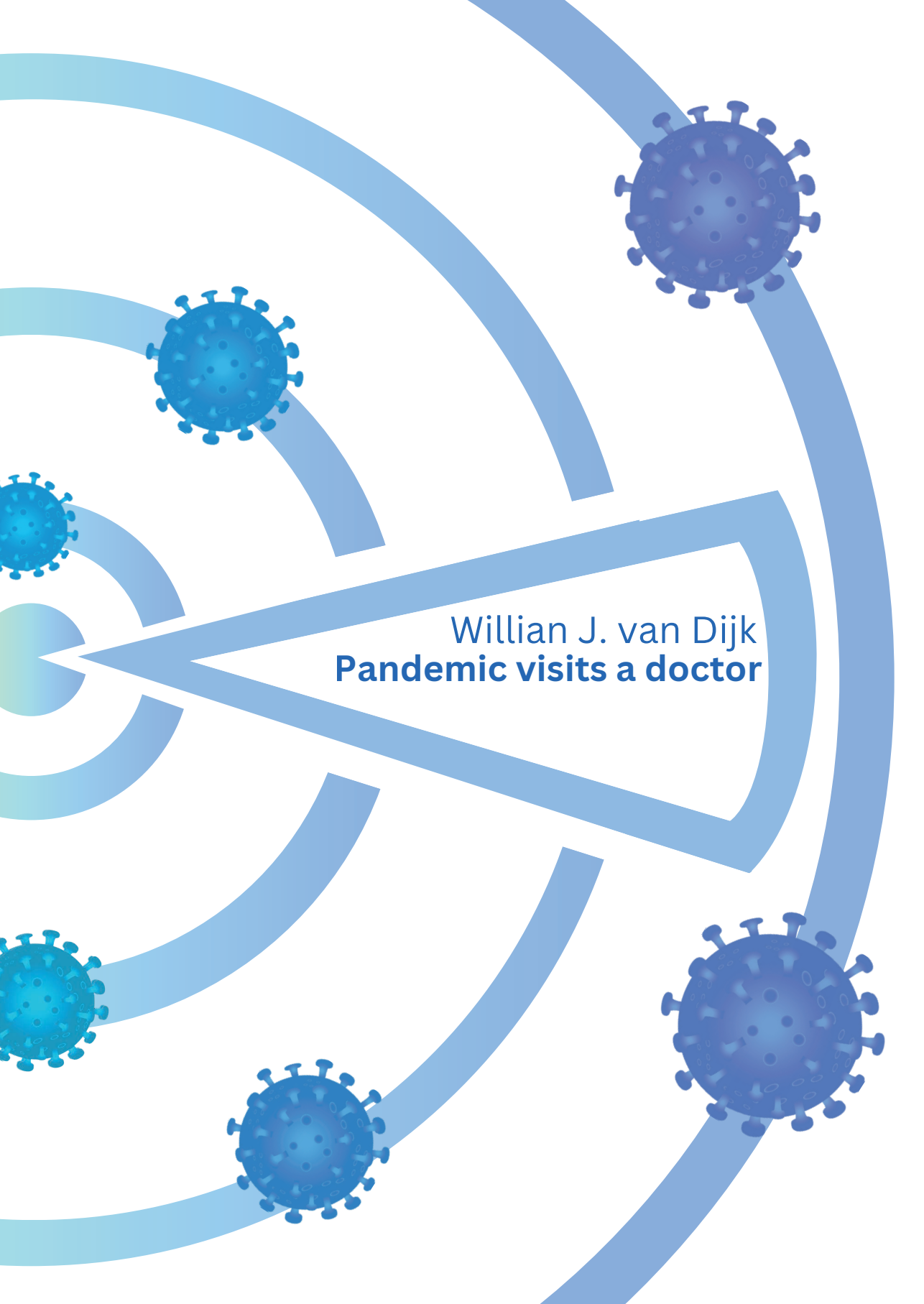
Pandemic visits a doctor

Dijk, W.J. van



Willian J. van Dijk

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PANDEMIC VISITS A DOCTOR

Wilhelm Jan van Dijk

Colophon

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Pandemic visits a doctor

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CHAPTER 1

Introduction

Pandemic Visits a Doctor

'How can I help you today?'
'I want to be most successful' answers Pandemic.
'Why?'
'It's in my nature, I have to.'
Doctor is silent for a moment.
'If I understood you correctly, you want to be the most successful pandemic of all time?'
'No, just of my time' answers Pandemic.
'Oh, that makes things easy. You just have to be slightly different from the others. But on most aspects just copy other Pandemics. Be the most infectious, not that deadly, and travel by air.'
'How to be a special one?' asks Pandemic.
Doctor takes a moment of reflection. Is it wise to inform Pandemic about this?
Eventually doctor replies:
'You should choose your timing. Start when they have forgotten your predecessor.'
'Thank you for this advice. See you next time.'

WHAT ARE PANDEMICS?

Pandemics are epidemics in multiple countries. Epidemics often refer to infectious diseases that suddenly occur with a frequency in excess of normal expectancy.(1) Infectious disease occur in many different types, ranging from a simple 'common cold' (often rhinovirus) to severe consequences of human immunodeficiency viruses (HIV). The diseases result from the invasion of a host by an infectious agent, which may be a virus, bacterium, or parasite. These agents most often originate from animals (zoonotic).(2) Compared with other diseases, infectious disease also distinguish themselves by the possibility of transmission and immunity.(1) Someone with the outcome (infection) is a risk factor for others, while someone with a past outcome (immunity from a previous infection or vaccination) is a protective factor for others (herd immunity). This is not the case in non-infectious disorders, as for example diabetes. From these distinctive properties one may argue that in infectious disease not the individual, but the *population* is 'the patient'.

Though infectious disease are common, they rarely develop into a pandemic. An important factor for an infectious disease to become a 'successful' pandemic is to be 'highly contagious', which means one patient infects many others. Mostly (but not always) this coincides with a mode of transmission by pathogen-laden liquid particles in the air(aerosols) and being infectious before having symptoms (asymptomatic transmission and a long incubation period). Though a high mortality will increase the impact and threat from a pandemic, it might reduce the 'spread' of a pandemic, because the period of transmission will be shortened due to early death of the host. Another key factor for an infectious disease to become a 'successful' pandemic is to evade the defensive immune response or to be 'novel'.(3)

The most recent pandemic may still be remembered. Many of us experience its after-effects; physically, socially, or economically. But can you remember the one before COVID-19? The most 'recent' pandemic resembling the scale and magnitude of COVID-19 was the Spanish Flu (1918-1920). Though data quality from that time may be questioned, it is estimated the Spanish Flu had a similar case count (~500 million cases, though in a world population of a quarter of today), but a higher death toll compared to COVID-19 (Spanish flu: ~40 million deaths (~10%); more than the first world war. COVID-19: ~700 million cases, ~7 million reported deaths (~1%)).(4-6) There are many similarities between the two pandemics: they share symptoms, the existence of bacterial co-infections, the role of coagulation, signs of 'overreaction of the immune system', the role of 'fake news' (either through media or by word of mouth), and the implementation of social distancing (including a firm debate about efficacy of facemasks).(7, 8) In addition, a secondary 'pandemic' of neurological symptoms (encephalitis lethargica, with symptoms like extreme fatigue) is described in the decennia after this pandemic and many other 'long term effects' are described.(9-11) With so many similarities one wonders: "*Why was society so surprised by the COVID-19 pandemic?"*

History shows that pandemics of varying scales have occurred at different intervals, with major ones such as the Plague and the Spanish Flu having significant global impacts.(12) However, population growth and intensification in global travel and of modern food industry increase the likelihood of new disease emergence and transmission.(13, 14) Given that the majority of infectious agents originate from animals, the prevention of zoonotic spillover events (transmission to humans) can be promoted through broad political and societal interventions—such as ensuring safe livestock trade, protecting ecosystems and biodiversity, and mitigating global warming—as well as through smaller-scale individual actions, including maintaining personal hygiene.(15-18) However, while it is not feasible to aim successfully for Pandemic Prevention, it is necessary to aim for Pandemic Preparedness. *What lessons can be derived from past pandemics to enhance preparedness for future outbreaks?*

PANDEMIC PREPAREDNESS

Pandemic Preparedness can be structured around **three pillars**. Firstly, if a pandemic has emerged, one should be able to limit *the speed* of the spread of the infective agent (the first pilar). The limitation of its spread is essential to minimize deaths due to shortage of medical care capacity. The second pillar includes optimal planning (in time and space) of healthcare resources to treat those most affected by this new pandemic. And lastly, the third pillar, it is essential to develop vaccines or treatment as soon as possible.

The deceleration of the spread of the infective agent, the **first pillar**, is made possible by measures that share the goal of limiting human interaction.(19) Although the exact characteristics of the infectious agent responsible for future pandemics may be unknown, we can gain insights into its spread by modeling human interactions, which drive the *speed* of transmission. The **second pillar**,

planning of healthcare resources, is crucial, given that lack of adequate healthcare is associated with worse health outcomes.(20) In these two pillars it is challenging to find the right balance, because measures have side effects in the social and economic domains and misallocation of healthcare resources can be costly. Because of the exponential growth of a pandemic, early timing of these measures and allocation of healthcare resources is both essential and difficult. (21) Therefore early in the pandemic, there is an urgent need for information about the spread and impact of the infective agent.

During the COVID-19 pandemic the **third pillar,** development of vaccines, was achieved in a record breaking time of one year. Though vaccination protects the vaccinated individual against infection or severe disease, the most important goal is to achieve herd immunity by vaccination of the majority of the population.(22) However, soon after the start of the worldwide vaccination campaign, reports of a rare potential lethal side-effect were published: venous thromboembolic events (VTE).(23-25) This is a pathological clotting of the blood resulting in blockage of veins, for example the lungs or veins in the brain. These reports have led to several changes in the vaccination campaigns.(26, 27)

POPULATION WIDE PANDEMIC SURVEILLANCE

To find the right balances in the **first** (restrictive measures) and **second** (allocation of healthcare) pillars, several surveillance systems are operating, to detect new emerging epidemics, but also monitoring ongoing pandemics. These surveillance systems can operate at different levels, ranging from the general population to severely ill or counting the number of individuals deceased due to the infectious agent (figure 1).

In the Netherlands examples of these surveillance systems include the measurement of viral particles in wastewater and the assembling of the results of viral tests of several Dutch laboratories from the National Institute of Public Health and the Environment (RIVM).(28) In addition, a subset of Dutch primary care physicians report their diagnoses and results of viral tests to the Netherlands Institute of Health Services Research (NIVEL).(29)

A limitation of these systems is that they detect novel infectious agents relatively late. A pandemic caused by a novel agent can only be discovered by syndromic surveillance, based on symptomatic patients in contact with healthcare, because testing the population or in sewage systems is not yet available. As visualized in figure 1, this is only a subset of the infected population, resulting in relatively late detection of this novel infective agent. For more timely monitoring of infections, first a test needs to be developed and subsequently needs to be scaled up at (inter)national level, which takes several months.

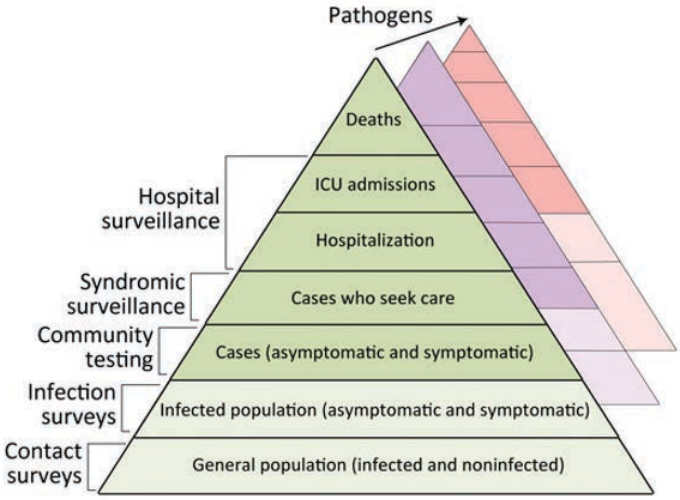

Figure 1: Pandemic surveillance levels; from: https://wwwnc.cdc.gov/eid/article/30/2/23-0768-f1

In December 2019, the late healthcare professional Li Wenliang was the first to report numerous cases of a novel type of pneumonia, which was later named COVID-19.(30, 31) Two months later, the World Health Organization declared COVID-19 a pandemic. In March SARS-CoV-2 was also introduced in the Netherlands, however, population based testing of symptomatic persons was not possible until June 2021.(32) During this period, several initiatives throughout the world, used e-health applications that measure self-reported symptoms instead of number of cases in contact with healthcare resulting in more timely monitoring of this infectious agents.(33, 34) Some of these pandemic surveillance applications focused on risk contacts or risk behavior (the bottom of the pyramid in figure 1).

While the COVID-19 pandemic led to the rapid expansion of novel surveillance methods, it is important to consider that many of these surveillance strategies have been designed from a Western perspective, focusing on high-income countries with well-developed healthcare infrastructures. However, infectious diseases such as malaria and tuberculosis, which are endemic rather than pandemic, continue to cause millions of deaths annually in low-income regions.(35, 36) Similarly, Ebola outbreaks occur regularly, and cholera resurges in conflict-affected areas, often with limited global attention.(37, 38) These examples highlight the need for a more inclusive and globally representative approach to pandemic preparedness, ensuring that surveillance systems are adapted to diverse healthcare settings and capable of addressing a broad range of infectious disease threats.

SURVEILLANCE OF ADVERSE EVENTS OF DRUGS: PHARMACOVIGILANCE OF VTE AFTER VACCINATION

Pandemic surveillance systems resemble systems that detect (novel) adverse effects of (new) pharmaceutical drugs. In the Netherlands, physicians are obliged to report possible drug related major adverse events they encounter during their clinical work.(39) These reports are processed by the Dutch Pharmacovigilance Centre Lareb that sounds an alarm when more reports are received than expected, based on historical background rates. During the COVID-19 vaccination campaign, Lareb (and the European Medicines Agency) received reports on VTE related to SARS-CoV-2 vaccines.(26, 40, 41) Because of these reports, the AstraZeneca vaccine was discontinued in individuals below the age of 60 in the Netherlands.(42)

However, this surveillance system of adverse events has several disadvantages. It depends on reports of physicians and patients and the comparison with background rates - either historical or concurrent- but has the limited ability to control for confounding factors. Variations in background rates exist during time and geographical region. This variation was especially present during the pandemic, during which healthcare utilization changed dramatically.(43)

In case of vaccines, it is crucial that potential adverse events are measured and reported correctly because a high (>80%) proportion of the population needs to be vaccinated to achieve herd immunity (third pillar). Uncertainty about adverse potential adverse events could fuel vaccine hesitancy, which could lower the possibility to achieve herd immunity. This thesis will reflect on the measurement and assessment of these adverse events of SARS-CoV-2 vaccines during the COVID-19 pandemic.

PRIMARY CARE AND SURVEILLANCE

In the Netherlands, Primary Care physicians serve as the 'gatekeepers' of Dutch healthcare. Almost all Dutch inhabitants are registered at their own general practitioner. General practitioners diagnose and treat many patients themselves, and refer severely ill patients to hospitals or other specialized care. In addition they perform prevention and chronic disease management in patients with, for example, cardiovascular diseases of chronic pulmonary disease.

Also during the COVID-19 pandemic Dutch general practitioners served as gatekeepers, and organized acute care consultation locations, often in collaboration with other general practitioners in the same area, to see patients suspected of COVID-19 separate from other patients. The composition of the workload in primary care changed substantially during the pandemic (e.g., shorter consults, more visits at home).(44, 45) At the start of the vaccination campaign, general practitioners performed the vaccination of patients with comorbidities or frailty. Because of the population-wide reach, data from primary care was used both for surveillance of the pandemic (29) and surveillance of (thrombotic) adverse events following vaccination.(46)

AIM AND OUTLINE

This thesis focusses on the three pillars in pandemic prevention, i.e., speed of spread, planning of healthcare resources, and vaccination, and is divided in two parts:

1. Surveillance of the spread of infectious disease using population derived data on symptoms and behavior and the application of these data. (e.g. surveillance of the pandemic)

Chapter 2 addresses the validation of data from a citizen science based app. The data of this app was used to predict COVID-19 related primary care workload in **chapter 3**. In **chapter 4** the data from this app were used to describe persisting symptoms following COVID-19 (Post-COVID).

2. Coagulation and venous thrombotic events (VTE) as adverse events following SARS-CoV-2 infection and SARS-CoV-2 vaccination. (e.g. surveillance of adverse events)

The association between intrinsic coagulation potential ('tendency to clot') and COVID-19 infections and its severity in the subsequent six months is described in **chapter 5**. In **chapter 6** changes in coagulation parameters following a SARS-CoV-2 vaccine are described and the association between the inflammatory response and coagulation is determined. **Chapter 7** entails a case-control study in which we assess the relative risks of developing a VTE after each type of SARS-CoV-2 vaccine.

This thesis concludes with a general discussion in **chapter 8** in which recommendations are made on the use of population based data and the suitability of several types of data and designs for surveillance for side-effects of vaccines and the surveillance of infectious agents and its impact on healthcare during a pandemic.

BUILDING BLOCKS

Before I proceed, several building blocks of the thesis should be considered: data from several sources used in this thesis will now be introduced briefly. The **COVID radar app** is an e-health application to gather population based data. Dutch citizens were invited to fill in a daily questionnaire about symptoms and risk behavior. Additionally users received feedback about their data relative to the national mean. The main aim of the app was to function as a population based surveillance tool, using symptoms and behavior, without the explicit need for a test result or contact with healthcare.

Electronic Healthcare Records (EHR) from the **ELAN** (Extramural Leiden Academic Network) contains information about patients and their health registered by general practitioners in the area of Leiden, The Hague, and Zoetermeer. These data were used to measure the number of COVID-19 related contact with primary care during the pandemic.

We further used data of two randomized trials to assess coagulation in relation with SARS-CoV-2 infection or vaccination. The **BCG-PRIME** trial was conducted to assess the risk of (severe) COVID-19 associated with the Bacillus Calmette-Guérin (BCG) vaccine. However, we related several measures of coagulation determined at baseline (prior to vaccination) with subsequent SARS-CoV-2 infection and severity of disease. The **IDSCOVA** trial compared the immunogenicity of fractional intradermal and full-dose intramuscular delivery of the mRNA-1273 (Moderna) vaccine. We assessed the change in coagulation parameters following these vaccinations.

The **TERA** (Thrombosis Etiology and Risk factors Assessment) study is based on both questionnaire data from patients and healthy individuals as well as data from **Dutch Statistics (CBS)** on risk factors for VTE. Within the data warehouse of CBS data were gathered from hospitals, insurance companies, and governmental sources. These data contain registrations of diagnoses, billings, reimbursement of medication, and administration of vaccines. These data were used to estimate the relative risk of VTE following SARS-CoV-2 vaccination and the absolute impact of vaccination on the number of VTE in the Netherlands in pandemic situation of 2021.

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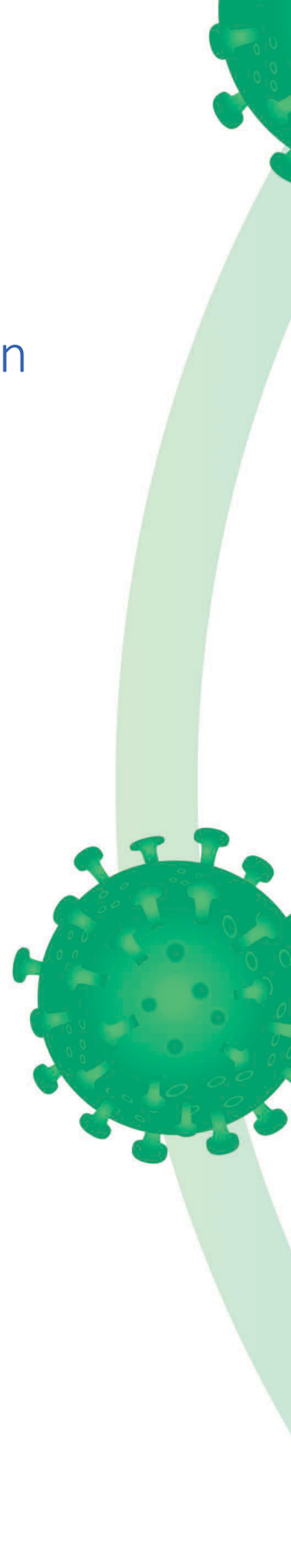
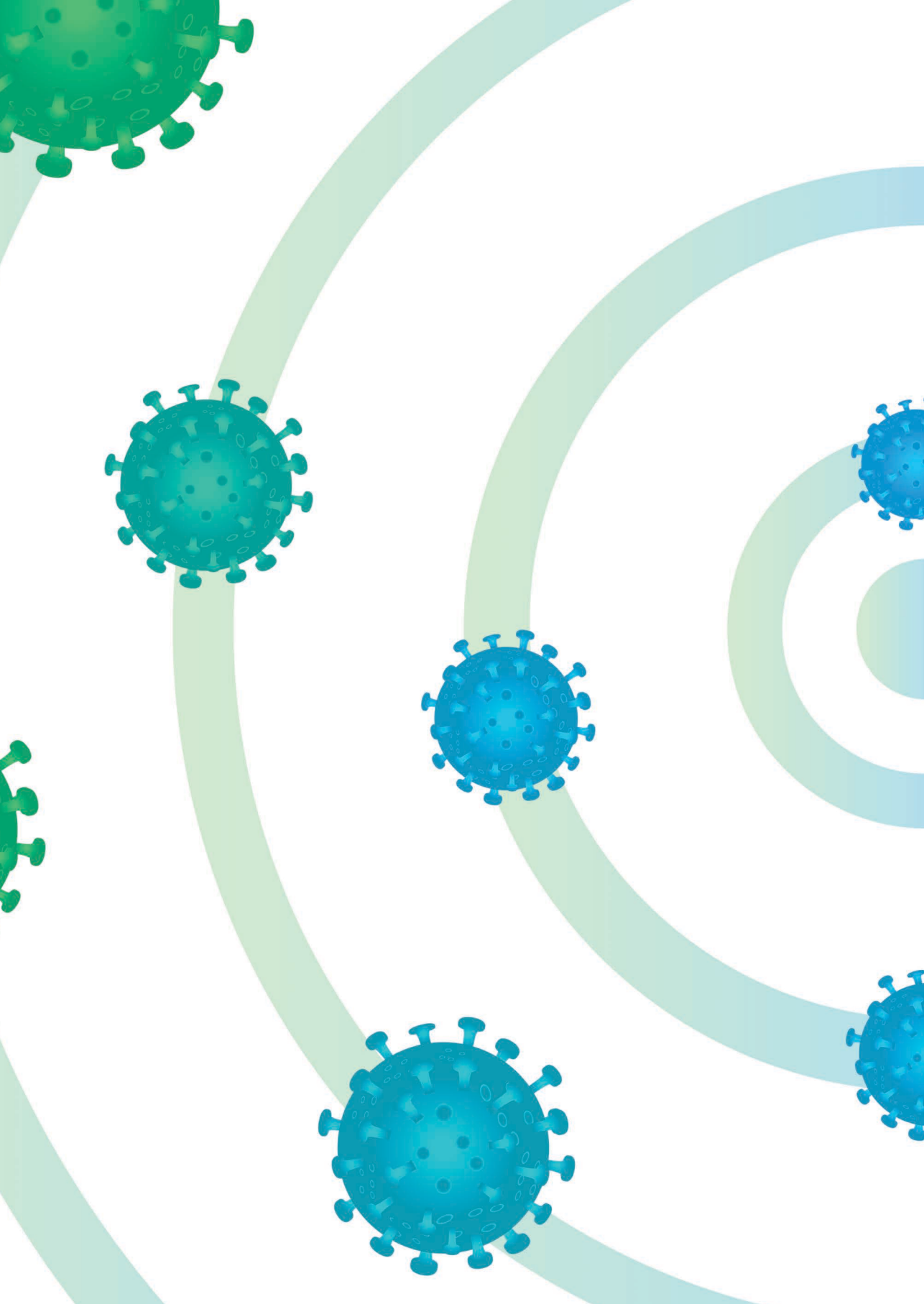
PART I

Surveillance of the pandemic with population derived data on symptoms and behavior and the application of these data





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CHAPTER 2

COVID RADAR app: Description and Validation of Population surveillance of Symptoms and Behavior in relation to COVID-19

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ABSTRACT

Background

Monitoring of symptoms and behavior may enable prediction of emerging COVID-19 hotspots. The COVID Radar smartphone app, active in the Netherlands, allows users to self-report symptoms, social distancing behaviors, and COVID-19 status daily. The objective of this study is to describe the validation of the COVID Radar.

Methods

COVID Radar users are asked to complete a daily questionnaire consisting of 20 questions assessing their symptoms, social distancing behavior, and COVID-19 status. We describe the internal and external validation of symptoms, behavior, and both user-reported COVID-19 status and state-reported COVID-19 case numbers.

Results

Since April 2nd, 2020, over 6 million observations from over 250,000 users have been collected using the COVID Radar app. Almost 2,000 users reported having tested positive for SARS-CoV-2. Amongst users testing positive for SARS-CoV-2, the proportion of observations reporting symptoms was higher than that of the cohort as a whole in the week prior to a positive SARS-CoV-2 test. Likewise, users who tested positive for SARS-CoV-2 showed above average risk social-distancing behavior. Per-capita user-reported SARS-CoV-2 positive tests closely matched government-reported per-capita case counts in provinces with high user engagement.

Discussion

The COVID Radar app allows voluntarily self-reporting of COVID-19 related symptoms and social distancing behaviors. Symptoms and risk behavior increase prior to a positive SARS-CoV-2 test, and user-reported case counts match closely with nationally-reported case counts in regions with high user engagement. These results suggest the COVID Radar may be a valid instrument for future surveillance and potential predictive analytics to identify emerging hotspots.

INTRODUCTION

The world is in the throes of the coronavirus-disease-2019 (COVID-19) pandemic with more than 100 million cases and over 2 million confirmed deaths worldwide as of December 2020 [1]**.** In the Netherlands, the first case of COVID-19 was diagnosed in February 2020 and since then over one million cases and 17,500 deaths have been confirmed [2]. To date more than 60,000 COVID-19 patients have been admitted to Dutch hospitals, with over 12,000 of these eventually admitted to intensive care [2] – this in a country with just over 1,000 intensive care beds [3]. The strategies of Test Trace and Isolate (TTI), and of measures intended to reduce social contact, have been widely adopted to "flatten the curve" [4, 5]. An important limitation of the TTI strategy is transmission of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) by COVID-19 carriers without symptoms. Given their lack of symptoms, they may not be tested and remain unidentified by the TTI process despite being a possible source of viral transmission [6]. Recent studies show that this subpopulation may account for as much as half of COVID-19 transmissions [6, 7].

An instrument to continuously monitor social-distancing behavior and symptoms in the population at a local level may support and improve the TTI process by decreasing the delay in identification of risk areas and populations. Research using voluntary symptom self-reporting apps performed in the United Kingdom, the United States of America, and Israel show promising results in the local prediction of COVID-19 using symptom-based tracking [8-10]. However, we find no apps using voluntary social-distancing behavior-reporting to track local COVID-19 hotspots.

During the first COVID-19 wave in the Netherlands, the Leiden University Medical Center (LUMC) and the tech company ORTEC developed and introduced the COVID Radar app. This questionnaire-based app allows individuals to anonymously report COVID-related symptoms and social-distancing behaviors on a regional and population level. The app provides users with direct feedback on, and peer comparison with, their reported social-distancing behavior and symptoms. Our theory is that tracking of symptom and social-distancing behavior data at a population level can be used to identify regions where more COVID-19 cases will subsequently occur, allowing (regional) policy makers and healthcare professionals to affect changes to regulations earlier, and thus more effectively.

In this first descriptive study, our aim is to observe the associations between self-reported symptoms, social-distancing behavior, and self-reported COVID-19 infection by the app's users (i.e. criterion validity), and the associations between these variables and state-reported COVID-19 infections by the National Institute for Public Health and the Environment (i.e. external validation).

METHODS

COVID Radar app

The COVID radar app was released on the 2nd of April 2020 following a short publicity campaign in the local and national media [11]. The app is free to download and allows for multiple user accounts

from the same household on one smartphone. The app is not age-limited, meaning children are allowed to download and use the app. Over 85% of the households in COVID radar's user population with minors under 18 years of age are linked to an adult smartphone. Upon first use of the app, users are asked to provide informed consent to share the following information with the research institution as stipulated by the conditions of the European General Data Protection Regulation. Users may opt out by either removing the app or by requesting the data manager to remove all data collected from that individual. Users are asked to register by entering the four digits of their postal code, gender (Male/Female/Other/Not Specified), age category (0-5, 6-11, 12-18, 19-29, ten-year increments from 30-80 and a category for 80+), and occupation (healthcare, education, catering industry, or other occupation with high risk of close contact). Following the initial setup, users are asked to report their symptoms and behavior daily via a questionnaire. A push-reminder is sent every-other day to users reminding them to do so. Figure 1 shows screenshots of the app and supplemental table 1 shows a list of the questions users are asked.

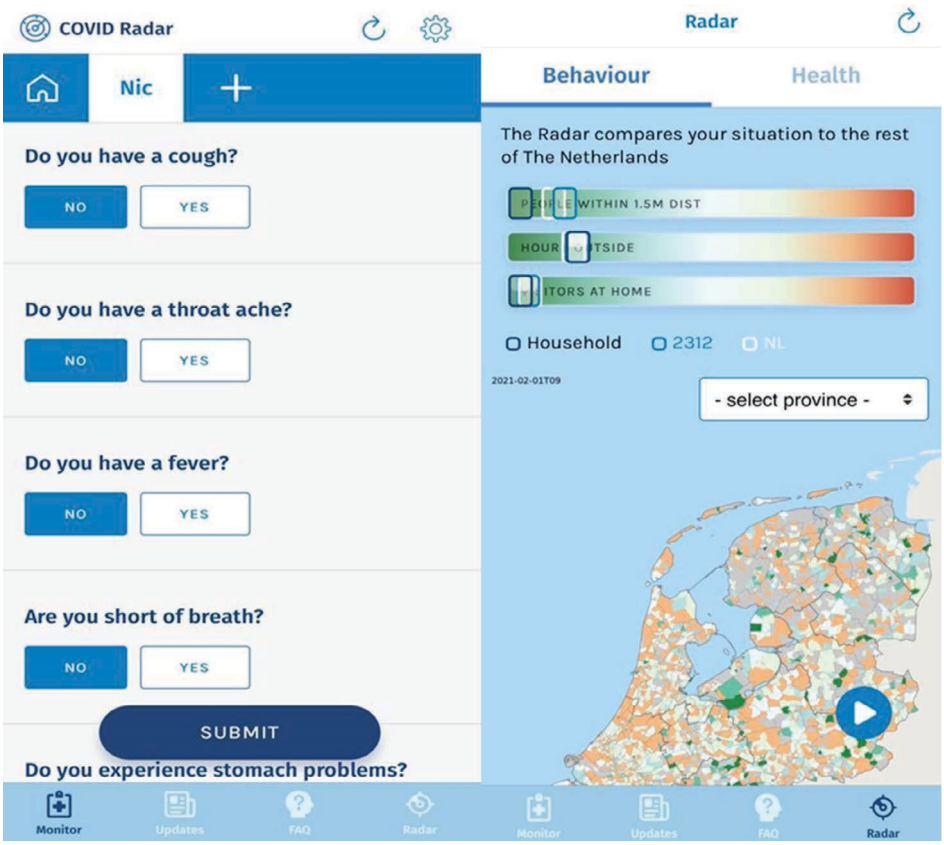

Figure 1: Screenshot of COVID radar app (Left: questions. Right: feedback) "Reprinted from "urlmaps.com" under the Creative Commons Attribution License , with permission from "i-mapping", original copyright 2021."

Each observation is comprised of questions assessing symptoms, social-distancing behavior, whether or not the user has been exposed to an individual with COVID-19 in the past 2 weeks, and a user's COVID-19 test history. The questions asked were periodically updated with the addition/removal dates of each question detailed in the online supplement. Via maps displayed within the app, users are presented with regional incidences of symptoms and personal feedback on their social-distancing behavior compared to regional and national means (figure 1).

Data are transferred daily to a safe data environment within the Information Technology system of the LUMC (supplemental Figure 2). Following importation of the daily data, we exclude observations from users who had requested to opt out, observations listing nonexistent postcodes, and double measurements within one user. Given users are asked if they have tested positive for SARS-CoV-2 within the past two weeks, we considered users SARS-CoV-2 positive/negative if they indicated a SARS-CoV-2 test result at least twice in the app, with the date of the first report used as day zero. More details on the development of the app, selection of the chosen questions, (external) data sources, and data cleaning is available in the supplement. Ethical approval was provided by the Medical Ethical Board of the LUMC (dossier number N20.067), which gave permission to refrain from obtaining consent from parents or guardians as data collection was anonymous. Only data on age category, profession and four digit postal code was collected rendering the data was untraceable to an individual.

Comparison of included/excluded observations

Following the data cleaning process detailed in the online supplement, we compared the available data in the excluded cohort with that of the included cohort. For each of the binary (symptom) variables collected by the app, we compared the proportion of excluded and included observations reporting this symptom. For each of the continuous social-distancing behavior variables, we compared the mean values for the included/excluded cohorts.

Descriptive statistics

To describe participant characteristics, we used histograms to explore age distributions of the app users, the number of times the app was used each day, and the number of times individual users used the app. We further compared age, gender, and profession for users ever having tested positive with those never having tested positive for SARS-CoV-2.

Validation testing

Given the eventual goal of the COVID Radar app is to predict emerging hotspots, we tested the expected associations between symptoms/behavior and SARS-CoV-2 test outcome. We used user-reported test results as our outcome measure for criterion validity testing and cases reported by the National Institute for Public Health and the Environment (RIVM) as our outcome measure for external validation [2].

Criterion validity

As a measure of criterion validity, we explored associations between the binary symptom variables (e.g., cough, sore throat, loss of smell/taste) and the continuous social-distancing behavior variables (e.g., number of house outside house, number of people within 1.5m) within the cohort of users ever reporting a SARS-CoV-2 test. For users within this ever-tested cohort, we used the date of the test as day 0 and observed the 21 days before and after the test. We calculated the daily mean or proportion for each variable for the entire user- cohort. We then calculated the difference between ever-positive or ever-negative users' reported values and the mean values for the entire user-cohort on that day. By comparing data from the same days, we eliminated bias introduced by variations in time due to the various lock-down measures implemented during the observation window, as well as seasonal effects on symptoms. The mean values and 95% confidence intervals for these differences were then plotted to show how the ever-positive and ever-negative cohorts compared to the cohort as a whole with regard to these variables in the days surrounding a test. Given the formulation of the question ("Have you tested positive/ negative for SARS-CoV-2 in the past two weeks"), the date of the test cannot be determined for those answering this question in the 14 days following the implementation of the question about testing in the app. Given this and the fact that this analysis involved looking at the 14 days prior to a test, users reporting a SARS-CoV-2 test in the 14 days following implementation of the question about testing were not included in this analysis.

External Validation

As a measure of external validation, we compared per-capita user-reported COVID-19 status among the 12 Dutch provinces with per-capita rates as reported by RIVM over the course of the pandemic [2]. Within each province, we plotted 7-day backward looking moving averages of the daily proportion of users reporting each symptom variable alongside the daily nationally reported COVID-19 case counts and the weekly proportions of users reporting each symptom variable alongside the number of Rhinovirus cultures reported by Dutch laboratories [12]. We further plotted daily means and 7-day backward looking moving averages of each social-distancing behavior variables and qualitatively observed how well they reflect nationally applied lockdown-measures and holidays.

Sensitivity analyses

We repeated the above-described analyses for (a) the cohort of users using the app an above-median number of times during the observation period, (b) the cohort excluding healthcare professionals, and (c) the cohort excluding inhabitants of the province 'Zuid-Holland', the home province of the LUMC where the app was created and users were most exposed to COVID Radar app-related media and advertisements. All statistical analyses were performed in STATA 16.1 (StataCorp, College Station, USA). STATA syntaxes for all analyses are provided in the online supplement.

RESULTS

In the period 2 April, 2020 to 31 January, 2021 (305 days), the COVID Radar app was downloaded by 278,523 unique users who filled in the in-app questionnaire 6,202,606 times. A total of 102,445 (1.65%) observations were excluded (Supplemental Figure 4).

Comparison of included/excluded observations

The data for the 102,445 (1.65%) excluded observations were fairly representative of the included observations' data in terms of symptoms and behavior. However, excluded observations were less often from a health professional and showed a slightly different age distribution (i.e. older age groups are over-represented in the excluded cohort) (see supplemental Table 2).

Descriptive statistics

The age distribution of the app's users showed a fairly consistent distribution of users 18-69 years old, and an under-representation of young (<18) and old (>70) users. Female users were overrepresented compared to national figures (See S. Figure 5). The number of observations (questionnaires answered) per day dropped from over 100,000 in the first week of the app to a steady-state of around 10,000 observations per day during the course of the observation window (2 April, 2020 to 31 January, 2021) (See Figure 2).

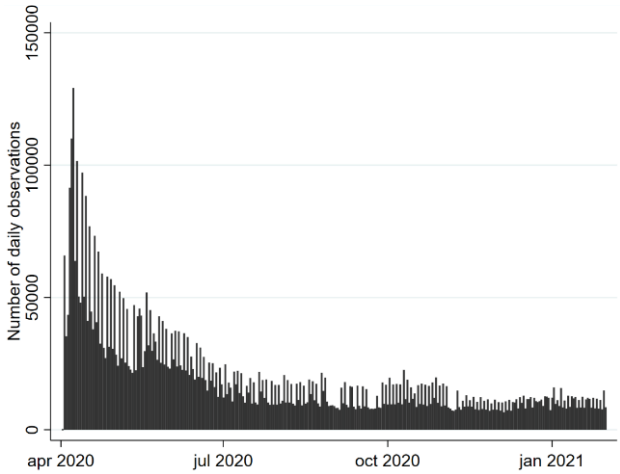

Figure 2: Number of observations per day

The effects of the push reminder sent every-other day to all users is seen in the periodicity in the number of observations between even and odd days. The number of daily observations was highest in the province Zuid-Holland, the home province of the LUMC where the app was conceived and advertised (see Figure 3).

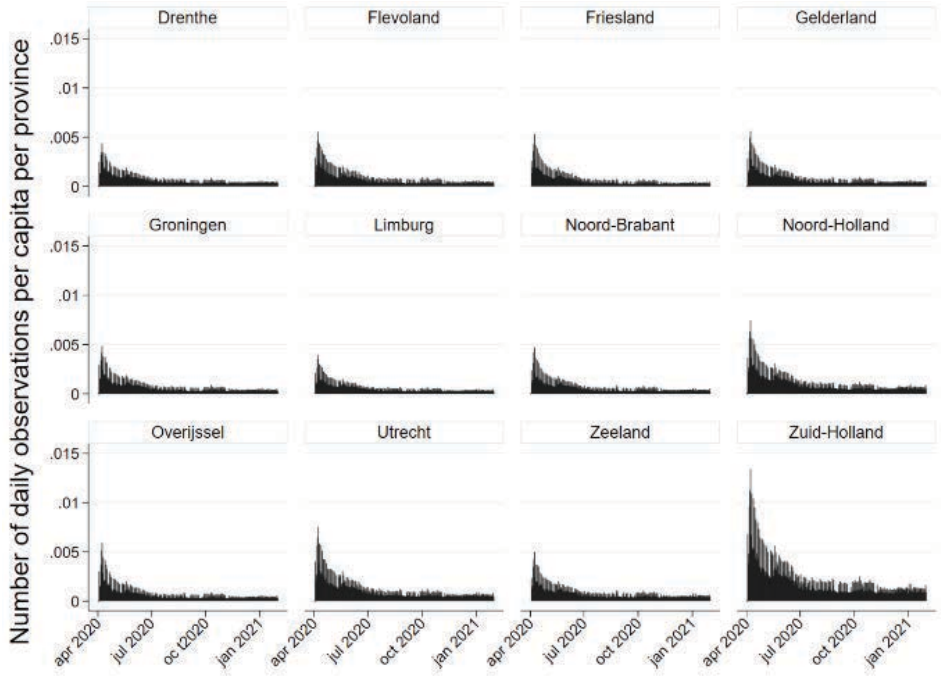

Figure 3: Number of observations per capita per province in the Netherlands

The number of observations per user ranged from 1 to 305 with a median value of 6 (25^{th},75^{th} percentiles [p_{25}, p_{75}] = 2, 21) (See Figure 4).

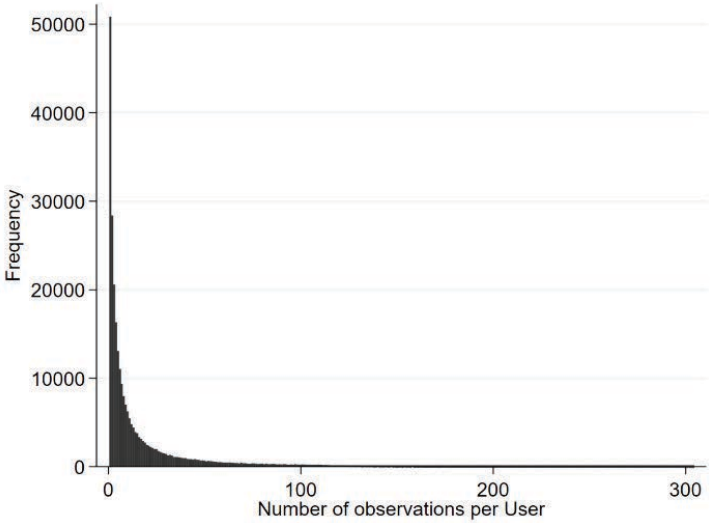

Figure 4: Distribution of number of observations per user

Criterion validation

From a total of 278,523 unique users, 1,981 (0.71%) reported ever testing positive and 1214 (0.44%) negative for SARS-CoV-2. Ever-positive users were more likely to be women, older than 40 years of age, and healthcare professionals (Table 1).

Table 1: Comparison of users never vs. ever tested SARS-CoV-2 positive

Number of days	305			
Number of users	278.523			
Number of observations	6.100.261			
Observations per user, median and Interquartile range (IQR)	6		[2 ; 21]	
	Never SARS-CoV-2 +		**Ever SARS-CoV-2+**	
	N	**(%)**	**N**	**(%)**
Total	276.542	99,3%	1.981	0,71%
Gender (female)	152.515	55,2%	1.201	60,6%
Profession				
Catering	259	0,1%	9	0,5%
Education	1.427	0,5%	77	3,9%
Healthcare	28.830	10,4%	372	18,8%
Other	16.510	6,0%	412	20,8%
Other_Contact	1.239	0,4%	35	1,8%
Missing	228.277	82,5%	1.076	54,3%
Age				
0-5	9.033	3,3%	1	0,1%
06-11	11.273	4,1%	17	0,9%
12-18	17.635	6,4%	72	3,6%
19-29	34.309	12,4%	102	5,1%
30-39	39.305	14,2%	157	7,9%
40-49	41.887	15,1%	327	16,5%
50-59	54.718	19,8%	604	30,5%
60-69	45.993	16,6%	476	24,0%
70-79	20.292	7,3%	208	10,5%
80+	2.097	0,8%	17	0,9%

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

The proportion of users reporting the eight symptom variables increased beginning approximately 7 days prior to a positive test. This increase was smaller in the cohort of negative tested users (Figures 5a and 5b).

The continuous social-distancing behavior-based variables likewise showed above-mean values in this ever-positive cohort until approximately 7 days prior to a positive test, at which point they sharply decreased to remain below-mean in the week before and after a positive test. These fluctuations were not seen in users testing negative for SARS-CoV-2 (see Figure 6 a and b).

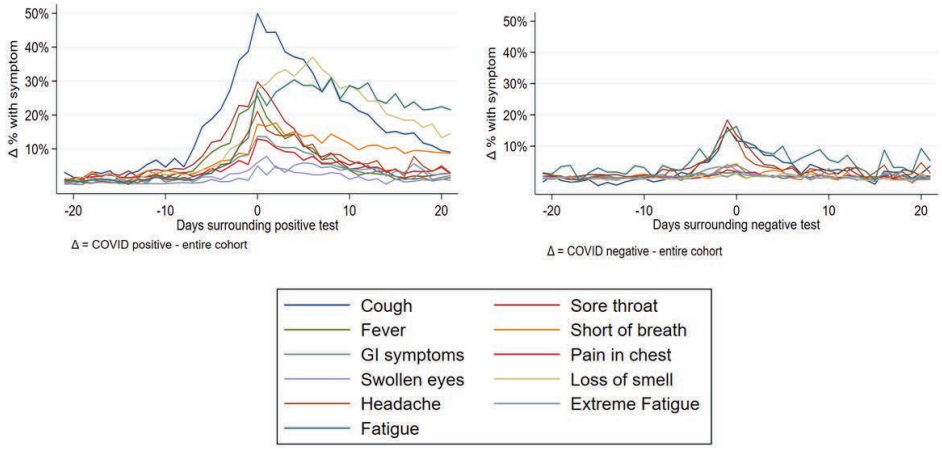

Figure 5: a) Daily reported proportion of users reporting symptoms surrounding positive SARS-CoV-2 test. b) Daily reported proportion of users reporting symptoms surrounding negative SARS-CoV-2 test.

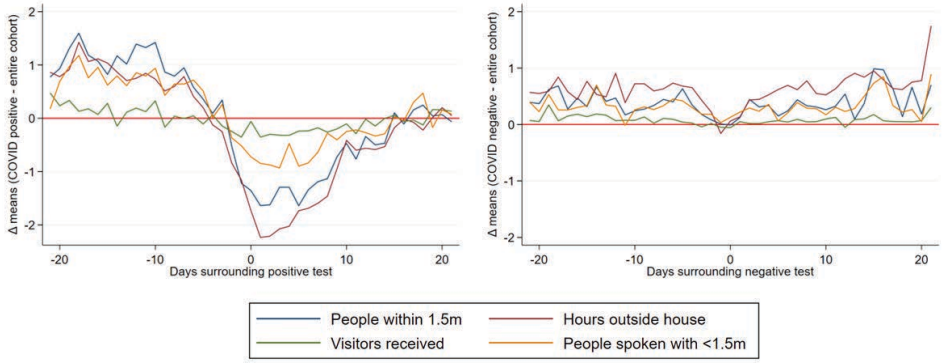

Figure 6: a) Mean difference between SARS-CoV-2 positive users and daily means for behavior variables in the days surrounding positive SARS-CoV-2 test. Red line: no difference. (example: on day 20 before test, on average a positive tested user reported one extra person within 1.5m (blue line) compared with the daily mean). B) Mean differences between SARS-CoV-2 negative users and daily means for behavior variables in the days surrounding negative SARS-CoV-2 test.

External Validation

As of early January 2021, almost one million cases of COVID-19 had been reported in the Netherlands by the National Institute for Public Health and the Environment (RIVM). The RIVM-reported daily case counts varied from 0 to over 13,000 cases per day. Positive SARS-CoV-2 tests reported in the COVID Radar app alongside the case count as reported by the RIVM for each province show that the association between these two is highest in provinces with a higher number of users, especially Zuid-Holland (Figure 7).

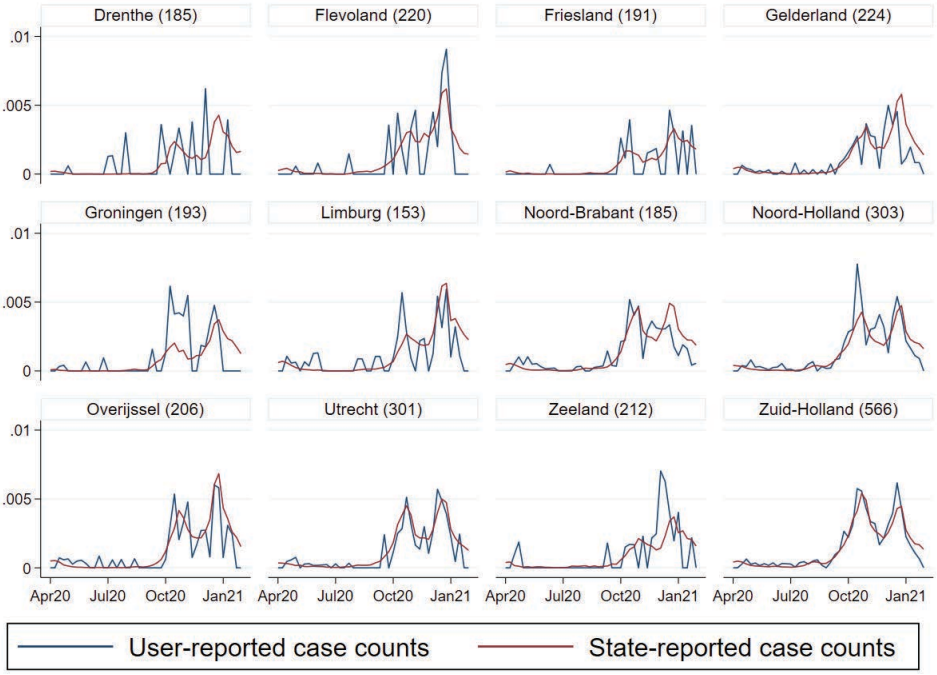

Figure 7: Weekly proportion of SARS-CoV-2 positive users reported in the COVID Radar app and case count per capita as reported by the RIVM for each province. (the number written beside the name of each province represents the mean number of weekly unique users per 100.000 inhabitants in that province during the study period).

Symptoms and social-distancing behavior varied over time, with both showing a clear temporal association with RIVM-reported case counts over time (Figure 8 and 9).

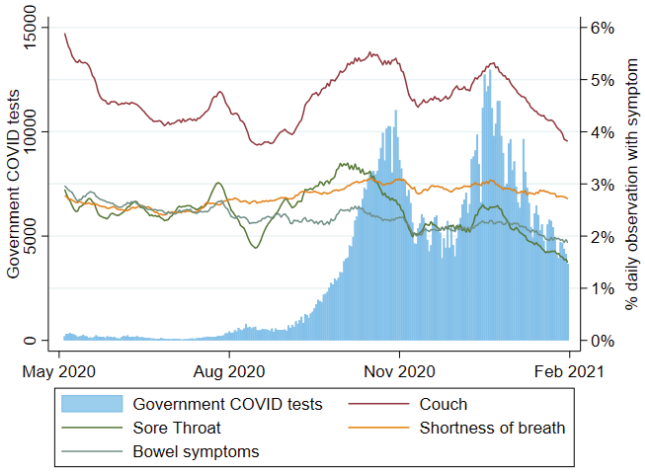

Figure 8: Symptoms vs. time alongside number of nationally reported positive SARS-CoV-2 tests (Percentage of users reporting cough, sore throat, GI (gastrointestinal) symptoms, and shortness of breath).

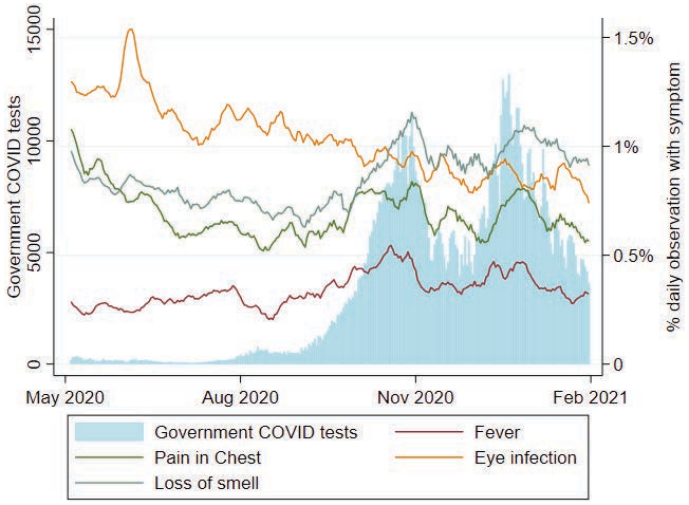

Figure 9: Symptoms vs. time alongside number of nationally reported positive SARS-CoV-2 tests (percentage of users reporting pain in chest, fever, loss of smell, swollen eyes)

Plotting the RIVM-reported number of reported positive cultures of Rhinovirus alongside our symptom data suggests variables 'fever', 'pain in the chest' and 'loss of smell' are associated with COVID-19 case count while variables 'coughing' and 'sore throat' correlated more closely with Rhinovirus cultures (Figure 10).

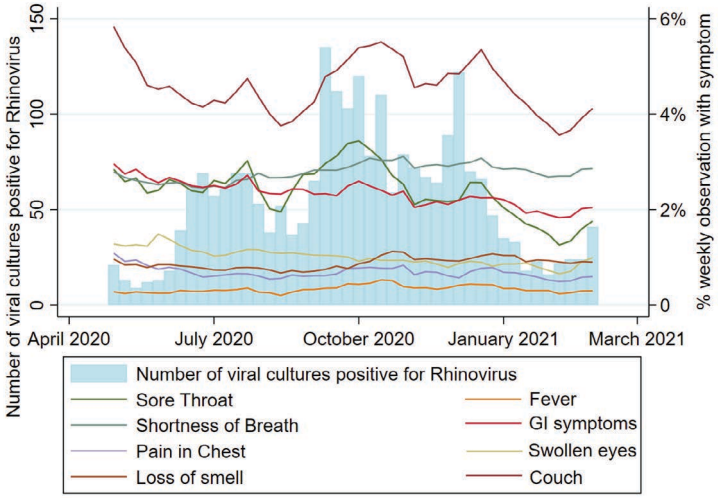

Figure 10: Symptoms vs. time (weekly) alongside number of nationally reported positive cultures of Rhinovirus (Percentage of users reporting symptoms)

The daily mean number of people within 1.5 meters declined sharply around the middle of September, reflecting the national lockdown measures introduced, and showed peaks during national holidays (Figure 11).

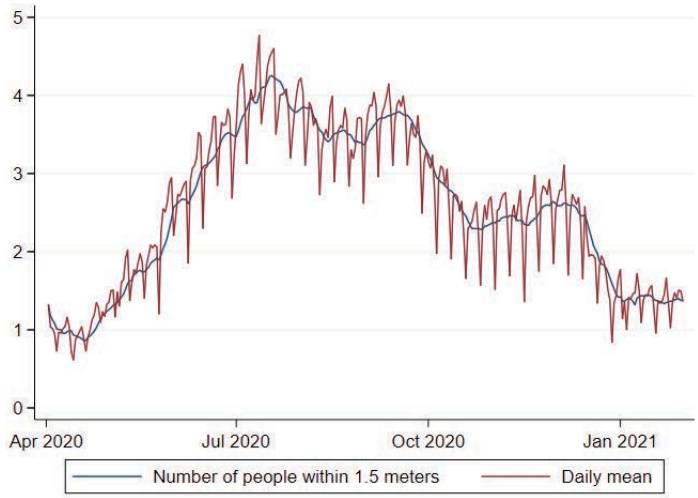

Figure 11: Daily mean number of people within 1.5 meters (with 7-day backward-looking moving average in black) September: closing of restaurants. December: holidays, followed by closing of non-essential shops.

The variable 'number of visitors' likewise showed peaks in the period around Christmas and New Year's Eve (Figure 12).

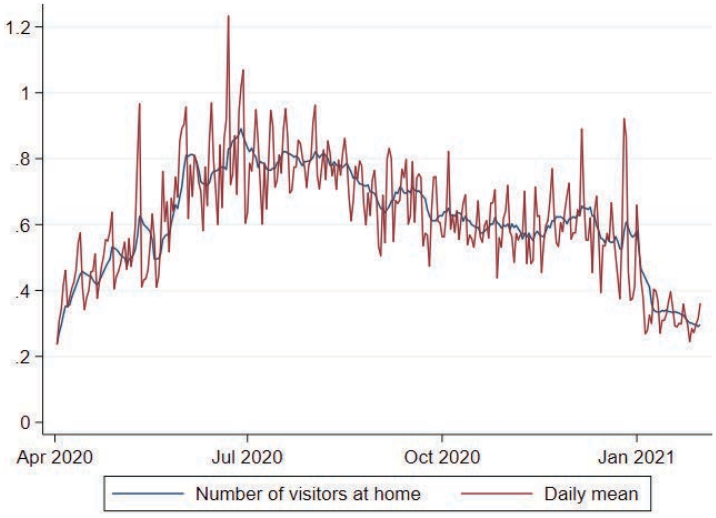

Figure 12: Daily mean number of visitors vs. time (with 7-day backwards moving average in black). Peaks are seen during Christmas and New Year's Eve. The fifth of December is a holiday in the Netherlands known as "Sinterklaasavond".

Sensitivity analyses

These analyses were repeated using (a) only users reporting an above median number of observations (referred to as 'faithful' users), (b) only users outside the province Zuid-Holland, and (c) only non-healthcare professionals. Differences in the results for these three sensitivity analyses were minimal and none of the trends seen here were reversed (data shown in supplements).

DISCUSSION

Since April 2020, the COVID Radar app has collected over 6 million user-provided questionnaires detailing COVID-related symptoms and social-distancing behaviors from over 275,000 unique users within the Netherlands. Symptom and behavior data were temporally associated with user-reported SARS-CoV-2 tests. A correlation between in-app reported case count and national-reported case counts was likewise seen, especially in provinces with high user-engagement. Social-distancing behavior variables showed the expected pattern in relation to national applied lockdown measures and holidays.

Criterion validity

Our qualitative (visual) association testing showed clear associations between both user-reported symptoms and user-reported social-distancing behavior, and user-reported SARS-CoV-2 test results. While not here quantified, some variables (e.g. 'fever', 'pain in the chest' and 'loss of smell') were more closely associated with case-count than others (e.g. 'coughing' and 'sore throat'), which seemed as associated with Rhinovirus as with SARS-CoV-2. These associations are supported by prior research [13-16]. The pattern of social-distancing behaviors within the cohort of users who eventually report a positive SARS-CoV-2 test was particularly interesting. This cohort showed above-mean risk social-distancing behavior (e.g. more people within 1.5m, more visitors at home) between 20 and 10 days prior to a positive test (i.e. the period during which transmission likely occurred), at which point their social-distancing behavior quickly drops to a below-mean value as they became symptomatic and decided to be tested. The extent of above mean risk behavior was lower in users eventually testing negative.

External validity

Comparing COVID radar data to external data sources showed logical (temporal) associations in symptoms, social-distancing behavior, and test results. The strongest associations were observed in regions with high user-engagement. Given the symptoms tracked by the app are common both to SARS-CoV-2 and other respiratory tract infections, future efforts directed at prediction will need to correct for Rhinovirus and other viruses using viral surveillance data from Dutch laboratories. The extent and types of restrictions imposed on the Dutch population varied during the observation period and their effects were clearly visible in the social-distancing variables reported by users.

Comparison of excluded and included observations showed slight differences in age distribution but relative consistency in other variables. The small size of the excluded cohort minimized the risk of bias being introduced via this exclusion step. There was a large variance in the number of observations per user, with some users answering questionnaires daily while others filled in the app only once during the observation period. While it is reasonable to assume more faithful users may provide more accurate data, sensitivity analyses performed using data from users with an above-median number of app entries show no significant differences as compared to our primary analyses. The lack of a clear difference in the results when analyzing users of different engagement-levels suggests any bias introduced by differences in the reporting habits of these users was small.

There was an overrepresentation of users from the province Zuid-Holland in our data, due to Zuid-Holland being the home province of the LUMC, the hospital in charge of app design/analysis. This also likely explains the over-representation of health care professionals, to whom the app was thoroughly advertised within the environment of the LUMC. Despite this overrepresentation, our sensitivity analyses excluding Zuid-Holland users and healthcare professionals showed similar results, suggesting any bias introduced by their overrepresentation is minimal. COVID radar users were more often female and middle-aged. This was due to the overrepresentation of healthcare workers (who were more often female and mid-aged). However the sensitivity analysis excluding healthcare workers resulted in no different conclusions.

Noteworthy too is the fact that fully 30% of those users reporting a positive SARS-CoV-2 test reported no symptoms on the day of the positive test (data shown in S. Figure 26). This is in line with the estimated number of COVID-19 carriers without symptoms, as reported by other studies [6, 7]. Our analysis likewise showed loss of smell and cough may continue for weeks following the positive SARS-CoV-2 test, as also confirmed in previous studies [17].

Limitations

All data in the app was self-reported and thus subjected to differences in personal interpretation of the questions. However, we do not expect differential misclassification as we see logical trends in symptoms and behavior on both individual and national levels. State-reported case counts were those reported by RIVM, whose data should include tests performed in private practices as they are required to be forwarded to RIVM. However, as there is no oversight for this process, the RIVM-reported case-counts likely represent under-estimates of the number of confirmed cases [18].

COVID radar additionally provided direct feedback to users on how their symptoms and behavior compared to their peers which likely has an effect on user behavior. This may bias the generalizability of COVID radar data, especially behavioral data. The effect of this feedback loop on users' behavior would be expected to lead to an overly conservative estimate of the behavior

of the population. Despite this, expected changes in reported behavior in the periods following national holidays and changes to social distancing policies are observed in COVID radar data. Additionally, altered behavior due to app-feedback would be expected to be observed in more loyal users of the app. Our sensitivity analysis on loyal users showed no significant difference in reported behavior. Given these realities, while we accept that app feedback altering user behavior has the potential to bias our results, we feel any bias introduced has been shown here to be small.

Testing capacity in the Netherlands was low during the developmental stage of the app and has increased during the study period. In the final months of 2020, testing was expanded to include those without symptoms. As a result, the prevalence of COVID-19 in the Netherlands could be underestimated. Because of this change in testing policy, the question regarding negative tests was implemented at a later date resulting in less data about negative tests compared to data on positive tests during a shorter period of time. Nonetheless, we were able to show that the association between symptoms and a negative test is less apparent than their associations with a positive test, suggesting our conclusions remain valid. Also, testing of the underaged (<12 years) was rare during the study period, resulting in a relatively old SARS-CoV-2 positive cohort in this study.

Future implications

Having validated the expected associations between symptoms, social-distancing behavior, and COVID case-count, our next steps will involve attempted prediction of emerging hotspots by combining symptom and social-distancing behavior data to quantify risk of COVID-19 cases. Such predictions could be used to help guide COVID-19 policy. Our study indicated the quality of the submitted data is best where user-engagement is high. Prediction-based goals will thus be aided by increasing user count. Regional predictions may additionally be improved through incorporation of data from general practitioners, more detailed demographic data, and mobility data using a machine learning based approach. Another possibility for further research is testing of associations between regional SARS-CoV-2 cases, symptoms, behavior and other regional data related, for example, to the physical environment.

Conclusion

The COVID Radar app successfully collects anonymous, user-reported data on COVID-19-related symptoms and social-distancing behavior. Initial validation showed symptoms and behavior reported within the app are correlated with in-app reporting of a SARS-CoV-2 test. The predictive potential of the COVID Radar is demonstrated as external validation showed in-app reported positive SARS-CoV-2 tests track well with state-reported case counts. Future research will focus on regional predictions using these data.

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SUPPLEMENTALS

See:

3

CHAPTER 3

Population-based data from the COVID RADAR app as a surveillance tool and predictor of COVID-19 related primary care demand

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Under Review

ABSTRACT

Introduction

Self-reported, population-based data may serve as a valuable surveillance tool for infectious disease outbreaks, especially when testing resources are limited. Using COVID-19 as a case study, we examined whether app-based reports on symptoms and behaviours could predict future primary care workload.

Methods

We analysed data from the COVID RADAR app (2020–2022), where users anonymously reported symptoms and COVID-related behaviours. These data were aggregated daily and linked to COVID-related primary care visits within the Extramural Leiden University Medical Center Academic Network Datawarehouse (≈250,000 patients). We employed recalibrated Poisson regression and Random Forest models, retrained quarterly, and validated performance using Root Mean Square Error (RMSE). Models were compared against those using only confirmed test counts.

Results

Over the study period, 9% of the population contacted primary care for COVID-related reasons. Both models showed moderate predictive accuracy, with the Random Forest model (RMSE ~22) underestimating early demand by a factor 2, and the Poisson model (RMSE ~71) overestimating during peaks by a factor 10. However, both outperformed test-only models (RMSE Poisson ~63,000; Random Forest ~36). The most important predictor was the proportion of app users reporting recent contact with a COVID-positive individual, increasing in importance over time.

Conclusion

These findings suggest that self-reported symptom and behaviour data may enhance surveillance and early prediction of healthcare demand during pandemics. Further improvement may be achieved through app refinement, integration of additional predictors, and enhanced modelling of transmission dynamics.

KEY MESSAGES

- In pandemics, surveillance is important for planning of healthcare demand
- In primary care, as the first line within the healthcare system, care demands are less predictable
- Population-based data from an app may offer valuable surveillance for prediction of primary care demand, particularly when testing capacity is limited.

INTRODUCTION

New infectious disease outbreaks present significant challenges to healthcare, due to limited knowledge and scarce data on disease characteristics, and exponentially increasing patient demand.(1, 2) During the latest pandemic, coronavirus disease (COVID-19), the demand for acute healthcare services (Intensive Care Unit beds) increased substantially, while the number of patients attending elective healthcare (e.g., chronic disease management, cancer screening) decreased.(3-5) Also in primary care this pandemic changed the workload.(6, 7) Although the number of short patient contacts by primary care physicians decreased during the COVID-19 pandemic, the number of contacts with patients in need of more intensive home visits increased in the Netherlands.(8) The limited availability of healthcare resources in general was associated with an increase of the mortality rate of COVID-19.(9) Therefore, careful planning and allocation of these resources is important during a rapidly evolving pandemic. To achieve this, an extensive surveillance system should be in place, i.e., a nationwide test-and-trace policy.

During the COVID-19 pandemic, an upsurge of the number of positive tests usually preceded an increase of hospital healthcare demand,(10, 11) and subsequently (approximately one week later) the number of Intensive Care admissions.(12, 13) In primary healthcare, as the first line within the healthcare system in the Netherlands, care demands are less predictable when based on the number of positive tests, as most patients visit their general practitioner (GP) before they test positive. Therefore, more rapidly available population-based data could support optimal resource distribution and workforce planning of COVID-19 related primary healthcare demand.

A nationwide smartphone app (COVID RADAR), collected anonymous individual data about COVID-19 related symptoms, risk behaviour, and positive test results among community-dwelling participants from April 2020 until February 2022.(14, 15) In prior research, these data were successful in predicting the replication rate of COVID-19.(16)

In this study we will describe the COVID-19 related primary care demand and show the value of population-based data during a pandemic by predicting COVID-19 related workload in primary care in the Leiden and The Hague area in the Netherlands.

METHODS

Study design

We used data from the COVID RADAR app as input to predict the COVID-19 related workload, as expressed by data from the Extramural Leiden University Medical Center Academic Network (ELAN) Datawarehouse. COVID RADAR app users were asked for informed consent upon first use of the app. Patients enlisted with ELAN primary care practice centres could withdraw via an informed opt-out procedure.(17) All data were aggregated by day and covered the region of Leiden and The Hague (with a total of 2 million inhabitants) from January 2020 until February

2022. Ethical approval was granted by the Medical Ethical Board of the LUMC (dossier number N20.070 and 20.080) and the ELAN scientific board (dossier number 895648).

COVID RADAR app

A comprehensive overview of the COVID RADAR app's operations, data collection, and data processing has been previously published.(14) In summary, the COVID RADAR app was a free app that prompted users to anonymously report COVID-19 symptoms and associated behaviours through a brief daily questionnaire. The questions covered symptoms as well as contacts with patients with COVID-19. Users also provided details on SARS-CoV-2 test results and vaccination status; the specific questions are outlined in supplemental table 1. Various national media campaigns, including social media, promoted app usage. The questionnaire design allowed for updates based on changes such as new mitigation measures and evolving scientific insights during the pandemic. No detailed personal information was registered in the app; age was registered in categories of decades and location was registered via the first four digits of the postcode, preserving anonymity of users.

COVID-19 related workload in primary care

The aggregated daily COVID RADAR data were linked to aggregated daily data from the ELAN Datawarehouse. The ELAN Datawarehouse contained electronic health records (EHRs) from approximately 250,000 persons, which was stable during the research period. Data from the ELAN Datawarehouse were extracted from the GP practices each 12 weeks.

We selected EHRs from patients with a respiratory complaint in 2020 - 2022, coded following the International Classification of Primary Care (ICPC). Within these patients we selected records with COVID-19 as the primary reason for contact, from free text and relevant codes (see supplemental document 1 for details about this selection). We included at most one contact per day per person. We excluded records reporting tests results, e-mail or notes, since this is not actual 'patient contact time'. We extracted the following data from the selected patients from the ELAN Datawarehouse: age, sex, type of contact (in-person or not (telephone, etc.), during in- or out-office hours), and if this contact resulted in referral to a hospital.

Descriptive analyses

Firstly, we described the temporal patterns of COVID-19 related workload registered in ELAN during the pandemic expressed in terms of the number and type of contacts, and associations with referral to the hospital.

Secondly, we described the daily temporal patterns of variables from the COVID RADAR about number of reports, symptoms (using the European Centre for Disease Prevention and Control

(ECDC) definition for COVID-19 like symptoms: Cough, fever, shortness of breath or anosmia),(18) behaviour and proportion of users reporting recent contact with a COVID-19 patient.

We compared both data sources (the app and ELAN) with state-reported numbers of positive test results and numbers of new hospitalizations. These data are publicly available and provided by the National Institute for Public Health and the Environment (RIVM).(19)

Predictive analyses

We smoothed the outcome (number of COVID-19 related primary care contacts) using the 3 days prior and 3 days after each point (7-day range) to account for the strong weekly pattern. We used two candidate predictors from the COVID RADAR app: (1) the daily proportion of users who had recent contact with a COVID-19 patient over the past seven days (risk contacts) and (2) the daily proportion of users reporting an ECDC-defined COVID-like symptom over the same period. We divided the study period in blocks of 12 weeks that corresponded with the frequency with which data were extracted from the ELAN Datawarehouse during the pandemic. We designed our model with a training and validation approach in the following way to mimic as much as possible the real world situation in which this model was developed during the pandemic. In a first step, we fitted a regression model on the COVID-19 related healthcare demand data of the first block of 12 weeks using predictors in this block and used this model to make a prediction for the demand of the subsequent block of 12 weeks using the predictors from the first block. Subsequently, this model was recalibrated using data from both the first and second blocks, and we used this new recalibrated model to make a prediction for the third block. This was repeated until February 2022 (end of COVID RADAR data collection), yielding for each of the periods a daily prediction made by a model fitted using data from all preceding periods.

We compared two types of models: a Poisson regression model and a Random forest model (using a grid search with prespecified limits to optimize hyperparameters, details in supplemental document 2). Model fits were compared with the root mean square error (RMSE). We report the coefficients of the Poisson model and the relative importance of the candidate predictors (based on the mean decrease in RMSE) in the Random forest model for each time block.(20)

To compare the added value of the app's data, we also performed the same prediction procedure including all three models with the number of state reported positive tests as a predictor. Statistical analyses were performed using STATA 16.1.

RESULTS

COVID-19 related primary care contacts

Over the research period (January 2020 until February 2022; ~750 days) in approximately 250,000 registered patients (in an area of 2.5 million inhabitants), 47,974 COVID-19 related primary care

contacts in 22,338 (9%) unique patients were registered (table 1). The GP referred 13% (n=2,901) of these patients for further workup, which could result in hospital admission. Patients referred to a hospital were more often older than 40 years of age. Contacts that resulted in referral were more often in-person during office hours (see table 1). Most COVID-19 related primary care contacts were in the second half of 2020. From the second half of 2021 onwards the proportion of patients who was referred to hospital increased. The number of COVID-19 related primary care contacts, number of positive tests and hospitalizations showed similar patterns through time (see figure 1).

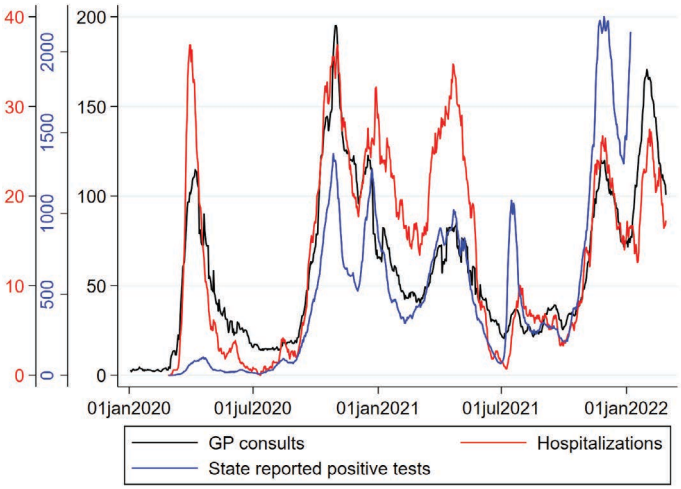

Figure 1: Temporal daily trends of primary care consults, hospitalizations and positive tests due to COVID-19 (all 7 days mean smoothed). Number of positive tests (blue line) stops one month earlier because of a large number of positive tests in February 2022 (35,000) that would have limited the informativeness of the scale.

Risk contact and symptom data

Over the research period, out of approximately 2.5 million inhabitants, 86,037 individuals used the COVID RADAR app in total 2.8 million times (see supplemental table 2). A median number of 7,800 (IQR 4,600; 10,800) unique individuals used the app each week. See for patterns of app usage, reports of positive tests and behaviour the supplementary document 3. Figure 2 shows the daily proportion of users reporting a recent contact with a COVID-19 patient, and figure 3 shows the proportion of users reporting symptoms defined as 'COVID-like symptoms' by the ECDC. The proportion with recent contact with a COVID-19 patient showed higher variability in accordance with the number of COVID-19 related primary care contacts than the proportion of users with symptoms.

Table 1: Patients and their contacts with their GP because of COVID-19, stratified by subsequent referral to the hospital

Referral to hospital		**No**	**Yes**
Number of Patients		19437 (87%)	2901 (13%)
Number of Contacts	1	12702 (97%)	425 (3%)
	2	3669 (88%)	490 (12%)
	3	1456 (74%)	499 (26%)
	>3	1610 (52%)	1487 (48%)
Sex	Female	11508 (87%)	1792 (13%)
Age, mean, SD		43.2 (21.2)	51.0 (19.1)
Age category	<18	2643 (95%)	125 (5%)
	18-39	5832 (90%)	653 (10%)
	40-59	6362 (84%)	1174 (16%)
	>60	4600 (83%)	949 (17%)
Number of contacts		42694 (92%)	3980 (9%)
Contact type	Phone, in office hours	26339 (95%)	1302 (5%)
	Consult, in office hours	12190 (83%)	2549 (17%)
	Phone, out of office hours	1253 (95%)	61 (5%)
	Consult, out of office hours	815 (93%)	60 (7%)
Quarter	2020q1	1732 (97%)	57 (3%)
	2020q2	3877 (93%)	310 (7%)
	2020q3	2261 (92%)	190 (8%)
	2020q4	10865 (95%)	512 (5%)
	2021q1	4475 (91%)	470 (9%)
	2021q2	4329 (87%)	638 (13%)
	2021q3	2280 (84%)	447 (16%)
	2021q4	6168 (89%)	727 (11%)
	2022q1	6655 (91%)	629 (9%)

SD: Standard deviation.

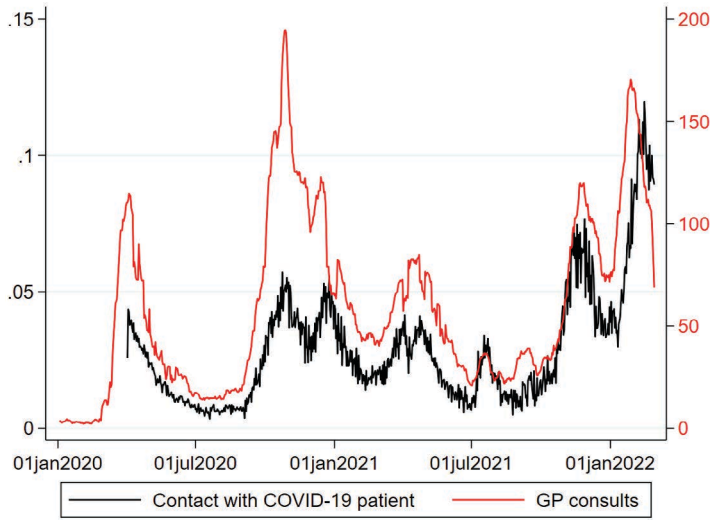

Figure 2: Daily proportion of app users reporting recent contact with COVID-19 patient (<14 days) Red: number of GP consultations because of COVID-19

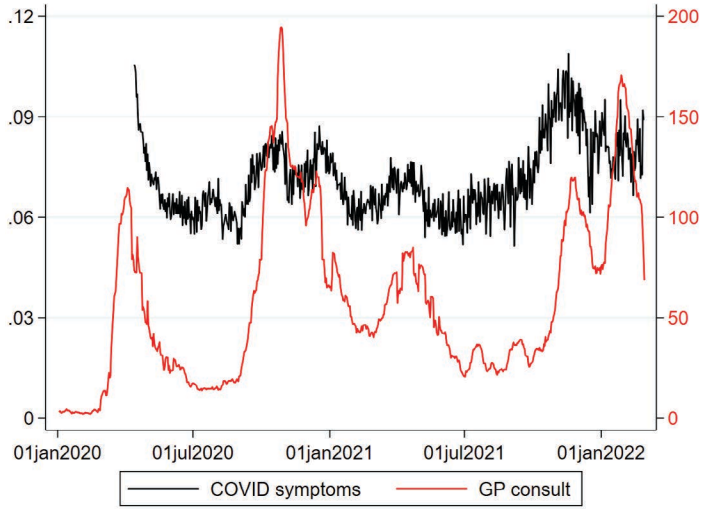

Figure 3: Daily proportion of app users reporting symptoms from ECDC definition of COVID-19 ECDC: (cough, fever, shortness of breath or loss of smell/taste) Red: number of GP consultations because of COVID-19

Prediction

Using a quarter-frequent recalibration, both the Poisson regression and the Random forest model showed patterns in their predictions in line with the healthcare demand outcome (see figures 4 and 5). Their performance, tabulated in table 2, was poor during sudden increases of COVID-19 related primary care demand (Q4 of 2020). Later during the pandemic the Poisson model overestimated the number of COVID-19 related primary care contacts by a factor of 10. The Random forest model underestimated the care outcomes during the first wave (predicting a peak of 100 daily COVID-19-related contacts during the first wave, whereas the actual number was nearly 200) but had more accurate prediction later in the pandemic. Both models anticipated more contacts during the 'Dansen met Janssen'-wave (a superspreader event in the Netherlands, during which predominantly young people were allowed to 'go dancing' directly after a single shot of the Johnson & Johnson vaccine in June 2021). All predictions with COVID RADAR data were superior to predictions using state reported test results (see table 2 and supplemental figure 1; RMSE COVID RADAR between 10 - 252 vs RMSE test between 22 - 441,297).

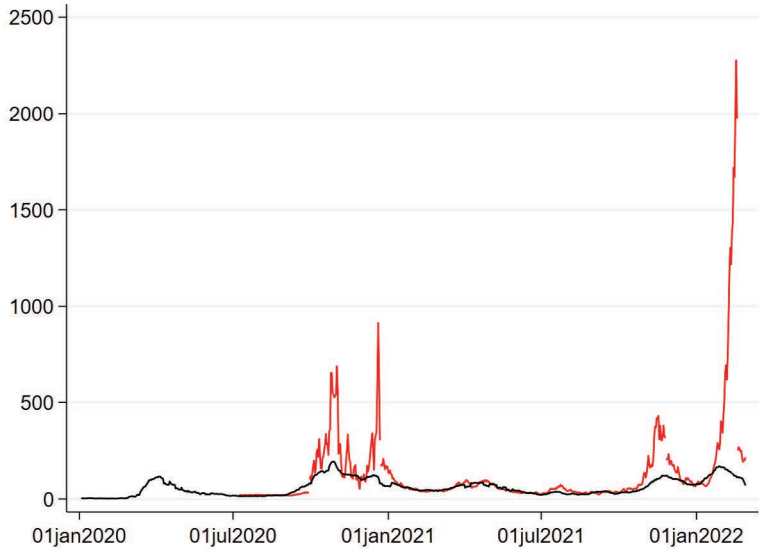

Figure 4: Predicted values for Poisson regression model (red) and actual number of COVID-19 related GP consults (black). Gaps because of recalibration of the model.

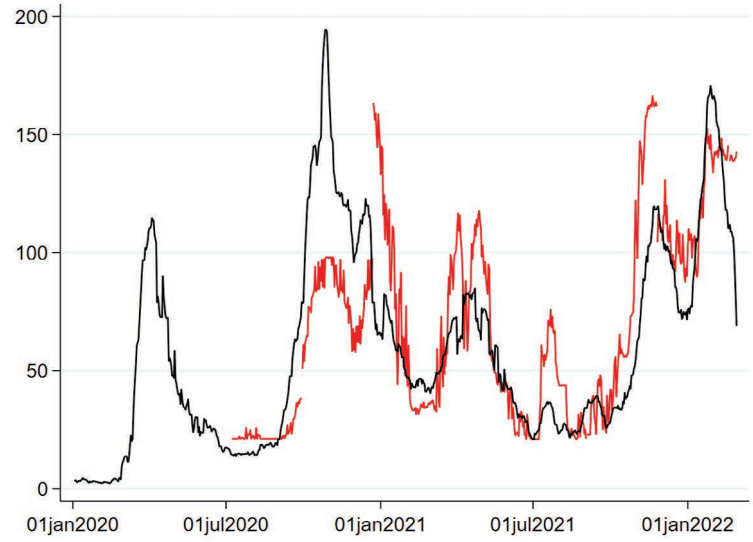

Figure 5: Predicted values for Random forest model (red) against actual number of COVID-19 related GP consults (black). Gaps because of recalibration of the model.

Table 2: Root mean square of the errors of predictions in the quarter after the training period. Quarters are not 'exact' quarters, but periods of 12 weeks, starting in July 2020. Last quarter starts end of November 2021 and ends in February 2022.

RMSE predictions outside training set		Poisson tests results	Random forest tests results	Poisson COVID RADAR	Random forest COVID RADAR
Quarter	2020q3	662	31.8	10.1	10.3
	2020q4	2837	67.3	130.6	48.2
	2021q1	25.7	42.1	19.2	23.0
	2021q2	21.9	30.5	10.4	16.5
	2021q3	26.6	31.5	10.8	11.3
	2021q4	52.4	21.9	62.2	24.5
	2022q1	441297	29.5	252	17.2

The variable 'risk contact' had the highest relative contribution in both models (see supplemental table 2). The contributions of the predictors were most stable in the Random Forest model, while the Poisson models exhibited unstable predictor coefficients. During the first 12 weeks symptoms had a slightly higher contribution to the Random forest model than later during the pandemic. No major shifts were seen in the selected days, whereas recent days were the most important.

DISCUSSION

We described COVID-19-related primary care volume in the Netherlands and evaluated whether population-based self-reported data could predict care demand over time. During the pandemic, 9% of the population contacted their GP for COVID-19-related concerns. Using simple models with quarterly recalibration, self-reported symptom and behaviour data moderately predicted care demand and outperformed models based solely on positive test results. While context-specific and in need of refinement, such data offer valuable predictive insights—especially early in a pandemic when testing is limited.

Capacity for testing was limited at the start of the pandemic in the Netherlands, resulting in a underestimation of SARS-CoV-2 infections.(21) Later in the pandemic, care demand declined relative to reported cases, likely due to increased testing, vaccination, new variants, and greater public awareness. Deviations in our predictions may also stem from limitations of models not tailored to capture pandemic complexity. The Poisson model's overestimations may reflect violations of event independence, reduced accuracy with common events, and overdispersion, where variance exceeds the mean.(22, 23) During the pandemic, the Random forest model showed improved performance; however, its performance was poor during the second wave (Q4 2020). Random forests predict within the range of observed training data, as they average target values within decision tree leaves. Thus, they cannot forecast values beyond prior extremes—e.g., the Q4 2020 ceiling of ~100 GP contacts reflected earlier data limits. Predictions near this maximum should be flagged as exceeding historical bounds. Future pandemic surveillance models

should adapt to the pandemic phase. Early on, extrapolative models (e.g., linear or Poisson regression) may be more suitable, while later phases could benefit from complex models (e.g., Random Forest) to capture interactions and improve precision. More frequent recalibration could help, though current data availability in quarterly blocks limits this. Incorporating assumptions about delays between risk behaviour, symptom onset, and GP visits—similar to compartmental models—may further enhance predictions.(16)

This study is the first to address prediction of COVID-19 related primary care workload using population-based data from an app. The use of an anonymous and voluntary-to-use app in pandemic surveillance has several advantages, especially during the start of a pandemic. It is easy to use, has privacy by design and operates instantaneously. To improve quality of surveillance and predictive performance in future pandemics, the questionnaire could be adapted, in such a way that the moment of contact with a COVID-19 patient is known more accurately. More complex models could be used, including predictors from other domains (such as the weather), tailored to future pandemics.

Data from a voluntary and anonymous app also has disadvantages. App users were not a random sample of the population and we know that demographics of users were not stable over time.(14) However using periodic recalibration, the impact of this bias was limited. The variable with highest predictive performance was 'risk contact with a COVID-19 patient'. However, knowledge about risk contacts is still partly dependent on testing capacity, which is limited during the start of a pandemic. This may explain why, in the early stages of the pandemic, symptoms carried relatively more weight in the models. Though variables about symptoms do not have this dependency, at the start of a future pandemic it may not yet be known which symptoms are predictive (such as loss of taste and/or smell during COVID-19). A questionnaire based app should be dynamic, allowing updates based on newest insights about disease characteristics. Self-reported data could suffer effects of misspecification of symptoms or underestimation of true contact with COVID-19 patients (because of unknown contacts). Other surveillance apps use Bluetooth or GPS for contact tracing, registering unknown contacts as well; however this approach raises privacy concerns and therefore limited usage uptake.(24-26)

During the latest pandemic, the COVID RADAR app was not used for proactive care planning, therefore its effectivity cannot be determined. Research on effects of digital contact tracing apps and digital surveillance is limited, but available studies show moderate effects.(27, 28) However, these studies focus on the effect of individual tracing on the growth of the pandemic, which is a different aim compared with the current study (pandemic surveillance and prediction of healthcare usage).

Conclusion

Concluding, population-based data from an instantaneously operating app has potential for use as a surveillance tool during a pandemic and prediction of (primary) healthcare demand and outperforms predictions based on the number of positive tests.

Disclosure statement: The authors report no conflict of interest

Data availability statement Raw data of the COVID RADAR app are available in a public, open access repository. Data are accessible on https://doi.org/10.17026/dans-zcd-m9dh

Data deposition Data are accessible on https://doi.org/10.17026/dans-zcd-m9dh

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SUPPLEMENTAL MATERIAL

See:

4

CHAPTER 4

Incidence, symptom clusters and determinants of post-acute COVID symptoms - a population based surveillance in community dwelling users of the COVID RADAR app

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ABSTRACT

Objectives: This study aims to describe the incidence, symptom clusters, and determinants of post-acute COVID symptoms using data from the COVID RADAR app in the Netherlands.

Design: Prospective cohort.

Setting: General population in the Netherlands from April 2020 to February 2022.

Participants: A total of 1478 COVID RADAR app users, with data spanning 40 days before to 100 days after positive SARS-CoV-2 test.

Outcome measures: Incidence and duration of 10 new symptoms that developed during acute infection, defined as ten days prior and ten days after positive test. Clustering of these post-acute COVID symptoms and associations between factors known in the acute phase and 100 day symptom persistence.

Results

The most frequent post-acute symptoms were cough, loss of smell or taste, and fatigue. At 100 days post-infection, 86 participants (8%) still experienced symptoms. Three post-acute COVID symptom clusters were identified: non-respiratory (headache and fatigue; 49% of participants with post-acute COVID symptoms); olfactory (15%) and respiratory (8%). Vaccination was associated with a lower risk of post-acute COVID symptoms 100 days after infection, albeit confidence intervals were wide (OR 0.5; 95%CI 0.2, 1.5), but not with non-respiratory symptoms (OR 1.0; 95%CI 0.3, 4.4). Severe acute disease increased the risk of post-acute COVID symptoms (OR 1.4; 95%CI 1.2, 1.5; per additional acute symptom).

Conclusions

In this cohort of infected community dwelling app users, 5-10% experienced post-acute COVID symptoms. The symptoms cluster in several distinct entities, which differ in incidence, patient characteristics and vaccination effects. This suggests multiple mechanisms underlying the development of post-acute COVID symptoms.

Strengths and limitations of this study

- This study uses data from community dwelling participants
- This study was able to measure newly developed symptoms on the individual level, by taking prior symptoms into account
- Detailed data collection that allowed adjustment for several possible confounders
- Participation was based on self-selection, which could result in oversampling of users experiencing symptoms.

INTRODUCTION

In the past years, millions of people have been infected with SARS-CoV-2.[1] An infection with SARS-CoV-2 can be followed by long lasting symptoms, with substantial impact on life.

These longlasting symptoms are referred to as “post-COVID”, “Post-acute sequelae of COVID-19” or “long Covid”, with fatigue as most frequently reported symptom.[2]

Several hypotheses about the pathogenesis of post-COVID include persistent presence or reactivation of viruses, tissue damage, auto-immunity or changes in the microbiome.[3] But also endothelial activation, coagulation activation and the formation of neutrophil extracellular traps (NET) are proposed mechanisms of post-COVID.[4, 5, 6, 7]

Research on post-COVID is challenging. Studies that have been performed on the subject varied in selection (many based on clinical cohorts), and length of follow-up. This heterogeneity, and that of definitions of post-COVID regarding type, duration, number, and severity of symptoms, has led to a wide range of prevalence estimates, from 5 to 50%.[8, 9] In addition, several subtypes of post-COVID have been proposed, increasing the complexity of studying these symptoms.[10]

The World Health Organization (WHO) defines post-COVID as symptoms not otherwise explained, persisting longer than two months following COVID-19 diagnosis in the past three months.[11] WHO emphasized that the definition is temporary as it is based on analyses of small studies with short follow-up in mostly hospitalized patients. Hence they advise to obtain new evidence from prospective studies with sufficient follow-up time, in less selected patient groups such as in primary care and community-dwelling people. An ideal study would be a large prospective longitudinal cohort of community-dwelling people with a sufficient number of repeated measurements for each patient before and after a SARS-CoV-2 test result.

In response to this, we utilized data from the COVID RADAR smartphone app in the Netherlands, active from April 2020 until February 2022, with which users anonymously answered a short daily questionnaire about their symptoms, SARS-CoV-2 test results, and vaccination status.[12] Using these data we were able to distinguish newly developed symptoms during acute SARS-CoV-2 infection from pre-existing symptoms.

In this study, our main objective was to identify symptoms persisting beyond the acute phase (post-acute COVID symptoms). Secondary, we aimed to identify clusters of post-acute COVID symptoms, i.e. possible subtypes of Post-COVID, and we investigated which factors in the acute phase (such as severity of disease and vaccination status) were associated with (clusters of) symptoms persisting at least 100 days after a positive test.

METHODS

COVID RADAR app

The COVID RADAR app was a free app through which users were asked to anonymously report on 10 different COVID-19 related symptoms by filling in a short daily questionnaire, with questions such as “Did you cough?” or “Did you have a fever?”.[12] In addition, users gave information about SARS-CoV-2 test results and vaccination status. See for details about the questions and other collected variables supplemental table 1. Participation in the app was voluntary; allowing participants to start, pause or stop using the app at their discretion. Different national (social) media campaigns encouraging usage of the app resulted in 284,000 individual users that filled out the questionnaire more than 8.5 million times between April 2020 and February 2022.

Ethical approval was provided by the Medical Ethical Board of the LUMC (dossier number N20.067). Upon first use of the app, users are asked to provide informed consent to share the information with the research institution. See the supplemental material and prior publication for more details.[12]

Patient and Public Involvement

Several focus groups interviews and qualitative thematic analysis on end-user emails were conducted.[13] Based on the experiences and feedback of these users we made several adjustment to the app. The app was dynamic, which allowed for updating questions in response to changes, for instance, changes in mitigation measures, but also improvements in user experience.

Definitions

We defined the acute phase of COVID-19 as the period between ten days prior and ten days after a report of a positive SARS-CoV-2 test. An acute symptom was defined as a symptom reported at least once during the acute phase.

The prior phase of COVID-19 was defined as the period between 40 and 11 days prior to a positive test result (see supplemental figure 1). A ‘prior symptom’ was defined as a symptom reported at >50% of a participant’s available observations during the prior phase. A symptom was considered ‘new’ if it developed during the acute phase, but was not a ‘prior symptom’.

For each symptom the day of recovery was defined as the day when this symptom was not reported by the participant in 14 consecutive days. The duration of symptoms was calculated for each symptom as the number of days between the onset of the symptom in the acute phase and the first day of recovery from the symptom. If this duration lasted longer than the ‘acute phase’, this symptoms was considered a ‘post-acute COVID symptom’.

Inclusion criteria

We included participants who reported their first positive test and had at least three prior app entries. They needed to answer the questionnaire for at least 100 days after their positive test or until they fully recovered from all new symptoms within those 100 days. Participants were considered lost to follow-up if they did not report for 14 days. We excluded those who were lost to follow-up before 100 days after positive test and were not recovered at their last report in the app. Since 26 October 2020 (7 months after the launch of the app) the symptoms 'fatigue' and 'headache' were added to the questionnaire. Given that these two symptoms were frequently reported in prior research as post-acute COVID symptoms,[11] we included only participants with reports of positive SARS-CoV-2 tests after 5 December 2020 (40 days after 26 October) in the present study, so all included participants could report all symptoms in the 'prior phase' and 'acute phase'.

Statistical analyses

To describe the incidence and duration of symptoms developed during the acute SARS-CoV-2 infection (new symptoms), we used histograms and median durations.

A correlation matrix was used to analyse which new post-acute COVID symptoms were associated with each other (e.g., symptom clusters). Correlations were based on the durations until recovery of each new symptom and clustered using agglomerative hierarchical clustering, with a complete linkage method. For this analysis data of participants were used when at least two symptoms were present for at least 15 days, to confirm they had symptoms lasting longer than the acute phase.

To analyse which factors in the acute phase of SARS-CoV-2 were associated with post-acute COVID symptoms, we focused on post-acute COVID symptoms that lasted until 100 days after SARS-CoV-2 test result. We included participants with a positive test between 6 December 2020 and 20 November 2021 (100 days before the end of data collection). We performed multivariate logistic regression analyses with the outcome "persistence of any new post-acute COVID symptom at 100 days after test result" and with the outcome "persistence of only post-acute COVID symptoms from symptom cluster X at 100 days after test result" (from the previous research question).

For the association between vaccination and new post-acute COVID symptoms at 100 days after test result we estimated odds ratios (adjusted for sex, age, livability index and period of infection). The primary aim of vaccination is to prevent severe COVID-19. It is likely that the mechanism by which vaccination affects persistence of post-acute COVID symptoms, might be through the effect of vaccination on severity of acute disease (mediation, see supplemental figure 2). To investigate this, we first used linear regression to assess the association between vaccination and number of newly developed acute symptoms (severity) and secondly we used logistic regression to assess the association between number of newly developed acute symptoms and persistence of new post-

acute COVID symptoms. Subsequently, the possibility of a mediating effect of severity of disease in the association between vaccination and persistence of new post-acute COVID symptoms was assessed by adding the number of newly developed acute symptoms to the model and compare the association between vaccination and persistence of new post-acute COVID symptoms with and without this adjustment. A difference between these associations indicates a possible mediating effect. In analyses with 'number of newly developed acute symptoms' we used only participants with at least one newly developed symptom in the acute phase.

The definitions of a 'prior symptom' (symptom reported at >50% of a participant's available observations during the prior phase) and recovery (no symptom in the 14 consecutive days) were based on clinical judgement and not on prior literature. We performed several sensitivity analyses with variations of abovementioned definitions of prior symptoms and recovery or with different lengths of follow-up. See for details about these analyses the supplementals. All statistical analyses were caried out in STATA 16.1, with the exception of the clustering analysis which was performed using Python (using the Scipy package). Data are accessible on https://doi.org/10.17026/dans-zcd-m9dh.[14]

RESULTS

During the research period 58,672 participants used the app (with in total 986,623 person days of data). From these, 3,642 participants reported a first positive test result of whom 1,478 participants met the inclusion criteria (see figure 1). 1,675 participants filled in the app fewer than three times in the prior phase and an additional 489 participants filled in the app for a period less than 100 days and stopped using the app before their recovery. Of those included 865 (59%) were female, 859 (58%) were 60 years or older and 614 (42%) were fully vaccinated before infection. Excluded participants were younger (709 (33%) was 60 years or older) and more frequently vaccinated (1081 (50%); see supplemental table 2). The pattern of app use is shown in supplemental figure 3.

New symptoms after acute SARS-CoV-2 infection and their duration

The majority of participants had at least one newly developed symptom (1,097, 74%). The most frequently reported symptom was cough (776, 53%). Symptoms that most frequently lasted longer than 90 days were shortness of breath, loss of smell or taste and fatigue (see figure 2 and supplemental table 3).

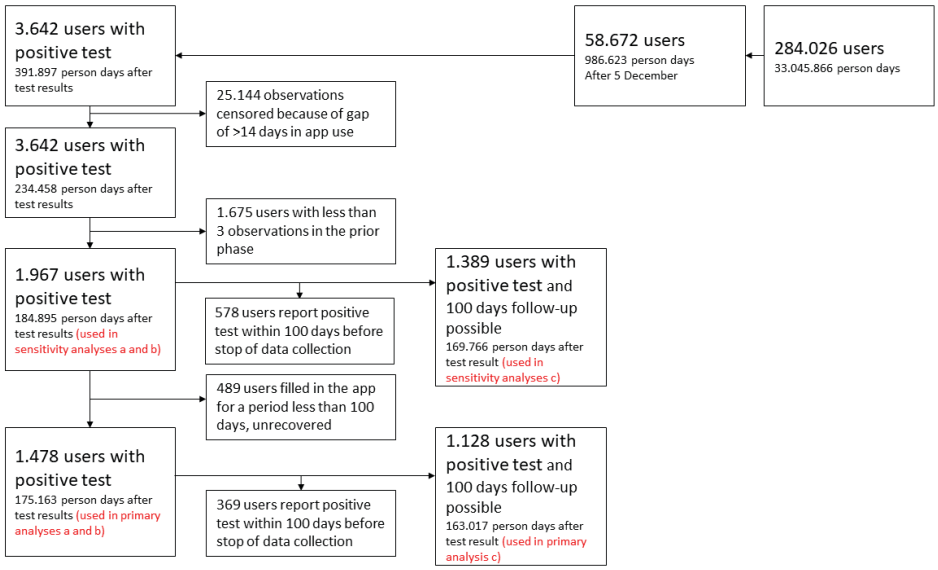

Figure 1: Flowchart of inclusion

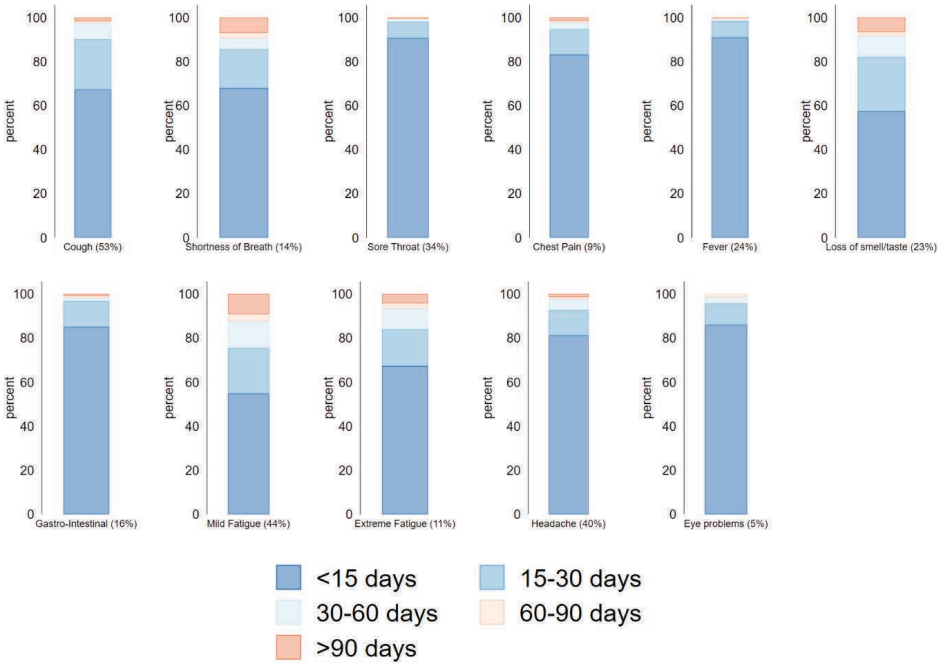

Figure 2: Duration of new symptoms Example: 783 (53%) of 1478 participants experience coughing during the acute phase (but not during the prior phase), of whom 65% (n=509) recovered within 15 days

Clustering of post-acute COVID symptoms

For 447 participants at least two symptoms lasted for over 15 days. Correlations between duration of post-acute COVID symptoms were low (see figure 3). The symptom 'Loss of taste or smell' (i.e., Olfactory symptoms) showed low correlations with all other post-acute COVID symptoms; and was subsequently clustered as a separate entity. The symptoms "Fatigue" and "Headache" (i.e., non-respiratory symptoms) had highest correlations, and were also clustered. "Headache" was also correlated with some post-acute COVID respiratory symptoms. The respiratory symptoms ("Cough", "Sore Throat" and "Shortness of breath"; i.e. Respiratory symptoms) were correlated and formed the third cluster of post-acute COVID symptoms.

The sensitivity analysis including the participants without recovery before loss to follow-up and assuming recovery at day of loss of follow-up (N=519), showed similar results (supplemental figure 4).

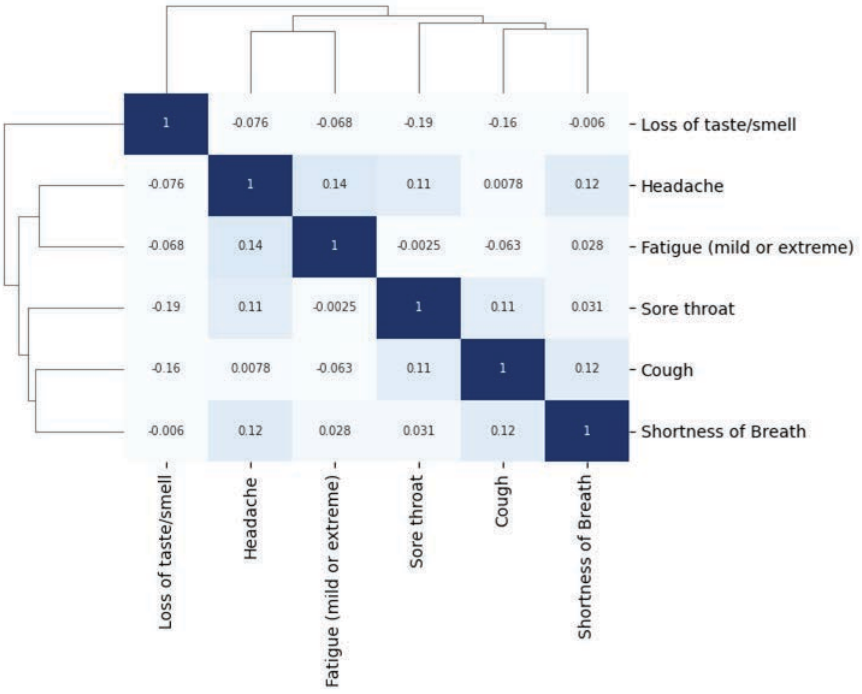

Figure 3: Correlation heatmap
Spearman correlations between duration of post-acute COVID symptoms in participants with at least two symptoms lasting for over 15 days. Using agglomerative hierarchical clustering with a complete linkage method three post-acute COVID symptom clusters were identified: "Olfactory symptoms", "Non-respiratory symptoms" (Fatigue and Headache) and "Respiratory symptoms".

Determinants associated with post-acute COVID symptoms at 100 days

Among the 1,478 participants, 1,128 reported a positive test before 20 November 2021 (100 days before end of data collection). Of these, 86 (7.8%) had new post-acute COVID symptoms persisting 100 days after a positive test. The proportion of patients with post-acute COVID symptoms was higher in the group infected during the period the alpha variant was most prevalent than during the delta variant period (proportion post-acute COVID symptoms at day 100 after infection with alpha variant: 8.7% vs 4.1% after delta variant). Patients with post-acute COVID symptoms persisting beyond 100 days were mostly female (61 (71%) with post-acute COVID symptoms vs 603 (58%) without post-acute COVID symptoms), of young age (47 (55%) vs 451 (43%) aged under 60 years), more often unvaccinated (76 (88%) vs 779 (75%)), and living in areas with a lower mean livability index (mean Z-score of 0.3 vs 0.5; see table 1).

Table 1: Characteristics of infected patients at the acute phase by new post-acute COVID symptoms at 100 days

Post-acute COVID symptoms at 100 days		**No**	**Yes**
Number		1042 (100%)	86 (100%)
Sex	Female	603 (58%)	61 (71%)
Age	<=18	45 (4.3%)	2 (2.3%)
	19-39	55 (5.3%)	8 (9.3%)
	40-59	351 (34%)	37 (43%)
	>60	591 (57%)	39 (45%)
Vaccinated		263 (25%)	10 (12%)
Time of infection			
Period 1 (Nov 20 – Jun 21)		787 (91%)	75 (8.7%)
Period 2 (Jul 21 – Nov 21)		255 (96%)	11 (4.1%)
Vaccination and time of infection			
Period 1 (Nov 20 – Jun 21) vaccinated		34 (94%)	2 (5.6%)
Period 1 (Nov 20 – Jun 21) unvaccinated		753 (91%)	73 (8.8%)
Period 2 (Jul 21 – Nov 21) vaccinated		229 (97%)	8 (3.4%)
Period 2 (Jul 21 – Nov 21) unvaccinated		26 (90%)	3 (10%)
Without prior symptoms		860 (83%)	69 (80%)
Newly developed acute symptoms, median (IQR)		2 (0, 4)	5 (4, 6)
Livability index (mean, SD)		0.05 (0.11)	0.03 (0.11)

SD: Standard Deviation. IQR: interquartile range

The risk of post-acute COVID symptoms at 100 days was lower in vaccinated participants compared to unvaccinated participants, though with wide confidence intervals (adjusted odds ratio (aOR) 0.5; 95% CI 0.2 , 1.5; adjusted for age, sex, time period and livability index, see table 2). This association was similar in both periods of infection. Vaccination was associated with fewer new symptoms during the acute phase, as a proxy for severity of acute infection (beta -0.9; 95% CI -1.6, -0.3), same adjustments as prior analysis), and was also associated with asymptomatic COVID-19 (aOR 2.8; 95% CI 1.6 , 5.1; same adjustments as prior analyses).

In participants with symptoms during the acute phase (n=819), the number of newly developed symptoms in the acute phase was positively associated with new post-acute COVID symptoms at 100 days after a positive test (aOR 1.4; 95% CI 1.2, 1.5 for each additional symptom in the acute phase, see table 2).

Including the number of newly developed symptoms during the acute phase (as a proxy for severity of acute infection) the association between vaccination and post-acute COVID symptoms at 100 days, changed from 0.6 to 0.7, which implies that severity of disease is a possible mediator in the effect of vaccination on post-acute COVID symptoms.

Table 2: Odds ratios for post-acute COVID symptoms at day 100

Symptoms at 100 days	**Odds ratio (95% CI) (adjusted 1)**	**Odds ratio (95% CI) (adjusted 2)**	**Odds ratio (95% CI) (adjusted 3)**
Vaccination			
In all included participants (86/1128)	0.4 (0.2 – 0.8)	0.5 (0.2 – 1.5)	0.6 (0.1 – 2.5)
In symptomatic participants (86/819)	0.5 (0.2 - 0.9)	0.6 (0.1 – 2.8)	0.7 (0.1 – 3.5)
Number of symptoms in acute phase			
In all included participants (86/1128)	1.5 (1.4 – 1.6)	1.5 (1.3 – 1.6)	-
In symptomatic participants (86/819)	1.4 (1.2 - 1.5)	1.4 (1.2 – 1.5)	-

CI: confidence interval
Adjusted 1) by age; sex and livability index
Adjusted 2) Adjustment 1 + period of infection
Adjusted 3) Adjustment 2 + number of new acute symptoms

Determinants associated with post-acute COVID symptom clusters

Within participants with post-acute COVID symptoms at day 100 (N=86), most had only non-respiratory symptoms at day 100 (42 (49%)), followed by only olfactory symptoms (12 (14%)) (see figure 4 and supplementary table 4). Participants with respiratory symptoms at day 100 (23 (27%)) often reported symptoms from the non-respiratory (i.e. headache and fatigue) cluster too (13 (15%)).

Only women reported post-acute COVID olfactory symptoms at day 100. Post-acute COVID respiratory symptoms were observed more in men than women (5 (71%) participants with post-acute COVID respiratory symptoms were men vs 14 (26%) in other symptom clusters) and in those who already reported other symptoms prior to infection (4 (57%) vs 5 (9%) in other symptom clusters reported prior symptoms). Post-acute COVID olfactory and post-acute COVID respiratory symptoms originated more frequently in the first half of 2021 (during the alpha variant) than during the second half of 2021 (during the delta variant) compared with post-acute COVID non-respiratory symptoms (18 (95%) vs 35 (84%) originated after the alpha variant respectively). Participants with non-respiratory symptoms at day 100 were in many ways similar to patients without symptoms at day 100, e.g., concerning vaccination status and period of infection (supplemental table 4).

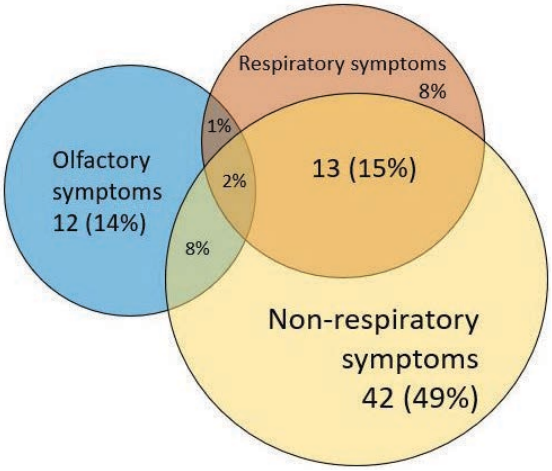

Figure 4: Distribution and overlap of three identified post-acute COVID symptom clusters. Total 86 participants with symptoms at day 100 (7.6%) Two of them were not part of any cluster.
Respiratory symptoms: cough, shortness of breath and sore throat; Non-respiratory symptoms: fatigue and headache; Olfactory symptoms: loss of taste or smell; For example: 42 participants (49%) reported only non-respiratory symptoms at day 100 (newly developed at the acute phase).

Vaccination in the acute phase were not associated with the presence of only post-acute COVID symptoms at day 100 from the non-respiratory cluster (aOR 1.0; 95%CI 0.3 , 4.4, see supplemental table 5). The number of symptoms in the acute phase was associated with the presence of only post-acute COVID symptoms at day 100 of the non-respiratory cluster (aOR 1.3; 95% CI 1.1, 1.5).

Sensitivity analyses

Sensitivity analyses using different definitions for prior symptoms (25% or 75% of prior observations with symptoms), different definitions for recovery (7 days or 21 days without symptoms), 60 days of follow-up instead of 100 days and including participants without recovery before loss to follow-up (assuming direct recovery or recovery at timepoint) showed small differences in estimates which did not alter conclusions (details reported in supplementals).

DISCUSSION

Using data from a population based app, with voluntary users, in the Netherlands we found that between 5% and 10% of participants still reported symptoms, newly developed during the acute phase, at 100 days after acute COVID-19. Most common post-acute symptoms were loss of smell and taste, fatigue, and shortness of breath.

Using a data driven approach we found three post-acute COVID symptom clusters: non-respiratory symptoms (headache and fatigue); olfactory symptoms and respiratory symptoms. The symptom

clusters differed in the moment that they originated (during the alpha or delta period), frequency, effect of vaccination, and characteristics of patients. This suggests that multiple mechanisms play a role in the development of post-acute COVID symptoms.

We found a negative association between vaccination and the persistence of post-acute COVID symptoms, however confidence intervals were wide indicting caution when interpreting these results. With the number of newly developed symptoms in the acute phase as a proxy for severity of disease, severity of disease was associated with persistence of post-acute COVID symptoms. Severity of acute disease is a possible mediator in the effect of vaccination in the persistence of symptoms after acute COVID-19. The negative association of vaccination was not seen in participants with only non-respiratory post-acute COVID symptoms, which was half of the participants with post-acute COVID symptoms.

Prior research from app data has shown a similar frequency of post-acute COVID symptoms as in our study, and also reported similar results with regard the influence of sex and age.[15, 16] Several meta-analyses have also led to incidence estimates for Post-COVID of 5-10%.[2, 3, 9] The positive association between severity of the acute disease and post-acute COVID symptoms was described in prior research.[17, 18] However, in most studies severe COVID-19 was considered after hospitalisation or admission to the Intensive Care Unit, while in our study, including only community dwelling patients, severity was measured in more detail. Still the risk for post-acute COVID symptoms at 100 days after infection increased with 40% per additional acute symptom, indicating that the association between severity of acute COVID-19 and post-acute COVID symptoms is also of relevance in outpatients. Antonelli et al. previously reported associations between vaccination status and fewer symptoms in the acute phase with fewer long lasting symptoms,[19] which is in line with our results, and has been confirmed with other data-sources and methods.[20, 21] We found a lower prevalence of post-acute COVID symptoms in the part of the research period in which the delta-variant was prevalent, which is consistent with prior reports.[22, 23] However, similar to our results, vaccination might have influenced this difference.

In an overview of seven studies on post-acute COVID symptom clusters, most studies clustered symptoms in neurologic, cardiorespiratory or systemic/inflammatory.[24] In the case of Post-COVID, in which several definitions exist, we encourage to extract data from population-based sources and utilize the duration of symptoms (excluding participants with only acute symptoms) for clustering, without restricting it to a specific timeframe. However, data-driven techniques should always be combined with clinical expertise as also discussed by Hulsen et al, to maximize their synergistic potential.[25]

Because the data were collected during the development of the epidemic, we were able to measure newly developed symptoms on the individual level, by taking prior symptoms into account. Another strength of this study is that participants were community-dwelling instead of selected from hospitals or from other healthcare resources. In addition, data from the app

were detailed, i.e., about symptomatology in the prior, acute and post-acute infection phase, and about many variables that allowed adjustment for confounding. We used several assumptions and definitions in our analyses, which did not appear crucial to the main results, as shown in sensitivity analyses.

This study has several limitations. The participants were self-selected app users, i.e., they started, paused and stopped the usage of the app voluntarily. This has resulted in oversampling of relatively older people (taking the time or considered it more important to participate) and oversampling of users experiencing symptoms. It is also possible that people with very severe (post-acute COVID) symptoms will be less likely to use the app. Because it is likely that participants with (post-acute COVID) symptoms will fill in the app for a longer period of time, we did not censor on duration of usage before 100 days and we performed a sensitivity analyses including participants who were lost to follow-up assuming immediate recovery or recovery at 100 days. Assuming recovery at 100 days in all participants who were lost to follow-up yielded higher estimates of post-acute COVID symptoms at day 100 (up to 30%), but this scenario is unlikely, since we believe that most of these participants stopped using the app because they did not experience symptoms anymore. Several post-acute COVID symptoms currently known (such as brain fog and depression) were not part of our survey and hence could not be used in our analyses. Further, the presence of multicollinearity arose due to the temporal concurrence of various SARS-CoV-2 variants and vaccination, in which vaccination and the variants were highly correlated (few participants were vaccinated during the period of the alpha variant and few unvaccinated during the period of the delta variant). Lastly we did not have details about comorbidities or BMI; and all data was self-reported with possible measurement error due to misinterpretation. As these factors influence the probability of development of severe COVID-19, they might be of relevance for targeting (preventive) therapies for Post-COVID.

In conclusion, in continuously data from the general population the incidence of post-acute COVID symptoms is between 5 and 10% and half of these patient suffer non-respiratory symptoms. A more severe acute infection is associated with a higher probability of prolonged post-acute COVID symptoms. In addition to the preventive effect of developing COVID-19, vaccination was associated with less post-acute COVID symptoms, but not with less post-acute COVID non-respiratory symptoms. Since evidence on aetiology of Post-COVID still needs to be built up, our findings might help to find subgroups at risk for developing specific kinds of Post-COVID for which eventually targeted interventions might become available.

Competing interests: non

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Author contributions: WJD wrote the manuscript, designed the methodology and performed the analyses. MLH, DMK, AHV advised and revised the manuscript. LGV, MEN, FRR and JCK were involved in development of the app and revised the manuscript. JCK is the guarantor.

Ethics Approval: Ethical approval was provided by the Medical Ethical Board of the LUMC (dossier number N20.067)

See supplemental material:

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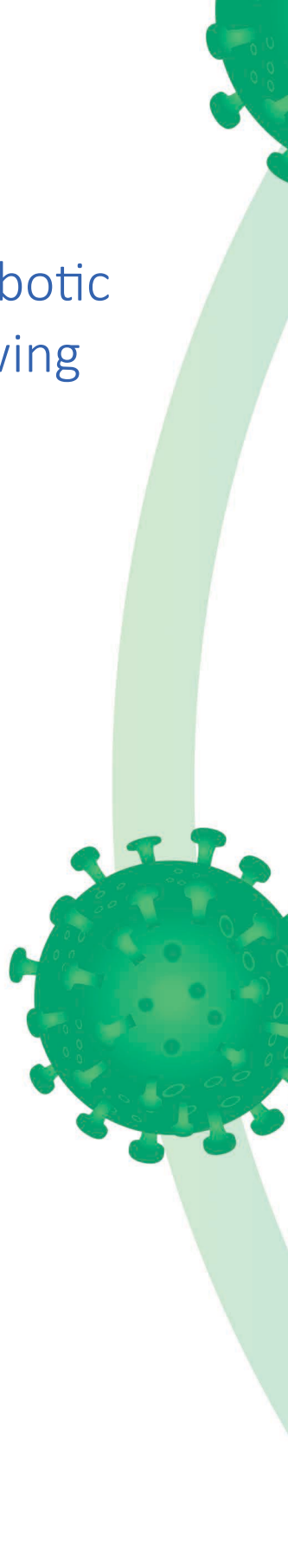
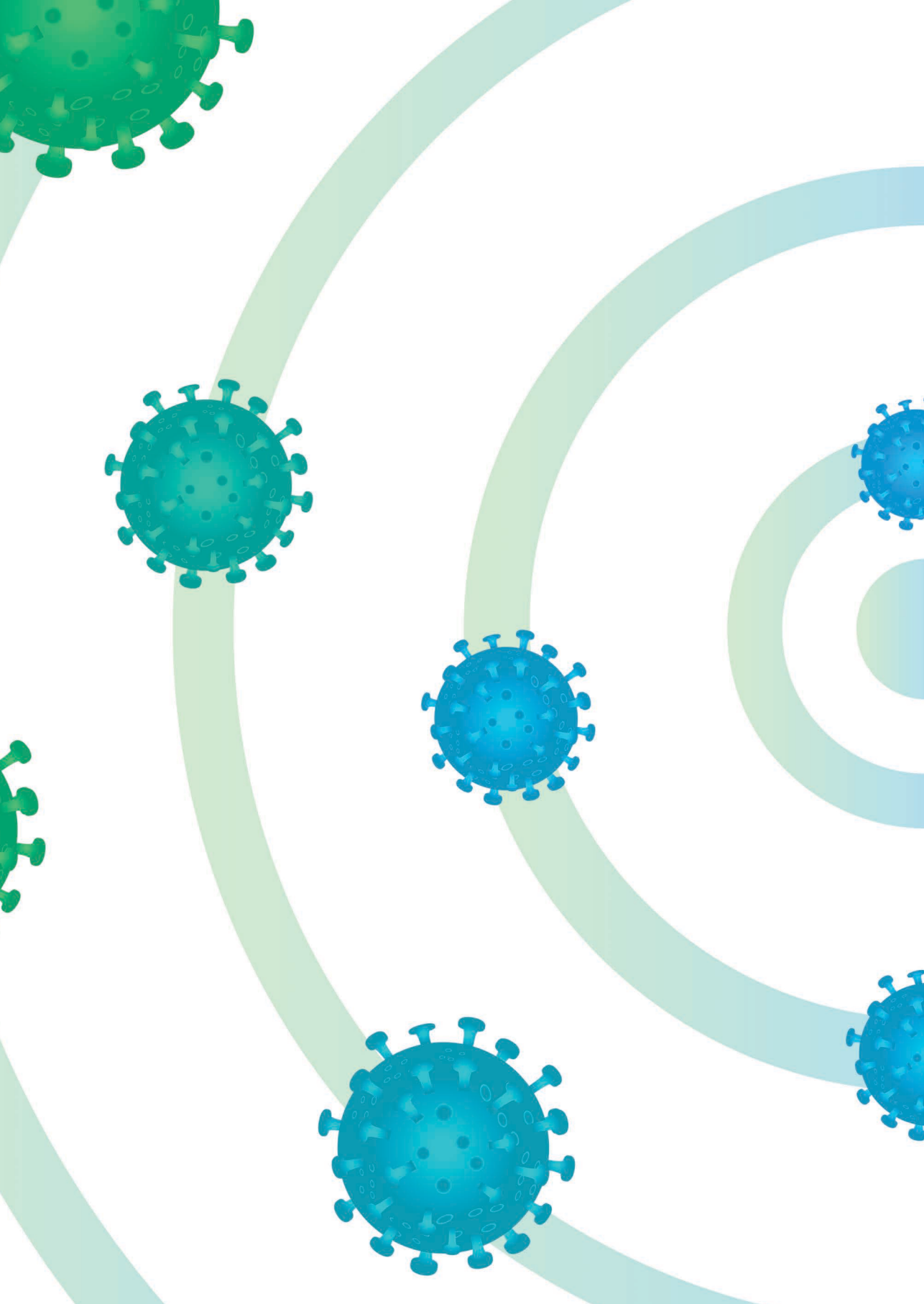
PART II

Coagulation and venous thrombotic events as adverse events following SARS-CoV-2 infection and SARS-CoV-2 vaccination





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CHAPTER 5

High thrombin potential prior to infection and severe COVID-19

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Under review

ABSTRACT

Introduction

Disease severity in infections varies, with severe SARS-CoV-2 infections often involving thrombosis.

Aim

To investigate whether high thrombin generation (TG) potential prior to infection is linked to SARS-CoV-2 infection or severe COVID-19.

Methods

This study was part of a randomized-controlled trial evaluating the BCG vaccine's effect on COVID-19 in individuals aged ≥60 with chronic comorbidities. Participants had no COVID-19 in the first 14 days after inclusion and did not use anticoagulants. Baseline blood samples were collected, and participants were followed for six months. TG peak height was the primary measure. An exploratory analysis of 159 proteins, including coagulation-related markers, was conducted using Quantitative Protein Mass Spectrometry (QPMS). Cox regression assessed associations with SARS-CoV-2 infection and severe COVID-19 (defined as requiring medication, hospitalization, ICU admission, or death), adjusting for age, sex, BMI, frailty, and comorbidities.

Results

Among 1,119 participants, 47 contracted SARS-CoV-2, with 10 developing severe disease. TG peak height was not associated with infection risk (adjusted Hazard ratio (aHR) 0.8; 95% CI: 0.4–1.7) but showed a potential link with severe COVID-19 (aHR 3.2; 95% CI: 0.4–28). Other TG parameters, excluding endogenous thrombin potential and start tail time, also indicated associations with severity. Several coagulation and immune-related proteins were linked to severe disease, though findings were limited by multiple testing.

Discussion

In older adults with comorbidities, higher TG potential was not linked to infection risk but may be associated with more severe COVID-19. These results could aid for further exploration of intrinsic risk factors and protective strategies for severe COVID-19 infections.

INTRODUCTION

COVID-19 has presented substantial clinical challenges, in a large part due to its highly heterogenous clinical presentation, which can range from asymptomatic or mild to severe disease or death.(1) A better understanding of the determinants associated with a severe course of COVID-19 may help to target treatment and preventive strategies.(2-11) Many of the severely ill COVID-19 patients suffer from venous thrombotic events.(12) Markers of increased coagulation (hypercoagulability), such as D-dimer, fibrinogen, factor VIII, von Willebrand factor, and thrombin generation (TG), have been associated with an increased risk of organ failure and mortality in patients with Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) infections.(7, 13-15) Severity of other infectious diseases, such as influenza, is also associated with dysregulation of coagulation.(16, 17) In addition, severity of sepsis can be predicted using coagulation parameters.(18, 19) The inflammatory and coagulation system are connected in multiple ways.(20) While coagulation markers have been associated with an increased risk of organ failure and mortality in individuals during a SARS-CoV-2 infection, it is currently unknown whether hypercoagulability prior to COVID-19 is associated with an increased risk of a SARS-CoV-2 infection or a more severe course of the disease, resembling an intrinsic susceptibility to severe infection.

Between September and December 2020, during the second wave of the COVID-19 pandemic prior to the approval of COVID-19 vaccines, a large double-blind placebo-controlled randomized controlled trial was conducted to assess the risk of COVID-19 associated with the Bacillus Calmette-Guérin (BCG) vaccine in individuals aged over 60 with comorbidities. Over 6000 individuals of 60 years of age were randomized 1:1 to receive either BCG or placebo. In a subset of the participants venous blood was drawn prior to randomization. At 6 months follow-up, no association was found between the BCG vaccine and the risk of COVID-19 infection, while no conclusions could be drawn regarding the effects of BCG on severity and mortality caused by the infection due to lack of power.(21) In a secondary analysis of these trial data we aimed to assess whether hypercoagulability, i.e., an increased TG potential, is associated with an increased risk of a SARS-CoV-2 infection or with a severe course of COVID-19.

METHODS

Trial design and selection of participants

This study was embedded in the BCG-PRIME study, a large placebo-controlled double-blind randomized controlled trial aimed to assess the risk of a SARS-CoV-2 infection or a severe course of COVID-19 associated with the BCG vaccine.(21) Between September and December 2020, participants were recruited from 20 hospitals in the Netherland or via self-report. Inclusion criteria were age 60 years or older, having one or more comorbidities, no contraindications to BCG vaccination, and no documented SARS-CoV-2 infection. Venous blood was drawn prior to BCG or placebo vaccination (1:1) in participants included in four of the 20 participating hospitals.

The primary trial was approved by the Utrecht Institutional Review Board (protocol NL74730.041.20) and registered in the European Clinical Trials Database (2020-003470-47). The current study was approved by the local Scientific Committee of the Department of Clinical Epidemiology of the Leiden University Medical Center (LUMC) (Protocol number A204).

For the current analysis, we included participants who donated a blood sample, excluding participants using anticoagulants (vitamin K antagonists, heparin or direct oral anticoagulants). Furthermore, we excluded participants with missing information on thrombin generation or details about comorbidities or with outliers for TG parameters (defined as five times the SD). Follow-up started two weeks after baseline, excluding participants with a positive SARS-CoV-2 test within the first two weeks that potentially influenced the baseline measurement of TG.

Follow-up consisted of questionnaires in a smartphone app (Research Follow App, Your Research, Huizen, the Netherlands) with bi-weekly reminders or by bi-weekly telephone consultations for a duration of six months. In addition to the questionnaires, information on the participants was retrieved from their general practitioner and hospital regarding SARS-CoV-2 infection, hospital visits, hospital admission, Intensive Care Unit (ICU) admission, or death.

Laboratory measurements

TG was assessed using the Calibrated Automated Thrombogram® (Diagnostica Stago, Asinères, France), according to the manufacturer's specifications.(22) In brief, coagulation was activated using a low amount of tissue factor and phospholipids, followed by continuous measurement of thrombin formation. The TG parameters measured were the endogenous thrombin potential (ETP), thrombin peak, time to peak, lag time, velocity index, and start tail time. We used peak height as the primary outcome of the TG assessment, as this is the most strongly associated with venous thrombosis in literature.(23, 24)

To explore possible pathophysiologic mechanisms, we analyzed 159 proteins with Quantitative Protein Mass Spectrometry (QPMS). These proteins are from a diverse range of physiological pathways, including 21 coagulation-related proteins, 18 complement pathway proteins and 14 apolipoproteins. The technical procedure of the QPMS and selection of these proteins is described by Van der Vliet et al.(25) In brief, using pre-synthesized $^{13}C^{15}N$ stable isotope labeled (SIL) peptides, quantification of several plasma proteins is possible in one measurement using liquid chromatography by a reversed-Phase UHPLC column (EclipsePlusC18 RRHD 150 x 2.1 m, 1.8 µm particles; Agilent Technologies, Santa Clara, USA) in combination with mass spectrometry analysis on a triple-quadrupole mass spectrometer (Agilent 6495C; Agilent Technologies, Santa Clara, CA, USA).(26) In each run the assay was calibrated using a pooled citrate sample of ten healthy individuals. We measured the proteins in all participants who developed COVID-19 during follow-up and 90 randomly selected participants (frequency matched by hospital of recruitment) without COVID-19 during follow-up for exploratory analyses into proteins or protein pathways associated with the development of severe COVID-19.

Outcomes

Two outcomes were defined. The first outcome was development of an infection with SARS-CoV-2 during follow-up. The second outcome was development of severe COVID-19, defined as requiring contact with a physician and need for medication, requiring hospital admission or admission to ICU, or COVID-19 resulting in dead. Both outcomes were adjusted for potential confounders, i.e., age, sex, BMI, clinical frailty score, and comorbidities (hypertension, cardiovascular disease, stroke, diabetes, chronic obstructive pulmonary disease, asthma, other pulmonary disease, chronic kidney disease and malignancy).

Statistical analysis

Baseline characteristics of participants are reported with proportions, means (with standard deviations (SD)) or medians (with interquartile ranges (IQR)). In addition, we compared included and excluded participants who were patients in the four participating hospitals.

All TG parameters and proteins were continuous variables. Because of inter-center differences in the blood sampling techniques, we normalized these continuous variables within each center. Incidence rates of infection were determined for quartiles of the TG parameters. We calculated hazard ratios for infection with SARS-CoV-2 with Cox-regression for each quartile with the first quartile as the reference, adjusted for confounders. As a sensitivity analysis we repeated this analysis after exclusion of participants who received a SARS-CoV-2 vaccination during follow-up. To study the association between TG parameters and the risk of a severe course of COVID-19, we calculated hazard ratios for severe disease with Cox-regression for tertiles of the TG parameters with the first tertile as the reference, adjusted for confounders.

In the exploratory analyses of the proteins measured with QPMS, we calculated hazard ratios with Cox-regression comparing above with below median values of these proteins for both outcomes. As this is an exploratory analysis with a large number of proteins, we only adjusted for confounders age and sex. We corrected for multiple testing in correlated data with the eigenvalues of a correlation matrix by an adapted Benjamini-Hochberg procedure with a false discovery rate of 25%.(27, 28) In addition we performed a hierarchical cluster analysis on the normalized protein levels with a Ward-linkage method, to find clusters of patients and compare them with patient characteristics and clinical outcomes.

RESULTS

In total, 6112 participants were randomized in the BCG-PRIME study, of whom 2221 participants were included in the four centers where blood samples were taken. Of these 2221 participants, 406 did not provide consent for a blood draw, four participants were excluded because of missing baseline characteristics, 668 participants were excluded because of use of anticoagulants at the time of the blood draw, five participants were excluded because of SARS-CoV-2 infection within two weeks after the blood draw, and 19 were excluded because of outliers in TG. This resulted in 1119 participants

eligible for analysis. Baseline characteristics of these participants are shown in Table 1. Comparison of excluded and included participants from the four participating hospitals (supplementary Table 1) showed no differences in outcomes, and only trivial differences in frequency of some comorbidities.

Table 1: Baseline characteristics of participants

N	1119
Sex, women (%)	473 (42)
Age, mean (SD)	69.3 (5.9)
BMI, mean (SD)	26.6 (4.1)
Frailty scale, median (IQR)	2.0 (1, 3)
Comorbidities, median (IQR)	2.0 (1, 2)
Hypertension (%)	602 (54)
Cardiovascular disease or stroke (%)	548 (49)
Diabetes Mellitus (%)	221 (20)
COPD (%)	122 (11)
Asthma (%)	159 (14)
Other pulmonary disease (%)	57 (5)
Chronic kidney disease (%)	46 (4)
Malignancy (%)	75 (7)
Vaccinated during follow-up (%)	390 (35)

COPD: chronic obstructive pulmonary disease. SD: standard deviation. IQR interquartile range

The participants were followed for a median of 170 days (range: 3 – 209 days), with a total of 508 patient-years; 47 (4%) participants tested positive for SARS-CoV-2 and ten participants (0.9%) developed severe COVID-19 during follow-up. Of these ten participants, two received antibiotics from a physician, three were hospitalized, three were admitted to the ICU, and two participants died with COVID-19 registered as the primary cause of death (see supplemental table 2 for baseline characteristics for participants who had severe or non-severe COVID-19). During follow-up 390 (35%) participants received a SARS-CoV-2 vaccine, but none of the participants who tested positive for SARS-CoV-2 received the vaccine prior to their infection.

Peak height of TG was not associated with the risk of a SARS-CoV-2 infection (risk of individuals with a peak height quartile 4 vs quartile 1: adjusted hazard ratio (aHR) 0.8; 95% CI 0.4, 1.7, see table 2 and figure 1). Similarly, velocity index was not associated with the risk of a SARS-CoV-2 infection. However, the fourth quartile of lag time, time to peak and start tail time, were associated with increased risk of a SARS-CoV-2 infection (with HR ranging between 1.4 and 1.8. Compared with the first quartile of ETP, all other quartiles of ETP had higher incidences of SARS-CoV-2 infections (aHR Q4 vs Q1 ETP: 1.9; 95%CI 0.7, 5.6). In an analysis restricted to participants who did not receive a SARS-CoV-2 vaccination during follow-up (N=729), TG showed similar associations with the risk of a SARS-CoV-2 infection (supplemental table 3).

Peak height appeared associated with an increased risk of a severe course of the disease (aHR tertile 3 vs tertile 1: 3.2; 95%CI: 0.4 – 29; see table 3 and figure 2). Out of the 10 people with

severe COVID-19, one had a peak height within tertile 1, five in tertile 2, and four in tertile 3 (see supplementary table 4). Like peak height, other TG parameters indicating hypercoagulability were associated with an increased risk of severe COVID-19, except for ETP and the start tail time (table 3). However, all effect estimates had wide confidence intervals. Distributions of TG parameters, stratified by outcome, showed small differences towards hypercoagulability in those with a severe outcome (mean Z-score Peak height no COVID-19: 0.04, SD 1,0; non-severe COVID-19: -0.1, SD 1.0; severe COVID-19: 0.3, SD 0.6, see figure 3).

To explore the relation between coagulation and development of COVID-19 in greater depth, we measured 159 proteins using QPMS. One protein had concentrations that fell below the assay's detection limit, leaving 158 proteins for analyses. After adjustment for confounding and multiple testing protein levels were not associated with SARS-CoV-2 infection (see supplementary table 5 and supplementary figure 1). Ten proteins were associated with SARS-CoV-2 infection, with a p-value <0.05 (unadjusted for multiple testing) (i.e., Hemopexin, Fibulin-1, Peroxiredoxin-2, Immunoglobulin G Fc-Binding Protein, Phospholipid transfer protein, Complement Factor B, Adhesion G protein-coupled receptor F5, Mannan binding lectin serine protease 1, Carbonic anhydrase 1 and Antithrombin).

Table 2: Association between trombin generation parameters at baseline and the development of COVID-19.

	HR (95% CI)				aHR (95% CI)			
	Q1	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Peak height (nmol)	Ref	0.8 (0.4; 1.9)	0.7 (0.3, 1.6)	0.9 (0.4, 1.9)	Ref	0.9 (0.4, 1.9)	0.7 (0.3, 1.5)	0.8 (0.4, 1.7)
Lag time (minutes)	Ref	0.5 (0.2, 1.3)	0.6 (0.2, 1.4)	1.5 (0.7, 2.9)	Ref	0.5 (0.2, 1.4)	0.6 (0.3, 1.5)	1.4 (0.7, 3.0)
Time to peak (minutes)	Ref	0.7 (0.3, 1.6)	0.6 (0.2, 1.85)	1.8 (0.9, 3.7)	Ref	0.7 (0.3, 1.6)	0.6 (0.2, 1.4)	1.8 (0.9, 3.7)
ETP (nmol x minutes)	Ref	3.2 (1.2, 8.7)	2.5 (0.9, 6.9)	2.3 (0.8, 6.4)	Ref	3.5 (1.3, 9.5)	2.4 (0.9, 6.8)	1.9 (0.7, 5.6)
Velocity index (minutes)	Ref	0.6 (0.3, 1.3)	0.5 (0.2, 1.1)	0.8 (0.4, 1.6)	Ref	0.6 (0.3, 1.3)	0.4 (0.2, 1.0)	0.7 (0.3, 1.5)
Start tail time (minutes)	Ref	0.5 (0.2, 1.2)	0.3 (0.1, 0.9)	1.7 (0.9, 3.4)	Ref	0.5 (0.2, 1.1)	0.3 (0.1, 0.8)	1.6 (0.8, 3.2)

Q: quartile. CI: confidence interval, ETP: endogenous thrombin potential, aHR: adjusted for sex, age, Body Mass Index (BMI), frailty score and comorbidities. Ref: reference

None of the proteins were significantly associated with a severe course of COVID-19, after adjustment of confounding and multiple testing (see supplementary table 4, supplemental figures 2 and 3). However, 18 proteins were associated with a severe course of SARS-CoV-2 infection with a p-value <0.05 (unadjusted for multiple testing) (i.e., Angiotensinogen, Kallistatin, Retinol Binding protein 4, Complement Component 7, Complement Component C5, Zinc-Alpha-2-Glycoprotein, Beta-2-Glycoprotein 1, Vitamin K dependent proteins 1, Z and C, Alpha-1-microglobulin, Serum Amyloid A4, Antithrombin III, Coagulation factor IX, Glycosylphosphatidylinositol-phospholipase D, Pigment Epithelium-Derived Factor, Alpha-1-acid glycoprotein and IgGFc-binding protein).

In the hierarchical cluster analysis, no clear clustering was seen of individuals who had a COVID-19 infection during follow-up (see supplemental figure 4). In the cluster analysis selecting only the 47 participants that developed COVID-19 during follow-up also no clear clustering was observed of participants who had a severe course of the disease (see supplementary figure 5).

Tabel 3: Association between trombin generation parameters at baseline and the development of severe COVID-19

	HR (95% CI)			aHR (95% CI)		
	T1	**T2**	**T3**	**T1**	**T2**	**T3**
Peak height (nmol)	Ref	4.8 (0.6, 41)	3.8 (0.4, 34)	Ref	4.6 (0.5, 40)	3.2 (0.4, 29)
Lag time (minutes)	Ref	0.7 (0.2, 3.3)	0.8 (0.2, 3.6)	Ref	0.7 (0.2, 3.3)	0.9 (0.2, 4.2)
Time to peak (minutes)	Ref	0.2 (0.02, 1.4)	0.5 (0.1, 2.1)	Ref	0.2 (0.02, 1.6)	0.6 (0.1, 2.3)
ETP (nmol x minutes)	Ref	1.9 (0.3, 10.4)	1.9 (0.3, 10.4)	Ref	1.8 (0.3, 10.2)	1.4 (0.2, 8.0)
Velocity index (minutes)	Ref	4.8 (0.6, 41)	3.8 (0.4, 34)	Ref	4.5 (0.5, 39)	3.3 (0.4, 30)
Start tail time (minutes)	Ref	(0.1, 2.0)	0.6 (0.2, 2.7)	Ref	0.4 (0.1, 2.2)	0.7 (0.2, 2.8)

T: tertile. CI: confidence interval. ETP: endogenous thrombin potential. Severe COVID-19: requiring contact with a physician and need for medication, requiring hospital admission or admission to ICU or COVID-19 resulting in dead. Ref: reference HR: Hazard ratio adjusted for sex, age, BMI, frailty score and comorbidities (except for pulmonary diseases and chronic kidney disease, because of non-positivity)

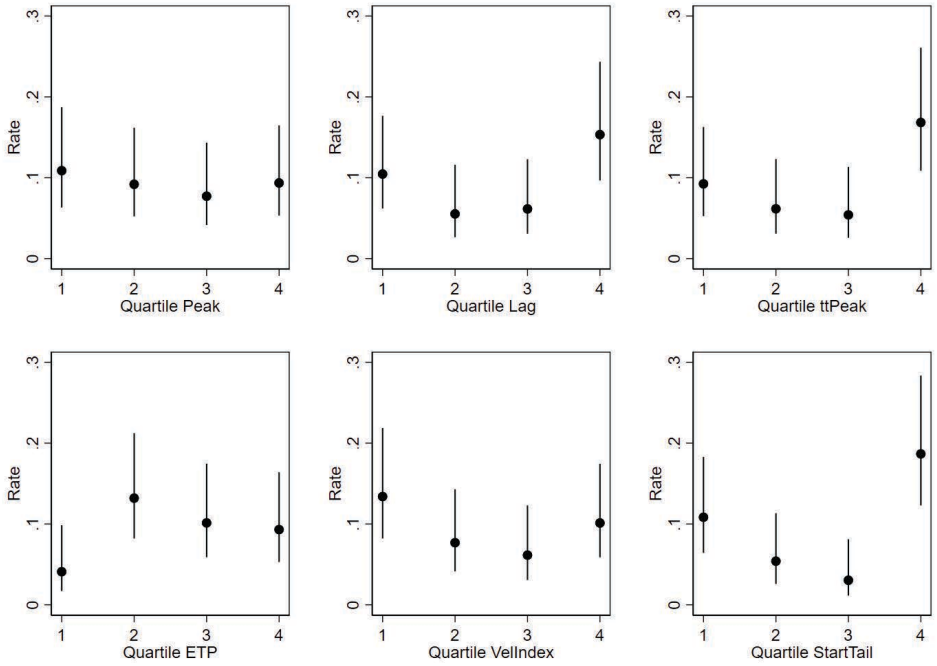

Figure 1: Incidence rate (per person year) and 95% confidence interval of development of an infection with SARS-CoV-2, by quartile of thrombin generation parameter at baseline. The confidence intervals are calculated using the quadratic approximation to the Poisson log likelihood for the log-rate parameter. ETP: endogenous thrombin potential. ttPeak: time to peak. VelIndex: Velocity index.

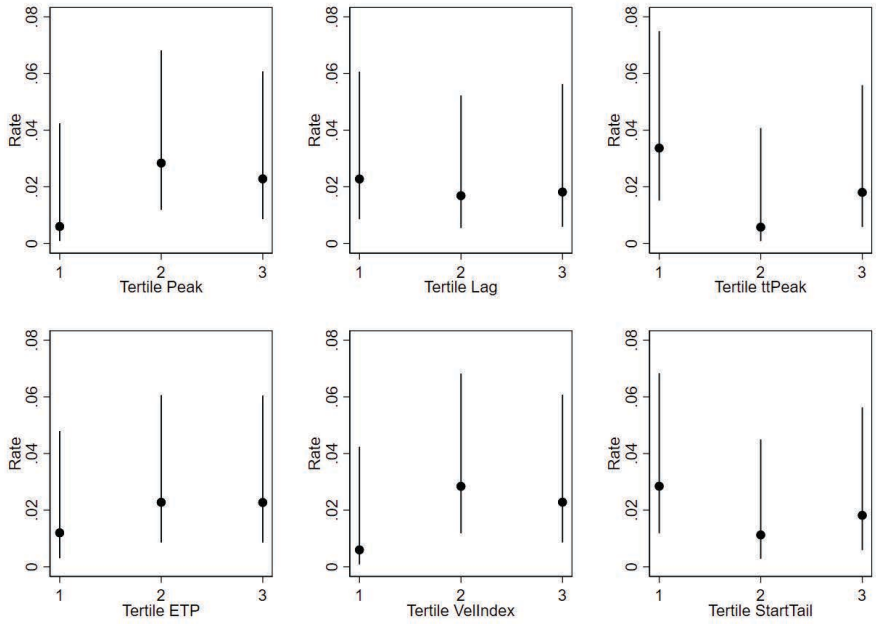

Figure 2: Incidence rate (per person year) and 95% confidence interval of development of severe COVID-19, by tertile of thrombin generation parameter at baseline. The confidence intervals are calculated using the quadratic approximation to the Poisson log likelihood for the log-rate parameter. ETP: endogenous thrombin potential. ttPeak: time to peak. VelIndex: Velocity index.

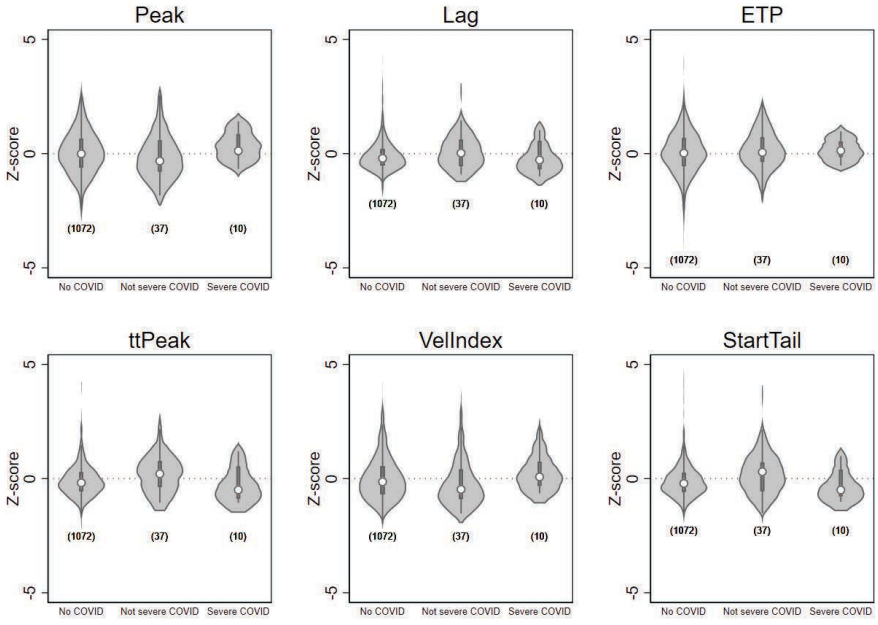

Figure 3: Plots of levels of thrombin generation parameters in participants without COVID-19 (1072), with non-severe COVID-19 (37) and severe COVID-19 (10). Values are normalized Z-values by center. ETP: endogenous thrombin potential. ttPeak: time to peak. VelIndex: Velocity index.

DISCUSSION

In a large cohort of older adults with comorbidities, we found that a high TG potential (expressed as a high peak height) prior to infection was not associated with a subsequent SARS-CoV-2 infection, but the data suggest an association with a severe course of the disease. In a quantitative protein assay, multiple proteins showed associations with SARS-CoV-2 infection, exhibiting diverse roles in coagulation, oxidative stress protection, immune system function, and inflammatory responses.

It is known that severe COVID-19 is often complicated by hypercoagulability.(7, 12-15) This may be a consequence of the severe infection, caused by hyperinflammation, hypoxia, immobilization, microvascular injury, or disseminated intravascular coagulation.(29, 30) However, our results show that also an intrinsic pre-existing hypercoagulant potential of the coagulation system may be associated with an increased risk of a more severe course of COVID-19.

In the exploratory analyses, after adjustment for confounding and multiple testing, none of the proteins measured with QPMS were associated with infection with SARS-CoV-2 or severe COVID-19. Some of the proteins with associations in this exploratory analyses (without adjustment for multiple testing) could be influenced by hemolysis caused by the differend blood collection (Hemopexin, Peroxiredoxin-2 and Carbonic anhydrase 1).(31) Other proteins associated with SARS-CoV-2 infection had diverse roles, e.g. in coagulation, oxidative stress protection, the immunesystem or inflammatory responses.(32-34) Regarding severe COVID-19, analyses unadjusted for multiple testing identified several proteins with functions related to inflammation, oxidative stress regulation, and coagulation.(35-38) The protein Angiotensinogen is part of the renin-angiotensin-aldosterone system, what is known to play a key part in COVID-19 disease progression, especially in patients with cardiovascular comorbidities.(39, 40) Complement component 7, together with components 5, 6, 8 and 9, is involved in lysis of pathogens by forming the Membrane Attack Complex and is involved in the pathogenesis of severe COVID-19.(34, 41) Zinc-alpha-2-glycoprotein is associated with severe COVID-19 via immunoregulatory pathways.(42-44) Alpha-1-acid glycoprotein plays significant roles in inflammation, immune response and is upregulated in severe COVID-19 in prior research.(45) The protein Retinol-binding protein 4 is known to be associated with severe COVID-19 via antiviral pathways.(46) Several antiphospholipid antibodies were associated with COVID-19 in prior research, suggesting the interplay between immunoinflammatory pathways and coagulation.(47) Although Serum Amyloid A4 itself is not described being associated with severe COVID-19, levels of other Serum Amyloid A proteins are known to be linked with severity of disease.(48)

To our knowledge this is the first study to measure TG in individuals prior to SARS-CoV-2 infection. There are studies in which TG was measured during COVID-19, where TG parameters were associated with severe COVID-19 or deterioration.(4, 5) In contrast, several studies in hospitalized COVID-19 patients did not find a prognostic value of TG for severity of disease.(49, 50) However, comparison with our results is difficult, because of the different timing of measurement of the

exposure, i.e., before or during COVID-19 infection, and in many prior studies patients used antitrombotic therapies during measurement of TG.

The strengths of this study include the large cohort of individuals with TG measurements performed before infection with SARS-CoV-2 and the detailed and extensive data collection, which allowed for adjustment for several potential confounders. Furthermore, the inclusion of participants in the study and the subsequent follow-up took place during the first half of the pandemic, during which period only one SARS-CoV-2 variant was present, which limited possible effect modification by variants.

Our study also has some limitations. The results are only applicable to older adults with comorbidities, without use of anticoagulants. However, older adults with comorbidities are most vulnerable and at risk of developing severe COVID-19, making them the most relevant population in this context. Our study was embedded in a randomized-controlled trial. Therefore a sample size calculation was not performed specifically for our research question. Particularly in the subgroup analysis of patients with severe COVID-19, sample size was small, resulting in wide confidence intervals and limited certainty in the results of this analysis. We considered several possible confounders in our analyses, i.e., age, sex, BMI, clinical frailty score, and comorbidities. However, for comorbidities and frailty, the direction of the association with TG is not clear, these could either be a cause or a consequence of an elevated TG. Infection with SARS-CoV-2 was based on self-reported test results. Even though all positive tests were confirmed by data from medical records, some infections could have been missed. We used a broad definition for severe COVID-19. This may have result in heterogeneity in severity in the patients classified as severe COVID-19. The measurements of TG were influenced by variations in blood collection methods, sample preparation and storage across the four hospitals.(51) However, all laboratory measurements were performed in one center using standardized procedures, and the data were normalized by hospital during analysis.

In conclusion, our results indicate that hypercoagulability, reflected by an increased peak TG, is not associated with an increased risk of a SARS-CoV-2 infection. However, when infected, pre-existant hypercoagulability may be associated with a more severe course of COVID-19. Several proteins related to the inflammation and coagulation were also associated with infection and COVID-19 severity. These results could aid further exploration of intrinsic risk factors for severe courses of an infectious disease.

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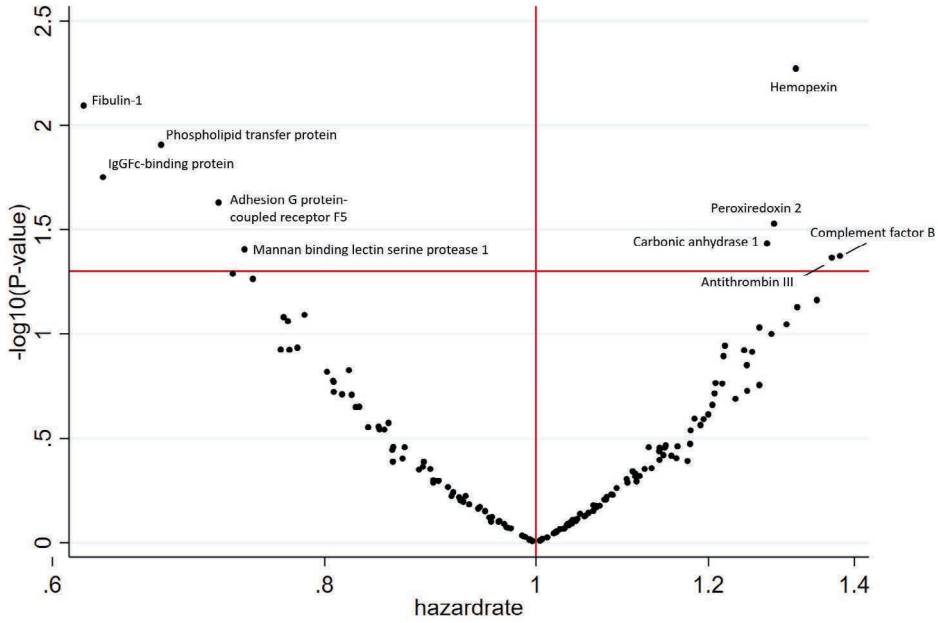
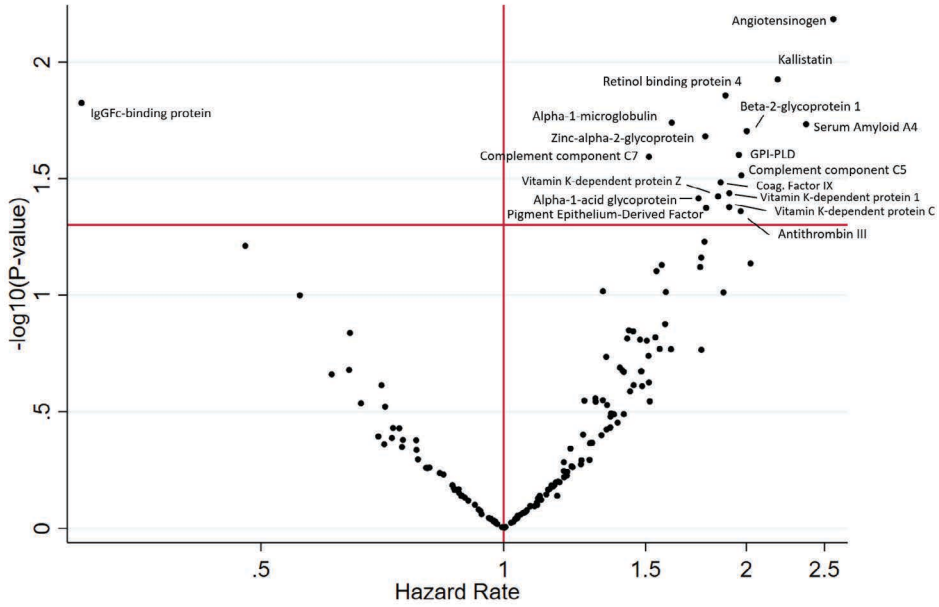
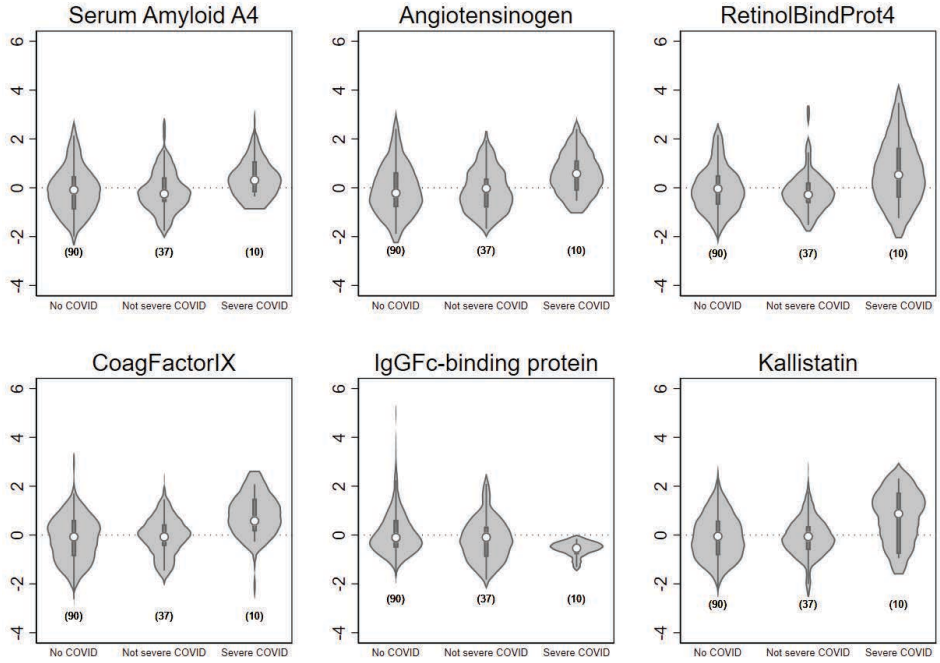
SUPPLEMENTALS



SFigure 1: Vulcanoplot of assocations of 158 proteins with development of subsequent SARS-CoV-2 infection, adjusted for age and sex. (Horizontal red line: unadjusted for multiple testing; p=0.05). After adjustment for multiple testing, non of the proteins remained statistically significant.



SFigure 2: Vulcanoplot of assocations between 158 proteins with development of subsequent severe COVID-19 (horizontal red line: p=0.05, unadjusted for multiple testing). After adjustment for multiple testing, non of the proteins remained statistically significant. GPI-PDL: Glycosylphosphatidylinositol-phospholipase D



SFigure 3: Plots of levels of six proteins in participants without COVID-19 (90), with non-severe COVID-19 (37) and severe COVID-19 (10). Values are normalized Z-values by center.

See other supplementals:

6

CHAPTER 6

Coagulation and inflammatory response after intramuscular or intradermal mRNA-1273 SARS-CoV-2 vaccine – secondary analysis of a randomized trial

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ESSENTIALS

1. Vaccination in the skin, instead of the muscle, produces antibodies at one fifth of the dose
2. Vaccination in the skin gave less systemic inflammation than intramuscular vaccination
3. The larger the inflammatory response following vaccination, the larger increase in coagulation
4. Intradermal vaccines may be more safe

ABSTRACT

Introduction

Fractional dosed intradermal vaccination produces antibody concentrations above the proposed proxy for protection against severe disease as compared with intramuscular vaccination and may be associated with a decreased prothrombotic effect.

Objectives

To assess changes in coagulation following standard dosed intramuscular or fractional dosed intradermal (1/5th of intramuscular) mRNA-1273 SARS-CoV-2 vaccine and to determine the association between the inflammatory response and coagulation.

Methods

This study was embedded in a randomized controlled trial assessing the immunogenicity of an intradermal fractional dosed mRNA-1273 vaccine. Healthy participants, aged 18-30 years, were randomized (2:1) to receive either two doses of intradermal or intramuscular vaccine. Blood was drawn prior to first and second vaccination dose, one and two weeks after the second dose. The outcomes were changes in coagulation parameters (primary endpoint peak height of the thrombin generation curve) and inflammation (high sensitive-CRP).

Results

123 Participants were included (81 intradermal; 42 intramuscular). Peak height increased after vaccination, (intramuscular 28.8 nmol; 95%CI: 6.3, 63.8; intradermal 17.3 nmol; 95%CI:12.5, 47.2) and recovered back to baseline within two weeks. Intramuscular vaccination showed a higher inflammatory response compared with intradermal vaccination (extra increase hs-CRP: 0.92 mg/L; 95%CI 0.2, 1.7). Change in Endogenous Thrombin Potential was associated with change in hs-CRP (Beta 28.0; 95%CI 7.6, 48.3).

Conclusions

A transient increase in coagulability after mRNA-1273 SARS-CoV-2 vaccination occurred, which was associated with the inflammatory response. While intradermal administration showed antibody concentrations above the proposed proxy for protection against severe disease, it was associated with less systemic inflammation. Hence, intradermal vaccination may be safer.

INTRODUCTION

Vaccination against COVID-19 has played a pivotal role in containment of the COVID-19 pandemic.[1] Soon after the implementation of large scale SARS-CoV-2 vaccination, thrombotic events were reported as possible side-effects.[2] First reports described a pattern of thrombosis after vaccination with the vector based ChAdOx1 nCoV-19 vaccine, associated with platelet factor four (PF4) autoantibodies, a low platelet count, increased D-dimer and decreased fibrinogen levels and was named vaccine induced thrombotic thrombocytopenia (VITT).[3, 4] In addition, increased rates of venous thrombotic events (VTE) without thrombocytopenia were reported in the weeks after vector based (ChAdOx1 nCoV-19 or Ad26.COV2.S) and (to a lesser extent) mRNA based vaccines (BNT162b2 or mRNA-1273).[5-9] This resulted in several changes in vaccination campaigns over the world.[10]

Several studies examining the effect of SARS-CoV-2 vaccination on coagulation parameters, such as the international normalized ratio (INR) and the activated partial thromboplastin time (APTT), showed contradictory results.[11-20] These parameters summarize only part of the coagulation system or are only relevant in patients receiving anticoagulant therapy, and may not accurately reflect alterations in the coagulation cascade. The thrombin generation assay (TGA) provides a global overview of both procoagulant and anticoagulant pathways.[21, 22]

Intradermal (ID) vaccination is a dose-sparing strategy, providing immune responses equivalent to intramuscular (IM) vaccination, while using smaller vaccine doses and with the benefits of fewer systemic side effects.[23, 24] A dose-sparing technique may particularly of interest to low- and middle- income countries.[25, 26] Intradermal administrations with fractional dose has been proven successful in the past for several vaccines, such as influenza, rabies or hepatitis B vaccines.[27] During the COVID-19 pandemic, we conducted a randomized controlled trial, comparing the immunogenicity of two 1/5th fractional ID doses and two full dose IM delivery of the mRNA-1273 (Moderna) vaccine, each 28 days apart, as a primary vaccination series.[28] Fractional dosing through ID vaccination showed antibody concentrations above the proposed proxy for protection against severe disease.[29]

It is possible that a fractional dose confers a lower thrombotic risk than a full dose vaccination. The aim of the present study was to assess the changes proxies for a prothrombotic change, i.e., levels of coagulation factor VIII, fibrinogen, and D-dimer levels, and thrombin generation parameters following mRNA-1273 vaccination, by dose, as well as the association between these changes and the inflammatory response.

METHODS

Trial design

This study was a secondary analysis of an open label, randomized controlled trial at the Leiden University Medical Center in the Netherlands. The trial was approved by the Medical Ethical

Committee Leiden, Den Haag, Delft (NL 76702.058.21) and registered in the International Clinical Trials Registry Platform (EUCTR2021-000454-26-NL). All participants provided written informed consent.

Procedures

Eligible participants were adults, aged 18-30 years and predominantly White or of European ancestry. Participants with a past or intercurrent SARS-CoV-2 infection, determined by a positive SARS-CoV-2 PCR or seropositivity (positivity SARS-CoV-2 anti-N), were excluded. Other main exclusion criteria were prior SARS-CoV-2 vaccination, use of anticoagulants or steroids, hematological disease, or pregnancy.

Participants were randomized into three groups. The control group received two standard doses of 100µg 28 days apart in the deltoid muscle (standard administration technique). Two experimental groups received two fractional doses (1/5th of the standard dose of 100µg mRNA-1273) 28 days apart in the dermis of the deltoid region, one with the classical Mantoux technique and the other with a small needle (Bella-mu) designed for ID administration. Since both experimental groups showed similar immunogenicity results, they were combined in further analyses.[29] The randomization was done using sealed envelopes. The participant and site staff were unblinded, as the administration route differs.[29]

Blood was collected at day 1 (D1; before first dose), 29 (D29, before second dose), 36 (D36; one week after second dose) and 43 (D43). Fibrinogen, factor VIII, and D-dimer levels (reported in ng/ml D-dimer units) were measured to assess changes in coagulation, using a coagulometric clot detection method on an ACL TOP 700 analyzer (Werfen, Barcelona, Spain) as previously described using designated reagent (D-dimer HS 500, HemosIL, Werfen, MN, USA).[30] Thrombin generation (lag time, endogenous thrombin potential (ETP), peak height, time to peak, velocity index and time until the start of the tail of the curve) was measured using the Calibrated Automated Thrombogram® (Diagnostica Stago, Asinères, France) as previously described. [31]. In the thrombin generation assay (TGA), coagulation is activated in plasma samples according to manufacturer's instructions, using a low amount of tissue factor and phospholipids followed by continuous measurement of thrombin formation and degradation. The ETP, which is the net result of pro- and anticoagulant potentials, is described by the height of the peak and the area under the curve. A higher peak height, ETP, start tail time or velocity index indicate hypercoagulability, while shorter lag time and time to peak represent hypercoagulability. The primary outcome of the TGA, measured in this study, was the peak height, as this is the most strongly associated with venous thrombosis risk.[32, 33]

Inflammation was assessed by high sensitive (hs)-CRP from serum using the immunoassay analyzer COBAS CORE (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Outliers for coagulation and inflammation parameters (defined as 5 times the standard deviation (SD)) were excluded. At baseline we collected self-reported data on age, sex, medication use, body mass index (BMI) and comorbidities.

The change in coagulation parameters and inflammatory response was expressed as the difference between D1 and D36 (one week after the second vaccination), as these are relatively 'fast' processes and we expected that alterations in coagulation and inflammation normalize quickly. Participants with missing data on D1 or D36 were excluded from the analyses. To assess whether changes in coagulation or inflammation persist for a prolonged period, we determined the levels of the affected parameters again at D43. All changes relative to baseline were analyzed by univariate linear regression analyses. In addition, we compared change in distribution, i.e., the SDs of the parameters before and after vaccination, of all parameters. To assess whether there were differences between changes in coagulation or inflammation parameters after vaccination, stratified for type of vaccine administration (ID or IM), we adjusted for baseline values by using the difference between the post value (D36) and baseline value (D1) as the dependent variable and both the assigned type of administration (IM or ID) and baseline value (D1) as the independent variables in a linear regression analysis.

The association between the inflammatory response and change in coagulation was assessed for ID and IM vaccination combined and visualized by scatter plots and tested using univariate linear regression analysis.

Statistical analyses were done using STATA 16.1 for Windows (StataCorp, College Station, USA). Sample size was calculated based on the original trial.

RESULTS

Between 14 June and 8 July 2021, 150 participants were enrolled, of whom 15 were excluded due to SARS-CoV-2 seropositivity at baseline or because of intercurrent SARS-CoV-2 infection before D29. Eleven additional participants were excluded because of missing coagulation data at baseline or D36 and one participant was excluded because of self-reported homozygosity for the Factor V Leiden mutation. Therefore, a total of 123 (82%) participants were included in the analyses of coagulation. Demographic characteristics of these participants are shown in table 1, stratified by group (ID vs IM). No major differences were observed between the ID and IM group, except a higher proportion of oral contraceptive use in the ID group than the IM group (26% vs 14%). For the analyses involving inflammatory markers, ten participants were excluded because of missing inflammation data at baseline or D36 and one participant was excluded because of a hs-CRP over 5 SD from the mean. The remaining 112 participants (75%) were included in the analysis on the association between coagulation and inflammation.

Table 1: Characteristics of participants

	Intradermal	
N	81	42
Age, mean (SD)	22.1 (3.2)	23.5 (3.7)
Sex (female)	34 (42%)	17 (40%)
BMI, mean (SD)	24.4 (4.7)	23.4 (3.7)
Comorbidity	38 (47%)	17 (40%)
Psychiatric	16 (20%)	8 (19%)
Pulmonal	2 (2%)	0 (0%)
Allergy	16 (20%)	5 (12%)
Neurological	5 (6%)	1 (2%)
Other	11 (14%)	5 (12%)
Medication use	27 (33%)	13 (31%)
Antihistamine	9 (11%)	4 (10%)
Methamphetamine	8 (10%)	3 (7%)
Oral contraceptives	21 (26%)	6 (14%)
Other	8 (10%)	5 (12%)

BMI: Body Mass index. SD: Standard Deviation

Differences between pre- and post-vaccination (D36-D1) for the coagulation and inflammation parameters are listed in table 2 and figure 1. The peak height increased in both the IM and ID groups (change in ID group: 17.3 nmol (95% Confidence Interval (CI): -12.5 , 47.2); change in IM group: 28.8 nmol (95%CI: -6.3 , 63.8)). The SDs were larger at D36 than at D1 and differed between the measurements and the two groups (Peak height SD in IM group: before vaccination (D1) 69.2; after vaccination (D36) 90.9 and peak height SD in ID group: before vaccination (D1) 93.1; after vaccination (D36) 99.1), indicating that the magnitude of the change in peak height is variable between study participants.

Changes between D1 and D36 were observed for other parameters of thrombin potential, fibrinogen and D-Dimer but not for FVIII levels (see supplemental figure 1). Most parameters were back to baseline levels at D43 (Peak height at D1: 219.4 nmol; at D36: 236.7 nmol; at D43: 223.6 nmol). Additionally, to confirm the quick normalization of coagulation after vaccination, we also compared coagulation parameters of D29 (just before second dose) to those of D1 and D43, to confirm they were similar (see table 2 and supplementals). Hs-CRP increased in IM vaccinated participants (D36 relative to D1) but remained stable after ID vaccination (change in ID group: -0.1 mg/L (95% CI: -0.8 , 0.6) and change in IM group: 1.1 mg/L (95% CI: 0.1 , 2.1; see table 2 and figure 1).

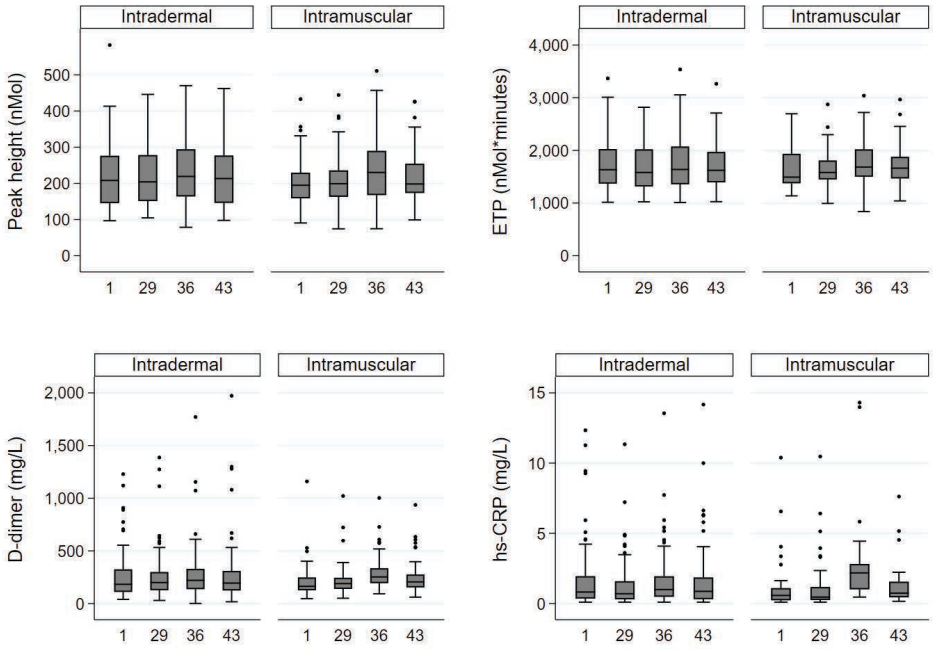

Figure 1:Distribution of peak height, ETP, D-dimer and hs-CRP at each timepoint, stratified for administration technique.
(1: baseline, 29: before 2nd dose, 36: one week after 2nd dose, 43: two weeks after 2nd dose) ETP: endogenous thrombin potential; Hs-CRP: High sensitive C-reactive protein

After adjustment for baseline values, IM administration was associated with mild increase in all coagulation parameters and with an increase in hs-CRP compared with ID administration (extra increase peak height: 8.4 nmol; 95% CI -16.9 , 33.7; and extra increase hs-CRP: 0.92 mg/L ;95% CI 0.2 , 1.7; see table 3).

Excluding participants using oral contraceptives did not alter these results (see supplemental table 3 and 4).

Table 2: Coagulation and inflammation at baseline, before 2nd vaccine; one and two weeks after 2nd vaccine.

	Day 1	Day 29	Day 36	Day 43	Change between
Intradermal, mean (SD)	**Baseline**	**Before 2nd vaccine**	**7 days after 2nd vaccine**	**14 days after 2nd vaccine**	**baseline and Day 36 (95% CI)**
N	81	80	81	81	
Peak height (nmol)	219.4 (93.1)	223.9 (87.5)	236.7 (99.1)	223.6 (94.2)	17.3 (-12.5 , 47.2)
Lag time (minutes)	5.8 (1.0)	5.9 (1.0)	5.6 (0.9)	5.7 (0.9)	-0.3 (-0.6 , 0.03)
ETP (nmol x minutes)	1719.2 (467.4)	1687.5 (433.5)	1760.0 (515.1)	1690.9 (439.5)	40.7 (-111.9 , 193.4)
Time to peak (minutes)	10.5 (1.7)	10.4 (1.8)	9.9 (1.7)	10.3 (1.9)	-0.6 (-1.1 , -0.03)
Start tail time (minutes)	27.6 (3.2)	27.0 (3.0)	26.9 (3.4)	27.1 (3.0)	-0.8 (-1.8 , 0.3)
Velocity index (nmol/minute)	52.2 (32.1)	56.5 (35.1)	63.9 (41.6)	58.5 (40.9)	11.7 (0.11 , 23.3)
Fibrinogen (mg/dL)	273.4 (57.9)	272.5 (60.3)	284.3 (64.8)	277.9 (71.5)	10.9 (-8.2 , 30.0)
Factor VIII (%)	100.1 (23.4)	101.5 (25.9)	99.6 (26.4)	100.8 (34.0)	-0.5 (-8.2 , 7.3)
D-dimer (ng/ml)	258.6 (236.6)	266.7 (242.4)	274.7 (253.2)	279.9 (300.1)	16.1 (-60 , 92.1)
Hs-CRP (ng/mL)	1.7 (2.5)	1.4 (1.8)	1.6 (2.0)	1.7 (2.4)	-0.1 (-0.8 , 0.6)
	Day 1	**Day 29**	**Day 36**	**Day 43**	**Change between**
Intramuscular, mean (SD)	**Baseline**	**Before 2nd vaccine**	**7 days after 2nd vaccine**	**14 days after 2nd vaccine**	**baseline and Day 36 (95% CI)**
N	42	42	42	42	
Peak height (nmol)	202.9 (69.2)	210.0 (76.6)	231.7 (90.9)	222.0 (77.4)	28.8 (-6.3 , 63.8)
Lag time (minutes)	5.8 (1.0)	5.9 (1.0)	6.0 (1.1)	5.8 (1.0)	0.1 (-0.3 , 0.6)
ETP (nmol x minutes)	1648.7 (386.0)	1666.7 (369.9)	1761.7 (433.7)	1748.2 (413.0)	113 (-65.2 , 291.2)
Time to peak (minutes)	10.8 (2.0)	10.8 (2.1)	10.4 (1.8)	10.4 (1.6)	-0.3 (-1.2 , 0.5)
Start tail time (minutes)	27.7 (3.0)	27.6 (3.1)	27.4 (2.8)	27.2 (2.7)	-0.2 (-1.6 , 1.1)
Velocity index (nmol/minute)	46.8 (28.9)	51.2 (33.0)	59.8 (39.1)	53.8 (31.5)	13.0 (-1.9 , 27.9)
Fibrinogen (mg/dL)	263.8 (57.4)	269.1 (58.7)	295.9 (59.0)	263.1 (43.7)	32.1 (6.8 , 57.4)
Factor VIII (%)	106.5 (24.1)	107.7 (25.4)	105.3 (25.9)	97.6 (26.9)	-1.1 (-12.0 , 9.7)
D-dimer (ng/ml)	215.8 (187.3)	233.2 (180.0)	299.2 (188.8)	261.6 (180.4)	83.5 (0.8 , 166.1)
Hs-CRP (ng/mL)	1.2 (2.0)	1.1 (1.5)	2.3 (2.3)	1.1 (1.1)	1.1 (0.1 , 2.1)

(ETP: endogenous thrombin potential; Hs-CRP: High sensitive C-reactive protein; SD: standard deviation)

Association between coagulation and inflammation

The association between changes (e.g., delta) of coagulation parameters and change in hs-CRP between D1 and D36 are shown in table 4 and figure 2. A positive association was found between delta lag time (beta 0.13; 95%CI: 0.06 , 0.2), delta ETP (beta 28.0; 95%CI: 7.6 , 48.3), delta time to peak (beta 0.13; 95%CI: 0.01 , 0.27), delta time to tail (beta 0.26; 95%CI 0.003 , 0.51), delta fibrinogen (beta 14.6; 95%CI: 11.4 , 17.7), and delta Factor VIII (beta 2.2; 95%CI: 0.8 , 3.6) with delta hs-CRP. No association was found between the changes in the other coagulation parameters and the change in hs-CRP. Excluding participants with a delta hs-CRP under -5 or above 5 did not alter these results (results in supplemental figure 2).

Table 3: Difference in change after vaccination (D1 vs D36) between intramuscular and intradermal vaccination; adjusted for baseline.

	Extra increase IM vs ID (95% CI)
	Adjusted for difference in baseline
Peak height (nmol)	8.4 (-16.9 , 33.7)
Lag time (minutes)	0.35 (0.04 , 0.7)
ETP (nmol x minutes)	66.7 (-36.0 , 169.5)
Time to peak (minutes)	0.35 (-0.2 , 0.9)
Start tail time (minutes)	0.54 (-0.6 , 1.7)
Velocity index (nmol/minute)	1.76 (-9.5 , 13.0)
Fibrinogen (mg/dL)	17.8 (-1.4 , 36.9)
Factor VIII (%)	0.46 (-6.2 , 7.1)
D-dimer (ng/ml)	49.9 (-24.1 , 124.0)
Hs-CRP (ng/mL)	0.93 (0.2 , 1.7)

(ETP: Endogenous Thrombin Potential; Hs-CRP: High sensitive C-reactive protein)

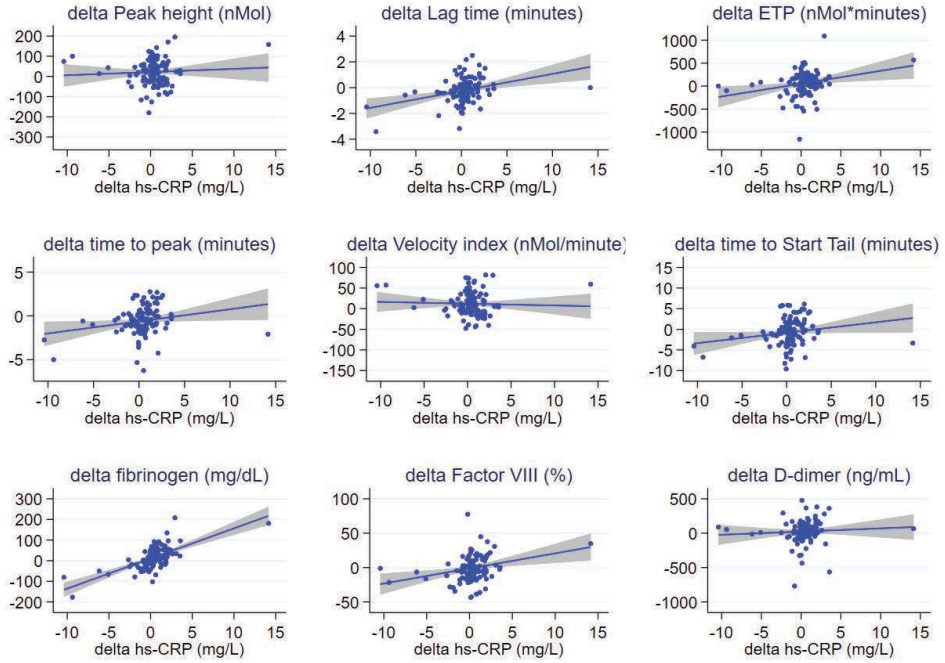

Figure 2: Scatter graphs for the association between the inflammatory response and changes after vaccination for each coagulation parameter; with fitted linear regression line. (ETP: Endogenous Thrombin Potential; Hs-CRP: High sensitive C-reactive protein)

Table 4: The association between the change of coagulation parameter and the change in the inflammatory response (delta hs-CRP) in all participants

	Units increase in the change in coagulation factors associated with one mmol increase in the change of hs-CRP (95% CI)
Peak height (nmol)	1.6 (-3.5 , 6.6)
Lag time (minutes)	0.13 (0.06 , 0.2)
ETP (nmol x minutes)	28.0 (7.6 , 48.3)
Time to peak (minutes)	0.13 (0.01 , 0.27)
Velocity index (nmol/minute)	-0.43 (-2.6 , 1.7)
Start tail time (minutes)	0.26 (0.003 , 0.51)
Fibrinogen (mg/dL)	14.6 (11.4 , 17.7)
Factor VIII (%)	2.2 (0.8 , 3.6)
D-dimer (ng/ml)	4.7 (-8.5 , 17.8)

(ETP: Endogenous Thrombin Potential; Hs-CRP: High sensitive C-reactive protein)

DISCUSSION

SARS-CoV-2 vaccination with full dose IM or fractional dose (1/5th of standard dose) ID of the mRNA-1273 vaccine results in a transient prothrombotic state as evidenced by changes in peak height, endogenous thrombin potential, levels of fibrinogen and D-Dimer. Particular the systemic inflammatory response was most pronounced in participants receiving the full dose of the vaccine intramuscularly as compared with the participants receiving the fractional dose intradermally. These changes in coagulation were associated with the inflammatory response.

Although no association was seen between the change in coagulation (D36-D1) and the inflammatory response after vaccination (D36-D1) in the primary coagulation endpoint (peak height), this was observed for multiple other coagulation endpoints, i.e., lag time, ETP, time to peak, the start tail time, fibrinogen and factor VIII. Particularly, the change in ETP was positively associated with the change in inflammation. This is most likely due to a longer time to complete inhibition of thrombin generation than a stronger and faster propagation of thrombin caused by inflammation (relative stronger association of start tail time with inflammation than lag time or time to peak or peak height). This suggests that inflammation causes the same amount of thrombin to be produced, however the inhibition of thrombin is slower. Fibrinogen and factor VIII are acute-phase proteins, which explains their association with inflammation. D-dimer was not associated with inflammation. This could be due to the relative shorter half-life of D-dimer (5 hours) as compared with CRP (19 hours), fibrinogen (40 hours) and factor VIII (12 hours), and therefore a possible change due to vaccination was not detectable anymore one week after vaccinations. It may seem contradictory that mean levels of FVIII do not change following vaccination (between D1 and D36), while there was an association between changes (e.g., delta) of FVIII and change in hs-CRP between D1 and D36. However, mean FVIII levels are measured on group level while the association between changes of FVIII and hs-CRP is assessed on an individual level.

Prior studies that evaluated coagulation parameters in SARS-CoV-2 vaccinated individuals using an unvaccinated control group also reported a change in the thrombohemorrhagic balance towards hypercoagulability.[16, 18, 19] Campello et al showed a transient increase in TGA at 3 days after vaccination, which normalized within 10 days.[14] Brambilla et al observed increased thrombin generation in 30 participants 8 days after receiving a mRNA vaccine.[13] Garabet et al. found no changes in thrombin generation or D-dimer in on average 11 days after vaccination, which might (similar to Campello et al.) be too long after vaccination to detect small and transient changes. [17] Despite an increased inflammatory response, Willems et al. found no changes in several activated coagulation factors in older participants in the 48 hours after vector-based vaccines. [20] However, all studies that reported an increase in coagulation parameters after vaccination concluded that these transient changes were not strong enough to be clinically relevant in an unselected population, and (similar to our study) no venous thrombotic events were observed.

The strengths of this study include the pre-post randomized design, preventing several possibilities of confounding. The only factor that could intervene in intra-individual change of coagulation may be an event of noticeable impact (e.g., infections, trauma). No such events were registered in the adverse event registration of the original randomized-controlled trial and participants with a SARS-CoV-2 infection were excluded. In addition, the route of vaccine administration (ID or IM) was randomized. The only difference by chance between the two groups (oral contraceptive use) did not affect the conclusions.

Our study has limitations. The limited sample size did not allow a stratified analysis for low and high risk venous thrombosis groups. This is particularly evident in the analyses comparing ID with IM, in which confidence intervals were wide. In addition, the timepoints on which coagulation and inflammation was measured (7 days after second dose of vaccination) could be too late, especially for the inflammatory response. Potentially, the effects of vaccination on coagulation and inflammation are different in the week directly after vaccination, however our blood sample was drawn not earlier than 7 days after the vaccination. Furthermore, loss to follow-up was about 25%. However, this was evenly distributed between the ID and IM group, and unlikely to have been related to these laboratory analyses. Additionally, the cohort consisted of young individuals (<30 years of age), limiting generalizability to middle-aged and older individuals. However, (thrombotic) side effects of SARS-CoV-2 vaccinations are more often reported in young people and are of relative higher importance for young people, because of a lower risk of severe COVID-19 infection in the young.[34] The cohort was predominantly White or of European ancestry**,** therefore it is unsure if our result apply to other ethnicities. It is known that TG is affected by differences in blood collection, sample preparation and storage.[35] One might say that this lack of official standardization and reference values of TG results in limited external replicability of our results. However, all blood collections and analyzes were standardized and performed in a single lab, preventing biased measurements. In addition, by focusing on within individual changes, we expect that the lack of standardization of TG does not influence external replicability of our results. No measurement of coagulation and

inflammation was performed in the first week after the first vaccine dose. Therefore, we do not know the effect of a single vaccination on coagulation and inflammation. However, most systemic side effects of the mRNA-1273 vaccine are reported especially after the second dose.[34] Von Willebrand Factor plays a key role in vascular inflammation and coagulation.[36] Unfortunately von Willebrand Factor was not measured in our study, which could have aided in the interpretation of our results. Because of the design of this study, we cannot conclude whether the smaller effect of ID vaccination than IM vaccination on coagulation and inflammation is caused by the administration technique, the fractional vaccine dose or both. Lastly, these results are only applicable for the mRNA-1273 vaccine. However, prior research suggests an even stronger effect on coagulation and inflammation after viral-vector based vaccines.[14, 19, 37]

Conclusion

We conclude that vaccination results in a transient prothrombotic state which is associated with the inflammatory response. ID vaccination with a 1/5th vaccine dose provokes a smaller systemic inflammatory response and might have a smaller effect on coagulation than IM vaccination, which indicates a benefit for ID administered or fractional dose (SARS-CoV-2) vaccines. Combined with other advantages of using ID fractional dose vaccines, e.g., economic, ecologic and on public health domains, our results support the additional potential benefit of further implementation of ID administered vaccines. Furter research, using larger cohorts, should be performed on the identification of subgroups with higher risk of vaccine induced thrombosis. These groups could potentially benefit the most of ID administered vaccines.

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COMPETING INTERESTS

All authors declare no financial or non-financial competing interests.

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SUPPLEMENTAL MATERIALS

See:

7

CHAPTER 7

Venous thrombosis associated with different types of SARS-CoV-2 vaccines in the Netherlands– results of the TERA case-control study

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ABSTRACT

Background

The magnitude of the risk of venous thromboembolism (VTE) after SARS-CoV-2 vaccines is debated.

Methods

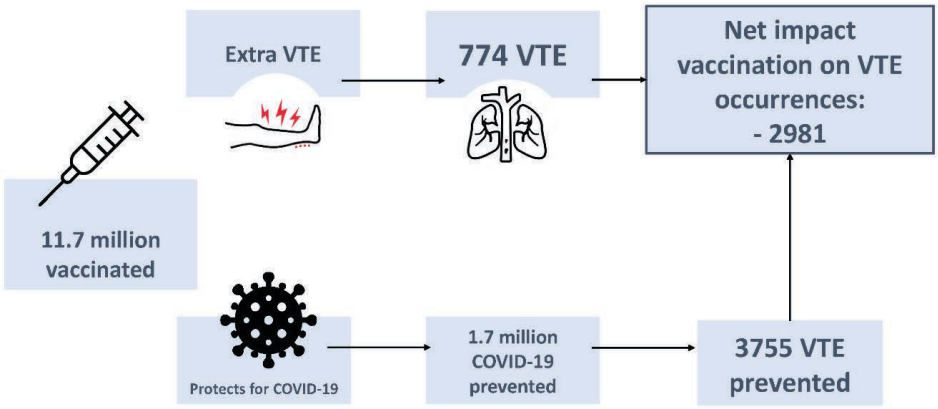
We included patients with a first VTE in 2021 and controls from a sample of Dutch citizens. Participants completed a questionnaire on VTE risk factors and vaccination, with data linked to Statistics Netherlands. Odds ratios (OR) with 95% confidence intervals (95%CI) expressed the relative rate of VTE within 28 days post-vaccination, adjusted for age, sex, BMI, month of index date and major VTE risk factors (COVID-19, surgery, cancer and immobilization). Using previously reported age-stratified VTE incidences, we estimated vaccination's net impact by comparing the number of events attributed to vaccination and prevented by vaccine-induced protection against COVID-19-associated VTE.

Results

We included 779 VTE patients and 5311 controls. mRNA-vaccines were not associated with VTE risk (BNT162b2 (Pfizer- BioNTech) vaccine OR 1.0; 95%CI 0.7-1.3, mRNA-1273 (Moderna) vaccine OR 1.4, 95%CI 0.8-2.4). Vector-based vaccines were associated with VTE risk (AZD1222 (AstraZeneca): OR 1.5, 95%CI 1.0-2.5, Ad26.COV2.S (Johnson & Johnson) OR 2.9, 95%CI 0.9-9.2). Excluding participants with major VTE risk factors, risks changed (BNT162b2: OR 1.5, 95%CI 1.1-2.1; mRNA-1273: OR 0.8, 95%CI 0.3-2.3; AZD1222: OR 2.0; 95%CI 1.0-3.9 and Ad26.COV2.S: OR 3.4; 95%CI 0.7-15.5). We estimated that SARS-CoV-2 vaccines attributed to ~700 VTEs but prevented ~3700 VTEs.

Conclusion

SARS-CoV-2 vaccines are associated with VTE, with varying risks between types of vaccines, and by sex and age. On a population level, in the Netherlands in 2021, SARS-CoV-2 vaccination resulted in a net benefit for the number of VTE events.



Visual summary. SARS-CoV-2 vaccinations were associated with venous thromboembolic events (VTE). However, on a population level, in the Netherlands in 2021, SARS-CoV-2 vaccination resulted in a net benefit on the number of VTE.

INTRODUCTION

During the year 2021, over 9 billion SARS-CoV-2 vaccines were administered worldwide, which were estimated to have prevented over ten million deaths.[1, 2] However, soon after the start of the vaccination campaign, several cases of venous thromboembolisms (VTE) following SARS-CoV-2 vaccination were reported.[3-5] This included reports of common manifestations of VTE, i.e., deep vein thrombosis and pulmonary embolism, which have a background incidence of 1-2 per 1000 per year, as well as an extremely rare (<1 per 25.000 vaccine doses) form of VTE characterized by thrombocytopenia, which has been called vaccine-induced immune thrombotic thrombocytopenia (VITT).[6, 7] These severe side effects were not detected in the primary randomized controlled trials (RCTs), because these RCTs were underpowered for this rare event.[8]

VTE is a relatively common and impactful disorder, and especially the reports on VITT led to changes in the vaccination strategy in many countries.[9] For example in the Netherlands, vaccination with the AZD1222 (AstraZeneca) vaccine was first paused and later discontinued in individuals younger than 60 years after reports of VITT in Denmark and Norway.[10] In addition a higher than expected number of spontaneous reports of VTE was received relative to background incidences.[11] Subsequently, large register-based studies showed increased rates of VTE following SARS-CoV-2 vaccines, in rare cases with thrombocytopenia but far more often without.[3, 12-22] This association was present in particular, but not limited to, the vector based vaccines (AZD1222 (AstraZeneca) and Ad26.COV2.S (Johnson & Johnson)).[23] In contrast, other studies showed no association between SARS-CoV-2 vaccines and VTE.[13, 20, 21, 24, 25]

Many of previous reports on the association between SARS-CoV-2 vaccines and VTE used self-controlled case series (SCCS) design.[13, 21, 26-29] One of the assumptions of this design is that the outcome (VTE) should not influence the subsequent probability of the exposure (vaccination).[26] In this specific situation, this assumption is unlikely to hold, i.e., a VTE may prompt the likelihood of subsequent vaccination. While there are methodological solutions for this in the SCCS design, other study designs may be better suited to provide a valid estimate of the VTE risk associated with SARS-CoV-2 vaccines.

To study the risk of VTE associated with different SARS-CoV-2 vaccines, we performed a case-control study including patients with a first VTE in 2021 and controls without VTE, i.e., The Thrombosis Etiology and Risk factor Assessment (TERA) study. Additional to the overall risk estimation, we assessed the risk of VTE after SARS-CoV-2 vaccination in different subgroups, defined by the presence or absence of additional VTE risk factors. Finally, we estimated the absolute number of VTE events attributed to and prevented by SARS-CoV-2 vaccinations in the Netherlands in 2021, to provide an estimate of the net effect of the vaccination campaign on VTE risk.

METHODS

Study design

Cases were patients selected from the files of 10 participating hospitals in the Netherlands (Leiden, The Hague, Amsterdam, Rotterdam (2), Nijmegen (2), Nieuwegein, Groningen, Eindhoven). We included patients who had a first VTE in 2021 based on diagnostic codes (DBC code: diagnosis-treatment combination) for pulmonary embolism or (deep) venous thrombosis. The selection of patients was performed between October 2022 and August 2023 using the diagnostic codes as listed in supplemental material A2. In eight of the hospitals an opt-in procedure was used, in which patients were invited to participate. After informed consent was obtained, patients were sent a detailed questionnaire containing questions on the VTE, co-morbidities, VTE related risk factors, and vaccination status (date, dose and type of vaccine) (supplemental B). In two hospitals (Leiden and The Hague), participants were invited via an opt-out procedure. Patients recruited from these hospitals received the questionnaire directly, without prior consent.

Controls were recruited from a population-based longitudinal cohort of 7000 randomly selected Dutch citizens from the online LISS (Longitudinal Internet studies for the Social Sciences) panel administered by Centerdata (Tilburg University, The Netherlands).[30] Individuals were not eligible as controls when they had suffered a VTE. In addition, we excluded controls with low quality data: when they had an unrealistic completion time (under 3 minutes; <5th percentile completion time) in combination with exclusively negative answers.

The questionnaire was sent to both the patients and the controls and was filled in between 2022 and 2023. The questionnaire was provided in Dutch or English, depending on the background of the participant, and could be filled in on paper or digitally depending on the preference of the participant. Importantly, the questionnaire was presented as a study on general risk factors for VTE and not specific for SARS-CoV-2 vaccination. With information provided by the participants in the questionnaire, the first VTE defined by the hospital diagnostic code, or the absence thereof was verified. Participants filling in at least 30% of the questions (including the primary exposure: SARS-CoV-2 vaccination) were included in the analyses.

Data of participants were enriched with healthcare information by linking it to data from Statistics Netherlands (CBS) for participants who consented to this linking. Informed consent for use and linking of their data was obtained digitally or in writing from all participants, at the start of the questionnaire.

Definitions

For the main analyses, we included patients who were alive at the time of the selection procedure, and were able to fill in the questionnaire. Confirmation of a first VTE was based on data from the questionnaire or CBS and when the event was not a first event but rather a recurrence, this patient was excluded from the analysis.

We defined the exposure, i.e., type of SARS-CoV-2 vaccination, as the vaccine registered in data from CBS. The National Institute for Public Health and the Environment collects COVID-19 vaccination data on a national level in the COVID Vaccination Information and Monitoring System (CIMS) for all persons who gave consent to share their data in CIMS (≈94%).[31, 32] The CIMS database contains vaccinations that were administered by diverse institutions, for example Municipal Health Services, general practitioners, and nursing homes, but are not entirely complete. Therefore, if no vaccination was registered in CIMS, we additionally used data from the questionnaire. In a similar way, we gather information on having had a SARS-CoV-2 infection, i.e., data from CBS and, in absence of information in CBS, supplemented with data from questionnaires.

To assess the risk of VTE associated with SARS-CoV-2 vaccination, we defined the risk period of a vaccination-associated VTE up to 28 days after vaccination. We therefore assessed whether patients and controls were vaccinated in the 28 days prior to the index date. For patients, the index date was the date of VTE diagnosis; for controls, a random index date in the year 2021 was generated. In the analysis on the risk of VTE associated with a specific vaccine dose, i.e., first or second, the most recent vaccine dose relative to the index date was used.

In addition, we assessed the risk of VTE associated with SARS-CoV-2 vaccination in several subgroups, defined by the presence or absence of additional VTE risk factors. We focussed on the major VTE risk factors, i.e., cancer (diagnosed less than 5 years ago), surgery in the past 90 days, COVID-19 in the past 60 days and immobilization for at least 3 days in the past 90 days (see supplemental document A4 and [33]). We considered a VTE risk factor present, when it was mentioned in the questionnaire or when it was registered in the healthcare data within CBS (from three datasets: two type of diagnostic codes and medication prescriptions, see supplemental materials A1 and A5). To prevent counting diagnoses of 'suspicion of cancer' as a cancer diagnosis, we considered a diagnosis for cancer present when this was registered in multiple data sources within CBS or when it was mentioned in the questionnaire.

Statistical analysis

Demographics of patients and controls were given as proportions or means. Self-reported weight and height were used to calculate body mass index (BMI). To estimate the relative risk of VTE, we used odds ratios, after adjustment for potential confounding factors. In addition to age, BMI (both as a continuous variable) and sex (categorical) we adjusted for calendar time with month of index date as a covariate in the multivariable logistic regression analyses. As endpoints we analysed all VTE as well as deep vein thrombosis (DVT) and pulmonary embolism (PE) separately.

Further analyses assessed risks for different SARS-CoV-2 vaccines (type and dose), and analyses in subgroups by sex, age (<=60 year; >60 year). We performed a sensitivity analysis in which different risk periods were applied, i.e., additional to defining a vaccination-associated VTE as a VTE occurring within 28 days after vaccination, we also used time periods of 14 and 180 days.

In the analysis on the combined effect of VTE risk factors and vaccination, only participants with non-missing data on BMI (0.1% missing) and VTE risk factor (3% missing), were analysed (complete case analysis). We performed for each VTE risk factor with missing information two sensitivity analyses assuming extreme answers when information on risk factors was missing (all missing values imputed as 'risk factor present or ''risk factor not present'). In addition, we performed two sensitivity analyses regarding the risk factor assessment: rather than combining both data sources, we analysed the data once using only risk factor data from CBS and once using only risk factor data from the questionnaire.

To estimate the impact of vaccination on VTE occurrence in 2021 in The Netherlands, we estimated the absolute number of events attributed to vaccination as well as the absolute number of events prevented by vaccine-induced protection against COVID-19 associated VTE. We stratified these calculations for age, i.e., <=60 years of age and >60 years of age.

To estimate the absolute number of VTE events attributed to SARS-CoV-2 vaccines, we first calculated the population attributable fraction (PAF) for each type of vaccine. The PAF is the fraction of total VTE events, attributed to this specific exposure. For the calculation of the PAF, we used the formula: PAF = pd * ((aOR-1 / aOR)), in which pd is the proportion of cases exposed to a type of vaccine and aOR the OR (adjusted for time, sex, BMI, age and the four VTE risk factors) for that type of vaccine.[34, 35] To calculate the absolute number of VTE events attributed to these vaccines, the PAF was multiplied by the number of registered VTE evens in healthcare data of CBS in 2021.

Subsequently we estimated the number of VTE events attributed to SARS-CoV-2 infection stratified by vaccination status. We considered an individual vaccinated, if a vaccine was administered between six and two months prior to SARS-CoV-2 infection (two months to prevent inclusion of vaccines causing VTE and six months because of waning effectiveness of vaccines for symptomatic SARS-CoV-2 infection).[36] We used the same formula for the PAF (in which pd now is the proportion of cases with a SARS-CoV-2 infection with or without prior vaccination, and the aOR is the relative risk of VTE after (un)vaccinated SARS-CoV-2 infection). This PAF was multiplied by number of registered VTE events in CBS in 2021, resulting in the number of VTE events following (un)vaccinated SARS-CoV-2 infections. Subsequently we calculated the percentage of individuals with (un)vaccinated SARS-CoV-2 infections who developed a VTE event.

To estimate the number of vaccine prevented SARS-CoV-2 infections we used the formula: PC = vaccinated SARS-CoV-2 infections in CBS * (1 / (1- VE)). In which PC is the number of SARS-CoV-2 infections that was prevented and VE is the vaccine effectiveness for SARS-CoV-2 infection, assuming situations with VE 50%, 75% and 90%.[37, 38] The number of prevented SARS-CoV-2 infections was multiplied by the ratio of VTE events following unvaccinated SARS-CoV-2 infections, resulting in the hypothetical number of SARS-CoV-2 related VTE that would have occurred without vaccination in 2021. Using the number of VTE attributed to the vaccines and the hypothetical

number of prevented VTE after SARS-CoV-2 without vaccination in 2021 the net number of VTE attributed to SARS-CoV-2 vaccines was estimated.

In an a priori power analysis with a power of 80%, an alpha of 5% and an exposure rate in the control group of 10% with 5 controls per case, at least 160 patients were needed to be included to detect an odds ratio of 2. The sample size for the analyses per type of vaccine (with a minimal exposure rate of 2%) was 650 patients for an odds ratio of 2. Statistical analyses were carried out with STATA 16.1 for Windows (StataCorp, College Station, USA).

RESULTS

In total, 4048 patients were selected from the files of the 10 Dutch hospitals. Of these patients, 555 (14%) were deceased before selection. Of the 3493 living patients who were invited to participate, 1016 (29%) returned the questionnaire. Of these, 744 (73%) gave permission for linkage to data of CBS, which was successful in 716 (96%). Of the 1016 individuals that returned a questionnaire 779 were eligible as cases (see figure 1). Of the cases that were eligible 579 patients gave permission for linkage and were successful linked to data of CBS (74%). In the control group, from the population-based longitudinal cohort of randomly selected Dutch citizens, 5540 out of 7056 (79%) individuals returned the questionnaire. From the in total 5311 eligible controls (see figure 1), 4709 (89%) participants from the control group were successfully linked to data of CBS.

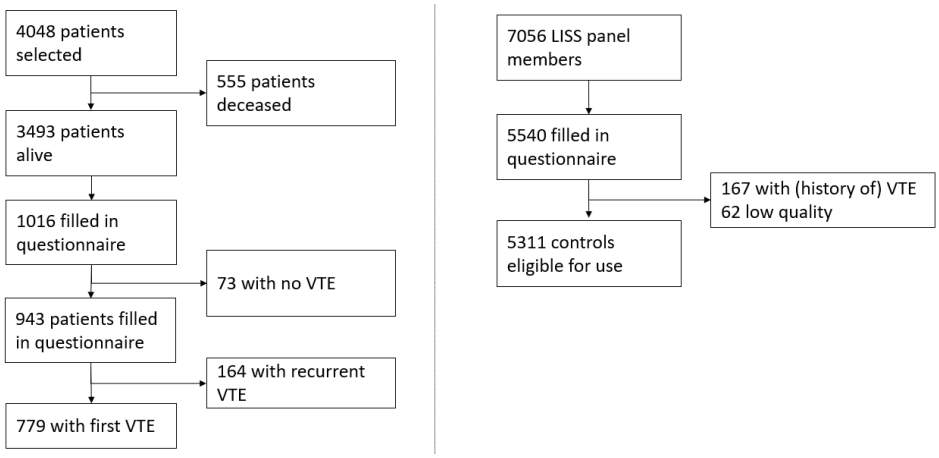


Figure 1: Flowchart of inclusion of eligible patients and controls

Demographics of the patients and controls are summarized in table 1. Patients were older, more frequently male, and had a higher BMI than the control subjects. Most of the VTE events were pulmonary emboli (54%), 35% were DVT and 11% were from atypical origin (such as VTE in the arm, portal vein or cerebral venous sinus). More information about the timing and distribution of

the SARS-CoV-2 vaccinations in the Netherlands is summarized in the supplemental document A7 & A8. Most of the vaccines were administered during the Spring of 2021 and the most commonly administered vaccine was BNT162b2 (Pfizer- BioNTech). The AZD1222 (AstraZeneca) vaccine was predominantly administered to individuals aged between 60 and 70 years of age and the Ad26. COV2.S (Johnson & Johnson) vaccine to individuals aged below 60 years of age.

Table 1: Demographics of included controls and patients with a first venous thromboembolic event

		Controls	Patients
Total		5311	779
Age	Mean (SD)	53.5 (18.2)	61.1 (14.1)
Sex	Men, n (%)	2476 (46.6)	438 (56%)
BMI	Mean (SD)	26.0 (4.7)	27.7 (5.2)
	Missing, n(%)		2 (0.3%)
VTE TYPE	Pulmonary Embolism, n (%)	-	422 (54%)
	DVT Leg, n (%)	-	273 (35%)
	Other, n (%)	-	84 (11%)
RIsk factor	Cancer (<5 year), n (%)	195 (3.7%)	102 (14%)
	Immobilization (<90 days), n (%)	99 (1.9%)	209 (28%)
	Surgery (<90 days), n (%)	125 (2.4%)	110 (15%)
	COVID-19 (<60 days), n (%)	167 (3.1%)	113 (15%)
	Any (of the above) , n (%)	513 (9.7%)	352 (45%)
	None, n (%)	4674 (88%)	311 (40%)
	Missing, n(%)	124 (2.3%)	116 (15%)

SD: standard deviation. BMI: Body mass index. DVT: Deep venous thrombosis. COVID-19: corona virus disease 2019

In the main analysis, when a SARS-CoV-2 vaccine exposure occurred within 28 days prior to VTE, the mRNA-type vaccines were not or at most mildly associated with an increased risk of VTE, (BNT162b2 (Pfizer): aOR 1.0 (95%CI 0.7, 1.3) and mRNA-1273 (Moderna): aOR 1.4 (95%CI 0.8, 2.4), see table 2). The vector based vaccine types were associated with an increased VTE risk (AZD1222: aOR 1.5 (95%CI 1.0, 2.5) and Ad26.COV2.S aOR 2.9 (95%CI 0.9, 9.2), see table 2). In the sensitivity analysis, when a vaccination-associated VTE was defined as a VTE occurring within 14, or within 180 days after vaccination, relative risks were highest in the first 14 days after vaccination (14 days: AZD1222: aOR 1.8 (95%CI 1.0, 3.3) and Ad26.COV2.S aOR 3.8 (95%CI 1.0, 15.4) see supplementary table 1a-c).

Relative risks varied between age groups and sexes. The AZD1222 vaccine was associated with VTE predominantly in young (<60 years: aOR 2.0 (95%CI 0.7, 5.7)) and in women (aOR 1.8 (95%CI 0.9, 3.7)). The Ad26.COV2.S vaccine, which was administered only to individuals aged <60 years old, was associated with a high relative risk of VTE in men (aOR 4.4 (95%CI 1.1, 18.1).

The risk of VTE differed per vaccine dose. For the vaccines associated with VTE risk (except for the Ad26.COV2.S vaccine which is administered only once), the risk of VTE was higher for the first

dose than for the second dose (mRNA-1273 first dose aOR 2.4 (95%CI 1.1, 5.5); second dose aOR 1.5 (95%CI 0.6, 4.1). AZD1222 first dose aOR 1.8 (95%CI 1.0, 3.3); second dose aOR 1.2 (95%CI 0.5, 2.0).

SARS-CoV-2 vaccination affected both the occurrence of DVT and PE (see supplementary table 1a-c). The Ad26.COV2.S vaccine showed an association with the risk of both PE and DVT (aOR PE: 3.8, 95%CI 1.1, 14) and aOR DVT: 2.1, 95%CI 0.3, 16.3). The AZD1222 was predominantly associated with DVT (aOR DVT 1.8, 95%CI 0.9, 3.7 and PE 1.3, 95%CI 0.7, 2.5).

Table 2: Odds ratios for the association between type and dose of vaccination and the risk of a first venous thromboembolic event within 28 days.

				mRNA						Vector					
28-day risk period		**Any first VTE**		**BNT162b2 (Pfizer-BioNTech)**			**mRNA-1273 (Moderna)**			**AZD1222 (AstraZeneca)**			**Ad26.COV2.S (Johnson & Johnson)[b]**		
		Cases	Controls	aOR[a]	95% CI		aOR	95% CI		aOR	95% CI		aOR	95% CI	
Any vaccine dose	**All**	779	5311	1.0	0.7	1.3	1.4	0.8	2.4	1.5	1.0	2.5	2.9	0.9	9.2
	Men	438	2476	0.8	0.6	1.2	1.7	0.9	3.3	1.3	0.7	2.6	4.4	1.1	18.1
	Women	341	2835	1.2	0.8	1.7	1.1	0.5	2.5	1.8	0.9	3.7	1.6	0.2	13.7
	<=60	327	3118	1.1	0.7	1.6	1.0	0.4	2.6	2.0	0.7	5.7	3.5	1.1	11.4
	>60	452	2193	0.9	0.6	1.4	1.5	0.8	2.9	1.2	0.7	2.1			
First vaccine dose	**All**	779	5311	1.1	0.8	1.6	2.4	1.1	5.5	1.8	1.0	3.3	3.0	0.9	9.3
	Men	438	2476	1.0	0.6	1.7	3.4	1.2	9.4	1.4	0.6	3.3	4.5	1.1	18.7
	Women	341	2835	1.2	0.7	2.0	1.3	0.3	5.8	2.3	1.0	5.4			
	<=60	327	3118	1.2	0.7	2.2	0.9	0.2	4.0	3.0	0.9	10.4	3.5	1.1	11.6
	>60	452	2193	1.1	0.7	1.8	5.4	1.7	17.3	1.3	0.6	2.6			
Second vaccine dose	**All**	779	5311	0.9	0.6	1.3	1.5	0.6	4.1	1.2	0.5	2.0			
	Men	438	2476	0.7	0.4	1.2	2.7	0.8	8.8	1.1	0.4	2.2			
	Women	341	2835	1.1	0.6	1.8	0.6	0.1	4.6	1.2	0.4	2.5			
	<=60	327	3118	0.9	0.5	1.6	1.2	0.3	4.1						
	>60	452	2193	0.9	0.5	1.5	1.8	0.3	9.0	1.0	0.4	1.9			

a) aOR: adjusted odds ratio. Adjusted for age, sex, BMI, calendar time (month). VTE: venous thromboembolic event

b) The Johnson & Johnson vaccine was administered only once per person.

We subsequently stratified according to the presence or absence of other VTE risk factors as shown in table 3. The presence of any of the included risk factors for VTE, i.e., cancer (diagnosed less than 5 years ago), surgery in the past 90 days, and immobilization for at least 3 days in the past 90 days, or COVID-19 in the past 60 days, was associated with a 10.8-fold increased risk of VTE (95%CI: 8.8, 13.2). In the absence of VTE risk factors, the AZD1222 and Ad26.COV2.S vaccines were still associated with VTE risk (AZD1222: aOR 2.0, 95%CI 1.0, 3.9; and Ad25.COV2.S: aOR 3.4, 95%CI 0.7, 15.5), while the risk appeared mildly increased for the BNT162b2 vaccine (BNT162b2: 1.5, 95%CI 1.1, 2.1) and not for the mRNA-1273 vaccine (aOR 0.8, 95%CI 0.3, 2.3). The combination of VTE risk factors and SARS-CoV-2 vaccination was not associated with a markedly further VTE

risk increase for any vaccine except Ad26.COV2.S (aOR 35.0, 95%CI 3.0, 1414.6), albeit confidence intervals were wide. See supplemental table 2 for combined effect of vaccines and individual VTE risk factors. Sensitivity analyses assuming extreme values for missing data about VTE risk factors or with only risk factor data from CBS or the questionnaire resulted in some changes in the estimates, but did not alter conclusions (see supplemental table 2a-d).

Table 3: Odds ratios describing association between venous thromboembolic event, venous thromboembolic event risk factors and vaccines in the past 28 days.

Risk factor [a]	Vaccination	BNT162b2 (Pfizer- BioNTech)	mRNA-1273 (Moderna)	AZD1222 (AstraZeneca)	Ad26.COV2.S (Johnson & Johnson)
-	-	1 [ref]	1 [ref]	1 [ref]	1 [ref]
+	-	10.8 (8.8 – 13.2)	10.8 (8.8 – 13.2)	10.8 (8.8 – 13.2)	10.8 (8.8 – 13.2)
-	+	1.5 (1.1 – 2.1)	0.8 (0.3 – 2.3)	2.0 (1.0 – 3.9)	3.4 (0.7 – 15.5)
+	+	6.2 (3.7 – 10.5)	15.1 (6.5 – 34.9)	12.1 (4.8 – 30.2)	35.0 (3.0 – 1414.6)

a) Cancer diagnosis not longer than 5 years ago, immobilization for at least 3 days in the past 90 days, surgery in the past 90 days, COVID-19 infection in the past 60 days
adjusted for age, sex and month
659 cases (349 (53%) with venous thromboembolic event risk factor) and 5177 controls (511 (10%) with venous thromboembolic event risk factor)

In addition to the relative risk of VTE after SARS-CoV-2 infections, we made an estimate of the absolute effect of vaccination in the context of a pandemic in the Netherlands in 2021. During that year, in the Netherlands approximately 22 500 VTE events were registered (with an overall annual incidence of 0.16%), of which 7 500 (annual incidence: 0.08%) occurred in individuals aged 60 or younger and approximately 15 000 (annual incidence: 0.32%) in individuals older than 60 years old. A total of 11 million individuals received one of the SARS-CoV-2 vaccines. The PAF of VTE of all SARS-CoV-2 vaccinations combined was 5.1% for individuals younger and 2.6% for individuals older than 60 years old. Based on this PAF and the absolute number of VTE registered, we estimated that 385 (95%CI -562, 781) VTE events in individuals aged <60 years and 389 (95%CI -1429, 1402) VTE events in >60 years were attributed to SARS-CoV-2 vaccines (see table 4 and details in supplementary table 3). However, vaccination also prevented SARS-CoV-2 infections, and subsequent VTE events. Assuming a vaccine effectiveness of 75%, vaccination prevented over 1.3 million SARS-CoV-2 infections in individuals aged <60 and over 300 000 infections in persons aged >60. This would have resulted in 1274 (95%CI 1164; 1342) VTE evens in individuals younger than 60 years and 2481 (95%CI 2306; 2581) VTE events in individuals older than 60 years. These numbers on VTE events attributed to vaccinations and VTE events prevented by vaccinations, lead to, on a population level, prevention by vaccination of over 800 VTE in individuals <60 years, and over 2000 in individuals older than 60 years, with a net beneficial effect of SARS-CoV-2 vaccines on VTE incidence (see table 4). Assuming a lower vaccine effectiveness, the net benefit of vaccination persists, even with a vaccine effectiveness as low as 50%.

Table 4: Absolute number (95% confidence intervals) of venous thromboembolic events caused and prevented by SARS-CoV-2 vaccines.

	Population (adult)	**VTE in 2021**	
<=60 years	9 568 997	7562	
> 60 years	4 597 642	14 854	
Population attributable fraction	Vaccination	COVID-19 vaccinated	COVID-19 unvaccinated
<=60 years	5.1% (-7.4; 10.3)	0.3% (-2.3; 1.0)	13.0% (11.9; 13.7)
> 60 years	2.6% (-9.6; 9.4)	1.9% (1.1; 2.3)	11.1% (10.3; 11.5)
Number of:	Vaccination	COVID-19 vaccinated	COVID-19 unvaccinated
<=60 years	7 531 592 (79%)	341 622 (3.6%)	1 054 177 (11.0%)
> 60 years	4 185 689 (91%)	92 139 (2.0%)	244 510 (5.3%)
VTE associated with:	Vaccination	COVID-19 vaccinated	COVID-19 unvaccinated
<=60 years	385 (-562; 781)	20 (-171; 74)	983 (898; 1035)
> 60 years	389 (-1429; 1402)	288 (162; 34)	1646 (1530; 1713)
Proportion with VTE	Vaccination	COVID-19 vaccinated	COVID-19 unvaccinated
<=60 years	0.005%	0.006%	0.093%
> 60 years	0.009%	0.312%	0.312%
SARS-CoV-2 infections prevented			
Assumed vaccine effectiveness	50%	75%	90%
<=60 years	683 244	1 366 488	3 416 220
> 60 years	184 278	368 556	921 390
VTE prevented by vaccines			
<=60 years	637 (582; 671)	1274 (1164; 1342)	3184 (2910; 3354)
> 60 years	1240 (1153; 1291)	2481 (2306; 2581)	6202 (5766; 6454)
Net effect of vaccination on VTE			
<=60 years	-251 (-1233; 199)	-888 (-1903; -383)	-2799 (-3916; -2130)
> 60 years	-851 (-2719, 248)	-2092 (-4010; -905)	-5813 (-7882; -4364)

*See for more details supplemental table 3; VTE: venous thromboembolic event

DISCUSSION

The aim of this study was to assess the risk of VTE associated with SARS-CoV-2 vaccines. Our results indicate that mRNA-type vaccines were not or at most mildly associated with an increased risk of VTE. The vector based vaccines were associated with an increased risk of VTE, with relative risk estimates ranging between 1.5 for the AZD1222 vaccine to 2.9 for the Ad26.COV2.S vaccine. The risk of VTE varied by sex and age, depending on the type of vaccine.

The increased relative risk of VTE indicates that vaccines potentially lead to an increase in the absolute number of VTE events in the population. However, the protection against SARS-CoV-2 infection, which in itself is a risk factor for VTE, will prevent VTE events. Therefore, we assessed the net effect of SARS-CoV-2 vaccinations on VTE occurrence. Regardless of age, SARS-CoV-2 vaccines had a net beneficial effect on the number of VTE events, i.e., vaccines prevented more VTE events than they caused. Of course, this calculation concerns the effect on VTE only, while vaccination also has other major advantages.[36]

In the absence of VTE risk factors, the mRNA-1273 vaccine was not associated with the risk of VTE. The increased risk after this vaccine in the overall analyses may be explained by the preferential vaccination of individuals with risk factors with this vaccine. Indeed, in the Netherlands, this vaccine was predominantly used in older and frail persons, i.e., also with an increased VTE risk .[32, 39] In contrast, the BNT162b2 vaccine was associated with a mildly increased risk of VTE in the absence of VTE related risk factors but not in the overall study population. In both circumstances (residual) confounding may have played a role.

To our knowledge this is the first case-control study estimating the relative risks for VTE after all types of SARS-CoV-2 vaccination using detailed information from multiple sources and adjustment for multiple confounding factors. In line with our result, many prior studies found an increased risk of VTE after SARS-CoV-2 vaccines.[3, 15, 19, 27, 28, 40-42] The relative risk was highest after vector-based[17, 19, 22, 27, 28, 40, 42], although some studies have reported also an increased risk for the BNT162b2 vaccine.[16, 27, 42] Comparing relative risks for VTE after SARS-CoV-2 vaccines, unadjusted for possible confounding VTE risk factors, is difficult, because of differences between countries in vaccination strategies. Countries differed in timing and type of vaccines administered to specific targeted populations, resulting in different impact of confounding per country.[43] The net protective effect of vaccination on (COVID-19 related) VTE was also confirmed in prior research.[44, 45]

The major strength of our study is the detailed data available about VTE risk factors (both self-reported and from different registries) and vaccination. Using these data, we were able adjust for many possible confounders. We combined self-reported data and registry-based data, which limited the impact of both recall bias and registry bias. We included cases from several hospitals across the country and the control group consisted of a random sample of Dutch citizens.

Our study also has limitations. Most of VTE cases were selected using hospital diagnostic codes, which may have resulted in missing less severe VTE cases or cases with a second, more important, diagnosis. However, in the Netherlands, all PE and most DVT are treated (or diagnosed) in hospitals. The response rate of the patients was low (29%), which may have resulted in inclusion of patients who were healthier than average. The higher response rate in the control group was most likely due to participants from the LISS panel being more accustomed to regularly completing questionnaires. It is possible that individuals who are part of such a panel exhibit above-average health-seeking behaviours, and are therefore more likely to receive vaccinations. In addition, the linkage with data from CBS was less successful for cases compared to controls. Both these effects may have led to an underestimation of the true effect of SARS-CoV-2 vaccination on VTE in our study. Unfortunately, the study was underpowered for detailed subgroup analyses (for example individuals with a minor VTE risk factor, such as hormonal contraceptives or atypical VTE, such as cerebral venous sinus thrombosis). Furthermore, sample size was small for the Ad26.COV2.S vaccine (the least frequently administered vaccine in the Netherlands). There were no blood measurements performed in this study, therefore we could not study the effects of

vaccination on coagulation parameters. However, several prior studies found small and transient increases in coagulation parameters.[46-48] We were unable to test for specific booster effects, because boosters were administered predominantly after the inclusion period of our study. In estimating the absolute number of VTE cases caused or prevented by SARS-CoV-2 vaccination, we could have compared the number of VTE cases as registered in CBS in 2019 to those in 2021. However, this approach would not allow us to differentiate between VTE cases attributable to SARS-CoV-2 vaccines and those resulting from SARS-CoV-2 infections, nor detect preventing excess cases. Moreover, numerous other factors changed between 2019 and 2021, such as public health measures, a reduced incidence of other infections, and more. Instead, by using the PAF, we were able to account for these confounding factors and provide a more accurate estimation. Furthermore, for this calculation, we assumed that the effectiveness of vaccination was consistent in both magnitude and duration, irrespective of the type of vaccine used. In addition these calculations are dependent on several contextual factors, e.g., status of the pandemic.

In conclusion, SARS-CoV-2 vaccines are associated with the risk of VTE after extensive adjustment for confounders, which varied between types of vaccines, sexes and age. Highest risks were seen after vector-based vaccine types. On a population level, in the Netherlands in 2021, SARS-CoV-2 vaccines had a net beneficial effect on the number of VTE events.

ETHICS APPROVAL

The study was approved by the scientific committee of the department of Clinical Epidemiology Leiden University Medical Center (reference number A175). The Medial Ethics Committee of the Leiden University Medical Center exempted this study for the Medical Research Involving Human Subjects Act (reference number nWMODIV2_2022026).

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SUPPLEMENTARY MATERIAL

See:

8

CHAPTER 8

Discussion

The recent pandemic of COVID-19 has significantly impacted public health and society, with some effects persisting long-term. It is unknown when future pandemics will occur, and it is essential to be prepared. The discussion of this thesis focuses on the three pillars of pandemic preparedness. The first part will focus on the **first pillar** (gaining insight in the spread of the disease) and the **second pillar** (healthcare resource planning) of the pandemic response. It further examines the association between coagulation, SARS-CoV-2 infection, and vaccination (**third pillar**). Insights gained from the COVID-19 pandemic may contribute to a broader understanding of population health and healthcare systems, which may enhance preparedness for future outbreaks.

PART 1: SURVEILLANCE OF THE SPREAD OF INFECTIOUS DISEASE WITH POPULATION DERIVED DATA ON SYMPTOMS AND BEHAVIOR AND THE APPLICATION OF THESE DATA.

Initially, the COVID RADAR app proved to be a success, attracting tens of thousands of participants. Early in the pandemic, it provided valuable insights, introduced innovative methods of delivering informative feedback to users, and achieved all of this while maintaining user privacy. It was particularly inspiring to witness the level of user engagement with the app, demonstrated through their responses to news updates and substantive questions sent via email.

"We use the COVID RADAR as supermarket radar.
We go to areas with low risk behavior and low symptom scores"

Chapter 2 demonstrates that the data from the COVID RADAR app were able to capture patterns similar to those observed in other data sources, such as number of nationally reported positive tests. This type of data is particularly relevant for primary care, as it captures early stages in the trajectory of an infection (during which a primary care physician is involved). In **chapter 3** we show with relatively simple models that, though not perfectly, this type of data is able to predict COVID-19 related primary care workload, outperforming predictions based solely on positive test results. Furthermore, the app also unintentionally served as a surveillance tool for tracking long-term symptoms following SARS-CoV-2 infection. In **chapter 4** we describe that 5% –10% of individuals experienced post-acute COVID symptoms, which persisted until at least 100 days after a positive test result. These symptoms clustered into distinct entities with varying incidences, patient characteristics and vaccination effects, suggestive of multiple mechanisms behind the development of post-acute COVID symptoms.

Though the data from the app yielded valuable insights, applying data from the COVID RADAR app as a surveillance method during the pandemic proved complex. This complexity stemmed from the selection of users and heterogeneity in user engagement, uncertainties about measurement validity, and challenges in effectively leveraging this type of data.

Selection of users and user engagement

As the pandemic progressed, the app's user cohort primarily consisted of individuals aged 60 years or older residing in the province South-Holland. This demographic skew naturally limited both the applicability and the generalizability of the app's data. Moreover, there was significant heterogeneity in user engagement, with varying levels of loyalty to app usage.

Rennie et al. investigated factors influencing engagement with this type of app by inviting participants from an existing cohort to use an app (Fenland cohort, N=4031, aged 45–70).(1) Their analysis revealed that app users were predominantly from higher socioeconomic classes and urban areas, a pattern consistent with selection observed in many health cohorts.(2) Qualitative analysis revealed that the primary factor driving participation was simply "familiarity with the app." Additionally, technical design played a critical role, with a streamlined and user-friendly onboarding process being essential. Sustained engagement was associated with age above 55, intermediate-level occupations, and above-average health but showed no correlation with gender or educational level. Notably, the severity of lockdown measures had no impact on engagement levels. Users emphasized the importance of integrating app usage into their daily routines.

These findings align with the result of several qualitative studies we conducted with end-users of the COVID RADAR app.(3) Another factor deemed valuable was the app's ability to provide personalized feedback, which likely enhanced public engagement during the pandemic. Small analyses, often conducted at users' request and subsequently shared within the app, were highly appreciated. This approach enabled the app to deliver nuanced and valuable information during the uncertainty of the pandemic.

In summary, sustained engagement with an app requiring regular questionnaire completion largely mirrors the selection patterns observed in other health cohorts. However, it also depends on factors such as the level of publicity, the accessibility of the application, and the ability to integrate its use into a daily routine. Monitoring this selection process with both quantitative and qualitative methods is crucial to optimize accessibility and inclusivity. These insights are valuable to take into account in the interpretation of data from such an app, or to decide on changes during usage, e.g. to include a broader set of participants.

Validity of measurements

Within the COVID RADAR app no standardized or validated questionnaires were employed. Symptom-related questions were based on expert knowledge and early descriptions of SARS-CoV-2 symptomatology. However, the included symptoms align with those identified as most predictive in several other studies,(4-6) although these vary by SARS-CoV-2 variant.(7) Furthermore, symptom trajectories corresponded to expected patterns around positive and negative test results **(chapter 2)**.

In the absence of validated instruments to assess the construct 'risk behavior,' the questionnaire was designed to quantify social distancing behavior (e.g., the number of persons within 1.5 meters per time period) and included questions about working from home, facemask usage, and exercise.(8) However, participants found it challenging to report the number of persons they had encountered within 1.5 meters, as reflected in numerous emails from users, as well as several other items. This limitation may have impacted the app's cognitive validity (i.e., whether the test or instrument is measuring what it is supposed to, based on how people understand and process the questions).

"What counts as 'a person within 1.5 meters'? Also cyclers passing by?"
"I've a question: Is walking the dog considered sporting?"

During the pandemic, instruments were developed to measure "risk" or "preventive" behaviors.(9, 10) These instruments often included not only social distancing but also hygiene behaviors (e.g., handwashing) and detailed questions about facemask usage. However, these questionnaires were typically based on local behavioral recommendations and were often too detailed for daily use, with a high burden on the users. Moreover, none of these instruments were designed to examine the relationship between measures of risk-enhancing behaviors and subsequent positive SARS-CoV-2 tests. However, other studies with less detailed repeated data compared with the COVID RADAR app found associations between risk-enhancing behaviors and subsequent positive SARS-CoV-2 tests.(11, 12)

The construct of "risk behavior" is more complex than represented in the COVID RADAR app. The probability of infection depends not only on the extent of social distancing but also on hygiene practices and the nature of contacts. The incorporation of additional elements into the app's instrument could have enhanced its value, particularly by exploring interactions between behavior and individual characteristics. However, such an expansion would likely make the questionnaire lengthier, reducing the app's usability.

In **chapter 2**, we observe an association between above-average risk behavior and a positive test, which suggests good criterion validity. However, it remains questionable whether this above-average risk behavior sufficiently discriminates between the two outcomes, and whether this association is truly causal. The actual result of this behavior—contact with a COVID-19 patient—correlated much more strongly with a positive test result and was therefore utilized in **chapter 3**. Although defining the relationship between risk behavior and infection risk at the individual level proved challenging, at the population level the link between measured behaviors and the proportion of individuals eventually exposed to a COVID-19 patient provides valuable insights into the current reproduction number and, consequently, the effectiveness of public health measures.(13)

In summary, despite that the questionnaire was not based on previously validated instruments, both symptom and behavior data in the COVID RADAR app proved valuable during the pandemic.

While symptom data were useful at both the individual and population levels, risk behavior data were most informative at the population level due to limited cognitive validity (i.e., whether users understand the questions correctly) and the narrow scope of items measured within the construct of 'risk behavior.' However, the outcome of such behavior—(recent) contact with a COVID-19 patient—proved highly valuable.

Application of app data

Beyond challenges inherent to the app itself, external factors also impeded the applicability and impact of the collected data. Collaboration with key institutions, such as the Municipal Health Services (GGD) and the National Institute for Public Health and the Environment (RIVM) which were tasked with managing the pandemic, was limited. Potential reasons for this, in addition to those mentioned earlier, included the overwhelming workload of these organizations, the presence of similar projects, and the possible misalignment of certain aspects of the COVID RADAR app with existing international definitions. Consequently, the integration of COVID RADAR data into pandemic policymaking was significantly constrained.

In Sweden and the United Kingdom, similar apps were developed to predict COVID-19 incidence and hospital admissions.(4, 5, 14) While these apps provided early insights into the pandemic's progression and highlighted differences in symptomatology between SARS-CoV-2 variants, their subsequent impact on policy remains unclear.(15) In the reports, data were used from app users who received test results to first fit a model predicting the individual probability of a positive SARS-CoV-2 test based on symptoms. These individual probabilities were then used to estimate the daily proportion of untested app users who might be infected.

At first glance, this methodology appears appropriate; however, it carries potential pitfalls, which is the reason we used a different approach in **chapter 3**. Specifically, transferring predicted probabilities of a positive test result from a tested population to an untested population introduces bias unless adjustments are made for the likelihood of being tested (which is not stable over time). Additionally, translating these individual probabilities into group risks introduces a form of 'reverse ecological fallacy' or 'exception fallacy'—when inferences about a group are based on observations of exceptional cases or individuals.(16-18) Instead, inferences about groups or populations should be drawn from data collected at the same level, appropriately weighted, standardized, and periodically recalibrated. Importantly, such inferences should never be interpreted as applying to an individual.

An unexpected valuable application of the app's data was its ability to identify patients experiencing long-term symptoms following an initial infection (**chapter 4**). Similar symptom monitoring apps also reported on the duration of symptoms after the acute phase.(19, 20) Additionally, this method of data collection provided insights into vaccine effectiveness and side effects.(21, 22) Furthermore, such data-gathering approaches could potentially be used to assess

the effects of public health measures, both on the pandemic's progression and broader aspects of psychosocial health.(23)

In summary, integration of data from a symptom-monitoring, population-based app into the existing information flow for policymakers is difficult to be effectively achieved during an acute event like a pandemic. Instead, systems should be designed and incorporated well in advance of such situations. Inferences should align with the level of analysis (individual to individual, population to population). Finally, these data platforms can be structured to allow secondary use, enabling the assessment of the (long-term) impacts of infections, measures, or the pandemic itself.

General considerations and recommendations for population based pandemic surveillance

It is important to emphasize that this method is simple, inexpensive, and accessible, and above all independent of healthcare utilization. This method of pandemic surveillance could be of high value in locations and periods with limited testing capacity, such as early in the pandemic or in lower income countries.(24) The voluntary self-reporting of symptoms and (risk) contacts naturally has drawbacks due to recall bias and missing data. However, when compared to other forms of surveillance (based on testing or healthcare utilization), it also offers several advantages. By focusing on symptoms instead of positive tests, surveillance bias is limited. An example of this type of bias is the increase in positive tests later in the pandemic, not as a result of a higher number of infections but due to the expansion of testing capacity. Later during the pandemic the proportion of positive tests increased substantially, not because of more SARS-CoV-2 but a change of behavior: self-testing prior to PCR testing. Additionally, self-reporting of symptoms and (risk) contacts provides an earlier picture of the pandemic's spread, as the data is not tied to healthcare utilization (situated lower in the pyramid, see Figure 1, p. 11).

Currently, the COVID RADAR app has been discontinued. However, the RIVM still maintains the "Infectieradar," a web-based platform, which shares many similarities with the COVID RADAR app.(25) Strong features of this website include the individual adaptability of the questionnaire and the ability to offer free testing provided by the RIVM. This testing option can be a significant factor in attracting more users, as it enhances the "What's in it for me" aspect. Furthermore, this data collection is effectively integrated into the overall data streams used by policymakers, which improves impact and utility of the data.

Building on the experiences with the COVID RADAR app, the "Infectieradar" can become even more valuable. Making the platform available as an app will lower the threshold for users to share data and improve overall usability. Additionally, a regular dialogue with users with individual feedback and small analyses upon request within the app enhances engagement. Insights from experts in citizen science may further support this approach by the incorporation of recent best

practices.(26) Although the current number of Infectieradar users is modest (10800 in April 2025), it can be easily scaled up in the event of a new pandemic. The presence of a pre-established communication plan for scaling up the application during such a time will prove prudent. The primary care network can play a key role in this expansion, as this type of data is particularly valuable in primary care settings.**(chapter 3)** The questionnaire can also be optimized for specific circumstances. It is crucial to strike the right balance between minimization of the burden on participants (keeping the questionnaire brief) and maximization of the relevance of the collected information. Our experience suggests that the question about "recent contact with a COVID-19 patient" provided more valuable data than all the questions related to the type of (risk-enhancing) behavior. This balance can be improved by a focus on the moment and type of contact with a potential contagious patient, and less on the different categories of risk-enhancing behavior.

PART 2: COAGULATION AND VENOUS THROMBOTIC EVENTS AS ADVERSE EVENTS FOLLOWING SARS-COV-2 VACCINATION AND SARS-COV-2 INFECTION.

During my internship as a general practitioner in 2021, I worked at a practice in The Hague, located in a beautiful neighborhood with an above average number of immigrants and people with a lower socioeconomic status. One of our tasks was vaccinating vulnerable groups against COVID-19.

A woman in her 50s from Sub-Saharan Africa came to the practice for her vaccine. Communication was challenging due to language barriers, particularly when explaining a consent form about sharing vaccination data with a thing called "the government". After some difficulty and several explanations from both the doctors as the supporting staff, she agreed.

Just before administering the vaccine, she asked, "Which vaccine will I get?"

When told it was AstraZeneca, she responded, in perfectly clear Dutch:

"Oh, I don't want that; it causes Cerebral Venous Sinus Thrombosis."

Venous thromboembolism and COVID-19 infection

Early in the pandemic a strong association between severe COVID-19 and venous thromboembolism (VTE) appeared evident, and the preventive use of anticoagulation in hospitalized acute COVID-19 patients was recommended.(27-29) A SARS-CoV-2 infection complicated by VTE is associated with a high rate of mortality, although it can be debated whether this high risk of death is a direct consequence of the VTE, or that the VTE is a sign of severe COVID-19.(30) The coagulation system is strongly linked to inflammatory responses, but other mediating mechanisms, such as hypoxia, immobilization, microvascular injury, or disseminated intravascular coagulation may also explain the strong association between COVID-19 and VTE.(31, 32) In **Chapter 5** we describe that in addition to these elements, an intrinsic hypercoagulable potential prior to infection is associated with an increased risk of severe disease. While these data specifically pertain to SARS-CoV-2

infection, intrinsic factors may also influence the severity of other infections, as thrombosis- and haemostasis-related complications were also previously observed during the Spanish Flu (33) and after current severe influenza infections.(34-36) Further research can expand our knowledge of this increased intrinsic risk, which may be influenced by genetic factors.(37, 38) Promising pathways from prior research are via ACE-2, Factor V Leiden (R506Q) and several other genes found via Mendelian Randomization (ABO, ADAMTS13, FUT2).(39-41)

Venous thromboembolism and SARS-CoV-2 vaccines

Later in the pandemic during the vaccination campaign, a potential link between COVID-19 vaccination and VTE emerged.(42-44) In **Chapter 6**, a small, transient increase in coagulation parameters is reported that was observed following administration of a mRNA-type SARS-CoV-2 vaccine. This increase was associated with the inflammatory response, underscoring the important link between the coagulation system and inflammation. Notably, reduction of the dose by a different route of administration diminished the vaccine's impact on both the inflammatory response and coagulation parameters, without compromising its protective efficacy. This finding may explain the lower incidence of systemic side effects observed with intradermal vaccination compared with intramuscular administration in this trial.(45) Future research should explore the potential for adapting current vaccine administration routes to reduce adverse events. Additionally, dose reduction can enhance vaccine availability, which was the original reason underlying the trial, to promote equity and accelerate progress toward herd immunity (**third pillar of pandemic preparedness:** fast development of vaccines).(46)

In Chapter 7 we present results of the TERA-study that showed that there is indeed an association between several SARS-CoV-2 vaccines and VTE. Our results indicate that mRNA-type vaccines were not or at most mildly associated with an increased risk of VTE. The vector based vaccines were associated with an increased risk of VTE, with relative risk estimates ranging between 1.5 for the AZD1222 vaccine to 2.9 for the Ad26.COV2.S vaccine. In addition, we show that confounding due to the selective vaccination of individuals with specific risk factors for both severe COVID-19 and VTE may explain the conflicting results observed in ours and other observational studies.(47-51) For example, the mRNA-1273 vaccine was associated with an increased risk of VTE; however, in the absence of VTE risk factors, this risk returned to baseline. The increased risk after this vaccine in the overall analyses may be explained by the preferential vaccination of individuals with risk factors with this vaccine, which was the case in the Netherlands.

The fact that there was no increased risk for these adverse events detected in the original randomized controlled trials could be explained by the low VTE risk and a lack of power.(52) Even in a meta-analysis, that combined six RCTs including ~70 000 participants per arm, the power to detect a risk ratio of 1.5 was less than 20%. Despite this, the authors concluded there was no increased risk of VTE after SARS-CoV-2 vaccination. From our study, it became clear that in 2021, under the circumstances in the Netherlands during a large pandemic, even though vaccination

did lead to thrombotic events, more cases of thrombosis were prevented by vaccination than caused by it. In fact, under the scenario that everyone had been vaccinated with the vaccine associated with the highest risk of VTE, VTEs were still prevented overall. This raises the question whether it is relevant to focus solely on this type of adverse event in the context of another more urgent and acute problem, such as a pandemic. Of course, this balance may be viewed differently when discussing it outside the context of an active pandemic, as is currently the case with booster vaccinations or with flu vaccinations.

Surveillance of adverse events of vaccines during a pandemic

In the Netherlands, the Lareb Institute is responsible for detecting and reporting drug- and vaccine-related adverse events. A reporting portal is in place through which both healthcare professionals and patients can report potential side effects. By comparing the number of reported cases with the expected incidence (background incidence) of the relevant symptoms or conditions, the Lareb Institute assesses whether a particular adverse event occurs more frequently than anticipated in individuals receiving the medication (observed vs expected analysis).(53)

While healthcare providers are legally obliged to report these adverse events (54), underreporting has been observed in practice.(55) This is taken into account in the analyses by Lareb by issuing a signal at a relative risk of >0.8 instead of >1.(56) However, increased media attention regarding the potential risk of VTE following vaccination may have made physicians more likely to report these adverse events, leading to an earlier detection of a potential signal.(57-59) The level of reporting is also affected by the "Weber-effect", i.e., an increase in adverse effect reporting in the first period after approval of a drug, resulting in earlier detection of a potential signal. (53, 60) However, there are more factors that may influence the degree of reporting. The likelihood of a healthcare professional identifying and reporting a potential adverse event increases when the same physician both prescribed the medication and diagnosed the adverse event. In the Netherlands, general practitioners administered the AstraZeneca vaccine. A case of VTE diagnosed by the general practitioner may have been more frequently perceived as associated with the AstraZeneca vaccine, and subsequently reported, than VTE following vaccination performed by other institutions.

Though the current system via Lareb is able to measure the number of reported adverse events after vaccination,(53) due to these biases it is not able to measure the causal link between vaccination and adverse events (such as VTE). For this, more in depth and detailed studies are needed, with adjustment for confounding factors and appropriate controls.

The case-control vs self-controlled case series vs. cohort

In studying the association between SARS-CoV-2 vaccines and the risk of VTE, we specifically opted for a case-control design, rather than the self-controlled case series (SCCS) design that has

been conducted frequently in recent years for studies on vaccine-related adverse events.(61) In the SCCS design, individuals who experience an event serve as their own controls by comparing the frequency of exposure during a specified period just before the outcome with the frequency during a period more distant in time from the outcome (either before or after). This design is efficient, and time-invariant confounding factors are inherently controlled. However, unadjusted time-varying factors may introduce bias in a SCCS design. More importantly, the design assumes the absence of specific associations between the outcome and the probability of subsequent exposure. If this assumption is violated, a common solution is to implement a 'pre-exposure period,' during which time is excluded from the reference category. However, determining the length of this period is arbitrary and context-dependent, yet it can influence the magnitude of the bias.(62) In the study the relationship between SARS-CoV-2 vaccination and VTE, this assumption is likely not to hold. After the potential link between SARS-CoV-2 vaccines and VTE became apparent, VTE (the outcome) may have emerged as a relative contraindication for SARS-CoV-2 vaccination (the exposure). However, individuals with VTE related to risk factors also associated with severe COVID-19 might also have been more likely to receive vaccination. And finally, VTE associated with SARS-CoV-2 infection may have led to a lower likelihood of vaccination after VTE, as immunity from infection would already protect these individuals. In summary, numerous factors could influence the relationship between the outcome and subsequent exposure in varying directions and over different time periods (which in addition could differ for each type of SARS-CoV-2 vaccine), which severely compromises the determination of an appropriate 'pre-exposure period'.

We opted for a case-control design, rather than a cohort design, because of two reasons: feasibility and the impact of misclassification. Starting a new cohort for this research question is not feasible. The incidence of VTE is approximately 1 in 1000 per year or 1 in 12 000 per month—the risk period following vaccination. To detect a twofold increase in risk after vaccination during a four weeks time interval in a cohort study design with 80% power, two groups of approximately 300 000 individuals each (one vaccinated and one unvaccinated) would be required. While such a study is generally impractical for most types of vaccinations, it was theoretically feasible during the SARS-CoV-2 vaccination campaign. About 80% of the population was vaccinated, and their vaccination was documented in a national register. Several studies with this design have been published, though some of them were not sufficiently powered.(63-66)

A cohort design is only feasible when based on register-based data. However, registration of the exposure, vaccination, is not perfect. In the Netherlands, the primary contributor to misclassification of vaccination was that vaccination registration was only possible with the patient's consent, which was not granted in approximately 7% of cases.(67) As a result, 7% of vaccinated individuals were not recorded as such and were instead classified as unvaccinated. In 2021, according to official records, 84% of adults in the Netherlands were vaccinated. However, the actual vaccination coverage was closer to 90% (+~7%). This misclassification had a particularly

significant impact on the unvaccinated cohort, reducing its proportion from 16% to 10%, meaning that, in reality, one in three individuals classified as unvaccinated had actually received a vaccine in 2021.

The impact of this misclassification on study results depends on the chosen control group. If the control group consists of individuals for whom no vaccination was registered in 2021,(65) in fact one third of them was exposed to a vaccine. In a design that uses person time, where unvaccinated and vaccinated individuals until they were vaccinated contributed to 'control time',(63) 6% of this control time would actually be risk time (see table 1). In both examples this would result in a underestimation of the true effect. In addition, misclassification of the outcome and confounders (which is also likely to happen in a register) would inevitably contribute to an increase of the magnitude of this bias.(68)

Table 1: Effect of exposure misclassification, assuming a true relative risk of 2. If 7% of vaccinated individuals are not registered as vaccinated, this result in a misclassification of 6% of 'control time'.

	Registered (93% correct)				
	28d risk		93%		
NL 18+	14 000 000	**Risk**	**Control**		
Vaccinated	11 700 000	84%	897 534	5 401 233	
Unvaccinated	2 300 000	16%	0	2 300 000	
	Total patient time		897 534	7 701 233	100
	True patient time		897 534	7 227 132	94%
	False patient time		0	474 101	**6%**
	Incidence rate true		0.002	0.001	
	Incidence rate false		0.001	0.002	
	Number of events		1795	8175	
	Incidence rate measured		0.002	0.00106	
	Incidence rate ratio (biased)			1.88	
	Reality (100% correct)				
	28d risk		100%		
NL 18+	14 000.000	**Risk**	**Control**		
Vaccinated	12 580 645	90%	965 091	5 807 777	
Unvaccinated	1 419 355	10%	0	1 419 355	
	Total patient time		965 091	7 227 132	
	True patient time		965 091	7 227 132	
	False patient time		0	0	
	Incidence rate true		0.002	0.001	
	Incidence rate false		0.001	0.002	
	Number of events		1930	7227	
	Incidence rate measured		0.002	0.001	
	Incidence rate ratio (true)			2	

To address the previously mentioned challenges—violations of assumptions inherent to the SCCS design, the effects of misclassification, and the superior efficiency—we opted for a case-control

design. However, it should be noted that this design is not without potential bias either. Controls were selected from a random sample of Dutch residents who participated in a cohort regularly responding to questionnaires. This may have resulted in a selection of people who do not fully represent the "source population." Additionally, because controls could only be selected if they survived, it was not possible to assess the risk of death due to (vaccine-induced) VTE, which may have led to the selection of less severe cases that survived after experiencing VTE.

Finally, as with all case-control studies, there is a potential for recall bias, which is also a misclassification of exposure. Recall bias means that cases are more likely to remember their exposure than controls, which will result in an overestimation of the true effect. However, in the TERA-study we primary used data from the national vaccination register. Only if no vaccination was registered, we used data from the questionnaire. This method reduced the potential impact of both recall bias from questionnaires and measurement errors leading to misclassification in registries.

In the TERA study, we found a discrepancy between hospital records and self-reported data from questionnaires. Therefore, we used a similar approach — combining data from questionnaires and registers — for confounding factors such as hospitalization, immobilization, and cancer. This discrepancy between recorded and self-reported data was also evident for major diagnoses, such as cancer, a finding consistent with prior research.(69) In the TERA study, to verify VTE diagnoses, we used both hospital data and questionnaire responses. Among 1016 individuals who completed the questionnaire and had a registered VTE diagnosis in 2021, over 150 reported no VTE. After confirmation through chart review, only 73 of these cases were found indeed not to have had a VTE. Combining multiple data sources can help mitigate this issue, though some measurement error will inevitably remain.

Comparison of methods of surveillance of adverse events of vaccines during a pandemic

In the study by Pottegård et al., a register-based cohort design was employed, and the researchers used a historical background incidence as a 'control group'.(42) The use of background incidences during a pandemic can be problematic, as the pandemic itself acts as a "background factor" that may influence the incidence of adverse events.(70) This is particularly pertinent for VTE following COVID-19 vaccinations. While Pottegård et al. relied on a historical background incidence, this approach does not account for the numerous secondary effects of the pandemic that could impact VTE incidence. These include lockdowns, changes in the incidence of other infectious diseases, and the consequences of delayed medical care. Failing to account for these effects—or their results—may lead to biased estimates. Despite these theoretical constraints, this study, limited to the AstraZeneca vaccine, identified relative risks similar to those reported in the TERA case-control study, with similar patterns observed across subgroups—higher risks noted particularly among younger individuals and women.

Using similar (Scandinavian) data sources as Pottegård et al. but with a SCCS design, Berild et al. also identified a substantially increased risk of VTE following administration of the AstraZeneca vaccine, particularly among young individuals and women.(71) Additionally, small risk increases were observed after administration of the Pfizer and Moderna vaccines, primarily among older individuals (aged >50) (see table 2). These patterns align with the estimates we found in the TERA case-control study. However, in a post-hoc analysis using femoral fracture as a "negative control" event, the authors demonstrated the impact of violations of the assumptions underlying the SCCS design, which resulted in estimates suggesting a doubling of the risk of femoral fracture following SARS-CoV-2 vaccination (which is unlikely). The authors caution that small positive associations in an SCCS design should be interpreted with care and argue that the observed associations for Pfizer and Moderna vaccines are likely invalid.

Comparing the signals identified by Lareb in their observed-versus-expected analysis with the relative risks found in the TERA case-control study, similar patterns of relative risks were observed for the AstraZeneca vaccine (overall increased risk, particularly among women and younger individuals; see table 2).(56) Additionally, for the Moderna vaccine, adverse events were reported more frequently among men than women in both the Lareb and TERA studies. However, compared with TERA, Lareb reports lower relative risks following almost all vaccines, accept the AstraZeneca vaccine. This can be explained by the difference in media coverage about the VTE side effects of AstraZeneca vaccine, compared with these other two vaccines, resulting in less underreporting for the AstraZeneca vaccine than the other vaccines.

Table 2: Relative risk estimates (Odds ratios, Observed/Expected, rate ratios) of the relation between SARS-CoV-2 vaccines and VTE in subsequent 28 days, in different studies using different design and data sources.

		Pfizer	Moderna	AstraZeneca	Johnson & Johnson
TERA	All	1.0	1.4	1.5	2.9
Odds ratios	Men	0.8	1.7	1.3	4.4
	Women	1.2	1.1	1.8	1.6
	<60	1.1	1.0	2.0	3.5
SCCS (71)	All	1.1	1.3	2.0	X
Rate ratios	Men	1.1	1.3	1.5	X
	Women	1.1	1.2	2.5	X
	<60	1.0	1.3	3.0	X
Lareb (56)	All	0.1	0.2	0.5	0.4
O/E	Men	0.1	0.3	0.4	0.4
	Women	0.1	0.2	0.6	0.4
	<60	0.2	0.2	1.2	0.4

General considerations and recommendations for the surveillance of adverse events of vaccines during a pandemic

As outlined in the previous section, in the surveillance of adverse events, the O/E (Observed-to-Expected) design tends to underestimate risks due to underreporting, the extent of which varies and can significantly affect estimates. As such, it is suitable primarily as a broad signal detection tool. For interventions administered to a large proportion of the population (e.g., vaccines, exceeding 80% coverage), even small differences in risk may have substantial impacts, with varying effects across population subgroups. A more precise estimation of such (stratified) risks can be achieved with a SCCS design. However, this approach is effective only for detecting large effects when assumptions are violated—something that frequently occurs. A prospective cohort design is often less feasible, and suffers problems due to the use of concurrent or historical background incidence rates and biases introduced by the misclassification in register data. A case-control approach, leveraging both register data and data collected for research purposes, is best suited for measuring and adjusting for confounding factors.

Effective communication of vaccination risks and benefits to the general public

Vaccination of the majority of the population is the most effective method to rapidly emerge from a pandemic situation (**third pillar**). A pandemic (such as COVID-19) will probably be caused by a novel opportunistic agent for which no vaccine is initially available. Such a vaccine must be newly developed. Consequently, precise information on the incidence of adverse events in specific populations associated with this newly developed vaccine is absent at the start of a campaign. However, given the international emergency, in the case of an effective vaccine, relatively rare adverse events, undetected in well-conducted randomized controlled trials, will rarely outweigh the substantial benefits of vaccination for the complete population. Thus, it would be unethical to delay vaccination until certainty about all possible (rare) adverse events is achieved. It is crucial that the public can understand this rationale and assess these risks themselves, ultimately leading to a willingness to be vaccinated.

Clear communication regarding the efficacy and potential side effects of vaccines is essential, as the effectiveness of vaccination programs relies on achieving high participation rates within the population. During the COVID-19 vaccination campaign, widespread concern arose among significant segments of the population concerning potential side effects of the vaccines. This concern delayed the campaign and may have contributed to a lower than desired vaccination rate. Furthermore, these concerns about the SARS-CoV-2 vaccines have also undermined trust in other vaccines, as evidenced by the declining participation rates in other vaccination campaigns in the Netherlands since the COVID-19 pandemic.(72)

Within the framework of shared decision-making, effectively communicating the benefits and risks of vaccination is complex, even in the absence of the urgency of a pandemic. A well-informed

patient must understand both relative and absolute risks, weigh individual risks against benefits for the broader population (a trait increasingly uncommon in today's individualistic society), and also resist the influence of the "prevention paradox." Even highly educated individuals often struggle with the latter:

"I've received the flu vaccine for years, but it only makes me sick, and I've never had the flu."
- Many of my colleagues working in hospitals and academia-

Since vaccination is one of the most cost-effective forms of modern healthcare, it is essential to prioritize research and education in the area or risk communication.(73, 74) Previous studies indicate that public discussions about specific adverse events should not focus on the accuracy or inaccuracy of their causal relationship with the vaccine. Instead, emphasis should be placed on the severity of the disease that is being prevented.(75) A compelling narrative is more impactful on the general public than presenting relative risk figures. Furthermore, it is crucial to build immunity against misinterpretation and misinformation.(76) As a familiar and trusted figure, the general practitioner could play a pivotal role in guiding and educating the public.

CONCLUDING REMARKS

Now that the COVID-19 pandemic is behind us, important work begins. We have learned that being well-prepared is essential. During the acute phase of the pandemic, it is difficult to establish a high-quality population-based syndromic surveillance system. However, such a system is immensely valuable for gaining early insights into the pandemic's progression (**first and second pillars**). These pillars prioritize collaboration and require the avoidance of competition. To ensure the **third pillar** is robust, a sufficiently large portion of the population must be willing to receive a vaccine. Clear communication about the (potential impact of) adverse events, but more importantly about the benefits of vaccination, should not be reserved for times of crisis.

A shift in mindset may also be necessary, moving the focus from the individual to the population. It is not "What's in it for me?" but "What's in it for us?" While the focus on the individual has brought remarkable advancements (personalized medicine, inclusive care), we must also recognize the limitations of this perspective. The individual perspective is constrained not only in scope but also in time. I may not personally benefit from the efforts we make now for pandemic preparedness, but for us as a society, these efforts will undoubtedly prove vital in the future. This need for collective thinking extends beyond infectious diseases to other health challenges and future threats, such as an aging population and issues related to planetary health.

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APPENDICES

SUMMARY

The COVID-19 pandemic exposed the global vulnerability to infectious diseases, despite clear historical precedents such as the Spanish Flu. It is not feasible yet to successfully achieve full Pandemic Prevention, but it is necessary to aim for Pandemic Preparedness. This can be structured around three pillars:

1. Limiting the speed of the spread of infection
2. Optimizing healthcare resource allocation during a pandemic
3. Ensuring effective and safe vaccination campaigns

This thesis focusses on these three pillars in pandemic prevention, i.e., speed of spread, planning of healthcare resources, and vaccination, and is divided in two parts.

Part 1: Surveillance of infectious disease using population derived data during the start of a newly emerging pandemic and the application of these data.

Chapter 2 presents the development and validation of the **COVID RADAR app**, which enabled large-scale reporting of symptoms and risk behavior by citizens. With the app we could show that self-reported data correlated well with national case numbers, demonstrating potential as an early warning tool during pandemics.

Chapter 3 shows how these symptom and behavior data can predict COVID-19 related **primary care demand** during the COVID-19 pandemic. This predictive capacity is particularly valuable at the onset of a pandemic when healthcare systems are under strain and diagnostics are scarce.

Chapter 4 presents data on community-dwelling COVID RADAR app users, revealing that 5%–10% experienced post-acute COVID symptoms 100 days after a positive test result. These symptoms clustered into distinct entities with varying incidences, patient characteristics, and vaccination effects, suggestive of multiple mechanisms behind the development of post-acute COVID symptoms.

Part 2: Coagulation and venous thrombotic events as adverse events following SARS CoV-2 vaccination and infection.

Chapter 5 explores the relationship between the pre-existing coagulation potential and COVID-19 severity. In older adults with comorbidities, a higher than average tendency to clot did not increase the risk of infection but could be associated with more severe outcomes. Concentrations of several proteins related to the inflammation and coagulation system were also linked with infection and COVID19 severity. These results could aid further exploration of intrinsic risk factors for severe courses of an infectious disease.

In **chapter 6** presents a secondary analysis of a trial comparing fractional intradermal (ID) and full intramuscular (IM) doses of the SARS-CoV-2 vaccine. Both administration methods resulted in transient increases in inflammatory and coagulation markers, with the effects being more pronounced following IM vaccination. A correlation was observed between the inflammatory response and changes in coagulation parameters.

Chapter 7 it is reported that SARS-CoV-2 vaccines are associated with venous thromboembolism (VTE), with variation in the incidence based on vaccine type, sex, and age. On a population level in the Netherlands in 2021, it was estimated that the benefits of vaccination resulted in a net reduction in VTE events, due to the reduction in COVID-19 incidence.

Based on the experiences with the COVID RADAR app several recommendations were made **in chapter 8** for syndromic surveillance tools. Challenges such as selection bias and variability in user engagement were encountered. Despite these limitations, the data demonstrated strong validity for symptom and behavior patterns. Nevertheless, this data collection method, being simple and inexpensive, could enhance pandemic preparedness by monitoring pandemic progression potentially leading to limiting pathogen spread and optimizing healthcare resource allocation (first two pillars of pandemic preparedness).

Chapter 8 proceeds discussing various methods for monitoring adverse events associated with vaccines during a pandemic. This chapter includes various methods for monitoring adverse events associated with vaccines during a pandemic. Commonly used methods, such as the Observed vs. Expected design, the self-controlled case series, and cohort designs, are effective at detecting signals but fail to provide unbiased relative risk estimates in a pandemic context. Challenges include selective underreporting of events, violations of methodological assumptions, and difficulties in establishing appropriate control groups. Although not free from bias, the case-control design appears to be superior in addressing these challenges, and offers a more robust approach to evaluate vaccine safety under pandemic conditions.

During the COVID-19 pandemic, vaccines were developed in record-breaking time, marking a significant achievement in medical science. However, achieving sufficient vaccination coverage depends on public willingness to be vaccinated—a challenge compounded by growing vaccine hesitancy. To address this, public communication must improve, emphasizing not only the risks but, more importantly, the substantial benefits of vaccination. This may require a shift in mindset, encouraging individuals to consider the collective impact of vaccination. The narrative should transition from “What’s in it for me?” to “What’s in it for us?” fostering a sense of shared responsibility and communal benefit. This need for collective thinking extends beyond infectious diseases to other health challenges and future threats, such as an aging population and planetary health.

NEDERLANDSE SAMENVATTING

De COVID-19-pandemie heeft wereldwijd laten zien dat we kwetsbaar zijn voor infectieziekten, net zoals we dat 100 jaar geleden waren voor de Spaanse Griep. Het is nog niet mogelijk om pandemieën volledig te voorkomen, maar het is wél noodzakelijk te streven naar pandemische paraatheid. Dit kan worden gestructureerd op drie pijlers:

1. Het beperken van de snelheid waarmee infecties zich verspreiden
2. Het optimaliseren van de inzet van zorgcapaciteit tijdens een pandemie
3. Het waarborgen van effectieve en veilige vaccinatiecampagnes

Dit proefschrift richt zich op deze drie pijlers van pandemische paraatheid – verspreidingssnelheid, planning van zorgcapaciteit en vaccinatie – en is verdeeld in twee delen.

Deel 1: Surveillance van infectieziekten met populatiegegevens bij het begin van een opkomende pandemie, en de toepassing van deze gegevens

Hoofdstuk 2 beschrijft de ontwikkeling en validatie van de COVID RADAR-app, waarmee burgers op grote schaal symptomen en risicogedrag konden rapporteren. De zelfgerapporteerde gegevens bleken goed te correleren met de landelijke besmettingscijfers, wat de waarde van de app als vroegtijdig waarschuwingsinstrument tijdens pandemieën onderstreept.

Hoofdstuk 3 laat zien dat symptomen en gedragsdata uit de app voorspellend waren voor de vraag naar huisartsenzorg gerelateerd aan COVID-19. Deze voorspellende waarde is met name belangrijk aan het begin van een pandemie, wanneer zorgsystemen zwaar belast zijn en diagnostiek beperkt beschikbaar is.

Hoofdstuk 4 presenteert gegevens van gebruikers van de app, waaruit blijkt dat 5%–10% van hen 100 dagen na een positieve test nog post-acute COVID-symptomen ervaarde. Deze symptomen clusteren in verschillende groepen met uiteenlopende frequenties, patiëntkenmerken en vaccinatie-effecten, wat wijst op verscheidene onderliggende mechanismen van de ontwikkeling van post-acute COVID-symptomen.

Deel 2: Stolling en veneuze trombose als bijwerkingen van SARS-CoV-2-infectie en -vaccinatie

Hoofdstuk 5 onderzoekt de relatie tussen een verhoogde stollingsneiging vóór infectie en de kans op ontwikkeling van COVID-19, en een ernstige vorm hiervan na besmetting. Bij oudere volwassenen met andere aandoeningen bleek een hoge stollingsneiging niet samen te hangen met een verhoogd infectierisico, maar mogelijk wél met ernstiger ziekteverloop. Verschillende eiwitten gerelateerd aan de bloedstolling en inflammatie hingen samen met de ontwikkeling en ernst van COVID-19. Deze resultaten kunnen bijdragen aan vervolgonderzoek naar intrinsieke risicofactoren van ernstige beloop van infecties.

In **hoofdstuk 6** bevat een secundaire analyse van een gerandomiseerde studie waarin intradermale (ID) versus intramusculaire (IM) toediening van het SARS-CoV-2-vaccin werd vergeleken. Beide toedieningsvormen leidden tot tijdelijke verhoging van ontstekings- en stollingsmarkers, met sterkere effecten bij intramusculaire vaccinatie. Er werd een correlatie gevonden tussen de ontstekingsreactie en veranderingen in stollingsparameters.

Hoofdstuk 7 wordt gerapporteerd dat SARS-CoV-2-vaccins geassocieerd zijn met het ontstaan van veneuze trombo-embolieën (VTE), met variatie in de incidentie afhankelijk van het type vaccin, en geslacht en leeftijd van de gevaccineerde. Op bevolkingsniveau werd in Nederland voor het vaccinatiejaar 2021 geschat dat vaccinatie desalniettemin leidde tot een netto afname van VTE-gevallen, vanwege de preventie van ernstige COVID-19, dat immers ook een riscofactor is voor trombose.

Op basis van de ervaringen met de COVID RADAR-app worden in **hoofdstuk 8** aanbevelingen gedaan voor syndroomsurveillance-instrumenten. Uitdagingen zoals selectiebias en variabele gebruikersbetrokkenheid kwamen naar voren. Ondanks deze beperkingen toonden de data een sterke validiteit voor symptomen en gedragingen. Deze eenvoudige en goedkope methode van dataverzameling kan bijdragen aan betere pandemische paraatheid, met name op het gebied van verspreidingsbeperking en zorgplanning (pijlers 1 en 2).

Daarnaast bespreekt dit hoofdstuk diverse methoden om bijwerkingen van vaccins te monitoren tijdens een pandemie. Veelgebruikte methoden zoals *Observed vs. Expected*, *self-controlled case series* en cohortonderzoek zijn effectief in het detecteren van signalen, maar bieden in een pandemische context geen betrouwbare schattingen van de relatieve risico's. Problemen zoals selectieve onderrapportage, schending van methodologische aannames en uitdagingen bij het vaststellen van geschikte controlegroepen spelen hierbij een rol. Hoewel ook niet vrij van bias, biedt het case-control design in deze context een meer robuuste aanpak.

Tijdens de COVID-19-pandemie werden vaccins in recordtijd ontwikkeld – een ongekende prestatie in de medische wetenschap. Toch hangt voldoende vaccinatiegraad af van de bereidheid van de bevolking om zich te laten vaccineren, iets wat wordt bedreigd door toenemende vaccinatie-argwaan. Publieke communicatie over vaccinatie moet daarom verbeteren, door meer nadruk te leggen op de substantiële voordelen van vaccinatie – naast de risico's. Dit vraagt om een mentale verschuiving van "What's in it for me?" naar "What's in it for us?", om zo collectieve verantwoordelijkheid en solidariteit te bevorderen. Deze noodzaak tot collectief denken reikt verder dan infectieziekten en is ook van belang bij toekomstige uitdagingen zoals vergrijzing en planetaire gezondheid.

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CURRICULUM VITAE

Wilhelm Jan (Willian) van Dijk werd geboren in 1994 in Kampen. Nadat hij het gymnasium afrondde in 2012 op de Gereformeerde Scholengemeenschap Randstad, haalde hij een propedeuse Biomedische Wetenschappen aan de Universiteit van Amsterdam. Vervolgens studeerde hij Geneeskunde aan de Erasmus Universiteit in Rotterdam, hetgeen hij afsloot als arts in 2019. Tijdens de opleiding Geneeskunde nam hij deel aan het onderzoeksteam bij de interventiecardiologie in het Erasmus MC en schreef hij een masterscriptie over bacteriëmie bij patiënten op de spoedeisende hulp. Aansluitend werkte hij tot eind 2020 als arts-assistent op de Intensive Care in het Albert Schweitzer ziekenhuis in Dordrecht.

Eind 2020 startte hij met zowel de opleiding tot huisarts als een PhD (AIOTO-traject) bij de afdeling Public Health en Eerstelijnsgeneeskunde aan het Leids Universitair Medisch Centrum (LUMC) in Leiden en de Health Campus in Den Haag. Zijn onderzoek richtte zich eerst op het gebruik van data uit een app tijdens de COVID-19 pandemie. Later verbreedde zijn onderzoek zich naar het optreden van trombose rondom een SARS-CoV-2 infectie en na vaccinatie tegen dit virus. Dit onderzoek voerde hij uit op de afdeling Klinische Epidemiologie aan het LUMC, waar hij ook de opleiding tot Epidemioloog B afrondde.

Op dit moment combineert hij de laatste fase van de opleiding tot huisarts met onderzoek binnen de Academische Werkplaats Huisartsenzorg in Leiden. Dit onderzoek richt zich op de implementatie van onderwijs over digitalisering binnen de opleiding tot huisarts en het behoud van bevlogen professionals in de eerstelijn. Daarnaast is hij redacteur digitale zorg bij Huisarts & Wetenschap.

A