BMJ Open

Rapid, early and accurate SARS-CoV-2 detection during a COVID-19 outbreak in Austria: Evidence of effective sentinel surveillance screening in primary care

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-045225
Article Type:	Original research
Date Submitted by the Author:	30-Sep-2020
Complete List of Authors:	Leber, Werner; Queen Mary University of London, Centre for Primary Care and Public Health Lammel, Oliver; Praxis Dr Lammel Redlberger-Fritz, Monika; Medical University of Vienna Mustafa-Korninger, Maria Elisabeth; 4 Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG Glehr, Reingard Christina; Medical University of Graz Camp, Jeremy; Medical University of Vienna Agerer, Benedikt; Research Center for Molecular Medicine of the Austrian Academy of Sciences Lercher, Alexander; Research Center for Molecular Medicine of the Austrian Academy of Sciences Popa, Alexandra; Research Center for Molecular Medicine of the Austrian Academy of Sciences Genger, Jakob-Wendelin; Research Center for Molecular Medicine of the Austrian Academy of Sciences Penz, Thomas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Aberle, Stephan; Medical University of Vienna Bock, Christoph; Research Center for Molecular Medicine of the Austrian Academy of Sciences Bergthaler, Andreas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Stiasny, Karin; Medical University of Vienna Hochstrasser, Eva-Maria; Praxis Dr Lammel Hoellinger, Christian; Paracelsus Medical Private University Siebenhofer, Andrea; Institute of General Practice and Evidence-based Health Services Research, Medical University Graz; Institute for General Practice, Goethe University Griffiths, Chris; Barts and The London School of Medicine and Dentistry, Panovska-Griffiths, Jasmina; NIHR CLAHRC North Thames, Department of Applied Health Research, University College London, London, United Kingdom, ; Department of Global Health and Development, London School of Hygiene and Tropical Medicine, 15-17 Tavistock Place, WC1H 9SH,
Keywords:	PRIMARY CARE, COVID-19, Public health < INFECTIOUS DISEASES, VIROLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Rapid, early and accurate SARS-CoV-2 detection during a COVID-19 outbreak in Austria: Evidence of effective sentinel surveillance screening in primary care

Date: September 30, 2020

Version: 1.0

Co-authors

Werner Leber[1,*,&]
Oliver Lammel[2]
Monika Redlberger-Fritz[3]
Maria Elisabeth Mustafa-Korninger[4]
Reingard Christina Glehr[5]
Jeremy V. Camp[3]
Benedikt Agerer[6]
Alexander Lercher[6]
Alexandra M. Popa[6]
Jakob-Wendelin Genger[6]
Thomas Penz[6]
Stephan Aberle[3]
Christoph Bock[6]
Andreas Bergthaler[6]
Karin Stiasny[3]
Eva-Maria Hochstrasser[2]
Christian Hoellinger[7]
Andrea Siebenhofer[5,8]
Chris Griffiths[1]
Jasmina Panovska-Griffiths[9,10,11,*,&]

[1] Centre for Clinical Effectiveness and Health Data Science, Institute of Population Health Sciences, Barts School of Medicine and Dentistry, Queen Mary University of London, London, UK

[2] Practice Dr. Lammel, Ramsau am Dachstein, Austria

[3] Center for Virology, Medical University of Vienna, Vienna, Austria

[4] Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG, Salzburg, Austria

[5] Institute of General Practice and Evidence-based Health Services Research, Medical University of Graz, Graz, Austria

[6] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

[7] Paracelsus Medical University, Salzburg, Austria

[8] Institute of General Practice, Goethe-University Frankfurt am Main, Frankfurt am Main, Germany

[9] Department of Applied Health Care, Institute of Epidemiology & Health Care, University College London, London

[10] Institute for Global Health, University College London, London, UK

[11] The Queen's College, University of Oxford, Oxford, UK

& these authors contributed equally

* Corresponding authors: w.leber@qmul.ac.uk, j.panovska-griffiths@ucl.ac.uk

ABSTRACT

Objectives: We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a regional COVID-19 outbreak in Austria.

Design: Prospective cohort study.

Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03 April, 2020.

Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase chain reaction (RT-qPCR).

Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

Results: Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent), 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Strengths and limitations of this study

- Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- Symptomatic patients received same-day appointments for nasopharyngeal swabs, and people testing RT-PCR reactive were notified within 24 hours.
- Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- The relatively small patient cohort from a single testing site limits conclusions on causality and generalisability.
- Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 25 million cases, and over 850,000 deaths reported as of August 31, 2020. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.[1] In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,[2] infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that,[3,4] and gradually declining over 7-10 days.[5,6]

SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a large number of people. Although most infections remain isolated cases, a small number of individuals (10%) may cause up to 80% of secondary transmissions.[7] Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all documented infections.[8] Superspreading events have been reported from across the globe, and countries achieving early viral suppression took rapid and decisive action to implement

comprehensive case identification and testing, combined with contact tracing and isolation.[9,10] For epidemic control of COVID-19, the effective reproduction number, R_e, needs to be less than 1; the presence of undetected and persistent infection within the population, even if very small, can increase R_e and induce a secondary peak of infections. Therefore, rapid identification and containment of infection is a key factor for the prevention of onward transmission and controlling the virus to protect the public.[11]

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.[12] Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.[12,13] Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.[14] The first death from COVID-19 associated complications occurred on March 12, 2020, and as of August 31, 27,166 cases and 733 COVID-19 related deaths have been reported.

General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.[15-17] The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."[18] However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.[19] In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.[20] The new service supplemented the existing national health hotline for people at risk of COVID-19.[21] RT-qPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.[22] Early detection of SARS-CoV-2 is essential for effective contact tracing,[23] and whole genome sequencing may provide data on dynamics of transmission.[13]

The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.

METHODS

Setting

This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort of Schladming-Dachstein, political subdistrict of Groebming (population 22,829), Austria. The study was conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria, people with flu-like symptoms were advised to call the national health hotline instead of directly presenting to the hospital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and home self-isolate and self-care.

Design

We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2 RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and seropositivity data were collected to compare two groups within this cohort of patients:

- Patients testing RT-qPCR reactive at presentation with acute disease
- Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).

We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR and positive serostatus; and confirmed infection as the presence of convalescent anti-SARS-CoV-2 antibody 3-6 weeks after the acute illness, irrespective of the RT-qPCR result.

Intervention

On February 24, 2020, one day before the first two cases were reported in Austria, the National Influenza Screening Network was enhanced to include SARS-CoV-2 RT-qPCR testing.

Patients with mild to moderate flu-like symptoms calling the study sentinel GP were offered same day appointments for SARS-CoV-2 RT-qPCR testing. RT-qPCR results were available within 24 hours, and those patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2 infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number of days from the first patient to the last patient testing RT-qPCR reactive at the GP.

Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening for patients attending sentinel GPs and paediatric practices. Between November and March of each year,

participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms. Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal influenza virus activity in the country.

Outcome measures

We characterise the outbreak using the following four testing, clinical and viral genomic outcomes: A) The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative; B) The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters); C) The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent seropositivity (confirmed infection) to determine any potential correlation between these stages of disease; and D) the viral clades detected in the outbreak.

Clinical data

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK.

Testing

RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).[22] RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection.

Enzyme linked immune assays (ELISA)

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),[24] and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.[25] Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of

SARS-CoV-2. Reagent wells of the EDI™ Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of CoV2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA, IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI).

Neutralising antibody assay

Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI_ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers ≥10 were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.[26]

Statistical analysis

We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following four outcomes:

Outcome A: We tested the diagnostic accuracy of RT-qPCR, by determining its sensitivity and specificity. To do this, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive, antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive).

Outcome B: We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) *vs*. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent).

Outcome C: Multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and

the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody positive) and all patients with confirmed infection by calculating the correlation coefficient r, and estimating the 95% CI.

Outcome D: For clade analysis, SARS-CoV-2 full genome sequencing was undertaken as part of a wider study covering the whole of Austria.[13] The full-length sequences were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) database (http://gisaid.org).[27] Sequences were aligned in MEGA7 and non-synonymous nucleotide variants were identified to determine the respective clades, following the GISAID classification scheme for lineages.[28]

RESULTS

Overall testing results

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)
Sex		
Female	14 (63.6%)	9 (60%)
Male	8 (36.4%)	6 (40%)
Age (years)		
16-24	4 (26.7%)	3 (20%)

25-34	4 (26.7%)	2 (13.3%(
35-49	6 (40%)	4 (26.7%)
>50	8 (36.4%)	6 (40%)
Ethnic origin		
White	22 (100%)	15 (100%)

Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the 3 patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and 7 were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-14) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

Regression analysis on confirmed infection

Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03;

p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible covariates of confirmed infection.

Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069

Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive, irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody confirmed positive) among 15 patients respectively.

Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

Correlation between acute and confirmed infection

Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI 0.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did not detect any case with convalescent infection (red curve on Figure 2D).

Viral clade analysis

Thirteen of 15 full-length genome sequences were available for clade analysis via GISAID (Table 3); and two sequences were not available at the time of analysis. Lineages of SARS-CoV-2 have been identified based on mutations in key amino acid positions.[28] Clade G is defined by the mutations S-D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[29] Accordingly, among the 13 viral isolates, three different clades were identified, including clade L (N=2), GR (N=4) and L (N=7).

Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2 classification.

Disease Classification	Virus Name (GISAID)	EPI_ISL_#	Date of RT-qPCR	Lineage	ORF 8: 84	ORF3a: 57	S:614*	N:203**	N:204**
Early acute	hCoV-19/Austria/CeMM0191/2020	438032	13/03/2020	B(L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0248/2020	438078	21/03/2020	B (L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0235/2020	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0250/2020	438080	21/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0222/2020	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
Early acute	hCoV-19/Austria/CeMM0249/2020	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0267/2020	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0276/2020	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0303/2020	475778	29/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0324/2020	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0337/2020	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G

Caption Table 3: SARS-CoV-2 clades are classified by The Global Initiative on Sharing All Influenza Data (GISAID) using specific non-synonymous mutations in the viral genome. Clade G is defined by the mutations D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[29]Whole genome data were available for 13/15 sequences; data for two sequences were not available at the time of analysis. Accordingly, among the 13 sequences analysed, three different clades were identified, including clades L (N=2), GR (N=4) and G (N=7). All three clades were detected in early acute infection, and clade G was additionally detected in late acute infection. *For simplicity reasons, only mutation D614G (grey background) in the Spike protein defining clade G is shown. **Additional mutations R203K and G204R in the Nucleocapsid protein N defining clade GR are also shown in grey. ORF, open reading frame.

DISCUSSION

Our results demonstrate that SARS-CoV-2 RT-qPCR testing, when added to a national influenza surveillance programme in primary care, can rapidly, early and accurately diagnose COVID-19 during an outbreak. Of the 73

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and 7 with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,[30] we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary as we move away from the first and potentially towards the second COVID-19 wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy.

Loss of taste of smell has been recognised as an important marker of COVID-19;[1] however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.[31] Furthermore, loss of taste could not be objectively confirmed in one third of people[31] suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.[32] Timely and accurate testing is also a prerequisite for effective contact tracing.[23]

The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. No further endemic cases were detected after the

outbreak. This suggests that combination prevention including rapid testing and case notification in primary care, contact tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening sites can effectively detect and control a regional outbreak.[18]

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.[33] The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.[13]

To our knowledge, this is the first study to show that primary care can contribute to early case detection and termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients, public health, and health systems; nationally and internationally for outbreak epidemiology and control. As countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the number of its sentinels sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.[34]

Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing.

CONCLUSIONS

RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study. OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay. JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL, MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final version.

Acknowledgments: We thank Evelyn Marktl for daily updates on the Christian Drosten's COVID-19 podcast (https://www.ndr.de/nachrichten/info/podcast4684.html). We are grateful to the team of Praxis Dr Lammel for their contributions, and in particular to the nurse Sabine Roiderer for providing direct patient care and help with administration. We thank the patients of Schladming-Dachstein for participating in the study.

Funding: This research was self-funded by each individual co-author. This project received funding from the Vienna Science and Technology Fund (WWTF) as part of the WWTF COVID-19 Rapid Response Funding 2020. Award/Grant number is not applicable.

Ethics approval: The study used secondary anonymised data for which approval was granted by the University of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).

Patient consent for publication: Consent may not be required as no identifiable details on individuals are reported in this manuscript.

Patient and public involvement: No patient involvement.

Data availability statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests: None declared.

References

1. World Health Organisation (WHO). Clinical management of severe acute respiratory infection when COVID-19 is suspected. 2020. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed July 02, 2020).
2. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Ann Intern Med* 2020; 172(9): 577-82.
3. Cheng HY, Jian SW, Liu DP, et al. Contact Tracing Assessment of COVID-19 Transmission Dynamics in Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. *JAMA Intern Med* 2020.
4. Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. *MMWR Morb Mortal Wkly Rep* 2020; 69(13): 377-81.
5. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. *Clin Infect Dis* 2020.
6. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020; 581(7809): 465-9.
7. Endo A, Abbott S, Kucharski AJ, et al. Estimating the overdispersion in COVID-19 transmission using outbreak sizes outside China. *Wellcome Open Res* 2020; 5: 67.
8. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science* 2020; 368(6490): 489-93.
9. European Centre for Disease Control and Prevention (ECDC). Rapid Risk Assessment: Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK– ninth update. 2020. https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-covid-19-pandemic-ninth-update (accessed July 02, 2020).
10. Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study. *Lancet Infect Dis* 2020.
11. Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of Severe Acute Respiratory Syndrome Coronavirus 2. *Emerg Infect Dis 2020;* 26(6): 1059-66.
12. Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in Austria. *Wien Klin Wochenschr* 2020: 1-8.
13. Popa A, Genger J-W, Nicholson M, et al. Mutational dynamics and transmission properties of SARS-CoV-2 superspreading events in Austria. *bioRxiv* 2020: 2020.07.15.204339.
14. Independent T. https://www.independent.co.uk/news/world/europe/coronavirus-austria-cases-covid-19-hospital-lockdown-latest-a9466281.html. 2020.(Accessed September 05, 2020)

15. (European Centre for Disease Control and Prevention (ECDC). Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK –ninth update, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-disease-2019-ninth-update-23-april-2020.pdf (Accessed July 02, 2020)

16. de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. *Eur J Gen Pract* 2020; 26(1): 58-60.

17. Hull SA, Williams C, Ashworth M, et al. Suspected COVID-19 in primary care: how GP records contribute to understanding differences in prevalence by ethnicity. *medRxiv* 2020: 2020.05.23.20101741.

18. European Centres for Disease Control (ECDC). Strategies for the surveillance of COVID-19, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-2020.pdf (accessed July 11, 2020).

19. de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. *Lancet Infect Dis* 2020.

20. Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (Accessed July 02, 2020).

21. Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020).

22. Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2 (accessed September 29, 2020).

23. Kretzschmar ME, Rozhnova G, Bootsma MCJ, et al. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. *Lancet Public Health* 2020.

24. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol* 2020; 57(1): e100.

25. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. *J Korean Med Sci* 2020; 35(14): e149.

26. Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation studies of complex interventions (StaRI): a systematic review and e-Delphi. *Implement Sci* 2015; 10(1): 42.

27. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. *Euro Surveill* 2017; 22(13).

28. Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. *Front Microbiol* 2020; 11(1800).

29. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in genomic epidemiology studies of active hCoV-19 viruses. 2020. https://www.gisaid.org/references/statements-clarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020).

30. Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening schools, the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic wave in the UK: a modelling study. *Lancet Child Adolesc Health* 2020.

31. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste in 2013 European Patients With Mild to Moderate COVID-19. *Ann Intern Med* 2020.

32. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nat Med* 2020.

33. Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper Airway Symptoms. *Ear Nose Throat J* 2020: 145561320920762.

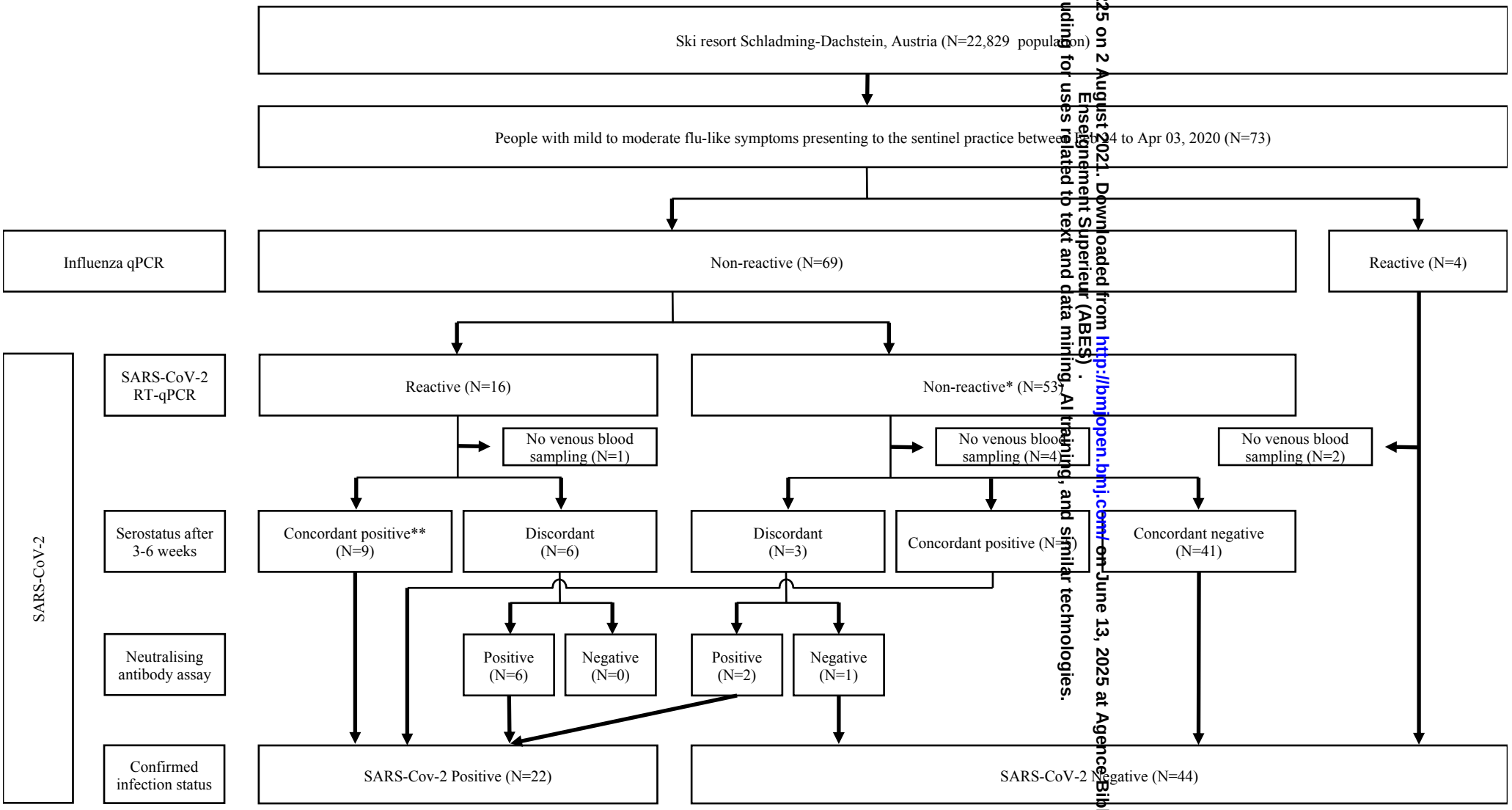
34. NHS England England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020. 2020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-june-2020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed September 09, 2020).

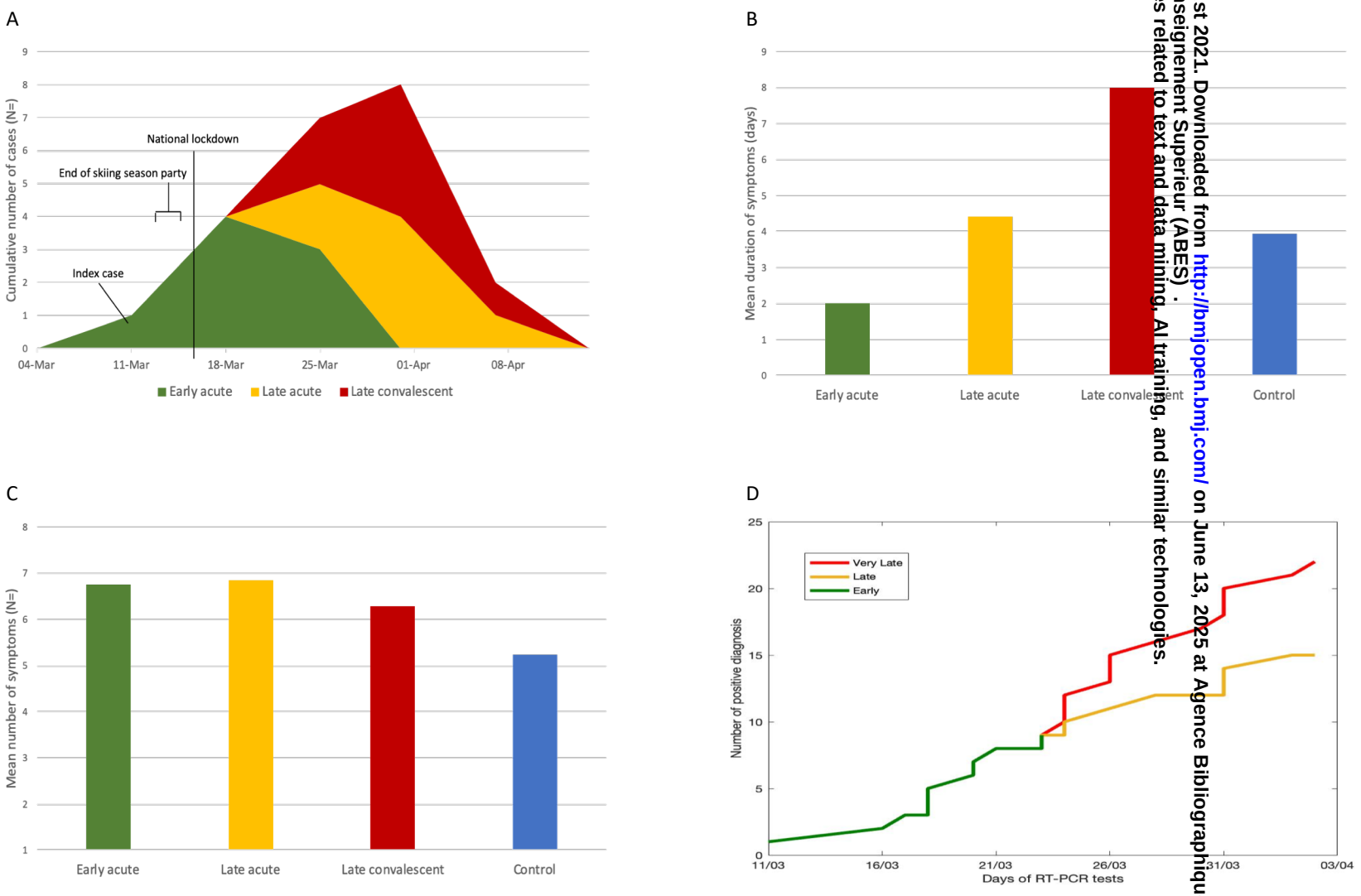
FIGURE LEGENDS

Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1 patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD (N=1, each). ***No concordant negatives.

Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive) in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B)

Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters; (C) Mean duration of symptoms; and (D): Mean number of symptoms.





STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6,7*
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5,6,7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	8
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Rapid, early and accurate SARS-CoV-2 detection during a COVID-19 outbreak in Austria: Evidence of effective sentinel surveillance screening in primary care (REAP-1)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-045225.R1
Article Type:	Original research
Date Submitted by the Author:	27-Jan-2021
Complete List of Authors:	Leber, Werner; Queen Mary University of London, Centre for Primary Care and Public Health Lammel, Oliver; Praxis Dr Lammel Redlberger-Fritz, Monika; Medical University of Vienna Mustafa-Korninger, Maria Elisabeth; 4 Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG Glehr, Reingard Christina; Medical University of Graz Camp, Jeremy; Medical University of Vienna Agerer, Benedikt; Research Center for Molecular Medicine of the Austrian Academy of Sciences Lercher, Alexander; Research Center for Molecular Medicine of the Austrian Academy of Sciences Popa, Alexandra; Research Center for Molecular Medicine of the Austrian Academy of Sciences Genger, Jakob-Wendelin; Research Center for Molecular Medicine of the Austrian Academy of Sciences Penz, Thomas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Aberle, Stephan; Medical University of Vienna Bock, Christoph; Research Center for Molecular Medicine of the Austrian Academy of Sciences Bergthaler, Andreas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Stiasny, Karin; Medical University of Vienna Hochstrasser, Eva-Maria; Praxis Dr Lammel Hoellinger, Christian; Paracelsus Medical Private University Siebenhofer, Andrea; Institute of General Practice and Evidence-based Health Services Research, Medical University Graz; Institute for General Practice, Goethe University Griffiths, Chris; Barts and The London School of Medicine and Dentistry, Panovska-Griffiths, Jasmina; NIHR CLAHRC North Thames, Department of Applied Health Research, University College London, London, United Kingdom, ; Department of Global Health and Development, London School of Hygiene and Tropical Medicine, 15-17 Tavistock Place, WC1H 9SH,
Primary Subject Heading:	General practice / Family practice

Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	PRIMARY CARE, COVID-19, Public health < INFECTIOUS DISEASES, VIROLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Rapid, early and accurate SARS-CoV-2 detection during a COVID-19 outbreak in Austria: Evidence of effective sentinel surveillance screening in primary care (REAP-1)

Date: January 22, 2021

Version: 2.0

Co-authors

Werner Leber[1,*,&]
Oliver Lammel[2]
Monika Redlberger-Fritz[3]
Maria Elisabeth Mustafa-Korninger[4]
Reingard Christina Glehr[5]
Jeremy V. Camp[3]
Benedikt Agerer[6]
Alexander Lercher[6]
Alexandra M. Popa[6]
Jakob-Wendelin Genger[6]
Thomas Penz[6]
Stephan Aberle[3]
Christoph Bock[6]
Andreas Bergthaler[6]
Karin Stiasny[3]
Eva-Maria Hochstrasser[2]
Christian Hoellinger[7]
Andrea Siebenhofer[5,8]
Chris Griffiths[1]
Jasmina Panovska-Griffiths[9,10,11,*,&]

[1] Centre for Clinical Effectiveness and Health Data Science, Institute of Population Health Sciences, Barts School of Medicine and Dentistry, Queen Mary University of London, London, UK

[2] Practice Dr. Lammel, Ramsau am Dachstein, Austria

[3] Center for Virology, Medical University of Vienna, Vienna, Austria

[4] Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG, Salzburg, Austria

[5] Institute of General Practice and Evidence-based Health Services Research, Medical University of Graz, Graz, Austria

[6] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

[7] Paracelsus Medical University, Salzburg, Austria

[8] Institute of General Practice, Goethe-University Frankfurt am Main, Frankfurt am Main, Germany

[9] Department of Applied Health Care, Institute of Epidemiology & Health Care, University College London, London

[10] Institute for Global Health, University College London, London, UK

[11] The Queen's College, University of Oxford, Oxford, UK

& these authors contributed equally

* Corresponding authors: w.leber@qmul.ac.uk, j.panovska-griffiths@ucl.ac.uk

ABSTRACT

Objectives: We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a regional COVID-19 outbreak in Austria.

Design: Prospective cohort study.

Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03 April, 2020.

Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase chain reaction (RT-qPCR).

Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

Results: Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent), 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Strengths and limitations of this study

- Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people testing RT-qPCR reactive were notified within 24 hours.
- Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.[1] In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,[2] infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that,[3,4] and gradually declining over 7-10 days.[5,6 7]

SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may cause up to 80% of secondary transmissions.[8] Although symptomatic infection is common (17 %, range 4-41%), the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.[9-11] Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all documented infections.[12] Superspreading events have been reported from across the globe, and countries

achieving early viral suppression took rapid and decisive action to implement comprehensive case identification and testing, combined with contact tracing and isolation.[13,14] For epidemic control of COVID-19, the effective reproduction number, R_e, needs to be less than 1; the presence of undetected and persistent infection within the population, even if very small, can increase R_e and induce a secondary peak of infections. Therefore, rapid identification and containment of infection is a key factor for the prevention of onward transmission and controlling the virus to protect the public.[15]

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.[16] Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.[16,17] Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.[18] The first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512 cases and 7.389 COVID-19 related deaths have been reported.

General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.[19-21] The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."[22] However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.[23] In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.[24] The new service supplemented the existing national health hotline for people at risk of COVID-19.[25] RT-qPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.[26] Early detection of SARS-CoV-2 is essential for effective contact tracing,[27] and whole genome sequencing may provide data on dynamics of transmission.[17,28]

The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.

METHODS

Setting

This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort of Schladming-Dachstein, political subdistrict of Groebming (population 22,829), Austria. The study was conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria, people with flu-like symptoms were advised to call the national health hotline instead of directly presenting to the hospital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and home self-isolate and self-care. Asymptomatic people were excluded from this study.

Design

We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2 RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and seropositivity data were collected to compare two groups within this cohort of patients:

- Patients testing RT-qPCR reactive at presentation with acute disease
- Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).

We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR and positive serostatus; and confirmed infection as the presence of convalescent anti-SARS-CoV-2 antibody 3-6 weeks after the acute illness, irrespective of the RT-qPCR result.

Intervention

On February 24, 2020, one day before the first two cases were reported in Austria, the National Influenza Screening Network was enhanced to include SARS-CoV-2 RT-qPCR testing.

Patients with mild to moderate flu-like symptoms calling the study sentinel GP were offered same day appointments for SARS-CoV-2 RT-qPCR testing. RT-qPCR results were available within 24 hours, and those patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2 infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number of days from the first patient to the last patient testing RT-qPCR reactive at the GP.

Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening for patients attending sentinel GPs and paediatric practices. Between November and March of each year, participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms. Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal influenza virus activity in the country.

Clinical data

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK.

Testing

RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).[26] RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection.

Enzyme linked immune assays (ELISA)

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),[29] and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.[30] Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of SARS-CoV-2. Reagent wells of the EDI™ Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA,

IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI).

Neutralising antibody assay

Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI_ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers ≥10 were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.[31]

Outcome measures and statistical analysis

We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following four testing, viral and genomic outcomes:

Outcome A: The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative. To determine the accuracy of RT-qPCR, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive, antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive).

Outcome B: The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters). We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) *vs*. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent).

Outcome C: The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent sero-positivity (confirmed infection) to determine any potential correlation between these stages of disease. We used multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody

positive) and all patients with confirmed infection by calculating the correlation coefficient *r*, and estimating the 95% CI.

Outcome D: The number of viral clades implicated in the outbreak. To do this, SARS-CoV-2 full genome sequencing was undertaken as part of a wider study covering the whole of Austria.[17,28] The full-length sequences were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) database (http://gisaid.org).[32] Sequences were aligned in MEGA7 and non-synonymous nucleotide variants were identified to determine the respective clades, following the GISAID classification scheme for lineages.[33]

RESULTS

Overall testing results

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)
Sex		
Female	14 (63.6%)	9 (60%)
Male	8 (36.4%)	6 (40%)
Age (years)		
16-24	4 (26.7%)	3 (20%)

25-34	4 (26.7%)	2 (13.3%(
35-49	6 (40%)	4 (26.7%)
>50	8 (36.4%)	6 (40%)
Ethnic origin		
White	22 (100%)	15 (100%)

Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-14) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

Regression analysis on confirmed infection

Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03;

p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible covariates of confirmed infection.

Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069

Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive, irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody confirmed positive) among 15 patients respectively.

Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

Correlation between acute and confirmed infection

Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI 0.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did not detect any case with convalescent infection (red curve on Figure 2D).

Viral clade analysis

Thirteen of 15 full-length genome sequences were available for clade analysis via GISAID (Table 3); and two sequences were not available at the time of analysis. Lineages of SARS-CoV-2 have been identified based on mutations in key amino acid positions.[33] Clade G is defined by the mutations D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[34] Accordingly, among the 13 viral isolates, three different clades were identified, including clade L (N=2), GR (N=4) and L (N=7).

Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2 classification.

Disease Classification	Virus Name (GISAID)	EPI_ISL_#	Date of RT-qPCR	Lineage	ORF 8: 84	ORF3a: 57	S:614*	N:203**	N:204**
Early acute	hCoV-19/Austria/CeMM0191/2020	438032	13/03/2020	B(L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0248/2020	438078	21/03/2020	B (L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0235/2020	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0250/2020	438080	21/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0222/2020	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
Early acute	hCoV-19/Austria/CeMM0249/2020	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0267/2020	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0276/2020	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0303/2020	475778	29/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0324/2020	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0337/2020	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G

Caption Table 3: SARS-CoV-2 clades are classified by The Global Initiative on Sharing All Influenza Data (GISAID) using specific non-synonymous mutations in the viral genome. Clade G is defined by the mutations D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[34] Whole genome data were available for 13/15 sequences; data for two sequences were not available at the time of analysis. Accordingly, among the 13 sequences analysed, three different clades were identified, including clades L (N=2), GR (N=4) and G (N=7). All three clades were detected in early acute infection, and clade G was additionally detected in late acute infection. *For simplicity reasons, only mutation D614G (grey background) in the Spike protein defining clade G is shown. **Additional mutations R203K and G204R in the Nucleocapsid protein N defining clade GR are also shown in grey. ORF, open reading frame.

DISCUSSION

Our results demonstrate that SARS-CoV-2 RT-qPCR testing, when added to a national influenza surveillance programme in primary care, can rapidly, early and accurately diagnose COVID-19 during an outbreak. Of the 73

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,[35] we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy.

Loss of taste of smell has been recognised as an important marker of COVID-19;[36,37] however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.[38] Furthermore, loss of taste could not be objectively confirmed in one third of people[38] suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.[39] Timely and accurate testing is also a prerequisite for effective contact tracing.[27]

The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or

did not attend the practice because of mild disease.[40] No further endemic cases were detected after the outbreak. This suggests that combination prevention including rapid testing and case notification in primary care, contact tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening sites can effectively detect and control a regional outbreak.[22]

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.[41] However, change or loss in smell/taste were not quantified using an established tool such as the visual analogue scale (VAS),[42,43] but rather assessed by simple "yes" and "no" answers using a standard clinical questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,[10] asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This potentially excludes an important segment of the infected population and future studies will focus on exploring this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.[28]

To our knowledge, this is the first study to show that primary care can contribute to early case detection and termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients, public health, and health systems; nationally and internationally for outbreak epidemiology and control. As

countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.[44] Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2 lateral flow antigen testing.

CONCLUSIONS

RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study. OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay. JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL, MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final version.

Acknowledgments: We thank Evelyn Marktl for daily updates on the Christian Drosten's COVID-19 podcast (https://www.ndr.de/nachrichten/info/podcast4684.html). We are grateful to the team of Praxis Dr Lammel for their contributions, and in particular to the nurse Sabine Roiderer for providing direct patient care and help with administration. We thank the patients of Schladming-Dachstein for participating in the study.

Funding: This research was self-funded by each individual co-author. This project received funding from the Vienna Science and Technology Fund (WWTF) as part of the WWTF COVID-19 Rapid Response Funding 2020. Award/Grant number is not applicable.

Ethics approval: The study used secondary anonymised data for which approval was granted by the University of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).

Patient consent for publication: Consent may not be required as no identifiable details on individuals are reported in this manuscript.

Patient and public involvement: No patient involvement.

Data availability statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests: None declared.

References

1. World Health Organisation (WHO). Clinical management of severe acute respiratory infection when COVID-19 is suspected. 2020. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed July 02, 2020).

2. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 2020; 172(9): 577-82.

3. Cheng HY, Jian SW, Liu DP, Ng TC, Huang WT, Lin HH. Contact Tracing Assessment of COVID-19 Transmission Dynamics in Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. JAMA Intern Med 2020.

4. Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. MMWR Morb Mortal Wkly Rep 2020; 69(13): 377-81.

5. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis 2020.

6. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581(7809): 465-9.

7. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-CoV viral load dynamics, duration of viral shedding and infectiousness: a living systematic review and meta-analysis. medRxiv 2020: 2020.07.25.20162107.

8. Endo A, Abbott S, Kucharski AJ, Funk S. Estimating the overdispersion in COVID-19 transmission using outbreak sizes outside China. Wellcome Open Res 2020; 5: 67.

9. Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus symptomatic COVID-19. Lancet 2021; 397(10269): 93-4.

10. Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis. Official Journal of the Association of Medical Microbiology and Infectious Disease Canada 2020; 5(4): 223-34.

11. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. Lancet Infect Dis 2020.

12. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science 2020; 368(6490): 489-93.

13. European Centre for Disease Control and Prevention (ECDC). Rapid Risk Assessment: Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK– ninth update. 2020. https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-covid-19-pandemic-ninth-update (accessed July 02, 2020).

14. Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study. Lancet Infect Dis 2020.

15. Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis 2020; 26(6): 1059-66.

16. Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in Austria. Wien Klin Wochenschr 2020: 1-8.

17. Popa A, Genger JW, Nicholson MD, et al. Genomic epidemiology of superspreading events in Austria reveals mutational dynamics and transmission properties of SARS-CoV-2. Sci Transl Med 2020; 12(573).

18. Independent T. https://www.independent.co.uk/news/world/europe/coronavirus-austria-cases-covid-19-hospital-lockdown-latest-a9466281.html. 2020.(Accessed September 05, 2020)

19. (European Centre for Disease Control and Prevention (ECDC). Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK –ninth update, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-disease-2019-ninth-update-23-april-2020.pdf (Accessed July 02, 2020)

20. de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. Eur J Gen Pract 2020; 26(1): 58-60.

21. Hull SA, Williams C, Ashworth M, Carvalho C, Boomla K. Prevalence of suspected COVID-19 infection in patients from ethnic minority populations: a cross-sectional study in primary care. British Journal of General Practice 2020; 70(699): e696-e704.

22. European Centres for Disease Control (ECDC). Strategies for the surveillance of COVID-19, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-2020.pdf (accessed July 11, 2020).

23. de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis 2020.

24. Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (Accessed July 02, 2020).

25. Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020).

26. Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2 (accessed September 29, 2020).

27. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert JHHM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. The Lancet Public Health.

28. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol 2020; 57(1): e100.

29. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. J Korean Med Sci 2020; 35(14): e149.

30. Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation studies of complex interventions (StaRI): a systematic review and e-Delphi. Implementation Science 2015; 10(1): 42.

31. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Eurosurveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2017; 22(13).

32. Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Frontiers in Microbiology 2020; 11(1800).

33. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in genomic epidemiology studies of active hCoV-19 viruses. 2020. https://www.gisaid.org/references/statements-clarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020).

34. Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening schools, the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic wave in the UK: a modelling study. The Lancet Child & Adolescent Health.

35. Aziz M, Goyal H, Haghbin H, Lee-Smith WM, Gajendran M, Perisetti A. The Association of "Loss of Smell" to COVID-19: A Systematic Review and Meta-Nnalysis. Am J Med Sci 2020.

36. von Bartheld CS, Hagen MM, Butowt R. Prevalence of Chemosensory Dysfunction in COVID-19 Patients: A Systematic Review and Meta-analysis Reveals Significant Ethnic Differences. ACS Chem Neurosci 2020; 11(19): 2944-61.

37. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste in 2013 European Patients With Mild to Moderate COVID-19. Ann Intern Med 2020.

38. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. Nat Med 2020.

39. Maltezou HC, Vorou R, Papadima K, et al. Transmission dynamics of SARS-CoV-2 within families with children in Greece: A study of 23 clusters. J Med Virol 2020.

40. Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper Airway Symptoms. Ear Nose Throat J 2020: 145561320920762.

41. Sung Y-T, Wu J-S. The Visual Analogue Scale for Rating, Ranking and Paired-Comparison (VAS-RRP): A new technique for psychological measurement. Behav Res Methods 2018; 50(4): 1694-715.

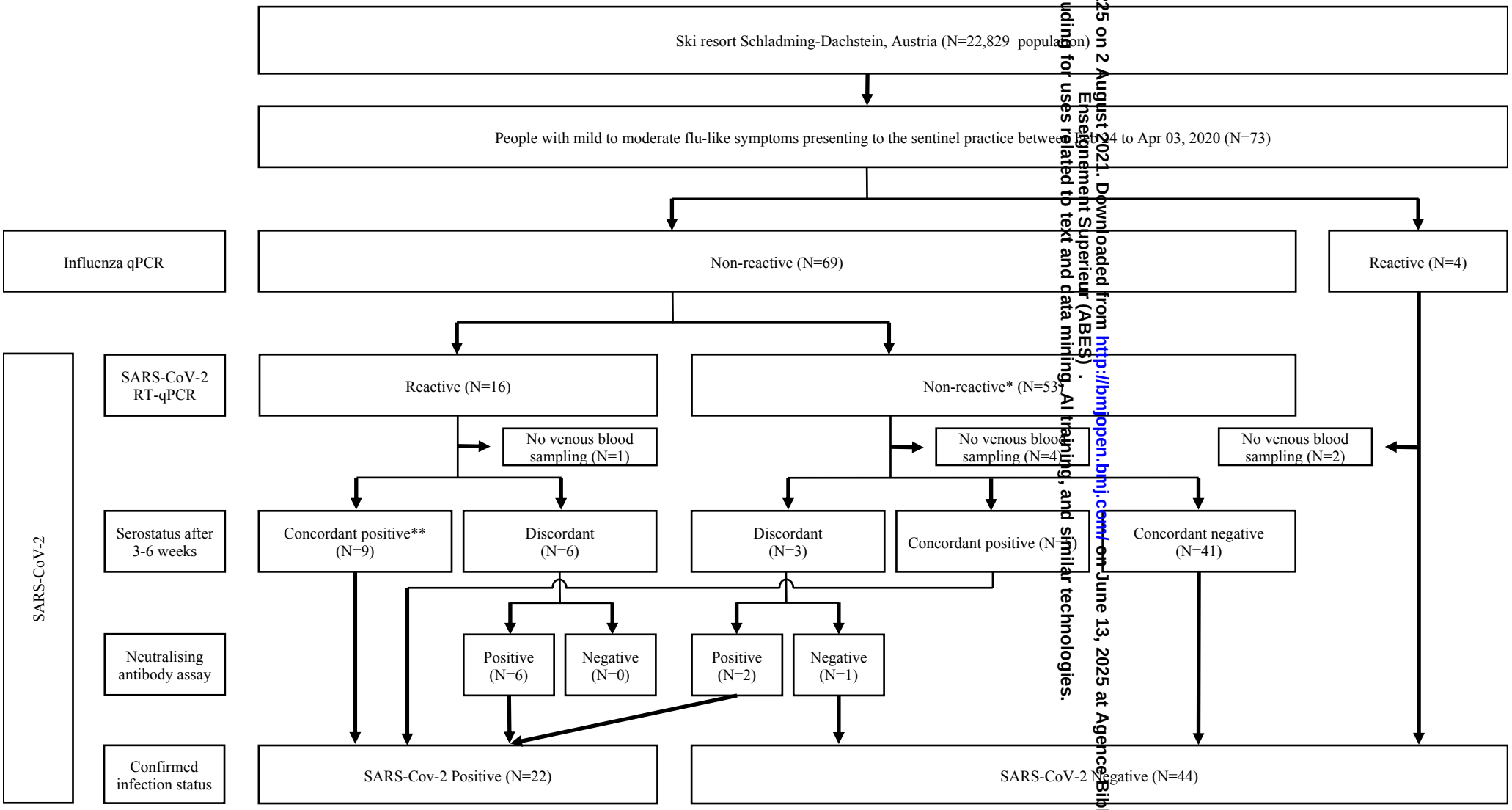
42. Rojas-Lechuga MJ, Izquierdo-Domínguez A, Chiesa-Estomba C, et al. Chemosensory dysfunction in COVID-19 out-patients. Eur Arch Otorhinolaryngol 2020: 1-8.

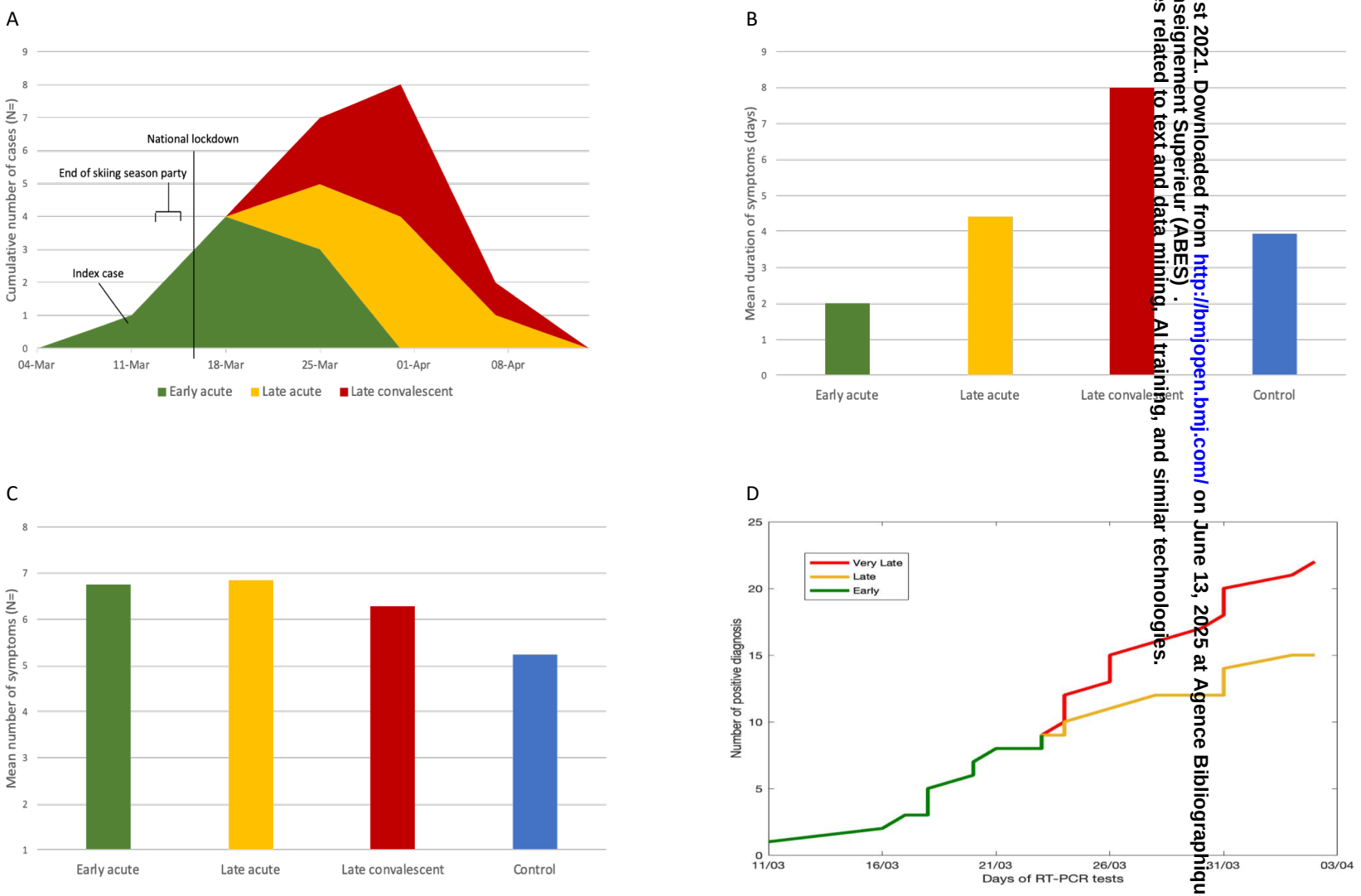
43. NHS England England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020. 2020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-june-2020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed September 09, 2020).

FIGURE LEGENDS

Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1 patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD (N=1, each). ***No concordant negatives.

Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive) in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B) Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters; (C) Mean duration of symptoms; and (D): Mean number of symptoms.





STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7,8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6,7*
Bias	9	Describe any efforts to address potential sources of bias	6,13
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Figure 1 attached
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations			13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12,13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A prospective cohort study (REAP-1)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-045225.R2
Article Type:	Original research
Date Submitted by the Author:	18-Mar-2021
Complete List of Authors:	Leber, Werner; Queen Mary University of London, Institute of Population Health Sciences, Centre for Clinical Effectiveness and Health Data Science Lammel, Oliver; Praxis Dr Lammel Redlberger-Fritz, Monika; Medical University of Vienna, Center for Virology Mustafa-Korninger, Maria Elisabeth; 4 Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG Glehr, Reingard Christina; Medical University of Graz, Institute of General Practice and Evidence-based Health Services Research Camp, Jeremy; Medical University of Vienna, Center for Virology Agerer, Benedikt; Research Center for Molecular Medicine of the Austrian Academy of Sciences Lercher, Alexander; Research Center for Molecular Medicine of the Austrian Academy of Sciences Popa, Alexandra; Research Center for Molecular Medicine of the Austrian Academy of Sciences Genger, Jakob-Wendelin; Research Center for Molecular Medicine of the Austrian Academy of Sciences Penz, Thomas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Aberle, Stephan; Medical University of Vienna, Center for Virology Bock, Christoph; Research Center for Molecular Medicine of the Austrian Academy of Sciences Bergthaler, Andreas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Stiasny, Karin; Medical University of Vienna, Center for Virology Hochstrasser, Eva-Maria; Praxis Dr Lammel Hoellinger, Christian; Paracelsus Medical Private University Siebenhofer, Andrea; Institute of General Practice and Evidence-based Health Services Research, Medical University Graz; Institute for General Practice, Goethe University Frankfurt Griffiths, Chris; Queen Mary University of London, Institute of Population Health Sciences Panovska-Griffiths, Jasmina; NIHR CLAHRC North Thames, Department of Applied Health Research, University College London, London, United Kingdom; University of Oxford, Wolfson Centre for Mathematical Biology & The Queen's College
Primary Subject Heading:	General practice / Family practice

Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	PRIMARY CARE, COVID-19, Public health < INFECTIOUS DISEASES, VIROLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A prospective cohort study (READ-1)

Date: March 17, 2021

Version: 3.0

Co-authors

Werner Leber[1,*,&]
Oliver Lammel[2]
Monika Redlberger-Fritz[3]
Maria Elisabeth Mustafa-Korninger[4]
Reingard Christina Glehr[5]
Jeremy V. Camp[3]
Benedikt Agerer[6]
Alexander Lercher[6]
Alexandra M. Popa[6]
Jakob-Wendelin Genger[6]
Thomas Penz[6]
Stephan Aberle[3]
Christoph Bock[6]
Andreas Bergthaler[6]
Karin Stiasny[3]
Eva-Maria Hochstrasser[2]
Christian Hoellinger[7]
Andrea Siebenhofer[5,8]
Chris Griffiths[1]
Jasmina Panovska-Griffiths[9,10,11,*,&]

[1] Centre for Clinical Effectiveness and Health Data Science, Institute of Population Health Sciences, Barts School of Medicine and Dentistry, Queen Mary University of London, London, UK

[2] Practice Dr. Lammel, Ramsau am Dachstein, Austria

[3] Center for Virology, Medical University of Vienna, Vienna, Austria

[4] Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG, Salzburg, Austria

[5] Institute of General Practice and Evidence-based Health Services Research, Medical University of Graz, Graz, Austria

[6] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

[7] Paracelsus Medical University, Salzburg, Austria

[8] Institute of General Practice, Goethe-University Frankfurt am Main, Frankfurt am Main, Germany

[9] Department of Applied Health Care, Institute of Epidemiology & Health Care, University College London, London

[10] Institute for Global Health, University College London, London, UK

[11] The Queen's College, University of Oxford, Oxford, UK

& these authors contributed equally

* Corresponding authors: w.leber@qmul.ac.uk, j.panovska-griffiths@ucl.ac.uk

ABSTRACT

Objectives: We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a regional COVID-19 outbreak in Austria.

Design: Prospective cohort study.

Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03 April, 2020.

Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase chain reaction (RT-qPCR).

Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

Results: Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent), 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Strengths and limitations of this study

- Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people testing RT-qPCR reactive were notified within 24 hours.
- Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.[1] In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,[2] infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that,[3,4] and gradually declining over 7-10 days.[5,6 7]

SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may cause up to 80% of secondary transmissions.[8] Although symptomatic infection is common (17 %, range 4-41%), the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.[9-11] Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all documented infections.[12] Superspreading events have been reported from across the globe, and countries

achieving early viral suppression took rapid and decisive action to implement comprehensive case identification and testing, combined with contact tracing and isolation.[13,14] For epidemic control of COVID-19, the effective reproduction number, R_e, needs to be less than 1; the presence of undetected and persistent infection within the population, even if very small, can increase R_e and induce a secondary peak of infections. Therefore, rapid identification and containment of infection is a key factor for the prevention of onward transmission and controlling the virus to protect the public.[15]

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.[16] Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.[16,17] Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.[18] The first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512 cases and 7.389 COVID-19 related deaths have been reported.

General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.[19-21] The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."[22] However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.[23] In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.[24] The new service supplemented the existing national health hotline for people at risk of COVID-19.[25] RT-qPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.[26] Early detection of SARS-CoV-2 is essential for effective contact tracing,[27] and whole genome sequencing may provide data on dynamics of transmission.[17,28]

The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.

METHODS

Setting

This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort of Schladming-Dachstein, political subdistrict of Groebming (population 22,829), Austria. The study was conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria, people with flu-like symptoms were advised to call the national health hotline instead of directly presenting to the hospital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and home self-isolate and self-care. Asymptomatic people were excluded from this study.

Design

We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2 RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and seropositivity data were collected to compare two groups within this cohort of patients:

- Patients testing RT-qPCR reactive at presentation with acute disease
- Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).

We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR and positive serostatus; and confirmed infection as the presence of convalescent anti-SARS-CoV-2 antibody 3-6 weeks after the acute illness, irrespective of the RT-qPCR result.

Intervention

On February 24, 2020, one day before the first two cases were reported in Austria, the National Influenza Screening Network was enhanced to include SARS-CoV-2 RT-qPCR testing.

Patients with mild to moderate flu-like symptoms calling the study sentinel GP were offered same day appointments for SARS-CoV-2 RT-qPCR testing. RT-qPCR results were available within 24 hours, and those patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2 infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number of days from the first patient to the last patient testing RT-qPCR reactive at the GP.

Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening for patients attending sentinel GPs and paediatric practices. Between November and March of each year, participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms. Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal influenza virus activity in the country.

Clinical data

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK.

Testing

RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).[26] RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection.

Enzyme linked immune assays (ELISA)

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),[29] and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.[30] Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of SARS-CoV-2. Reagent wells of the EDI™ Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA,

IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI).

Neutralising antibody assay

Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI_ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers ≥10 were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.[31]

Outcome measures and statistical analysis

We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following four testing, viral and genomic outcomes:

Outcome A: The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative. To determine the accuracy of RT-qPCR, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive, antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive).

Outcome B: The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters). We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) *vs*. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent).

Outcome C: The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent sero-positivity (confirmed infection) to determine any potential correlation between these stages of disease. We used multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody

positive) and all patients with confirmed infection by calculating the correlation coefficient *r*, and estimating the 95% CI.

Outcome D: The number of viral clades implicated in the outbreak. To do this, SARS-CoV-2 full genome sequencing was undertaken as part of a wider study covering the whole of Austria.[17,28] The full-length sequences were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) database (http://gisaid.org).[32] Sequences were aligned in MEGA7 and non-synonymous nucleotide variants were identified to determine the respective clades, following the GISAID classification scheme for lineages.[33]

RESULTS

Overall testing results

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)
Sex		
Female	14 (63.6%)	9 (60%)
Male	8 (36.4%)	6 (40%)
Age (years)		
16-24	4 (26.7%)	3 (20%)

25-34	4 (26.7%)	2 (13.3%(
35-49	6 (40%)	4 (26.7%)
>50	8 (36.4%)	6 (40%)
Ethnic origin		
White	22 (100%)	15 (100%)

Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-14) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

Regression analysis on confirmed infection

Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03;

p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible covariates of confirmed infection.

Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069

Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive, irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody confirmed positive) among 15 patients respectively.

Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

Correlation between acute and confirmed infection

Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI 0.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did not detect any case with convalescent infection (red curve on Figure 2D).

Viral clade analysis

Thirteen of 15 full-length genome sequences were available for clade analysis via GISAID (Table 3); and two sequences were not available at the time of analysis. Lineages of SARS-CoV-2 have been identified based on mutations in key amino acid positions.[33] Clade G is defined by the mutations D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[34] Accordingly, among the 13 viral isolates, three different clades were identified, including clade L (N=2), GR (N=4) and L (N=7).

Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2 classification.

Disease Classification	Virus Name (GISAID)	EPI_ISL_#	Date of RT-qPCR	Lineage	ORF 8: 84	ORF3a: 57	S:614*	N:203**	N:204**
Early acute	hCoV-19/Austria/CeMM0191/2020	438032	13/03/2020	B(L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0248/2020	438078	21/03/2020	B (L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0235/2020	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0250/2020	438080	21/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0222/2020	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
Early acute	hCoV-19/Austria/CeMM0249/2020	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0267/2020	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0276/2020	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0303/2020	475778	29/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0324/2020	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0337/2020	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G

Caption Table 3: SARS-CoV-2 clades are classified by The Global Initiative on Sharing All Influenza Data (GISAID) using specific non-synonymous mutations in the viral genome. Clade G is defined by the mutations D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[34] Whole genome data were available for 13/15 sequences; data for two sequences were not available at the time of analysis. Accordingly, among the 13 sequences analysed, three different clades were identified, including clades L (N=2), GR (N=4) and G (N=7). All three clades were detected in early acute infection, and clade G was additionally detected in late acute infection. *For simplicity reasons, only mutation D614G (grey background) in the Spike protein defining clade G is shown. **Additional mutations R203K and G204R in the Nucleocapsid protein N defining clade GR are also shown in grey. ORF, open reading frame.

DISCUSSION

Our results demonstrate that SARS-CoV-2 RT-qPCR testing, when added to a national influenza surveillance programme in primary care, can rapidly, early and accurately diagnose COVID-19 during an outbreak. Of the 73

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,[35] we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy.

Loss of taste of smell has been recognised as an important marker of COVID-19;[36,37] however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.[38] Furthermore, loss of taste could not be objectively confirmed in one third of people[38] suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.[39] Timely and accurate testing is also a prerequisite for effective contact tracing.[27]

The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or

did not attend the practice because of mild disease.[40] No further endemic cases were detected after the outbreak. This suggests that combination prevention including rapid testing and case notification in primary care, contact tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening sites can effectively detect and control a regional outbreak.[22]

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.[41] However, change or loss in smell/taste were not quantified using an established tool such as the visual analogue scale (VAS),[42,43] but rather assessed by simple "yes" and "no" answers using a standard clinical questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,[10] asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This potentially excludes an important segment of the infected population and future studies will focus on exploring this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.[28]

To our knowledge, this is the first study to show that primary care can contribute to early case detection and termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients, public health, and health systems; nationally and internationally for outbreak epidemiology and control. As

countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.[44] Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2 lateral flow antigen testing.

CONCLUSIONS

RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study. OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay. JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL, MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final version.

Acknowledgments: We thank Evelyn Marktl for daily updates on the Christian Drosten's COVID-19 podcast (https://www.ndr.de/nachrichten/info/podcast4684.html). We are grateful to the team of Praxis Dr Lammel for their contributions, and in particular to the nurse Sabine Roiderer for providing direct patient care and help with administration. We thank the patients of Schladming-Dachstein for participating in the study.

Funding: This research was self-funded by each individual co-author. This project received funding from the Vienna Science and Technology Fund (WWTF) as part of the WWTF COVID-19 Rapid Response Funding 2020. Award/Grant number is not applicable.

Ethics approval: The study used secondary anonymised data for which approval was granted by the University of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).

Patient consent for publication: Verbal consent was received from patients for study participation.

Patient and public involvement: No patient involvement.

Data availability statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests: None declared.

References

1. World Health Organisation (WHO). Clinical management of severe acute respiratory infection when COVID-19 is suspected. 2020. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed July 02, 2020).

2. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med 2020; 172(9): 577-82.

3. Cheng HY, Jian SW, Liu DP, Ng TC, Huang WT, Lin HH. Contact Tracing Assessment of COVID-19 Transmission Dynamics in Taiwan and Risk at Different Exposure Periods Before and After Symptom Onset. JAMA Intern Med 2020.

4. Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. MMWR Morb Mortal Wkly Rep 2020; 69(13): 377-81.

5. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clin Infect Dis 2020.

6. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581(7809): 465-9.

7. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-CoV viral load dynamics, duration of viral shedding and infectiousness: a living systematic review and meta-analysis. medRxiv 2020: 2020.07.25.20162107.

8. Endo A, Abbott S, Kucharski AJ, Funk S. Estimating the overdispersion in COVID-19 transmission using outbreak sizes outside China. Wellcome Open Res 2020; 5: 67.

9. Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus symptomatic COVID-19. Lancet 2021; 397(10269): 93-4.

10. Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis. Official Journal of the Association of Medical Microbiology and Infectious Disease Canada 2020; 5(4): 223-34.

11. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. Lancet Infect Dis 2020.

12. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science 2020; 368(6490): 489-93.

13. European Centre for Disease Control and Prevention (ECDC). Rapid Risk Assessment: Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK– ninth update. 2020. https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-covid-19-pandemic-ninth-update (accessed July 02, 2020).

14. Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study. Lancet Infect Dis 2020.

15. Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis 2020; 26(6): 1059-66.

16. Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in Austria. Wien Klin Wochenschr 2020: 1-8.

17. Popa A, Genger JW, Nicholson MD, et al. Genomic epidemiology of superspreading events in Austria reveals mutational dynamics and transmission properties of SARS-CoV-2. Sci Transl Med 2020; 12(573).

18. Independent T. https://www.independent.co.uk/news/world/europe/coronavirus-austria-cases-covid-19-hospital-lockdown-latest-a9466281.html. 2020.(Accessed September 05, 2020)

19. (European Centre for Disease Control and Prevention (ECDC). Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK –ninth update, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-disease-2019-ninth-update-23-april-2020.pdf (Accessed July 02, 2020)

20. de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. Eur J Gen Pract 2020; 26(1): 58-60.

21. Hull SA, Williams C, Ashworth M, Carvalho C, Boomla K. Prevalence of suspected COVID-19 infection in patients from ethnic minority populations: a cross-sectional study in primary care. British Journal of General Practice 2020; 70(699): e696-e704.

22. European Centres for Disease Control (ECDC). Strategies for the surveillance of COVID-19, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-2020.pdf (accessed July 11, 2020).

23. de Lusignan S, Dorward J, Correa A, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of General Practitioners Research and Surveillance Centre primary care network: a cross-sectional study. Lancet Infect Dis 2020.

24. Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (Accessed July 02, 2020).

25. Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020).

26. Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2 (accessed September 29, 2020).

27. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert JHHM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. The Lancet Public Health.

28. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. Curr Protoc Microbiol 2020; 57(1): e100.

29. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. J Korean Med Sci 2020; 35(14): e149.

30. Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation studies of complex interventions (StaRI): a systematic review and e-Delphi. Implementation Science 2015; 10(1): 42.

31. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. Eurosurveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin 2017; 22(13).

32. Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. Frontiers in Microbiology 2020; 11(1800).

33. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in genomic epidemiology studies of active hCoV-19 viruses. 2020. https://www.gisaid.org/references/statements-clarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020).

34. Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening schools, the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic wave in the UK: a modelling study. The Lancet Child & Adolescent Health.

35. Aziz M, Goyal H, Haghbin H, Lee-Smith WM, Gajendran M, Perisetti A. The Association of "Loss of Smell" to COVID-19: A Systematic Review and Meta-Nnalysis. Am J Med Sci 2020.

36. von Bartheld CS, Hagen MM, Butowt R. Prevalence of Chemosensory Dysfunction in COVID-19 Patients: A Systematic Review and Meta-analysis Reveals Significant Ethnic Differences. ACS Chem Neurosci 2020; 11(19): 2944-61.

37. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste in 2013 European Patients With Mild to Moderate COVID-19. Ann Intern Med 2020.

38. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. Nat Med 2020.

39. Maltezou HC, Vorou R, Papadima K, et al. Transmission dynamics of SARS-CoV-2 within families with children in Greece: A study of 23 clusters. J Med Virol 2020.

40. Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper Airway Symptoms. Ear Nose Throat J 2020: 145561320920762.

41. Sung Y-T, Wu J-S. The Visual Analogue Scale for Rating, Ranking and Paired-Comparison (VAS-RRP): A new technique for psychological measurement. Behav Res Methods 2018; 50(4): 1694-715.

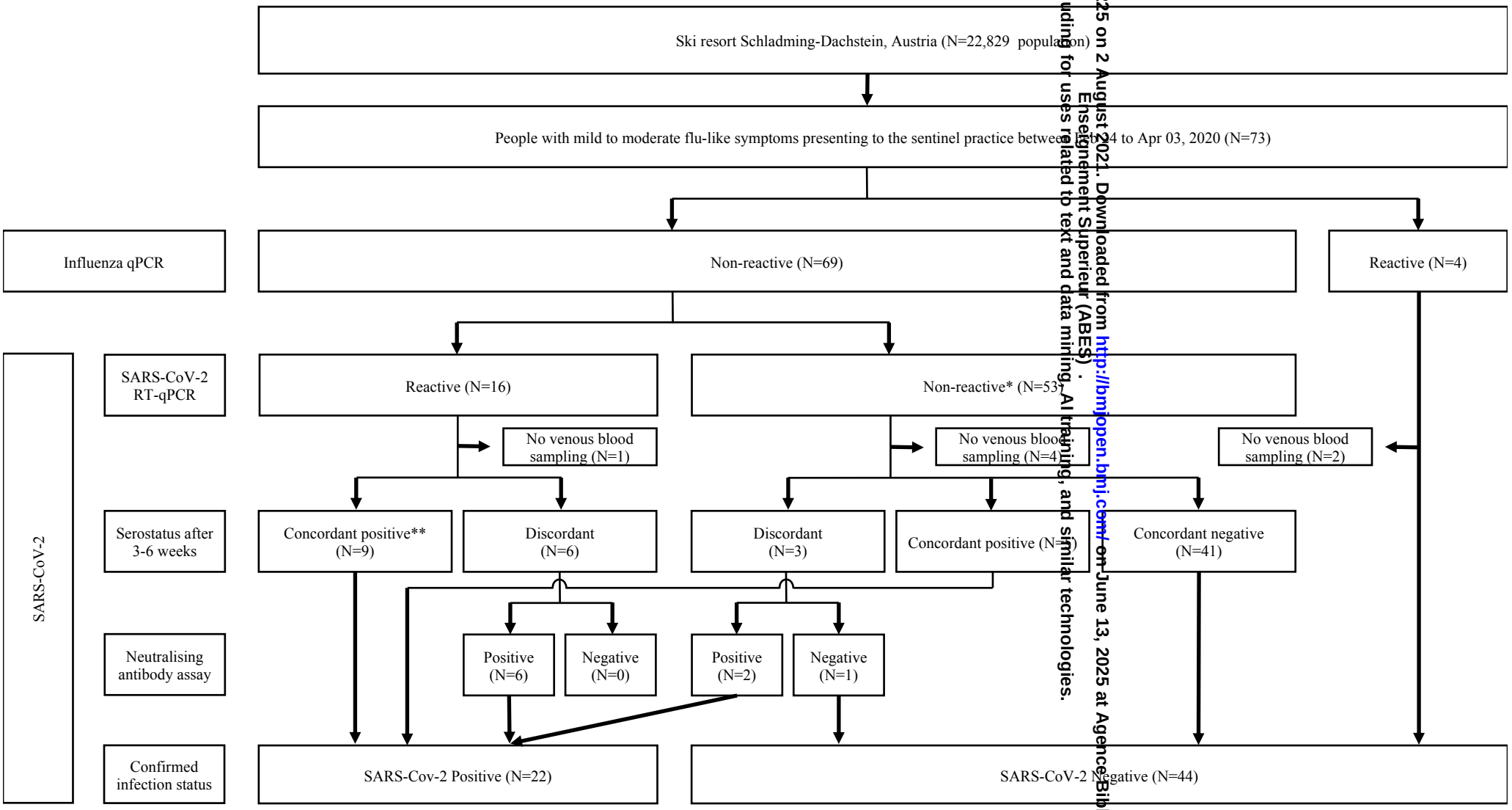
42. Rojas-Lechuga MJ, Izquierdo-Domínguez A, Chiesa-Estomba C, et al. Chemosensory dysfunction in COVID-19 out-patients. Eur Arch Otorhinolaryngol 2020: 1-8.

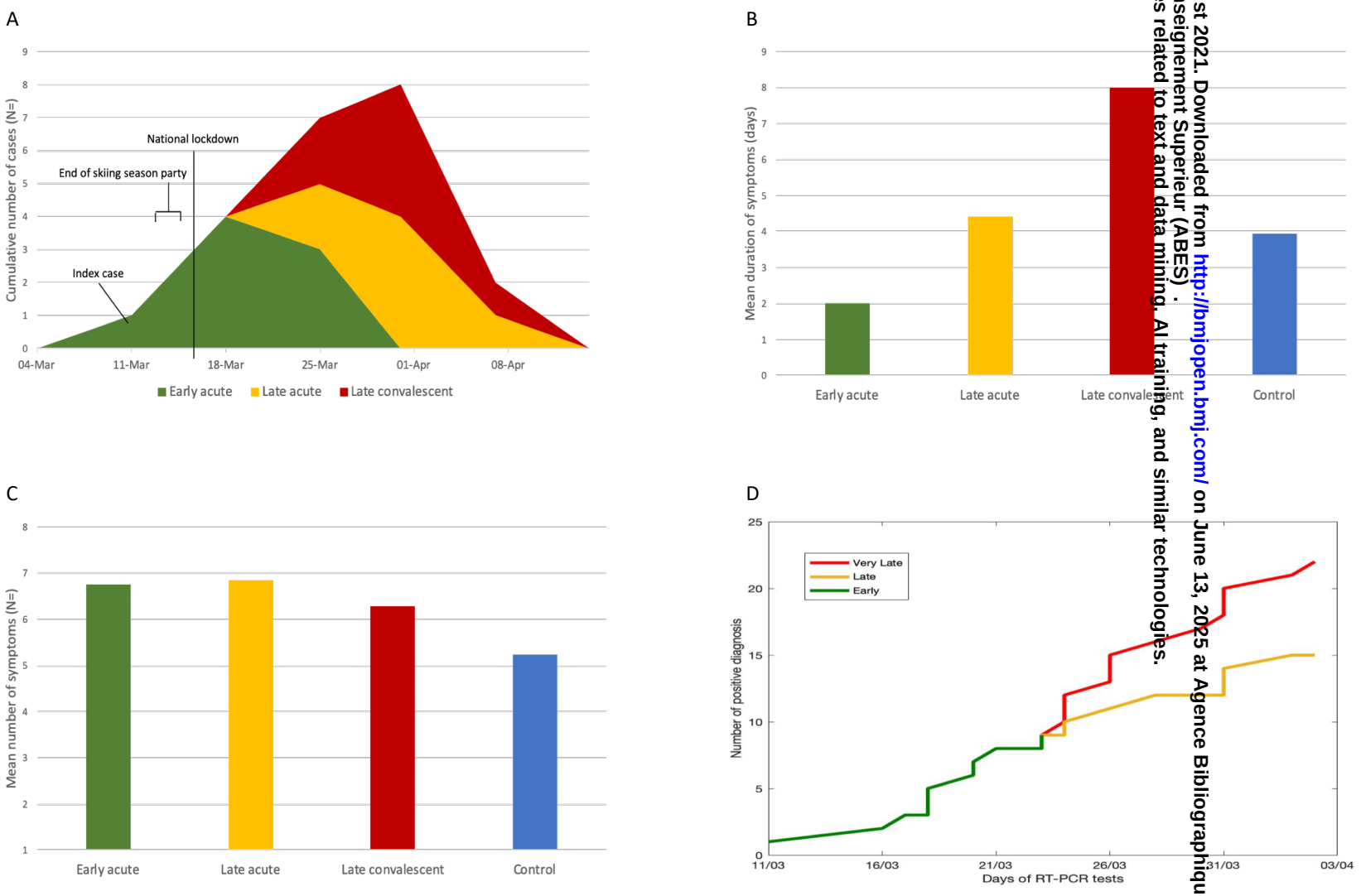
43. NHS England England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020. 2020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-june-2020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed September 09, 2020).

FIGURE LEGENDS

Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1 patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD (N=1, each). ***No concordant negatives.

Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive) in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B) Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters; (C) Mean duration of symptoms; and (D): Mean number of symptoms.





STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7,8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6,7*
Bias	9	Describe any efforts to address potential sources of bias	6,13
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Figure 1 attached
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations			13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12,13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A prospective cohort study (REAP-1)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-045225.R3
Article Type:	Original research
Date Submitted by the Author:	12-May-2021
Complete List of Authors:	Leber, Werner; Queen Mary University of London, Institute of Population Health Sciences, Centre for Clinical Effectiveness and Health Data Science Lammel, Oliver; Praxis Dr Lammel Redlberger-Fritz, Monika; Medical University of Vienna, Center for Virology Mustafa-Korninger, Maria Elisabeth; 4 Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG Glehr, Reingard Christina; Medical University of Graz, Institute of General Practice and Evidence-based Health Services Research Camp, Jeremy; Medical University of Vienna, Center for Virology Agerer, Benedikt; Research Center for Molecular Medicine of the Austrian Academy of Sciences Lercher, Alexander; Research Center for Molecular Medicine of the Austrian Academy of Sciences Popa, Alexandra; Research Center for Molecular Medicine of the Austrian Academy of Sciences Genger, Jakob-Wendelin; Research Center for Molecular Medicine of the Austrian Academy of Sciences Penz, Thomas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Aberle, Stephan; Medical University of Vienna, Center for Virology Bock, Christoph; Research Center for Molecular Medicine of the Austrian Academy of Sciences Bergthaler, Andreas; Research Center for Molecular Medicine of the Austrian Academy of Sciences Stiasny, Karin; Medical University of Vienna, Center for Virology Hochstrasser, Eva-Maria; Praxis Dr Lammel Hoellinger, Christian; Paracelsus Medical Private University Siebenhofer, Andrea; Institute of General Practice and Evidence-based Health Services Research, Medical University Graz; Institute for General Practice, Goethe University Frankfurt Griffiths, Chris; Queen Mary University of London, Institute of Population Health Sciences Panovska-Griffiths, Jasmina; NIHR CLAHRC North Thames, Department of Applied Health Research, University College London, London, United Kingdom; University of Oxford, Wolfson Centre for Mathematical Biology & The Queen's College
Primary Subject Heading:	General practice / Family practice

Secondary Subject Heading:	Infectious diseases, Public health
Keywords:	PRIMARY CARE, COVID-19, Public health < INFECTIOUS DISEASES, VIROLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Title: Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A prospective cohort study (READ-1)

Date: May 09, 2021

Version: 3.0

Co-authors

Werner Leber[1,*,&]
Oliver Lammel[2]
Monika Redlberger-Fritz[3]
Maria Elisabeth Mustafa-Korninger[4]
Reingard Christina Glehr[5]
Jeremy V. Camp[3]
Benedikt Agerer[6]
Alexander Lercher[6]
Alexandra M. Popa[6]
Jakob-Wendelin Genger[6]
Thomas Penz[6]
Stephan Aberle[3]
Christoph Bock[6]
Andreas Bergthaler[6]
Karin Stiasny[3]
Eva-Maria Hochstrasser[2]
Christian Hoellinger[7]
Andrea Siebenhofer[5,8]
Chris Griffiths[1]
Jasmina Panovska-Griffiths[9,10,11,*,&]

[1] Centre for Clinical Effectiveness and Health Data Science, Institute of Population Health Sciences, Barts School of Medicine and Dentistry, Queen Mary University of London, London, UK

[2] Practice Dr. Lammel, Ramsau am Dachstein, Austria

[3] Center for Virology, Medical University of Vienna, Vienna, Austria

[4] Medizinisch-chemisches Labor Dr. Mustafa, Dr. Richter OG, Salzburg, Austria

[5] Institute of General Practice and Evidence-based Health Services Research, Medical University of Graz, Graz, Austria

[6] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

[7] Paracelsus Medical University, Salzburg, Austria

[8] Institute of General Practice, Goethe-University Frankfurt am Main, Frankfurt am Main, Germany

[9] Department of Applied Health Care, Institute of Epidemiology & Health Care, University College London, London

[10] Institute for Global Health, University College London, London, UK

[11] The Queen's College, University of Oxford, Oxford, UK

& these authors contributed equally

* Corresponding authors: w.leber@qmul.ac.uk, j.panovska-griffiths@ucl.ac.uk

ABSTRACT

Objectives: We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a regional COVID-19 outbreak in Austria.

Design: Prospective cohort study.

Setting: A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

Participants: All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03 April, 2020.

Intervention: Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase chain reaction (RT-qPCR).

Outcome measures: We compared RT-qPCR at presentation with confirmed antibody status. We split the outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

Results: Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent), 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or cough. Transmission clusters of three viral clades (G, GR and L) were identified.

Conclusions: RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Strengths and limitations of this study

- Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people testing RT-qPCR reactive were notified within 24 hours.
- Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly among those people presenting late.

INTRODUCTION

The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and death.[1] In studies to date, the reported time for the infection to become symptomatic (incubation period) varies among different cohorts and settings, with a median incubation period around 5.1 days,[2] infectivity starting 2.3 days before symptom onset, peaking 1-2 days before that, and gradually declining over 7-10 days.[3-6]

SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may cause up to 80% of secondary transmissions.[7] Although symptomatic infection is common (17 %, range 4-41%), the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.[8-10] Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all documented infections.[11] Superspreading events have been reported from across the globe, and countries

achieving early viral suppression took rapid and decisive action to implement comprehensive case identification and testing, combined with contact tracing and isolation.[12,13] For epidemic control of COVID-19, the effective reproduction number, R_e, needs to be less than 1; the presence of undetected and persistent infection within the population, even if very small, can increase R_e and induce a secondary peak of infections. Therefore, rapid identification and containment of infection is a key factor for the prevention of onward transmission and controlling the virus to protect the public.[14]

In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on February 25, 2020.[15] Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts, including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries, including Germany, Denmark and Sweden.[15,16] Austria was one of the first countries to adopt comprehensive lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.[17] The first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512 cases and 7.389 COVID-19 related deaths have been reported.

General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of equitable care.[18-20] The European Centre for Disease Prevention and Control (ECDC) recommended integration of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."[21] However, in some countries, like the UK and the USA, primary care has been largely excluded from the national TTI strategy.[22,23] In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.[24] The new service supplemented the existing national health hotline for people at risk of COVID-19.[25] RT-qPCR is an established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.[26] Early detection of SARS-CoV-2 is essential for effective contact tracing,[27] and whole genome sequencing may provide data on dynamics of transmission.[28]

The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of symptoms among patients presenting during the first half (early presenters) and the second half (late presenters) of the outbreak, measured by the number of days from the first to the last case detected and dividing that period by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.

METHODS

Setting

This study was set in a sentinel GP participating in the National Influenza Surveillance Network in the ski-resort of Schladming-Dachstein, political subdistrict of Groebming (population 22,829), Austria. The study was conducted during a local COVID-19 outbreak in March and April 2020, during which 29 cases were detected by RT-qPCR locally. The bulk of the outbreak occurred after a 3-day party (March 13-15) prior to implementation of the national lockdown policy on March 16, which led to premature termination of the skiing season. All patients presenting with mild to moderate flu-like illness were included. Following the report of the first cases in Austria, people with flu-like symptoms were advised to call the national health hotline instead of directly presenting to the hospital or GP. Patients were advised to phone the GP or receive in-home testing by mobile testing units, and home self-isolate and self-care. Asymptomatic people were excluded from this study.

Design

We conducted a longitudinal evaluation comprising a prospective cohort to examine the impact of SARS-Cov-2 RT-qPCR testing on COVID-19 case detection. Between February 24 and April 03, 2020, RT-qPCR testing and seropositivity data were collected to compare two groups within this cohort of patients:

- Patients testing RT-qPCR reactive at presentation with acute disease
- Patients confirmed anti-SARS-CoV-2 antibody positive during the convalescence phase (confirmed infection).

We define acute disease as the presence of flu-like symptoms combined with reactive SARS-CoV-2 RT-qPCR and positive serostatus; and confirmed infection as the presence of convalescent anti-SARS-CoV-2 antibody 3-6 weeks after the acute illness, irrespective of the RT-qPCR result.

Intervention

On February 24, 2020, one day before the first two cases were reported in Austria, the National Influenza Screening Network was enhanced to include SARS-CoV-2 RT-qPCR testing.

Patients with mild to moderate flu-like symptoms calling the study sentinel GP were offered same day appointments for SARS-CoV-2 RT-qPCR testing. RT-qPCR results were available within 24 hours, and those patients with a reactive outcome were immediately notified by a clinician and advised to self-isolate for a minimum of two weeks following national policy at that time. Repeat follow-up RT-qPCR was arranged by the local public health authority (District Commissioner of Liezen, Austria), and people testing non-reactive on repeat RT-qPCR were released from self-isolation. After 3-6 weeks, venous blood was obtained to confirm SARS-CoV-2 infection using ELISA IgG and neutralizing antibody assay. We defined the period of the outbreak as the number of days from the first patient to the last patient testing RT-qPCR reactive at the GP.

Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening for patients attending sentinel GPs and paediatric practices. Between November and March of each year, participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms. Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal influenza virus activity in the country.

Clinical data

We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL) generated a clinical master case report form before extracting pseudonymised patient records into an Excel spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the University of Oxford, UK.

Testing

RT-qPCR

SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical University of Vienna on a Roche LightCycler (http://www.roche.com; Switzerland) using a primer-set provided by TIB MOLBIOL (https://www.tib-molbiol.com/; Germany).[26] RT-qPCR targeting the E-gene was considered reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent RNA polymerase (RdRP) gene detection.

Enzyme linked immune assays (ELISA)

IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies: EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),[29] and EPITOPE DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.[30] Reagent wells of the Anti-SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of SARS-CoV-2. Reagent wells of the EDI™ Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, https://www.diasorin.com/home) performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C). LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA,

IgM or IgG but detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI).

Neutralising antibody assay

Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI_ISL: 438123) for 1h at 37 °C. The mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects. NT titers ≥10 were considered positive. The study has been reported in accordance with STARI reporting guidelines for implementation studies.[31]

Outcome measures and statistical analysis

We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following four testing, viral and genomic outcomes:

Outcome A: The diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients testing SARS-CoV-2 negative. To determine the accuracy of RT-qPCR, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive, antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive).

Outcome B: The earliness of RT-qPCR testing by comparing the duration and number of symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late presenters). We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection: early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed infection according to the date of presentation to the GP during the outbreak as follows: people presenting with acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute disease) *vs*. those people presenting during the second half of the outbreak (late acute); and those people presenting with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak (late convalescent).

Outcome C: The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent sero-positivity (confirmed infection) to determine any potential correlation between these stages of disease. We used multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR reactivity at presentation and among all patients with confirmed infection. We reported the odds ratios (ORs) and the significance value (p) of each covariate on testing RT-qPCR reactive, and confirmed positive antibody status respectively. We quantified the association between patients with reactive RT-qPCR (and confirmed antibody

positive) and all patients with confirmed infection by calculating the correlation coefficient *r*, and estimating the 95% CI.

Outcome D: The number of viral clades implicated in the outbreak. To do this, SARS-CoV-2 full genome sequencing was undertaken as part of a wider study covering the whole of Austria.[28] The full-length sequences were matched to patient records by an anonymized unique identifier and uploaded to the Global Initiative on Sharing All Influenza Data (GISAID) database (http://gisaid.org).[32] Sequences were aligned in MEGA7 and non-synonymous nucleotide variants were identified to determine the respective clades, following the GISAID classification scheme for lineages.[33]

RESULTS

Overall testing results

Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%) tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis, 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were reported.

Table 1: Summary of the demographic characteristics of COVID-19 cases.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)	People with acute infection (RT-qPCR reactive and seropositive) (N=15)
Sex		
Female	14 (63.6%)	9 (60%)
Male	8 (36.4%)	6 (40%)
Age (years)		
16-24	4 (26.7%)	3 (20%)

25-34	4 (26.7%)	2 (13.3%(
35-49	6 (40%)	4 (26.7%)
>50	8 (36.4%)	6 (40%)
Ethnic origin		
White	22 (100%)	15 (100%)

Specificity and sensitivity of RT-qPCR

In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart, Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results. Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%), but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

Earliness of RT-qPCR testing

The mean duration of symptoms was 2 days (range 1-4) among early acute presenters, 4.4 days (range 1-7) among late acute presenters, 8 days (range 2-12) among people with late convalescent infection, and 3.9 days (range 1-14) among non-COVID-19 controls (Figure 2B). The mean number of symptoms was 6.75 (range 4-9) among early acute presenters, 6.86 (3-12) among late acute presenters, 6.3 (1-11) among people with convalescent infection, and 5.23 (range 2-11) among non-COVID-19 controls (Figure 2C).

Regression analysis on confirmed infection

Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of taste, but not loss of smell, was the key covariate significantly associated with positive serostatus (ORs=6.03;

p=0.047) (Table 2). Breathlessness (OR=6.9, p=0.054) and cough (OR=0.12, p=0.053) were also possible covariates of confirmed infection.

Table 2: Regression analysis on symptoms reported by patients diagnosed with COVI-19.

	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
Clinical symptom	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Change in taste	6.02	(1.02,35.51)	0.047	571.72	(1.92,170629.2)	0.029
Nausea/vomiting	4.42	(0.748,26.09)	0.101	370.11	(2.71,50429.42)	0.018
Sore throat	0.36	(0.067,1.93)	0.233	0.002	(0.000006,0.74)	0.039
Myalgia	1.15	(0.24,5.51)	0.865	121.82	(1.52,9749.08)	0.032
Breathlessness	6.90	(0.96,49.40)	0.054	134.46	(1.02,17796.87)	0.049
Change in smell	0.77	(0.098,6.15)	0.811	0.37	(0.008,15.87)	0.607
Fever	2.97	(0.44,20.35)	0.266	1.44	(0.057,36.66)	0.825
Cough	0.12	(0.014,1.03)	0.053	0.011	(0.00008,1.42)	0.069

Caption to Table 2: Symptoms associated with confirmed SARS-CoV-2 infection (antibody confirmed positive, irrespective of RT-qPCR result) among 22 patients, and with acute infection (RT-qPCR reactive, antibody confirmed positive) among 15 patients respectively.

Regression analysis on acute disease

All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis; and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11; p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002, p=0.039); and but not loss of smell (OR=0.37, p=0.607), fever (OR=1.44, p=0.825) or cough (OR=0.01, p=0.069).

Correlation between acute and confirmed infection

Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection (r=0.77, 95%CI 0.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did not detect any case with convalescent infection (red curve on Figure 2D).

Viral clade analysis

Thirteen of 15 full-length genome sequences were available for clade analysis via GISAID (Table 3); and two sequences were not available at the time of analysis. Lineages of SARS-CoV-2 have been identified based on mutations in key amino acid positions.[33] Clade G is defined by the mutations D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[34] Accordingly, among the 13 viral isolates, three different clades were identified, including clade L (N=2), GR (N=4) and L (N=7).

Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2 classification.

Disease Classification	Virus Name (GISAID)	EPI_ISL_#	Date of RT-qPCR	Lineage	ORF 8: 84	ORF3a: 57	S:614*	N:203**	N:204**
Early acute	hCoV-19/Austria/CeMM0191/2020	438032	13/03/2020	B(L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0248/2020	438078	21/03/2020	B (L)	L	Q	D	R	G
Early acute	hCoV-19/Austria/CeMM0018/2020	419671	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0228/2020	438061	18/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0235/2020	438066	19/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0250/2020	438080	21/03/2020	B.1.1 (GR)	L	Q	G	K	R
Early acute	hCoV-19/Austria/CeMM0222/2020	438056	17/03/2020	B.1.8 (G)	L	Q	G	R	G
Early acute	hCoV-19/Austria/CeMM0249/2020	438079	21/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0267/2020	438096	24/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0276/2020	438103	25/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0303/2020	475778	29/03/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0324/2020	475794	01/04/2020	B.1.8 (G)	L	Q	G	R	G
Late acute	hCoV-19/Austria/CeMM0337/2020	475800	03/04/2020	B.1.8 (G)	L	Q	G	R	G

Caption Table 3: SARS-CoV-2 clades are classified by The Global Initiative on Sharing All Influenza Data (GISAID) using specific non-synonymous mutations in the viral genome. Clade G is defined by the mutations D614G, C241T, C3037T and A23403G in the Spike protein; and clade GR by additional RG203KR mutations in the Nucleocapsid protein N; clade L is most closely related to the Wuhan reference strain (NC_045512.2).[34] Whole genome data were available for 13/15 sequences; data for two sequences were not available at the time of analysis. Accordingly, among the 13 sequences analysed, three different clades were identified, including clades L (N=2), GR (N=4) and G (N=7). All three clades were detected in early acute infection, and clade G was additionally detected in late acute infection. *For simplicity reasons, only mutation D614G (grey background) in the Spike protein defining clade G is shown. **Additional mutations R203K and G204R in the Nucleocapsid protein N defining clade GR are also shown in grey. ORF, open reading frame.

DISCUSSION

Our results demonstrate that SARS-CoV-2 RT-qPCR testing, when added to a national influenza surveillance programme in primary care, can rapidly, early and accurately diagnose COVID-19 during an outbreak. Of the 73

patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms, including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.

Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing the outbreak without intermittent lockdowns,[35] we suggest that systemic changes may also be needed. For example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and should be something that governments should consider as an additional strategy.

Loss of taste of smell has been recognised as an important marker of COVID-19;[36,37] however, more than half of patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be reduced.[38] Furthermore, loss of taste could not be objectively confirmed in one third of people[38] suggesting self-assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of people presenting with acute disease.[39] Timely and accurate testing is also a prerequisite for effective contact tracing.[27]

The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at the party causing acute illness among early presenters and second, low level home transmission of party goers to (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or

did not attend the practice because of mild disease.[40] No further endemic cases were detected after the outbreak. This suggests that combination prevention including rapid testing and case notification in primary care, contact tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening sites can effectively detect and control a regional outbreak.[21]

Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria; and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled accurate interpretation of RT-qPCR results.

There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP, potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among all people confirmed with infection (when including those with negative RT-qPCR), compared to those people presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and data collection was completed prior to publication of the first systematic review of altered taste and smell in the media.[41] However, change or loss in smell/taste were not quantified using an established tool such as the visual analogue scale (VAS),[42,43] but rather assessed by simple "yes" and "no" answers using a standard clinical questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,[9] asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This potentially excludes an important segment of the infected population and future studies will focus on exploring this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their worldwide spread.[28]

To our knowledge, this is the first study to show that primary care can contribute to early case detection and termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients, public health, and health systems; nationally and internationally for outbreak epidemiology and control. As

countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.[44] Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2 lateral flow antigen testing.

CONCLUSIONS

RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national sentinel surveillance of coronavirus.

Authors' Contributions: WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study. OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay. JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL, MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final version.

Acknowledgments: We thank Evelyn Marktl for daily updates on the Christian Drosten's COVID-19 podcast (https://www.ndr.de/nachrichten/info/podcast4684.html). We are grateful to the team of Praxis Dr Lammel for their contributions, and in particular to the nurse Sabine Roiderer for providing direct patient care and help with administration. We thank the patients of Schladming-Dachstein for participating in the study.

Funding: This research was self-funded by each individual co-author. This project received funding from the Vienna Science and Technology Fund (WWTF) as part of the WWTF COVID-19 Rapid Response Funding 2020. Award/Grant number is not applicable.

Ethics and consent: The Medical University of Graz Research Ethics Committee (reference number: 32-429 ex 19/20) approved collection of anonymised RT-PCR and antibody status data, and the Medical University of Vienna Research Ethics Committee (reference number: EK1339/2017) additionally approved usage of anonymised RT-PCR data collected as part of the National Influenza Surveillance Network including generation

of secondary genomic data. Written consent was obtained from all participating patients agreeing on anonymised data collection for data validation, quality control and research purposes.

Patient and public involvement: No patient involvement.

Data availability statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests: None declared.

References

1. World Health Organization (WHO). Clinical management of severe acute respiratory infection when COVID-19 is suspected, 2020. https://www.who.int/publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (Accessed May 05, 2021).
2. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Ann Intern Med* 2020; **172**(9): 577-82.
3. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020; **581**(7809): 465-9.
4. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-CoV viral load dynamics, duration of viral shedding and infectiousness: a living systematic review and meta-analysis. *medRxiv* 2020: 2020.07.25.20162107.
5. Byrne AW, McEvoy D, Collins AB, et al. Inferred duration of infectious period of SARS-CoV-2: rapid scoping review and analysis of available evidence for asymptomatic and symptomatic COVID-19 cases. *BMJ Open* 2020; **10**(8): e039856.
6. Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. *Clin Infect Dis* 2020.
7. Endo A, Abbott S, Kucharski AJ, Funk S. Estimating the overdispersion in COVID-19 transmission using outbreak sizes outside China. *Wellcome Open Res* 2020; **5**: 67.
8. Sayampanathan AA, Heng CS, Pin PH, Pang J, Leong TY, Lee VJ. Infectivity of asymptomatic versus symptomatic COVID-19. *Lancet* 2021; **397**(10269): 93-4.
9. Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: Systematic review and meta-analysis. *Official Journal of the Association of Medical Microbiology and Infectious Disease Canada* 2020; **5**(4): 223-34.
10. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. *Lancet Infect Dis* 2020.
11. Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). *Science* 2020; **368**(6490): 489-93.
12. European Centre for Disease Prevention and Control (ECDC). Rapid Risk Assessment: Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK– ninth update, 2020. https://www.ecdc.europa.eu/en/publications-data/rapid-risk-assessment-coronavirus-disease-2019-covid-19-pandemic-ninth-update (accessed May 05, 2020).
13. Koo JR, Cook AR, Park M, et al. Interventions to mitigate early spread of SARS-CoV-2 in Singapore: a modelling study. *Lancet Infect Dis* 2020.

14. Frieden TR, Lee CT. Identifying and Interrupting Superspreading Events-Implications for Control of Severe Acute Respiratory Syndrome Coronavirus 2. *Emerg Infect Dis* 2020; **26**(6): 1059-66.

15. Kreidl P, Schmid D, Maritschnik S, et al. Emergence of coronavirus disease 2019 (COVID-19) in Austria. *Wien Klin Wochenschr* 2020: 1-8.

16. Popa A, Genger J-W, Nicholson M, et al. Mutational dynamics and transmission properties of SARS-CoV-2 superspreading events in Austria. *bioRxiv* 2020: 2020.07.15.204339.

17. The Independent. 2020. https://www.independent.co.uk/news/world/europe/coronavirus-austria-cases-covid-19-hospital-lockdown-latest-a9466281.html (accessed May 05, 2020).

18. European Centre for Disease Prevention and Control (ECDC). Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK –ninth update, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-disease-2019-ninth-update-23-april-2020.pdf (accessed June 02, 2020).

19. de Sutter A, Llor C, Maier M, et al. Family medicine in times of 'COVID-19': A generalists' voice. *Eur J Gen Pract* 2020; **26**(1): 58-60.

20. Hull SA, Williams C, Ashworth M, Carvalho C, Boomla K. Suspected COVID-19 in primary care: how GP records contribute to understanding differences in prevalence by ethnicity. *medRxiv* 2020: 2020.05.23.20101741.

21. European Centre for Disease Prevention and Control (ECDC). Strategies for the surveillance of COVID-19, 2020. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-surveillance-strategy-9-Apr-2020.pdf (accessed July 11, 2020).

22. Roehr B. Covid-19 is threatening the survival of US primary care. *BMJ* 2020; **369**: m2333.

23. Harding-Edgar L, McCartney M, Pollock AM. Test and trace strategy has overlooked importance of clinical input, clinical oversight and integration. *Journal of the Royal Society of Medicine* 2020; **113**(11): 428-32.

24. Zentrum für Virologie Medizinische Universität Wien. Projekt Diagnostisches Influenzanetzwerk Österreich (DINÖ). https://www.virologie.meduniwien.ac.at/wissenschaft-forschung/virus-epidemiologie/influenza-projekt-diagnostisches-influenzanetzwerk-oesterreich-dinoe/ (accessed May 28, 2020).

25. Federal Ministry of Social Affairs H, Care and Consumer Protection, Republic of Austria. National Health Hotline 1450. 2019. https://www.1450.at/1450-die-gesundheitsnummer/ (accessed May 28, 2020.

26. Corman V, Bleicker T, Brünink S, et al. Diagnostic detection of 2019-nCoV by real-time RT-PCR, 2020. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2 (accessed July 02, 2020).

27. Kretzschmar ME, Rozhnova G, Bootsma MCJ, van Boven M, van de Wijgert JHHM, Bonten MJM. Impact of delays on effectiveness of contact tracing strategies for COVID-19: a modelling study. *The Lancet Public Health*.

28. Popa A, Genger JW, Nicholson MD, et al. Genomic epidemiology of superspreading events in Austria reveals mutational dynamics and transmission properties of SARS-CoV-2. *Sci Transl Med* 2020; **12**(573).

29. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol* 2020; **57**(1): e100.

30. Ahn JY, Sohn Y, Lee SH, et al. Use of Convalescent Plasma Therapy in Two COVID-19 Patients with Acute Respiratory Distress Syndrome in Korea. *J Korean Med Sci* 2020; **35**(14): e149.

31. Pinnock H, Epiphaniou E, Sheikh A, et al. Developing standards for reporting implementation studies of complex interventions (StaRI): a systematic review and e-Delphi. *Implementation Science* 2015; **10**(1): 42.

32. Shu Y, McCauley J. GISAID: Global initiative on sharing all influenza data - from vision to reality. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin* 2017; **22**(13).

33. Mercatelli D, Giorgi FM. Geographic and Genomic Distribution of SARS-CoV-2 Mutations. *Frontiers in Microbiology* 2020; **11**(1800).

34. Global Initiative on Sharing All Influenza Data (GISAID). Clade and lineage nomenclature aids in genomic epidemiology studies of active hCoV-19 viruses. 2020. https://www.gisaid.org/references/statements-clarifications/clade-and-lineage-nomenclature-aids-in-genomic-epidemiology-of-active-hcov-19-viruses/ (accessed September 05, 2020.

35. Panovska-Griffiths J, Kerr CC, Stuart RM, et al. Determining the optimal strategy for reopening schools, the impact of test and trace interventions, and the risk of occurrence of a second COVID-19 epidemic wave in the UK: a modelling study. *The Lancet Child & Adolescent Health*.

36. Aziz M, Goyal H, Haghbin H, Lee-Smith WM, Gajendran M, Perisetti A. The Association of "Loss of Smell" to COVID-19: A Systematic Review and Meta-Nnalysis. *Am J Med Sci* 2020.

37. von Bartheld CS, Hagen MM, Butowt R. Prevalence of Chemosensory Dysfunction in COVID-19 Patients: A Systematic Review and Meta-analysis Reveals Significant Ethnic Differences. *ACS Chem Neurosci* 2020; **11**(19): 2944-61.

38. Lechien JR, Chiesa-Estomba CM, Hans S, Barillari MR, Jouffe L, Saussez S. Loss of Smell and Taste in 2013 European Patients With Mild to Moderate COVID-19. *Ann Intern Med* 2020.

39. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nat Med* 2020.

40. Maltezou HC, Vorou R, Papadima K, et al. Transmission dynamics of SARS-CoV-2 within families with children in Greece: A study of 23 clusters. *J Med Virol* 2020.

41. Lovato A, de Filippis C. Clinical Presentation of COVID-19: A Systematic Review Focusing on Upper Airway Symptoms. *Ear Nose Throat J* 2020: 145561320920762.

42. Sung Y-T, Wu J-S. The Visual Analogue Scale for Rating, Ranking and Paired-Comparison (VAS-RRP): A new technique for psychological measurement. *Behav Res Methods* 2018; **50**(4): 1694-715.

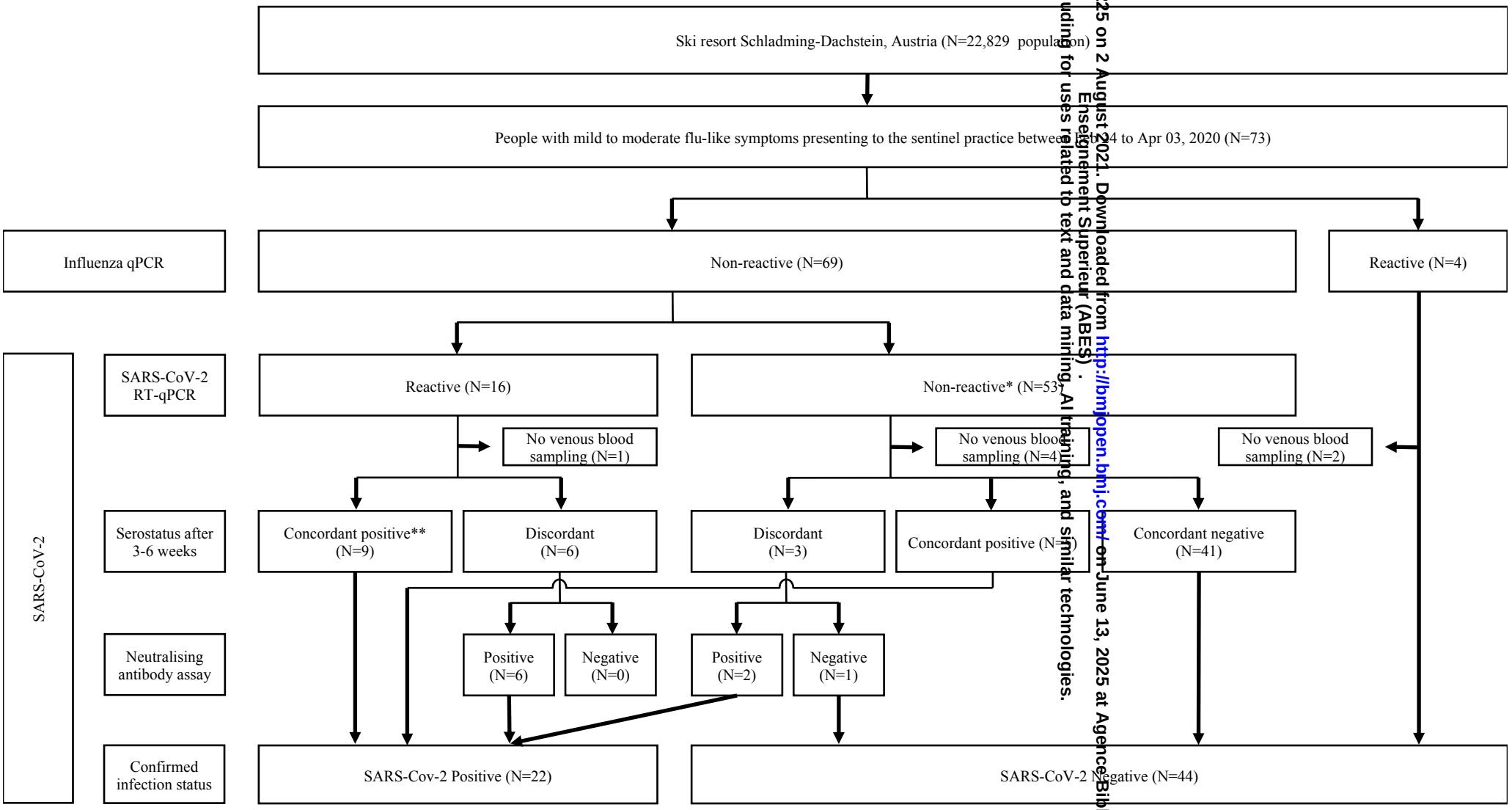
43. Rojas-Lechuga MJ, Izquierdo-Domínguez A, Chiesa-Estomba C, et al. Chemosensory dysfunction in COVID-19 out-patients. *Eur Arch Otorhinolaryngol* 2020: 1-8.

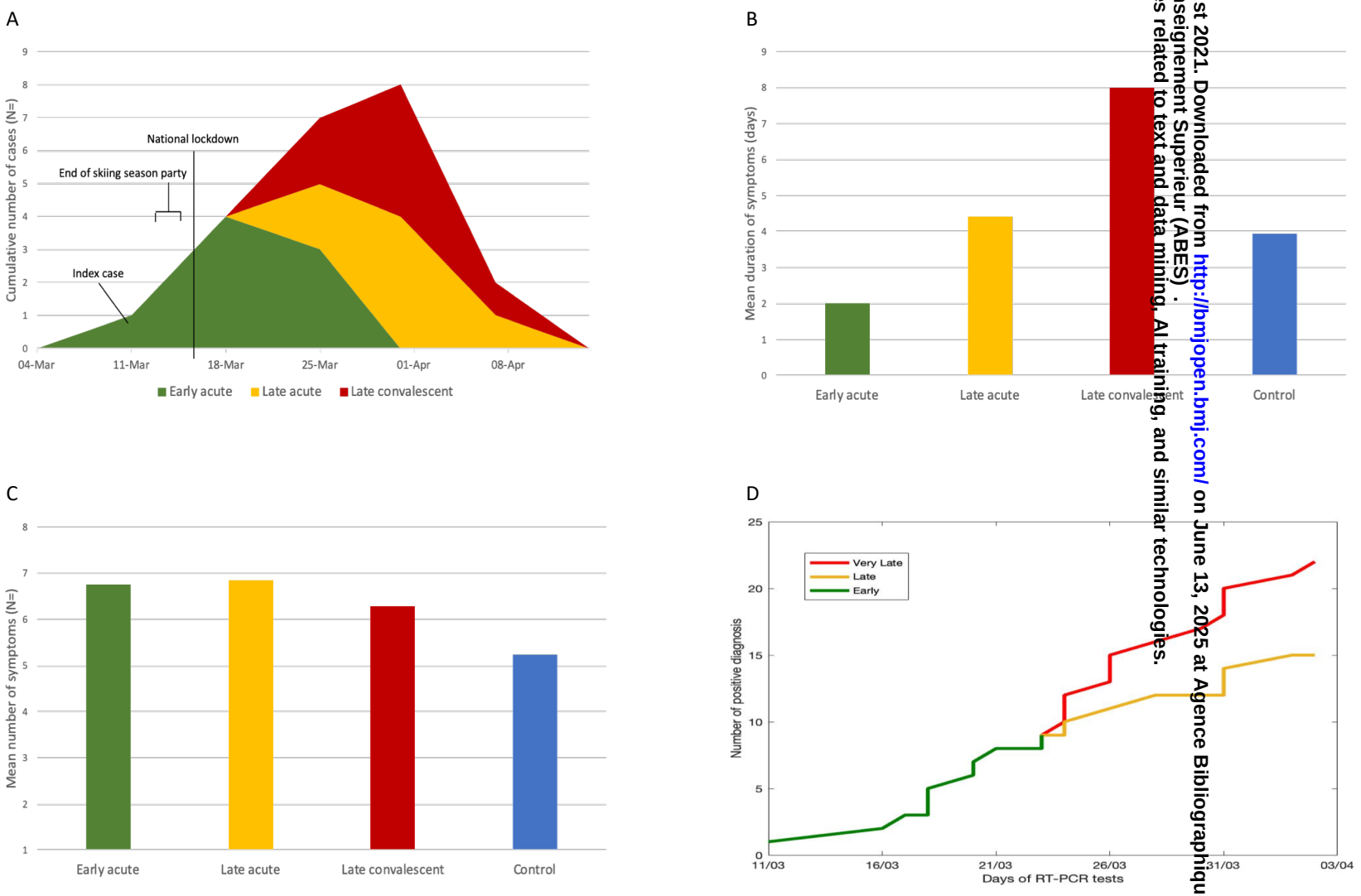
44. NHS England. NHS Test and Trace – week 4 of contact tracing, England: 18 to 24 June 2020. 2020. https://www.gov.uk/government/publications/nhs-test-and-trace-statistics-england-18-june-to-24-june-2020/weekly-nhs-test-and-trace-bulletin-england-18-24-june-2020 (accessed May 05, 2021).

FIGURE LEGENDS

Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1 patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD (N=1, each). ***No concordant negatives.

Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive) in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B) Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters; (C) Mean duration of symptoms; and (D): Mean number of symptoms.





STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2,3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3,4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7,8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*6,7*
Bias	9	Describe any efforts to address potential sources of bias	6,13
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7,8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7,8
		(*b*) Describe any methods used to examine subgroups and interactions	7,8
		(*c*) Explain how missing data were addressed	8
		(*d*) If applicable, explain how loss to follow-up was addressed	8
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	Figure 1 attached
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	8
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9,10
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	9,10
Discussion			
Key results	18	Summarise key results with reference to study objectives	11,12
Limitations			13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12,13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.