patients with COVID-19, ambulatory COVID-19 was associated with a substantial increase in excess VTE. This risk was much higher among unvaccinated individuals and increased with older age, in men, and in patients with obesity. Factor V Leiden thrombophilia further doubled VTE risk, comparable to a 10-year ageing effect. My findings call for targeted prevention and tailored thromboprophylaxis strategies for post-COVID-19 VTE in outpatient settings and suggest an aetiological role of inherited thrombophilia.

8.6.Supplements

sFigure 8 1 Flow-chart for the study design and participants' eligibility



sTable 8 1 Baseline characteristics of participants stratified by the VTE outcome during the follow-up period.

	People without VTE outcome	People with VTE outcome	SMD
Number	336653	137	
Age, mean (sd)	67.75 (8.08)	69.61 (7.87)	0.233
Sex, male (%)	140218 (41.7)	75 (54.7)	0.264
Ethnicity, White (%)	314064 (93.3)	121 (88.3)	0.173
Index of multiple deprivation, mean (sd)	17.19 (13.59)	21.76 (15.70)	0.311
Body mass index, mean (sd)	27.08 (4.63)	29.19 (5.36)	0.423
Vaccination status (%)			
Not or partially vaccinated	206624 (61.4)	106 (77.4)	0.352
Recent medications (%)			
Lipid lowering drugs	91756 (27.3)	42 (30.7)	0.075
RAS inhibitors	61092 (18.1)	36 (26.3)	0.197
Other anti-hypertensives	27305 (8.1)	23 (16.8)	0.265
Proton pump inhibitors	83280 (24.7)	63 (46.0)	0.456
Diabetes medicines	17208 (5.1)	12 (8.8)	0.144
Antidepressants	46470 (13.8)	28 (20.4)	0.177
Systemic glucocorticoids	15184 (4.5)	25 (18.2)	0.443
Immunosuppressants	3739 (1.1)	5 (3.6)	0.167
Antineoplastic agents	192 (0.1)	0 (0.0)	0.034
Recent orthopaedic surgery (%)	6382 (1.9)	7 (5.1)	0.175
Recent hospital admissions, mean (sd)	0.29 (1.51)	1.56 (3.68)	0.452
Coexisting conditions (%)			
Fracture	60922 (18.1)	32 (23.4)	0.13
Fall	25425 (7.6)	19 (13.9)	0.205
Cancer	32719 (9.7)	28 (20.4)	0.303
Malignant cancer	1179 (0.4)	3 (2.2)	0.165
Diabetes (uncomplicated)	24731 (7.3)	15 (10.9)	0.125
Diabetes (end-organ damage)	7122 (2.1)	7 (5.1)	0.161
Congestive heart failure	1753 (0.5)	2 (1.5)	0.095
Myocardial infarction	691 (0.2)	1 (0.7)	0.077
Cerebrovascular disease	2637 (0.8)	1 (0.7)	0.006
Peripheral vascular disease	1426 (0.4)	2 (1.5)	0.107
Liver disease (mild)	1586 (0.5)	1 (0.7)	0.034
Liver disease (moderate to severe)	653 (0.2)	0 (0.0)	0.062
COPD	53121 (15.8)	22 (16.1)	0.008
Chronic kidney disease	13529 (4.0)	9 (6.6)	0.114
Peptic ulcer	6667 (2.0)	8 (5.8)	0.2
Rheumatoid arthritis	8864 (2.6)	10 (7.3)	0.216
Dementia	2105 (0.6)	2 (1.5)	0.082
Hemiplegia	257 (0.1)	0 (0.0)	0.039
AIDS	326 (0.1)	0 (0.0)	0.044

SMD: Standardised mean difference, COPD: Chronic obstructive pulmonary disease, AIDS: Acquired immune deficiency syndrome

sTable 8 2 ICD-10 codes for the identification of venous thromboembolism

"I26"	Pulmonary embolism
"I260"	Pulmonary embolism with mention of acute cor pulmonale
"I269"	Pulmonary embolism without mention of acute cor pulmonale
"I801"	Phlebitis and thrombophlebitis of femoral vein
"I802"	Phlebitis and thrombophlebitis of other deep vessels of lower extremities
"I803"	Phlebitis and thrombophlebitis of lower extremities, unspecified
"I81"	Portal vein thrombosis
"I82"	Other venous embolism and thrombosis
"I820"	Budd-Chiari syndrome
"I822"	Embolism and thrombosis of vena cava
"I823"	Embolism and thrombosis of renal vein
"I828"	Embolism and thrombosis of other specified veins
"I829"	Embolism and thrombosis of unspecified vein

Chapter 9

Discussion

9. Discussion

In this concluding chapter, I initially present a systematic synthesis of the principal findings. Subsequently, I elaborate on the methodological rigor applied across the studies to ensure the accuracy, reliability, and trustworthiness of these research outcomes. Thirdly, I reflect the overarching limitations and strengths of this thesis. Lastly, I discuss the implications of my findings for evidence-based clinical and regulatory decision-making, and paint avenues for future real world data based research.

9.1.Main findings

In this thesis, I studied opioid utilisation in Catalonia. According to the study in Chapter 3, the use of opioids remained steady between 2007 and 2013 but saw a significant increase after that until 2019. The increase was most prominent in older adults and strongly correlated with the strength of the opioids used. Additionally, I found a rising trend in the use of strong opioids to treat non-cancer-related painful conditions. The association between incident use of Tramadol vs Codeine and clinical adverse events was examined in Chapter 4. In this retrospective, the dispensation of tramadol prescriptions was significantly associated with increased risks of mortality, cardiovascular events, and fractures compared to codeine prescription dispensation. No significant differences were observed in the risks of constipation, delirium, falls, opioid abuse/dependence, or sleep disorders within one year after treatment initiation. In Chapter 5, I assessed COVID-19 risk in relation to use of Ibuprofen and observed no differential risks of incident COVID-19 diagnosis or hospitalisation between ibuprofen users and users of other ns-NSAIDs, COX-2i, or paracetamol. The effectiveness of the SARS-COV-2 BNT162b2 vaccine in comparison with ChAdOx1 vaccines in real-world setting was investigated in Chapter 6. I found that, among adults aged 50 years and older, individuals who received two doses of

the BNT162b2 vaccine had a 30% lower risk of both COVID-19 diagnosis and related hospital admission compared to those who received the ChAdOx1 vaccine. I, in Chapter 7, replicated earlier findings that HLA-DBQ1*06 alleles positively correlate with antibody positivity to COVID-19 vaccines[232] and discovered unreported HLA allelic variant associations with antibody seropositivity. Utilising Mendelian randomisation techniques, I estimated the effectiveness of vaccine-induced antibody response in reducing breakthrough infection, hospitalisation, and mortality, providing first genetic evidence of the protective effects of vaccination against COVID-19 outcomes. The association between COVID-19 infection and acute risk of VTE was investigated in Chapter 8, the study shown that SARS-CoV-2 infection was associated with a more than 20-fold increased VTE risk within 30 days of a positive test. However, this risk was substantially reduced in fully vaccinated individuals experiencing breakthrough infections. Known VTE determinants, such as older age, male sex, and obesity, were also applicable to post-COVID-19 VTE. Factor V Leiden thrombophilia carriers exhibited had the post-COVID-19 VTE risk compared to non-carriers.

9.2.Methodological considerations

9.2.1. Sample size

It is recognized that weak exposures prevalent in the community can potentially lead to more relevant public health implications compared to strong associations with infrequently occurring agents.[233,234] Traditionally, establishing such associations required a meta-analysis of numerous small studies or, preferably, a collaborative reanalysis of a multitude of studies in a unified basis.[235] In this thesis, the SIDIAP and UK Biobank, along with two large US health claims databases, were leveraged to enable the identification of weak associations between the exposure/s and outcome/s of interest.

For example, the substantial size of US claims data not only allowed a comprehensive examination of the correlation between prevalent ibuprofen use and severe outcomes of COVID-19, such as hospitalization —a subject possibly underpowered in previous studies[148,155,168–170,236]— but also the analyses of comparisons with multiple other analgesic drugs, a matter of greater clinical relevance (Chapter 5). Nonetheless, it is still expected that for certain outcomes, such as rare adverse events of delirium or opioid abuse/dependence in relation to tramadol use, the available data for my research was insufficient to yield precise estimates, a limitation reflected by the notably broad confidence intervals (Chapter 4). This limitation was particularly evident in the analysis of vaccine comparative effectiveness, where the most paramount clinical outcome, COVID-19-related mortality, could not be thoroughly investigated in this thesis due to the low incidence of deaths post-vaccination.

9.2.2. Selection bias

Selection bias arises when the individuals under study do not adequately represent the target population, thereby potentially distorting the conclusions drawn. This is a common issue in epidemiological research, given that it is practically inevitable to study a sample rather than the entire population.[237–239] In general, improper selection procedures might introduce bias more relevant in descriptive studies, such as drug utilization patterns, compared to analytic studies focused on evaluating adverse drug effects. In my research, I estimated the annual incidence of opioid use over several years based on data from the SIDIAP database (Chapter 3). The results could ensure high internal validity due to the substantial number of participants and the representativeness of SIDIAP participants compared to the broader population, as corroborated by previous studies.[71]

Empirically, case-control studies are more prone to selection bias compared to cohort studies.[240] One primary reason is the retrospective nature, which often rely on

participants' recall of past exposures. This can lead to imprecise measurements and skewed distribution of exposure variables, thereby compromising the study's validity.[241,242] However, in Chapter 7 of this thesis, which used a case-control design, the exposure variable—specifically, a genetic variant—is not subjectively collected but quantitatively measured. This minimizes the risk of selection bias associated with inaccurate measurements. Another common source of selection bias in the literature arises from the use of control groups that are not representative of the population from which the cases originated, such as studies based on only hospitalized patients.[243] In contrast, the present study utilizes a nested Case-Control design, where both cases and controls are drawn from the same large, community-based cohort. This approach could effectively mitigate the issue of an unrepresentative control group.[244] Nevertheless, it is important to note that the entire UKBB cohort has been previously reported to be susceptible to healthy volunteer bias, another form of selection bias. Therefore, while the study design minimizes certain biases, it is not entirely free from the influence of selection bias.

Prevalent user bias

In analysing the causal effect of a medicine or vaccine using a prevalent cohort design (Chapter 5), a common form of selection bias is "prevalence bias".[245,246] This occurs when the analysis includes individuals who have been using the drug for an extended period before the follow-up started, potentially leading to a "survivor" effect or "depletion of susceptibles".[247] Additionally, this situation can create a complex confounding structure where the covariates are affected by prior drug use.[248] Despite recognizing this potential pitfall, I applied this methodology to investigate the effects of ibuprofen on COVID-19 outcomes for two primary reasons. Firstly, ibuprofen has a long history of widespread use, and its safety profile is well-established, making the occurrence of "survivor" effects

in the early stages of its pharmacotherapy possibly unlikely. Secondly, this design directly aligned with the central research question: assessing whether chronic (prevalent) ibuprofen users faced an increased risk of adverse COVID-19 outcomes.

Collider bias

Collider bias, also historically known as Berkson's bias[249] or Simpson's paradox bias[250] or M-bias,[251] represents a specialized form of selection bias that is highly relevant to recent studies aimed at identifying risk factors for COVID-19 using observational data.[252] This bias occurs when a variable, known as a collider, is influenced by at least two other variables.[253] For instance, being a healthcare worker (exposure) and the severity of a COVID-19 infection (outcome) may both influence the likelihood of an individual being sampled, included or studied in various COVID-19 studies. Bias therefore could be introduced when researchers restricted analysis to individuals who have either been hospitalized due to COVID-19, undergone testing for active infection, or voluntarily participated. In Chapter 8 of this thesis, I investigated potential risk factors for COVID-19 related venous thromboembolism only in those who tested PCR positive for COVID-19. However, because all events of venous thromboembolism in my study were ascertained days after infection, the notion that the outcome (post-COVID-19 venous thromboembolism) could influence the likelihood of being test positive is untenable due to this well-established temporal sequence, precluding the concern of collider bias. In summary, collider bias is a particular issue that should be carefully considered in observational research methodologies like cross-sectional surveys or case-control studies, where the temporal relationship between exposure and outcome is less clearly specified.

9.2.3. Confounding bias

Indication bias

Confounding bias comes when there is a variable correlated with both the exposure and the outcome, while not being a part of the causal pathway in the estimation of an association.[254] 'Confounding by indication', sometimes known as ''confounding by severity", is a specific subtype[255] where the determinant of the outcome is more prevalent in individuals perceived to be at high risk or with poor prognosis, thereby being treated as an indication for intervention, such as drugs.[256] To mitigate such bias, existing methods have been systematically reviewed[257–259] and can be broadly categorized into two primary approaches: selecting an appropriate study design and employing statistical-analytical techniques.

In this thesis, I have utilized both strategies synergistically to create more balanced comparison groups even in the context that exposures in observational data are inherently non-randomized. For example, in Chapter 4, where I investigated potential adverse events linked to tramadol use, I included codeine as an active comparator.[260] Both opioids are recommended by the WHO for second-step chronic pain management, thereby ensuring some level of prognostic similarity between treatment groups. This methodology is also adopted in Chapters 5 and 6. However, it is crucial to understand that this approach unavoidably shifts the research focus from quantifying the effect of a single drug to its comparative effect.[261] If the comparator drug is not naïve to the outcome of interest, the estimates could be distorted and should be interpreted accordingly. Ultimately, the extent to which this methodological modification may compromise the core question of interest highly depends on target drugs and choices of alternatives prescribed for the same medical indication in various clinical practices.[262] Moreover, the validity of the comparison can be further improved by limiting individuals to those who meet the certain criteria for treatment as outlined in clinical guidelines.[263] A prime example is presented in Chapter 5 of this thesis, where the study population is restricted

people with a medical history of either osteoarthritis or back pain, conditions for which ibuprofen is commonly prescribed.

In my thesis, I primarily used matching and stratification as bias-reducing data-analytical techniques,[263] such as in Chapters 4, 5, and 8. These methods are favoured over traditional model-based approaches such as regression for several reasons: they are straightforward for interpretation and communication of results to a non-specialist audience, they do not require assumptions about the relationships, such as linearity and independence, and they are robust to the inclusion of a large number of covariates.

Residual confounding

In addition to the confounding by indication, residual confounding represents another source of bias due to either unadjusted or unmeasured confounders, which is difficult to eliminate entirely in observational studies.[264] In Chapter 5, I applied a large-scale propensity score method to balance thousands of characteristics across comparison groups. This data-driven approach does not require all confounders to be pre-specified based on directed acyclic graph (DAG); it assumes that unmeasured confounders will be to some extent corrected with those adjusted covariates. Several simulation and empirically studies[161,265] have supported the potential for mitigating residual confounding through this statistical method. However, the methodological challenges remain before this approach gains more widespread acceptance in medical research.[266]

Instrumental variable-based methods offer another avenue for addressing confounding.[267,268] As shown in Chapter 7. I utilized genetic variants as instruments for antibody positivity (exposure). This method can theoretically mimic randomisation process based on the knowledge that the allocation of genetic variants from parents is largely independent of post-birth factors, thereby nullifying confounders as well. Nevertheless, the primary limitation persists in the 'exclusion restriction' assumption,

which posits that the instrumental variable influences the outcome solely through its role in treatment assignment. This assumption is not only difficult to satisfy but also practically unverifiable. Moreover, the treatment effect derived from this method may not be directly applicable a broader population, an issue also seen seen in RCTs.[269,270]

9.2.4. Information bias

Information bias arises when incorrect information about either exposure or outcome or any covariates is incorrectly categorized or defined.[271] The impact of information bias is particularly relevant in pharmacoepidemiological studies in my thesis based on routinely collected data, such as electronic health records or insurance claims databases.[272] The manifestation of bias varies according to the specific context, with typical examples in my thesis including misclassification bias, protopathic bias, and detection bias.

Misclassification

The robustness of observational research depends on accurate assessment of exposure, outcomes, and confounding variables. Measurement errors, such as misclassification of categorical variables or inaccurate quantification of continuous variables, can introduce bias.[273,274] In my thesis, misclassification of exposure is an inherent challenge, particularly in studies solely reliant on secondary data. For instance, in Chapter 4, I used dispensed medications data to define exposure to tramadol versus codeine, which could promise a more reliable measure of actual opioid use compared to prescription records.[275,276] In Chapter 6, the evaluation of COVID-19 vaccine effectiveness is less susceptible to exposure misclassification, as vaccines are administered within healthcare settings, mitigating the common issue that prescribed medications are not always consumed. Conversely, as discussed in Chapter 5, the use of ibuprofen, identified through claims records, is more prone to misclassification bias due to its widespread over-the-counter availability.[277] Outcome misclassification is another concern. To address this, I

managed to develop comprehensive code lists for outcome ascertainment and consulted clinicians with expertise in the relevant coding systems. While chart reviews or electronic free-text entries could improve outcome identification, they were not feasible given the scope and resource constraints of my research involving hundreds of thousands of participants. As a consequence, some of my research outcomes may be subject to misclassification to some extent. Specifically, as discussed in Chapter 8, venous thromboembolism events identified solely through hospital data may disproportionately represent more severe cases. This is supported by research indicating that hospital data often captures patients with significant risk factors for thromboembolism.[278,279] Additionally, the accuracy of hospital data in identifying this outcome can be affected by various factors such as practice variations and institutional characteristics.[280]

Unless explicitly stated, I have assumed that misclassification is non-differential, leading to bias towards the null hypothesis. However, it is crucial to be aware that this epidemiologic 'mantra' is not universally applicable with important exceptions, as evidenced by recent literature. Overall, my thesis pursued more on qualitatively assessment the impact of misclassification on association estimates. Further investigation is still needed focusing on explicit and quantitative analysis of misclassification bias.[281]

Detection bias

Surveillance or detection bias occurs when individuals within one exposure group are more or less likely to have the study outcome identified due to changed chance of screening, diagnostic testing, or because of a related symptom,[282,283] which is a nonrandom type of information bias and generally refers to the idea that "the more you look, the more you find".[284]

In Chapter 8, I assessed the risk of post-acute sequelae of SARS-CoV-2 infection among individuals who tested positive for COVID-19, comparing them with those who were either never tested or tested negative. Because, during the initial phase of the pandemic, patients diagnosed with COVID-19 could be prioritized for hospital referral,[285] there was an increased likelihood for them of detecting asymptomatic conditions including the studied venous thromboembolism in my thesis. To counteract this bias, I used rigorous propensity score and exact matching based on patient characteristics, time periods, and limited the study to those tested in community settings. Despite these measures, the observed association may still be inflated due to the non-differential detection rate of the outcome between comparison groups.[286] Future research should consider selecting an unexposed comparator group with a comparable possibility of undergoing diagnostic testing such as those tested negative controls, or if possible, adjusting for surveillance rates in the outcome model, to decouple risk of disease from risk of disease diagnosis.[287]

Protopathic bias

Protopathic bias, also known as reverse causation bias, should be a concern when a medication (the exposure) is administered in reaction to a symptom of a yet-to-be-diagnosed disease (the outcome).[288] This form of bias is historically problematic in drug-cancer association studies[289] and other long-latency outcomes,[290] but it can also compromise in my studies focusing on short-term drug effects.[291] For instance, in Chapter 5, ibuprofen usage for fever relief could be a precursor to an undiagnosed COVID-19 infection. Consequently, a subsequent COVID-19 diagnosis following ibuprofen use may represent protopathic bias rather than a genuine safety concern associated with the drug. To mitigate this bias, I constructed a cohort in which the exposure was temporally anchored in December 2019 before the emergence of SARS-CoV-2, a period during which it would be implausible to attribute ibuprofen use to COVID-19 symptoms.

9.3.Overall strengthens and limitations

In this thesis, the specific strengths, limitations , as well as their potential impact on the findings presented has been explicitly discussed in relative chapter of each individual study. A summary of these key aspects that apply to a number of studies within this thesis is provided in this section.

Primarily, all studies have been conducted utilizing reliable data sources. The study participants were generally representative of the source population with respect to major demographic factors.[71,231] Of note, the potential impact of non-randomly selected samples on the estimates, particularly in association analyses, has been a subject of substantial discussions among scholars, and it is widely acknowledged that its relevance much relies on the objectives of the study.[292] Research that aim to ascertain the prevalence of a particular exposure or illness within a population undeniably need a more representative sample to enable convincing generalization of findings to the target population.[293] Nonetheless, this criterion is not universally applicable to most of my studies trying to delineate the associations between exposures and diseases. For instance, although a prior study has identified evidence of a "healthy volunteer" selection bias in the UK Biobank, the authors posited that valid evaluations of exposure-disease relationships may still be broadly generalizable.[188,294]

Another significant strength of these studies is the substantial size of the cohorts, which permits robust and precise risk estimates even for rare outcomes.[295] In the investigation of Ibuprofen's association with COVID-19, over 10 million individuals were included for analysis, thus enabling the detection of minimal differences with greater precision than previously smaller studies. Additionally, the integration of routine medical records with bespoke, in-depth health data, such as lifestyle factors, biomarkers, and genetic information, allows for examination of research questions comprehensively.[296] A prime

example in my thesis is the identification of novel HLA gene allele associations with antibody response and breakthrough infections, which was realized by integrating data from primary care, COVID-19 surveillance, serum antibody biomarkers, and genotypes. Such a study would not been feasible with any of the single data source alone.

Also, by combining a suitable study design with cutting-edge statistical methodologies, this dissertation advances causal inference in observational data.[297,298] The new-user cohort design was employed to assess vaccine efficacy, diverging from the nested case-control design conventionally utilized for real-world vaccine effectiveness research pre-COVID-19 pandemic.[299,300] The latter design, while optimizing study efficiency in smaller sample, is vulnerable to significant biases, such as difficulty in establishing temporal relationships, constrained generalizability due to case and control selection from specific settings, and inadequate control of confounders during the matching process.[301,302] The unparalleled vaccination campaigns during the pandemic motivate me to implement cohort designs, typically requiring substantial sample sizes and extended follow-up periods for sufficient outcome observation but providing strong evidence for causal relationships between exposures and outcomes.

Lastly, the timely acquisition of evidence is of particular importance in informing public health practices[303] and regulatory decisions during the ongoing pandemic.[304] For instance, my study into the acute VTE risk addressed a pressing need in the initial guidelines about prophylaxis for patients with less severe COVID-19. During the early period, the optimal management of VTE risk among non-hospitalized individuals continued to pose a considerable clinical challenge.[305] Overall, my research presented here remains updated with the evolving data related to COVID-19 and vaccines, making it both unique and highly relevant to the current crisis.

Despite the noted strengths, it is important to recognize potential limitations when interpreting the results of this research. One concern is the generalizability of epidemiological findings to broader populations, as cohort curation and recruitment in my studies are not entirely random. For example, registered individuals may have better access to healthcare services compared to those unregistered. Generally, it is expected that SIDIAP, built up on national primary care systems, would have less selective bias than US Claims, where socioeconomic status plays a significant role in the data collection process. A similar, albeit less pronounced, concern arises in UK Biobank cohort-based studies, given that it is only comprised of a contemporary middle-aged population residing in the UK and the participants was found to be healthier and lack of ethnic diversity particularly in non-White ethnic populations. A significant challenge in deriving causality from observational data is confounding. Pharmacoepidemiology studies, which are centered on medications, vaccines, and therapies, are particularly prone to a unique form of bias termed 'confounding by indication,' unless appropriately addressed. As defined in Brian L. Strom's book on pharmacoepidemiology,[306] confounding by indication occurs when the risk of an adverse event is linked to the reason for medication use rather than the medication itself. Hence, this bias emerges when the prescription rationale correlates with the outcome under investigation. I have managed to mitigate this potential bias by comparing the drug under study with an alternative sharing similar indications for prescription, such as Tramadol versus Codeine, or Ibuprofen versus other NSAIDs. In the vaccine effectiveness study, despite both targeting SARS-COV-2, a direct comparison of BNT162b2 and ChAdOx1 vaccines was undertaken, considering the UK's vaccination strategy that prioritized the earlier post-marketing vaccine, BNT162b2, for the most vulnerable.[307] Nevertheless, residual confounding, which could bias observed associations, cannot be entirely dismissed. In my research, those using exclusively real-

world data such as Chapter 4 and 5, inaccurately measured covariates like socioeconomic factors and lifestyle could introduce residual confounding, as most EHR or administrative databases were not designed to capture such data. Furthermore, erroneous modeling or categorization of continuous confounders could also result in residual confounding, highlighting the necessity to validate modeling assumptions and explore alternative ways. In my thesis, the adoption of advanced methods, such as large-scale propensity scoring, specialized sensitivity analyses, and rigorous empirical calibration through negative control experiments, can potentially minimize the impact of confounding bias. In summary, there is a growing acceptance of evidence from pharmacoepidemiology studies using read world data, and more recent investigations reveal that well-executed observational analyses can emulate desirable attributes of randomized trials.[308,309]

9.4.Implications

My thesis can offer novel and significant insights that are of relevance to clinicians and policymakers in the field of public health as they navigate the complexities of the ongoing opioid epidemic. For example, my findings have been advertised to broader clinical audiences through the BMJ Medical Education, which has highlighted that tramadol, initially introduced as a seemingly safer alternative to other opioids, is now increasingly prescribed for chronic non-cancer pain, and may need to be treated as carefully as other stronger opioids such as Morphine.[310] Also, the research results on opioid trends indicate a low risk of the US-originated opioid epidemic spreading to Europe, the evidence for the persistent upward trajectory of opioid use over the years, as well as the concerning initiation of weak opioids for chronic non-cancer pain in my study, should be considered seriously by the decision makers. Moreover, there is a scarcity of high-quality data on opioid-related emergency department visits, opioid-related overdose deaths, and addiction treatment, which hinders a better understanding of the true nature and extent

of the opioid crisis in Europe. Urgent attention from authorities is warranted to address this knowledge gap.

During the early stages of the COVID-19 pandemic, widespread concerns arose regarding the potential exacerbation of COVID-19 symptoms by NSAIDs, particularly ibuprofen. My study findings of over 10 million individuals from US claims-based databases is consistent with a growing body of evidence, supporting no increased risk of NSAIDs treatment in COVID-19 patients as indicated. Thus, previous cautious measures for prescribing NASIDs recommended by many regulatory organizations may need to be reconsidered and updated.[311] In addition, vaccine comparative effectiveness research is a cornerstone for the planning of vaccination strategies. My study is the first one to provides a quantitative metrics of two most widely administrated vaccines in real-world settings (30% lower risks of both infection and hospitalisation among individuals vaccinated BNT162b2 vs ChAdOx1). It should be noted that the US CDC has disseminated my findings as a way to inform targeted immunization schedules, to prioritize different groups for vaccination, to communicate about vaccine, and finally to maximize public health benefits.[312] Another policy reflection to this may be that from May 2021, ChAdOx1-S primary immunization was not recommended as first-line vaccination for individuals not in a clinical risk group aged under 40 years. Also, ChAdOx1-S vaccine were only recommended by EMA as booster vaccination for those clinically contraindicated with BNT162b2 or mRNA-1273.[313] Moreover, my study showed that VTE hazard increase substantially and shortly after SARS-COV-2 infection and identified several independent risk factors, which can be used to stratify patients with different risk profiles for post–COVID-19 VTE. Although existing trials (e.g., ETHIC, ACTIV-4B) did not generally support routine pharmacologic thromboprophylaxis for outpatients with COVID-19, the results should be interpreted as inconclusive given the great statistical uncertainty and

underrepresentation of older patients. Therefore, my results can guide the use of thromboprophylaxis in selected outpatient subpopulations, particularly among those with a high risk of developing VTE, as referred by the recent clinical consensus.[314]

9.5.Future research

In today's eras, the demand for timely, transparent, and comprehensive evidence is increasingly important in tackling the public health crises, which will drive and direct future medical research. To meet these requirements, challenges in real-world evidence generation life cycle have to be addressed to uncover its power and promise its high-quality nature. First and foremost, data collected in real-world is not for non-research purposes can be unstructured, heterogeneous, and prone to measurement errors. Massive efforts have been made to improve data quality at the source data before it can be used for research purposes. In recent years, the concept of Common Data Model (CDM) has been increasingly implemented, as it can transform data stored in various formats, database systems, and information models into a unified format and representation (terminologies, vocabularies, coding schemes). However, the impact of low data quality during the data collection process is still a question of unknown and warrant more investigations. Building upon CDM, distributed networks for pharmacoepidemiologic studies have emerged, allowing investigators to execute standardized procedures to answer common topics in a transparent way. Several networks have been established worldwide, such as the US Sentinel, the Observational Medical Outcomes Partnership, the Asian Pharmacoepidemiology Network (AsPEN), and the recently initiated Data Analysis and Real-World Interrogation Network (DARWIN EU®). It is foreseeable that improved data quality standards and active engagement of key stakeholders (e.g., regulatory agencies, research institutes, industries, etc.) will prosperous studies utilizing real-world data. Lastly, linking comprehensive biomedical data from the existing biobank with real-

world data further boosts the scope and depth of medical research and triangulate the real-world evidence.

9.6.Conclusion

In conclusion, this thesis exemplifies modern observational studies by leveraging real-world and bespoke multiple-layer biomedical data. The findings have provided valuable insights that can inform clinical decision-making and guide policy recommendations in response to the ongoing opioid epidemic, COVID-19 pandemic and potentially other global health challenges. The vision of effective, robust and almost real-time evidence generation in modern medicine is being accelerated with rapid methodological advancements, as they contribute to a profound understanding of increasing abundance of large-scale data for the improvement of population health.

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