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

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2 Austria: Evidence of effective sentinel surveillance screening in primary care

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36 49 **ABSTRACT**

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38 50 **Objectives:** We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
39 51 regional COVID-19 outbreak in Austria.

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41 52 **Design:** Prospective cohort study.

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43 53 **Setting:** A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

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45 54 **Participants:** All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
46 55 April, 2020.

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48 56 **Intervention:** Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
49 57 chain reaction (RT-qPCR).

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51 58 **Outcome measures:** We compared RT-qPCR at presentation with confirmed antibody status. We split the
52 59 outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
53 60 with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
54 61 convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
55 62 accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

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3 63 **Results:** Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent),
4 64 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all
5 65 acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of
6 66 symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days
7 67 (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was
8 68 associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
9 69 cough. Transmission clusters of three viral clades (G, GR and L) were identified.

10 68 associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
11 69 cough. Transmission clusters of three viral clades (G, GR and L) were identified.
12 70
13 71 **Conclusions:** RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
14 72 with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
15 73 surveillance of coronavirus.
16 74

17 74 **Strengths and limitations of this study**

- 18 75 • Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian
19 76 National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- 20 77 • Symptomatic patients received same-day appointments for nasopharyngeal swabs, and people testing RT-PCR
21 78 reactive were notified within 24 hours.
- 22 79 • Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- 23 80 • The relatively small patient cohort from a single testing site limits conclusions on causality and
24 81 generalisability.
- 25 82 • Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly
26 83 among those people presenting late.
27 84

28 85 **INTRODUCTION**

29 86 The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus
30 87 2 (SARS-CoV-2), continues to spread globally with more than 25 million cases, and over 850,000 deaths reported
31 88 as of August 31, 2020. Undetected infection and delays in implementing an effective test-trace-isolate (TTI)
32 89 strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide spectrum
33 90 of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like illness,
34 91 loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure and
35 92 death.¹ In studies to date, the reported time for the infection to become symptomatic (incubation period) varies
36 93 among different cohorts and settings, with a median incubation period around 5.1 days,² infectivity starting 2.3
37 94 days before symptom onset, peaking 1-2 days before that,^{3,4} and gradually declining over 7-10 days.^{5,6}
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39 96 SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a
40 97 large number of people. Although most infections remain isolated cases, a small number of individuals (10%)
41 98 may cause up to 80% of secondary transmissions.⁷ Undocumented infection may constitute the majority of cases
42 99 (86%), causing more than half (55%) of all documented infections.⁸ Superspreading events have been reported
43 100 from across the globe, and countries achieving early viral suppression took rapid and decisive action to implement

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3 101 comprehensive case identification and testing, combined with contact tracing and isolation.^{9,10} For epidemic
4 102 control of COVID-19, the effective reproduction number, R_e , needs to be less than 1; the presence of undetected
5 103 and persistent infection within the population, even if very small, can increase R_e and induce a secondary peak of
6 104 infections. Therefore, rapid identification and containment of infection is a key factor for the prevention of onward
7 105 transmission and controlling the virus to protect the public.¹¹
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13 107 In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on
14 108 February 25, 2020.¹² Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts,
15 109 including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries,
16 110 including Germany, Denmark and Sweden.^{12,13} Austria was one of the first countries to adopt comprehensive
17 111 lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching
18 112 self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.¹⁴ The
19 113 first death from COVID-19 associated complications occurred on March 12, 2020, and as of August 31, 27,166
20 114 cases and 733 COVID-19 related deaths have been reported.
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26 116 General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of
27 117 equitable care.¹⁵⁻¹⁷ The European Centre for Disease Prevention and Control (ECDC) recommended integration
28 118 of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."¹⁸
29 119 However, in some countries, like the UK and the USA, primary care has been largely excluded from the national
30 120 TTI strategy.¹⁹ In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase
31 121 chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92
32 122 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.²⁰ The new service
33 123 supplemented the existing national health hotline for people at risk of COVID-19.²¹ RT-qPCR is an established
34 124 technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.²² Early detection of
35 125 SARS-CoV-2 is essential for effective contact tracing,²³ and whole genome sequencing may provide data on
36 126 dynamics of transmission.¹³
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45 128 The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and
46 129 timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-
47 130 CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the
48 131 accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute
49 132 infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically
50 133 defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of
51 134 symptoms among patients presenting during the first half (early presenters) and the second half (late presenters)
52 135 of the outbreak, measured by the number of days from the first to the last case detected and dividing that period
53 136 by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a
54 137 correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.
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139 **METHODS**

140 **Setting**

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

151 **Design**

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

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

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

161 **Intervention**

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

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

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

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3 175 participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms.
4 176 Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue
5 177 cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal
6 178 influenza virus activity in the country.
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11 180 **Outcome measures**

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13 181 We characterise the outbreak using the following four testing, clinical and viral genomic outcomes: A) The
14 182 diagnostic accuracy (using sensitivity and specificity) of SARS-CoV-2 RT-qPCR among patients with mild to
15 183 moderate flu-like symptoms at presentation by comparing molecular diagnosis with anti-SARS-CoV-2 antibody
16 184 testing during convalescence, and hospital admission and death, including any alternative diagnoses for patients
17 185 testing SARS-CoV-2 negative; B) The earliness of RT-qPCR testing by comparing the duration and number of
18 186 symptoms during the first half of the outbreak (early presenters) and during the second half of the outbreak (late
19 187 presenters); C) The key clinical symptoms associated with RT-qPCR reactivity (acute infection) and convalescent
20 188 seropositivity (confirmed infection) to determine any potential correlation between these stages of disease; and
21 189 D) the viral clades detected in the outbreak.
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28 191 **Clinical data**

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30 192 We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL)
31 193 generated a clinical master case report form before extracting pseudonymised patient records into an Excel
32 194 spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a
33 195 secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of
34 196 Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the
35 197 University of Oxford, UK.
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41 199 **Testing**

43 200 **RT-qPCR**

44 201 SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical
45 202 University of Vienna on a Roche LightCycler (<http://www.roche.com>; Switzerland) using a primer-set provided
46 203 by TIB MOLBIOL (<https://www.tib-molbiol.com/>; Germany).²² RT-qPCR targeting the E-gene was considered
47 204 reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent
48 205 RNA polymerase (RdRP) gene detection.
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52 206 **Enzyme linked immune assays (ELISA)**

53 207 IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial
54 208 test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies:
55 209 EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),²⁴ and EPITOPE
56 210 DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.²⁵ Reagent wells of the Anti-
57 211 SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of
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3 212 SARS-CoV-2. Reagent wells of the EDI™ Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-
4 213 19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT
5 214 LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, <https://www.diasorin.com/home>)
6 215 performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-
7 216 CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG
8 217 against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C).
9 218 LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative
10 219 detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary
11 220 units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence
12 221 immunoassay (ECLIA) for qualitative detection of CoV2 antibodies in human serum against a recombinant
13 222 nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA, IgM or IgG but
14 223 detecting IgG predominantly. Results are reported as numeric values in form of signal sample/cutoff (COI).

21 Neutralising antibody assay

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23 225 Samples with discordant antibody results (see below) were further evaluated using an in-house neutralising
24 226 antibody assay as follows: Serial dilutions of heat-inactivated serum samples were incubated with 50-100 TCID50
25 227 SARS-CoV-2 (hCoV-19/Austria/CeMM0360/2020; GISAID EPI_ISL: 438123) for 1h at 37 °C. The mixture was
26 228 added to Vero E6 (ATCC ® CRL-1586) cell monolayers and incubation was continued for two to three days. NT
27 229 titers were expressed as the reciprocal of the serum dilution that protected against virus-induced cytopathic effects.
28 230 NT titers ≥ 10 were considered positive. The study has been reported in accordance with STARI reporting
29 231 guidelines for implementation studies.²⁶

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35 233 **Statistical analysis**

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37 234 We present a descriptive statistics of patient demographics including age, gender and ethnicity; and the following
38 235 four outcomes:

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40 236 **Outcome A:** We tested the diagnostic accuracy of RT-qPCR, by determining its sensitivity and specificity. To do
41 237 this, we stratified RT-qPCR results in four groups: true reactive (RT-qPCR reactive and confirmed antibody
42 238 positive); false reactive (RT-qPCR reactive, antibody negative); true non-reactive (RT-qPCR non-reactive,
43 239 antibody negative); and false non-reactive (RT-qPCR non-reactive, antibody positive).

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46 240 **Outcome B:** We calculated the earliness of RT-qPCR testing by determining the mean duration of symptoms, in
47 241 days (range), and mean number of symptoms (range), across the three cohorts of patients with confirmed infection:
48 242 early acute, late acute and late convalescent. The three cohorts were obtained by stratifying people with confirmed
49 243 infection according to the date of presentation to the GP during the outbreak as follows: people presenting with
50 244 acute infection (RT-qPCR reactive, confirmed antibody positive) during the first half of the outbreak (early acute
51 245 disease) vs. those people presenting during the second half of the outbreak (late acute); and those people presenting
52 246 with previous disease (RT-qPCR non-reactive but confirmed antibody positive) in the second half of the outbreak
53 247 (late convalescent).

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58 248 **Outcome C:** Multivariate logistic regression tested the association of 15 clinical symptoms with RT-qPCR
59 249 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%)

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279 **Specificity and sensitivity of RT-qPCR**

280 In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR
 281 outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient
 282 who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with
 283 mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart,
 284 Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six
 285 were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant
 286 negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza
 287 who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients
 288 with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with
 289 reactive RT-qPCR were neutralising antibody positive; and of the 3 patients with non-reactive RT-qPCR, two
 290 were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and
 291 neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive
 292 (true reactive) and 7 were non-reactive (false non-reactive). There were no false reactive RT-qPCR results.
 293 Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of
 294 RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%),
 295 but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all
 296 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

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298 **Earliness of RT-qPCR testing**

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

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305 **Regression analysis on confirmed infection**

306 Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of
 307 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 COVID-19.

Clinical symptom	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
	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).

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330 **Viral clade analysis**

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

337 **Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2**
 338 **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

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

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350 **DISCUSSION**

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

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3 353 patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute
4 354 disease and 7 with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were
5 355 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first
6 356 half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean
7 357 duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a
8 358 quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing
9 359 rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms,
10 360 including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and
11 361 cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with
12 362 convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2
13 363 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.
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20 365 Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results
21 366 suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains
22 367 within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks
23 368 early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing
24 369 the outbreak without intermittent lockdowns,³⁰ we suggest that systemic changes may also be needed. For
25 370 example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they
26 371 may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to
27 372 social distancing and reduced physical contacts is necessary as we move away from the first and potentially
28 373 towards the second COVID-19 wave. Enhanced testing is an important factor, and our study suggests that testing
29 374 in primary care at symptom onset is highly accurate and should be something that governments should consider
30 375 as an additional strategy.
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38 377 Loss of taste of smell has been recognised as an important marker of COVID-19;¹ however, more than half of
39 378 patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be
40 379 reduced.³¹ Furthermore, loss of taste could not be objectively confirmed in one third of people³¹ suggesting self-
41 380 assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of
42 381 people presenting with acute disease.³² Timely and accurate testing is also a prerequisite for effective contact
43 382 tracing.²³
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49 384 The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was
50 385 brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was
51 386 diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it
52 387 is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading
53 388 events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces
54 389 and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters
55 390 observed in this study may represent different types of viral exposure. First, inhalation of high density aerosols at
56 391 the party causing acute illness among early presenters and second, low level home transmission of party goers to
57 392 (late presenting) friends and family during the lockdown. No further endemic cases were detected after the
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3 393 outbreak. This suggests that combination prevention including rapid testing and case notification in primary care,
4 394 contact tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge,
5 395 our study is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza
6 396 screening sites can effectively detect and control a regional outbreak.¹⁸
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11 398 Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating
12 399 in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national
13 400 SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria;
14 401 and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were
15 402 detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and
16 403 result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab
17 404 Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled
18 405 accurate interpretation of RT-qPCR results.
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25 407 There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP,
26 408 potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven
27 409 patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our
28 410 COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend
29 411 emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather
30 412 than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested
31 413 by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among
32 414 all people confirmed with infection (when including those with negative RT-qPCR), compared to those people
33 415 presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and
34 416 data collection was completed prior to publication of the first systematic review of altered taste and smell in the
35 417 media.³³ The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we have
36 418 not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the data
37 419 presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and their
38 420 worldwide spread.¹³
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48 422 To our knowledge, this is the first study to show that primary care can contribute to early case detection and
49 423 termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients,
50 424 public health, and health systems; nationally and internationally for outbreak epidemiology and control. As
51 425 countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the
52 426 disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases
53 427 should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the
54 428 number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential
55 429 partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly
56 430 enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.³⁴
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3 431 Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the
4 432 Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability
5 433 of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome
6 434 sequencing to support complex contact tracing.
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11 436 **CONCLUSIONS**

13 437 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with
14 438 mild-to-moderate illness in a heterogenous viral community outbreak. This study demonstrates high rates of
15 439 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19
16 440 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national
17 441 sentinel surveillance of coronavirus.
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23 444 OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG
24 445 submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and
25 446 JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay.
26 447 JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL,
27 448 MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final
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42 457 **Ethics approval:** The study used secondary anonymised data for which approval was granted by the University
43 458 of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).
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46 459 **Patient consent for publication:** Consent may not be required as no identifiable details on individuals are
47 460 reported in this manuscript.
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50 461 **Patient and public involvement:** No patient involvement.
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53 462 **Data availability statement:** The datasets used and/or analysed during the current study are available from the
54 463 corresponding author on reasonable request.
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56 464 **Competing Interests:** None declared.
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554 FIGURE LEGENDS

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35 555 Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15
36 556 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-
37 557 qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing
38 558 antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant
39 559 antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44
40 560 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1
41 561 patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with
42 562 Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious
43 563 mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD
44 564 (N=1, each). ***No concordant negatives.
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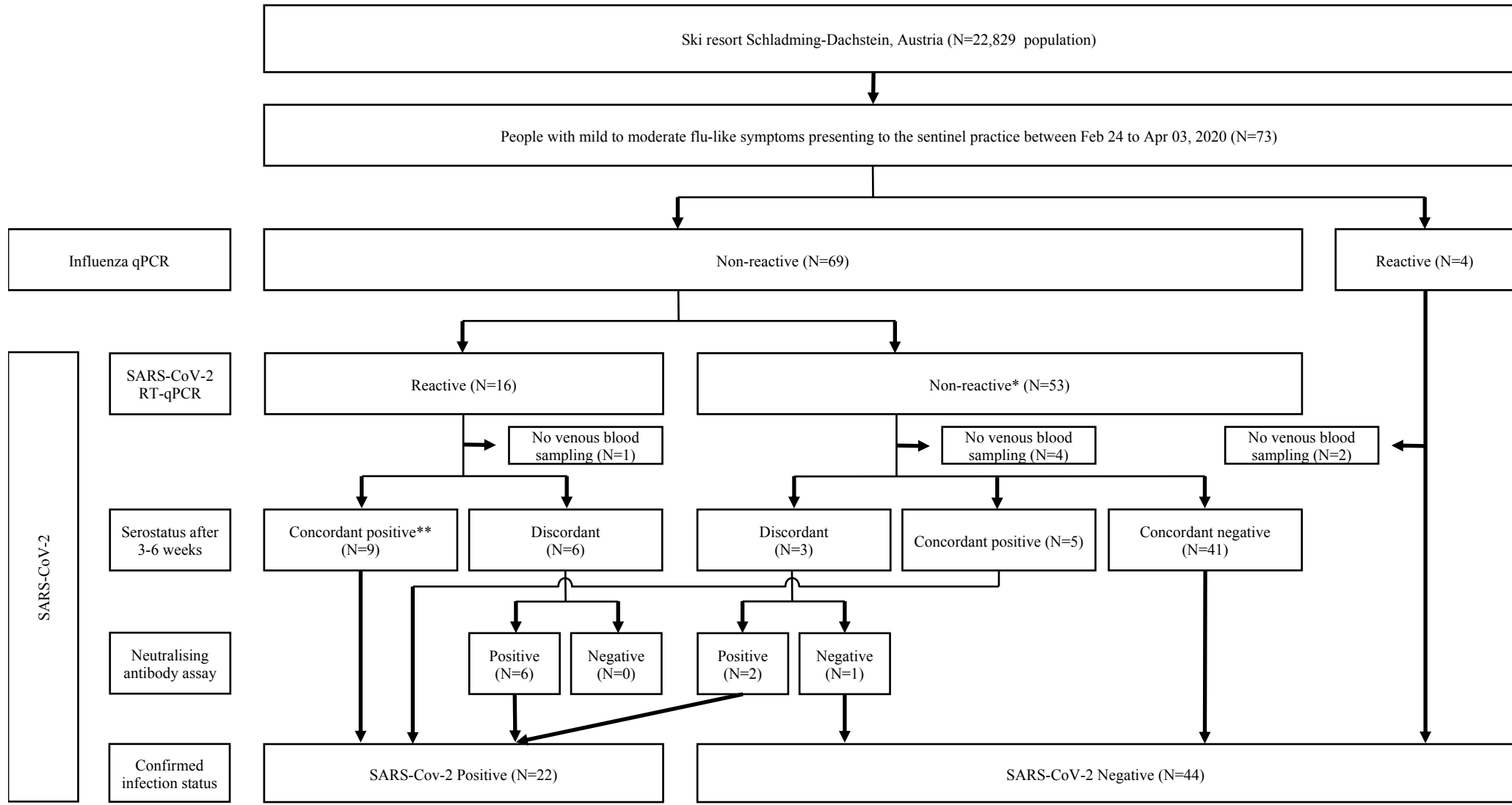
51 566 Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main
52 567 outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing
53 568 season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last
54 569 endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive)
55 570 in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute
56 571 infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B)

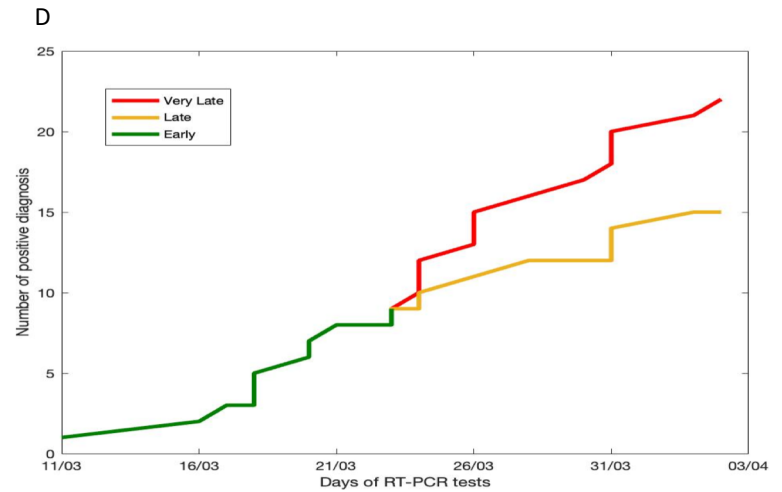
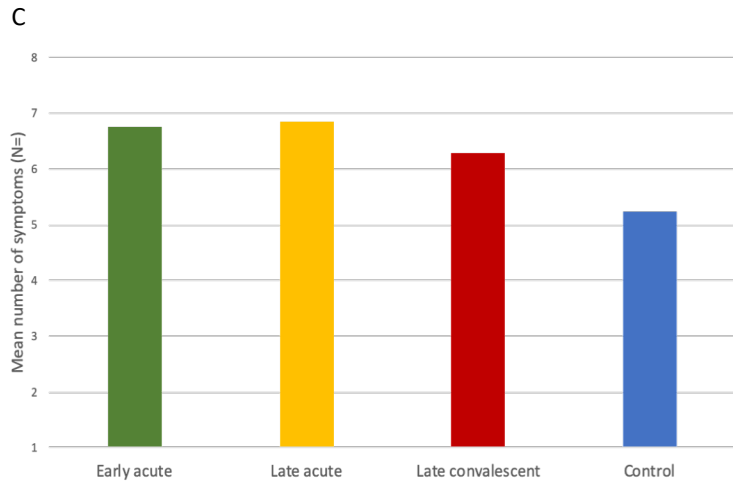
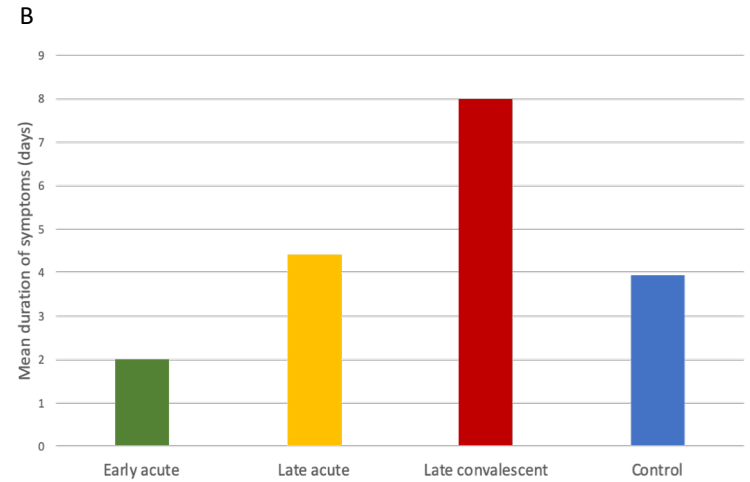
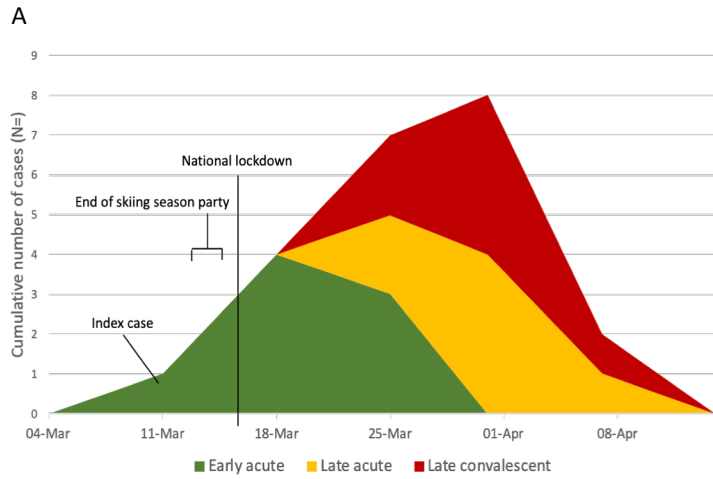
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3 572 Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive
4 573 among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters;
5 574 (C) Mean duration of symptoms; and (D): Mean number of symptoms.
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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)

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Primary Subject Heading:	General practice / Family practice

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





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

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35
36 49 **ABSTRACT**

37
38 50 **Objectives:** We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
39 51 regional COVID-19 outbreak in Austria.

40
41 52 **Design:** Prospective cohort study.

42
43 53 **Setting:** A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

44
45 54 **Participants:** All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
46 55 April, 2020.

47
48 56 **Intervention:** Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
49 57 chain reaction (RT-qPCR).

50
51 58 **Outcome measures:** We compared RT-qPCR at presentation with confirmed antibody status. We split the
52 59 outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
53 60 with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
54 61 convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
55 62 accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

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3 63 **Results:** Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent),
4 64 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all
5 65 acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of
6 66 symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days
7 67 (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was
8 68 associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
9 69 cough. Transmission clusters of three viral clades (G, GR and L) were identified.

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13 70
14 70 **Conclusions:** RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
15 71 with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
16 72 surveillance of coronavirus.
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18 73

19 74 **Strengths and limitations of this study**

- 20 74
- 21 74
- 22 75 • Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian
23 76 National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- 24 76
- 25 77 • Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people
26 78 testing RT-qPCR reactive were notified within 24 hours.
- 27 78
- 28 79 • Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- 29 79
- 30 80 • The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- 31 80
- 32 81 • Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly
33 82 among those people presenting late.
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36 84 **INTRODUCTION**

37 85 The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus
38 86 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths
39 86 reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate
40 87 (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide
41 87 spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like
42 88 illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ
43 89 failure and death.¹ In studies to date, the reported time for the infection to become symptomatic (incubation period)
44 89 varies among different cohorts and settings, with a median incubation period around 5.1 days,² infectivity starting
45 90 2.3 days before symptom onset, peaking 1-2 days before that,^{3,4} and gradually declining over 7-10 days.^{5,6 7}
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53 95 SARS-CoV-2 has the potential for ‘superspreading’ events, resulting in clusters of disease outbreaks among a
54 96 large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may
55 96 cause up to 80% of secondary transmissions.⁸ Although symptomatic infection is common (17 %, range 4-41%),
56 97 the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.⁹⁻¹¹
57 98 Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all
58 99 documented infections.¹² Superspreading events have been reported from across the globe, and countries
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3 101 achieving early viral suppression took rapid and decisive action to implement comprehensive case identification
4 102 and testing, combined with contact tracing and isolation.^{13,14} For epidemic control of COVID-19, the effective
5 103 reproduction number, R_e , needs to be less than 1; the presence of undetected and persistent infection within the
6 104 population, even if very small, can increase R_e and induce a secondary peak of infections. Therefore, rapid
7 105 identification and containment of infection is a key factor for the prevention of onward transmission and
8 106 controlling the virus to protect the public.¹⁵
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14 108 In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on
15 109 February 25, 2020.¹⁶ Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts,
16 110 including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries,
17 111 including Germany, Denmark and Sweden.^{16,17} Austria was one of the first countries to adopt comprehensive
18 112 lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching
19 113 self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.¹⁸ The
20 114 first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512
21 115 cases and 7.389 COVID-19 related deaths have been reported.
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28 117 General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of
29 118 equitable care.¹⁹⁻²¹ The European Centre for Disease Prevention and Control (ECDC) recommended integration
30 119 of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."²²
31 120 However, in some countries, like the UK and the USA, primary care has been largely excluded from the national
32 121 TTI strategy.²³ In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase
33 122 chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92
34 123 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.²⁴ The new service
35 124 supplemented the existing national health hotline for people at risk of COVID-19.²⁵ RT-qPCR is an established
36 125 technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.²⁶ Early detection of
37 126 SARS-CoV-2 is essential for effective contact tracing,²⁷ and whole genome sequencing may provide data on
38 127 dynamics of transmission.^{17,28}
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46 129 The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and
47 130 timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-
48 131 CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the
49 132 accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute
50 133 infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically
51 134 defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of
52 135 symptoms among patients presenting during the first half (early presenters) and the second half (late presenters)
53 136 of the outbreak, measured by the number of days from the first to the last case detected and dividing that period
54 137 by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a
55 138 correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.
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140 METHODS

141 Setting

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

151

152 Design

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

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

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

161

162 Intervention

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

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

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3 174 Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening
4 175 for patients attending sentinel GPs and paediatric practices. Between November and March of each year,
5 176 participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms.
6 177 Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue
7 178 cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal
8 179 influenza virus activity in the country.

12 180 **Clinical data**

14 181 We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL)
15 182 generated a clinical master case report form before extracting pseudonymised patient records into an Excel
16 183 spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a
17 184 secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of
18 185 Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the
19 186 University of Oxford, UK.

23 187

25 188 **Testing**

27 189 RT-qPCR

28 190 SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical
29 191 University of Vienna on a Roche LightCycler (<http://www.roche.com>; Switzerland) using a primer-set provided
30 192 by TIB MOLBIOL (<https://www.tib-molbiol.com/>; Germany).²⁶ RT-qPCR targeting the E-gene was considered
31 193 reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent
32 194 RNA polymerase (RdRP) gene detection.

36 195 Enzyme linked immune assays (ELISA)

37 196 IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial
38 197 test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies:
39 198 EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),²⁹ and EPITOPE
40 199 DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.³⁰ Reagent wells of the Anti-
41 200 SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of
42 201 SARS-CoV-2. Reagent wells of the EDITM Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-
43 202 19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT
44 203 LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, <https://www.diasorin.com/home>)
45 204 performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-
46 205 CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG
47 206 against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C).
48 207 LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative
49 208 detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary
50 209 units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence
51 210 immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a
52 211 recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA,

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

214 Neutralising antibody assay

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

222

223 **Outcome measures and statistical analysis**

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

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

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

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

249 positive) and all patients with confirmed infection by calculating the correlation coefficient r , and estimating the
250 95% CI.

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

258 RESULTS

259 Overall testing results

260 Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients
261 were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for
262 the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received
263 SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%)
264 tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling
265 (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive
266 vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis,
267 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were
268 confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the
269 outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April
270 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative
271 diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis
272 (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive
273 pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were
274 reported.

275 **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%)

276

277 **Specificity and sensitivity of RT-qPCR**

278 In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR
 279 outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient
 280 who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with
 281 mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart,
 282 Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six
 283 were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant
 284 negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza
 285 who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients
 286 with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with
 287 reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two
 288 were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and
 289 neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive
 290 (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results.
 291 Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of
 292 RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%),
 293 but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all
 294 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

295

296 **Earliness of RT-qPCR testing**

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

302

303 **Regression analysis on confirmed infection**

304 Multivariate regression on all 66 patients, including 22 (31.9%) with confirmed infection, suggested that loss of
 305 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 COVID-19.

Clinical symptom	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
	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).

327

328 **Viral clade analysis**

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

335 **Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2**
 336 **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

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

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348 **DISCUSSION**

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

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3 351 patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute
4 352 disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were
5 353 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first
6 354 half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean
7 355 duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a
8 356 quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing
9 357 rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms,
10 358 including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and
11 359 cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with
12 360 convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2
13 361 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.
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21 363 Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results
22 364 suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains
23 365 within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks
24 366 early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing
25 367 the outbreak without intermittent lockdowns,³⁵ we suggest that systemic changes may also be needed. For
26 368 example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they
27 369 may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to
28 370 social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an
29 371 important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and
30 372 should be something that governments should consider as an additional strategy.
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37 374 Loss of taste of smell has been recognised as an important marker of COVID-19;^{36,37} however, more than half of
38 375 patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be
39 376 reduced.³⁸ Furthermore, loss of taste could not be objectively confirmed in one third of people³⁸ suggesting self-
40 377 assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of
41 378 people presenting with acute disease.³⁹ Timely and accurate testing is also a prerequisite for effective contact
42 379 tracing.²⁷
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47 381 The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was
48 382 brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was
49 383 diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it
50 384 is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading
51 385 events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces
52 386 and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters
53 387 observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at
54 388 the party causing acute illness among early presenters and second, low level home transmission of party goers to
55 389 (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among
56 390 children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or
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3 391 did not attend the practice because of mild disease.⁴⁰ No further endemic cases were detected after the outbreak.
4 392 This suggests that combination prevention including rapid testing and case notification in primary care, contact
5 393 tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study
6 394 is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening
7 395 sites can effectively detect and control a regional outbreak.²²
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13 397 Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating
14 398 in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national
15 399 SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria;
16 400 and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were
17 401 detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and
18 402 result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab
19 403 Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled
20 404 accurate interpretation of RT-qPCR results.
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26 406 There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP,
27 407 potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven
28 408 patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our
29 409 COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend
30 410 emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather
31 411 than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested
32 412 by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among
33 413 all people confirmed with infection (when including those with negative RT-qPCR), compared to those people
34 414 presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and
35 415 data collection was completed prior to publication of the first systematic review of altered taste and smell in the
36 416 media.⁴¹ However, change or loss in smell/taste were not quantified using an established tool such as the visual
37 417 analogue scale (VAS),^{42,43} but rather assessed by simple “yes” and “no” answers using a standard clinical
38 418 questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,¹⁰
39 419 asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This
40 420 potentially excludes an important segment of the infected population and future studies will focus on exploring
41 421 this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we
42 422 have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the
43 423 data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and
44 424 their worldwide spread.²⁸
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56 426 To our knowledge, this is the first study to show that primary care can contribute to early case detection and
57 427 termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients,
58 428 public health, and health systems; nationally and internationally for outbreak epidemiology and control. As
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3 429 countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the
4 430 disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases
5 431 should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the
6 432 number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential
7 433 partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly
8 434 enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.⁴⁴
9 435 Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the
10 436 Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability
11 437 of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome
12 438 sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2
13 439 lateral flow antigen testing.
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441 CONCLUSIONS

23 442 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with
24 443 mild-to-moderate illness in a heterogeneous viral community outbreak. This study demonstrates high rates of
25 444 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19
26 445 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national
27 446 sentinel surveillance of coronavirus.
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33 448 **Authors' Contributions:** WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study.
34 449 OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG
35 450 submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and
36 451 JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay.
37 452 JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL,
38 453 MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final
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55 463 of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).
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57 464 **Patient consent for publication:** Consent may not be required as no identifiable details on individuals are
58 465 reported in this manuscript.
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3 466 **Patient and public involvement:** No patient involvement.

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5 467 **Data availability statement:** The datasets used and/or analysed during the current study are available from the
6 468 corresponding author on reasonable request.

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8 469 **Competing Interests:** None declared.
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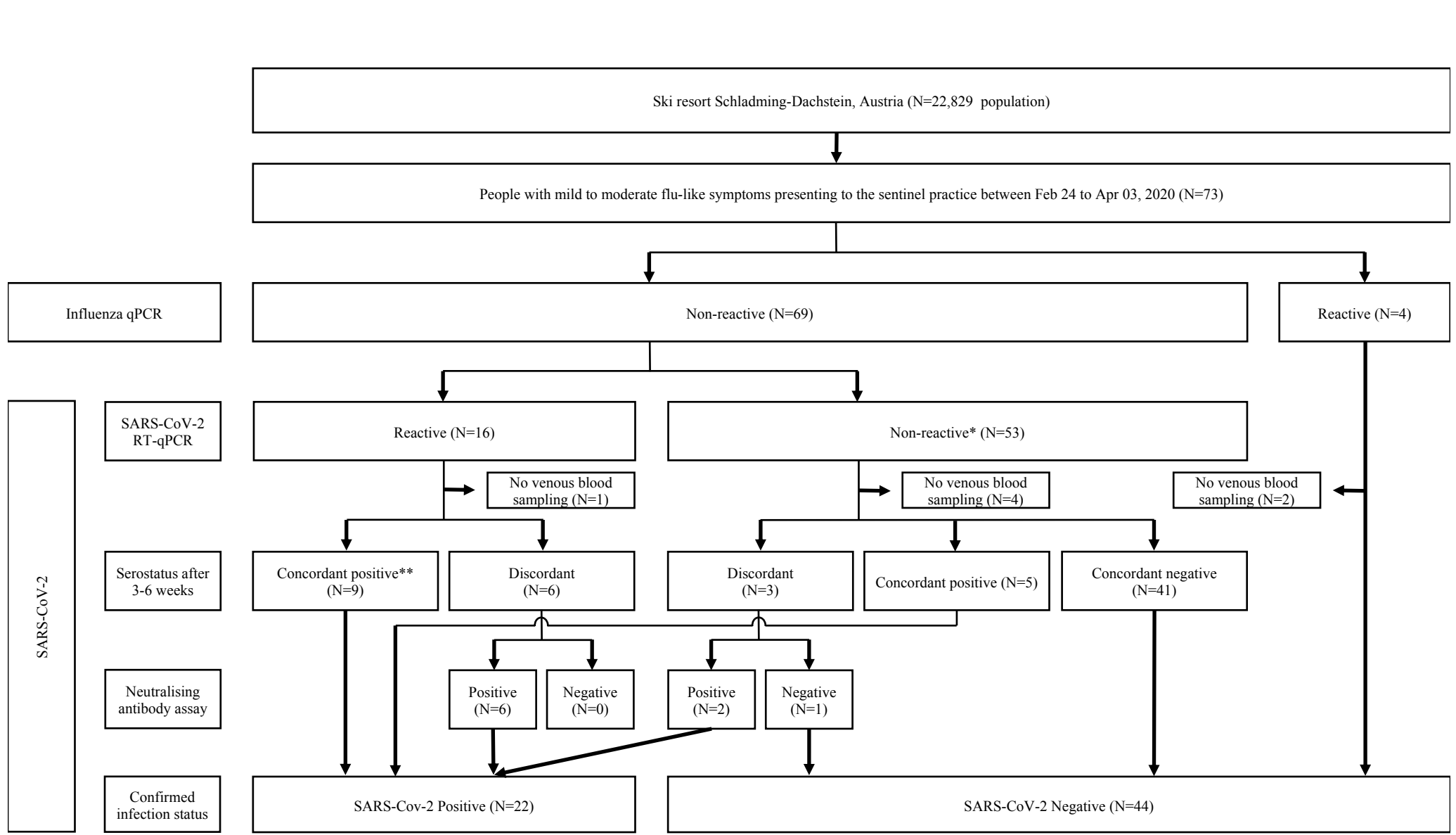
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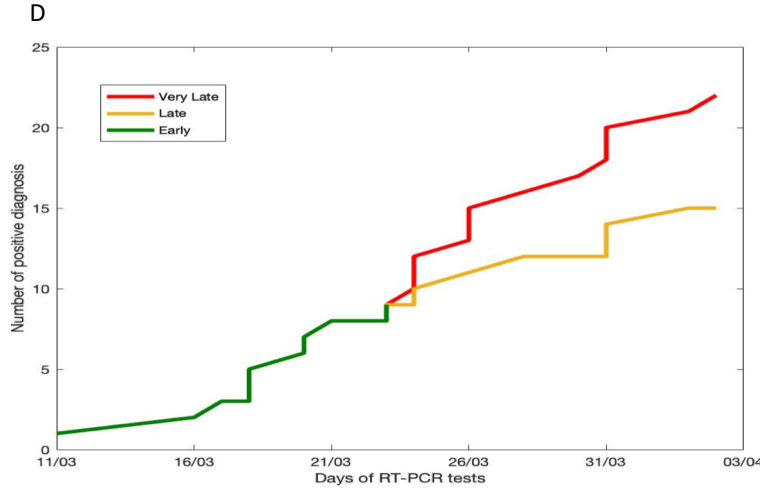
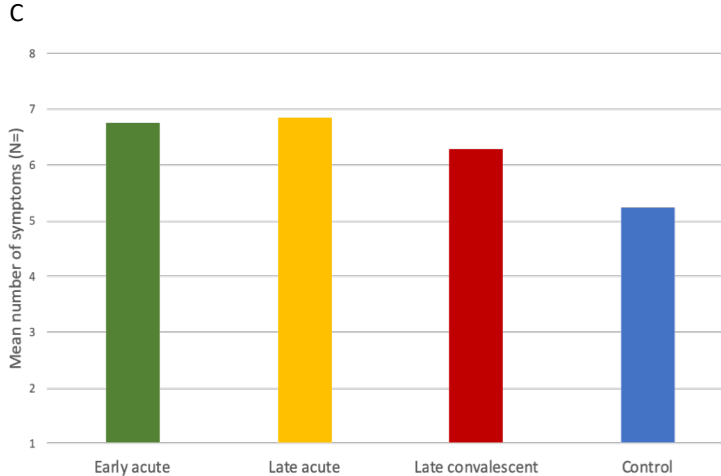
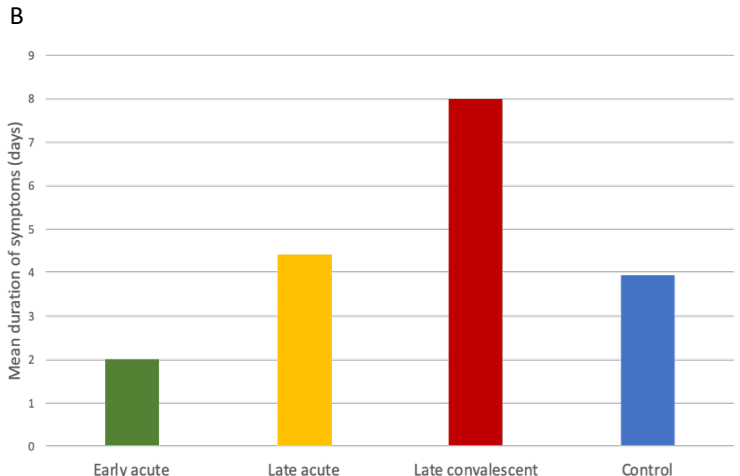
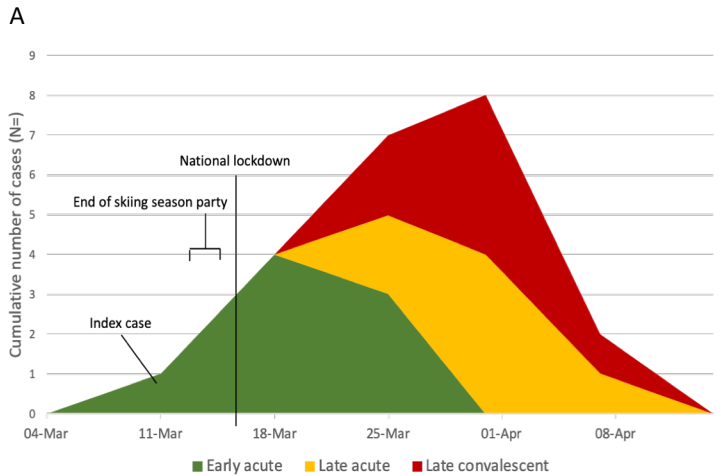
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19 578
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22 580 **FIGURE LEGENDS**

24 581 Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15
25 582 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-
26 583 qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing
27 584 antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant
28 585 antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44
29 586 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1
30 587 patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with
31 588 Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious
32 589 mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD
33 590 (N=1, each). ***No concordant negatives.

39 591
40 592 Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main
41 593 outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing
42 594 season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last
43 595 endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive)
44 596 in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute
45 597 infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B)
46 598 Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive
47 599 among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters;
48 600 (C) Mean duration of symptoms; and (D): Mean number of symptoms.





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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)

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Primary Subject Heading:	General practice / Family practice

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





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4 1 Title: Rapid, early and accurate SARS-CoV-2 detection using RT-PCR in primary care: A
5 2 prospective cohort study (REAP-1)
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36 49 **ABSTRACT**

37
38 50 **Objectives:** We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
39 51 regional COVID-19 outbreak in Austria.

40
41 52 **Design:** Prospective cohort study.

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43 53 **Setting:** A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

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45 54 **Participants:** All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
46 55 April, 2020.

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48 56 **Intervention:** Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
49 57 chain reaction (RT-qPCR).

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51 58 **Outcome measures:** We compared RT-qPCR at presentation with confirmed antibody status. We split the
52 59 outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
53 60 with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
54 61 convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
55 62 accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

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3 63 **Results:** Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent),
4 64 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all
5 65 acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of
6 66 symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days
7 67 (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was
8 68 associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
9 69 cough. Transmission clusters of three viral clades (G, GR and L) were identified.

10 68 associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
11 69 cough. Transmission clusters of three viral clades (G, GR and L) were identified.
12 70
13 71 **Conclusions:** RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
14 72 with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
15 73 surveillance of coronavirus.
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17 74 **Strengths and limitations of this study**

- 18 75 • Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian
19 76 National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- 20 77 • Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people
21 78 testing RT-qPCR reactive were notified within 24 hours.
- 22 79 • Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- 23 80 • The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- 24 81 • Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly
25 82 among those people presenting late.
26 83

27 84 **INTRODUCTION**

28 85 The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus
29 86 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths
30 87 reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate
31 88 (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide
32 89 spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like
33 90 illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ
34 91 failure and death.¹ In studies to date, the reported time for the infection to become symptomatic (incubation period)
35 92 varies among different cohorts and settings, with a median incubation period around 5.1 days,² infectivity starting
36 93 2.3 days before symptom onset, peaking 1-2 days before that,^{3,4} and gradually declining over 7-10 days.^{5,6 7}
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38 95 SARS-CoV-2 has the potential for ‘superspreading’ events, resulting in clusters of disease outbreaks among a
39 96 large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may
40 97 cause up to 80% of secondary transmissions.⁸ Although symptomatic infection is common (17 %, range 4-41%),
41 98 the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.⁹⁻¹¹
42 99 Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all
43 100 documented infections.¹² Superspreading events have been reported from across the globe, and countries

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3 101 achieving early viral suppression took rapid and decisive action to implement comprehensive case identification
4 102 and testing, combined with contact tracing and isolation.^{13,14} For epidemic control of COVID-19, the effective
5 103 reproduction number, R_e , needs to be less than 1; the presence of undetected and persistent infection within the
6 104 population, even if very small, can increase R_e and induce a secondary peak of infections. Therefore, rapid
7 105 identification and containment of infection is a key factor for the prevention of onward transmission and
8 106 controlling the virus to protect the public.¹⁵

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14 108 In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on
15 109 February 25, 2020.¹⁶ Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts,
16 110 including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries,
17 111 including Germany, Denmark and Sweden.^{16,17} Austria was one of the first countries to adopt comprehensive
18 112 lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching
19 113 self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.¹⁸ The
20 114 first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512
21 115 cases and 7.389 COVID-19 related deaths have been reported.

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28 117 General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of
29 118 equitable care.¹⁹⁻²¹ The European Centre for Disease Prevention and Control (ECDC) recommended integration
30 119 of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."²²
31 120 However, in some countries, like the UK and the USA, primary care has been largely excluded from the national
32 121 TTI strategy.²³ In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-polymerase
33 122 chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to any of the 92
34 123 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.²⁴ The new service
35 124 supplemented the existing national health hotline for people at risk of COVID-19.²⁵ RT-qPCR is an established
36 125 technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.²⁶ Early detection of
37 126 SARS-CoV-2 is essential for effective contact tracing,²⁷ and whole genome sequencing may provide data on
38 127 dynamics of transmission.^{17,28}

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46 129 The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and
47 130 timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-
48 131 CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the
49 132 accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute
50 133 infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically
51 134 defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of
52 135 symptoms among patients presenting during the first half (early presenters) and the second half (late presenters)
53 136 of the outbreak, measured by the number of days from the first to the last case detected and dividing that period
54 137 by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a
55 138 correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.

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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.

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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.

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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.

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3 174 Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening
4 175 for patients attending sentinel GPs and paediatric practices. Between November and March of each year,
5 176 participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms.
6 177 Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue
7 178 cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal
8 179 influenza virus activity in the country.

12 180 **Clinical data**

14 181 We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL)
15 182 generated a clinical master case report form before extracting pseudonymised patient records into an Excel
16 183 spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a
17 184 secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of
18 185 Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the
19 186 University of Oxford, UK.

23 187

25 188 **Testing**

27 189 RT-qPCR

28 190 SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical
29 191 University of Vienna on a Roche LightCycler (<http://www.roche.com>; Switzerland) using a primer-set provided
30 192 by TIB MOLBIOL (<https://www.tib-molbiol.com/>; Germany).²⁶ RT-qPCR targeting the E-gene was considered
31 193 reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent
32 194 RNA polymerase (RdRP) gene detection.

36 195 Enzyme linked immune assays (ELISA)

37 196 IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial
38 197 test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies:
39 198 EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),²⁹ and EPITOPE
40 199 DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.³⁰ Reagent wells of the Anti-
41 200 SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of
42 201 SARS-CoV-2. Reagent wells of the EDITM Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-
43 202 19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT
44 203 LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, <https://www.diasorin.com/home>)
45 204 performed on the LIAISON® platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-
46 205 CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG
47 206 against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C).
48 207 LIAISON® SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative
49 208 detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary
50 209 units (AU/mL). Elecsys® Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence
51 210 immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a
52 211 recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA,

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

214 Neutralising antibody assay

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

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223 **Outcome measures and statistical analysis**

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

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

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

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

249 positive) and all patients with confirmed infection by calculating the correlation coefficient r , and estimating the
250 95% CI.

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

257 258 RESULTS

259 Overall testing results

260 Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients
261 were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for
262 the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received
263 SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%)
264 tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling
265 (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive
266 vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis,
267 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were
268 confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the
269 outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April
270 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative
271 diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis
272 (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive
273 pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were
274 reported.

275 **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%)

276

277 **Specificity and sensitivity of RT-qPCR**

278 In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR
 279 outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient
 280 who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with
 281 mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart,
 282 Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six
 283 were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant
 284 negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza
 285 who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients
 286 with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with
 287 reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two
 288 were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and
 289 neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive
 290 (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results.
 291 Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of
 292 RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%),
 293 but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all
 294 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

295

296 **Earliness of RT-qPCR testing**

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

302

303 **Regression analysis on confirmed infection**

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

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

308 **Table 2: Regression analysis on symptoms reported by patients diagnosed with COVID-19.**

Clinical symptom	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
	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

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

313 Regression analysis on acute disease

314 All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis;
 315 and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed
 316 (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates
 317 were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11;
 318 p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002,
 319 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,
 320 p=0.069).

322 Correlation between acute and confirmed infection

323 Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection ($r=0.77$, 95%CI
 324 0.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber
 325 in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did
 326 not detect any case with convalescent infection (red curve on Figure 2D).

327

328 **Viral clade analysis**

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

335 **Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2**
 336 **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

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

347

348 **DISCUSSION**

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

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3 351 patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute
4 352 disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were
5 353 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first
6 354 half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean
7 355 duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a
8 356 quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing
9 357 rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms,
10 358 including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and
11 359 cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with
12 360 convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2
13 361 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.
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20 363 Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results
21 364 suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains
22 365 within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks
23 366 early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing
24 367 the outbreak without intermittent lockdowns,³⁵ we suggest that systemic changes may also be needed. For
25 368 example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they
26 369 may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to
27 370 social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an
28 371 important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and
29 372 should be something that governments should consider as an additional strategy.
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36 374 Loss of taste of smell has been recognised as an important marker of COVID-19;^{36,37} however, more than half of
37 375 patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be
38 376 reduced.³⁸ Furthermore, loss of taste could not be objectively confirmed in one third of people³⁸ suggesting self-
39 377 assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of
40 378 people presenting with acute disease.³⁹ Timely and accurate testing is also a prerequisite for effective contact
41 379 tracing.²⁷
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47 381 The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was
48 382 brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was
49 383 diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it
50 384 is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading
51 385 events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces
52 386 and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters
53 387 observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at
54 388 the party causing acute illness among early presenters and second, low level home transmission of party goers to
55 389 (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among
56 390 children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or
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3 391 did not attend the practice because of mild disease.⁴⁰ No further endemic cases were detected after the outbreak.
4 392 This suggests that combination prevention including rapid testing and case notification in primary care, contact
5 393 tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study
6 394 is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening
7 395 sites can effectively detect and control a regional outbreak.²²
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13 397 Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating
14 398 in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national
15 399 SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria;
16 400 and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were
17 401 detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and
18 402 result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab
19 403 Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled
20 404 accurate interpretation of RT-qPCR results.
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26 406 There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP,
27 407 potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven
28 408 patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our
29 409 COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend
30 410 emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather
31 411 than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested
32 412 by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among
33 413 all people confirmed with infection (when including those with negative RT-qPCR), compared to those people
34 414 presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and
35 415 data collection was completed prior to publication of the first systematic review of altered taste and smell in the
36 416 media.⁴¹ However, change or loss in smell/taste were not quantified using an established tool such as the visual
37 417 analogue scale (VAS),^{42,43} but rather assessed by simple “yes” and “no” answers using a standard clinical
38 418 questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,¹⁰
39 419 asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This
40 420 potentially excludes an important segment of the infected population and future studies will focus on exploring
41 421 this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we
42 422 have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the
43 423 data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and
44 424 their worldwide spread.²⁸
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56 426 To our knowledge, this is the first study to show that primary care can contribute to early case detection and
57 427 termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients,
58 428 public health, and health systems; nationally and internationally for outbreak epidemiology and control. As
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3 429 countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the
4 430 disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases
5 431 should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the
6 432 number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential
7 433 partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly
8 434 enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.⁴⁴
9 435 Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the
10 436 Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability
11 437 of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome
12 438 sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2
13 439 lateral flow antigen testing.
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22 441 CONCLUSIONS

23 442 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with
24 443 mild-to-moderate illness in a heterogeneous viral community outbreak. This study demonstrates high rates of
25 444 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19
26 445 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national
27 446 sentinel surveillance of coronavirus.
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33 448 **Authors' Contributions:** WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study.
34 449 OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG
35 450 submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and
36 451 JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay.
37 452 JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL,
38 453 MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final
39 454 version.
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52 461 2020. Award/Grant number is not applicable.
53

54 462 **Ethics approval:** The study used secondary anonymised data for which approval was granted by the University
55 463 of Graz Research Ethics Committee, Austria (reference number: 32-429 ex 19/20).
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57 464 **Patient consent for publication:** Verbal consent was received from patients for study participation.
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3 466 **Patient and public involvement:** No patient involvement.

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5 467 **Data availability statement:** The datasets used and/or analysed during the current study are available from the
6 468 corresponding author on reasonable request.

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8 469 **Competing Interests:** None declared.
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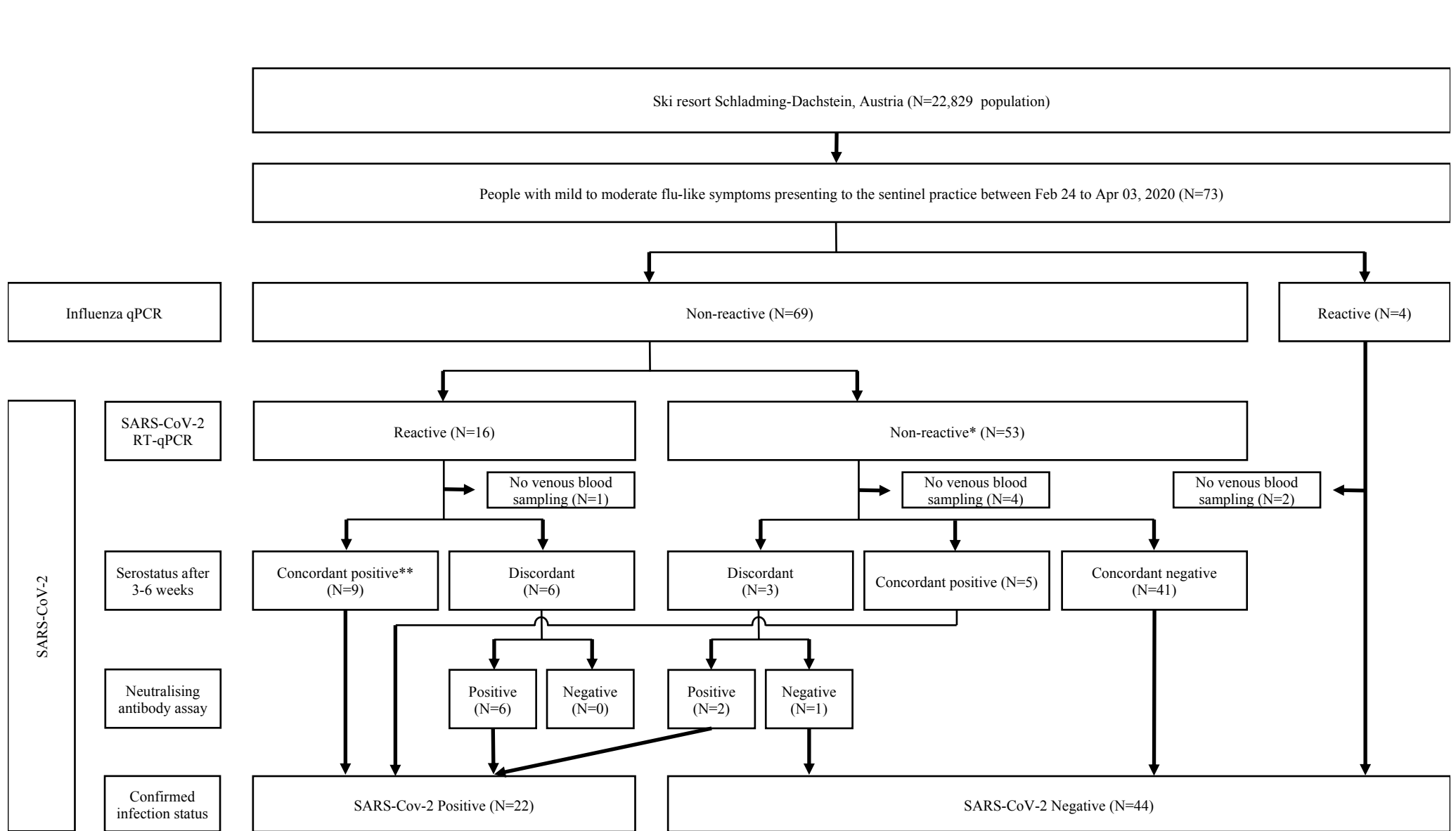
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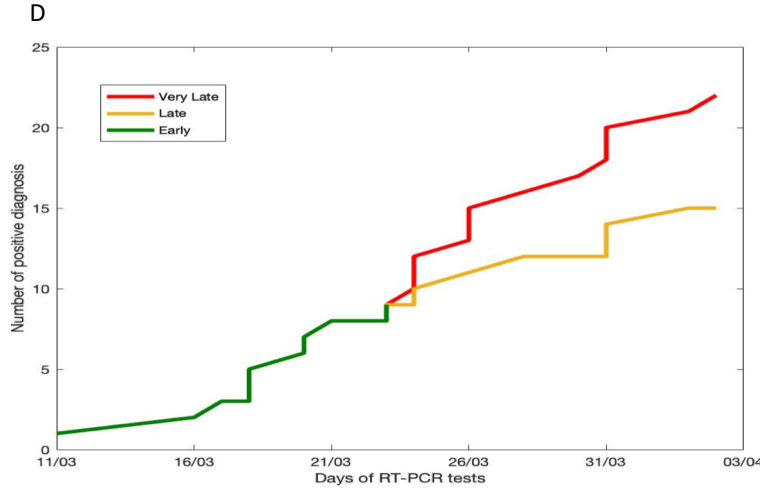
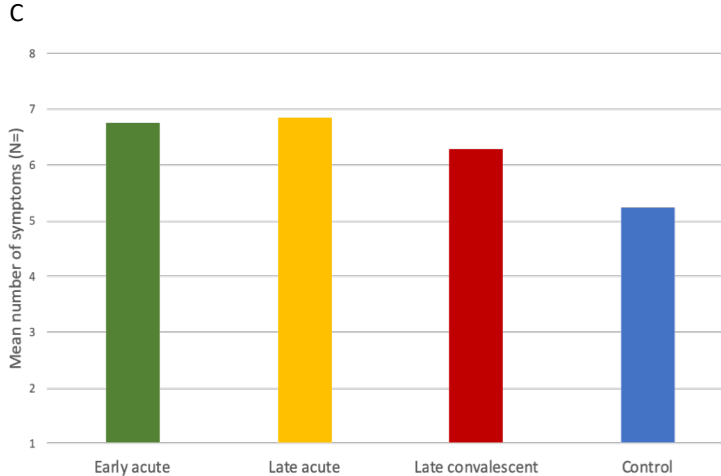
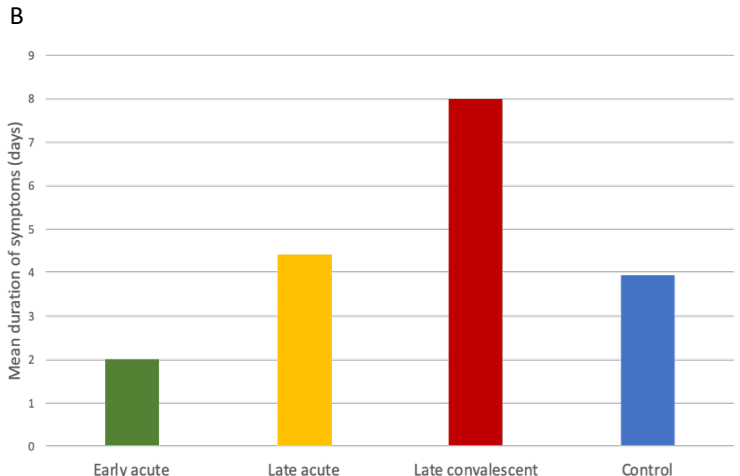
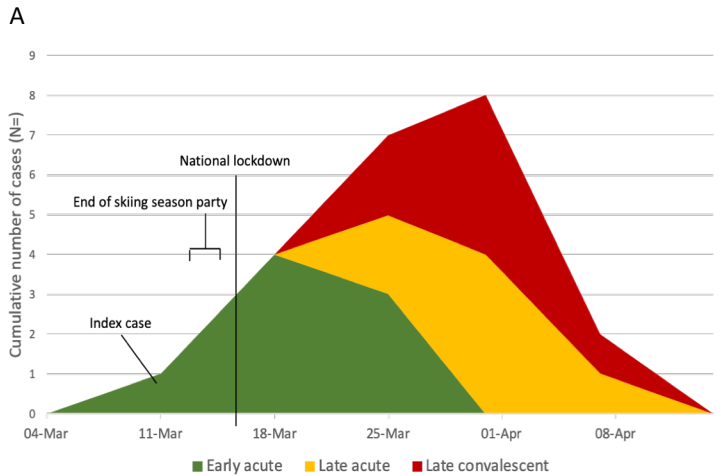
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22 580 **FIGURE LEGENDS**

24 581 Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15
25 582 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-
26 583 qPCR); among the former, 9 patients tested concordant antibody positive and 6 patients tested neutralizing
27 584 antibody positive following discordant ELISA result; and among the latter, 5 patients tested concordant
28 585 antibody positive and 2 patients tested neutralizing antibody positive following discordant ELISA result. 44
29 586 patients with non-reactive RT-qPCR tested antibody negative, including 41 with concordant negative ELISA, 1
30 587 patient with negative neutralizing antibody after discordant ELISA result and 2 patients diagnosed with
31 588 Influenza. Antibody status was not available for 7 patients. **Final clinical diagnoses included infectious
32 589 mononucleosis (N=2); bacterial tonsillitis, bacterial pneumonia, and bronchitis and exacerbation of COPD
33 590 (N=1, each). ***No concordant negatives.

39 591
40 592 Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main
41 593 outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing
42 594 season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last
43 595 endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive)
44 596 in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute
45 597 infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B)
46 598 Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive
47 599 among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters;
48 600 (C) Mean duration of symptoms; and (D): Mean number of symptoms.





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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.

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

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Primary Subject Heading:	General practice / Family practice

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35
36 49 **ABSTRACT**

37
38 50 **Objectives:** We explore the importance of SARS-CoV-2 sentinel surveillance testing in primary care during a
39 51 regional COVID-19 outbreak in Austria.

40
41 52 **Design:** Prospective cohort study.

42
43 53 **Setting:** A single sentinel practice serving 22,829 people in the ski-resort of Schladming-Dachstein.

44
45 54 **Participants:** All 73 patients presenting with mild-to-moderate flu-like symptoms between 24 February and 03
46 55 April, 2020.

47
48 56 **Intervention:** Nasopharyngeal sampling to detect SARS-CoV-2 using real-time reverse transcriptase-polymerase
49 57 chain reaction (RT-qPCR).

50
51 58 **Outcome measures:** We compared RT-qPCR at presentation with confirmed antibody status. We split the
52 59 outbreak in two parts, by halving the period from the first to the last case, to characterise three cohorts of patients
53 60 with confirmed infection: early acute (RT-qPCR reactive) in the first half; and late acute (reactive) and late
54 61 convalescent (non-reactive) in the second half. For each cohort we report the number of cases detected, the
55 62 accuracy of RT-qPCR, the duration and variety of symptoms, and the number of viral clades present.

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3 **63 Results:** Twenty-two patients were diagnosed with COVID-19 (8 early acute, 7 late acute and 7 late convalescent),
4 **64** 44 patients tested SARS-CoV-2 negative, and 7 were excluded. The sensitivity of RT-qPCR was 100% among all
5 **65** acute cases, dropping to 68.1% when including convalescent. Test specificity was 100%. Mean duration of
6 **66** symptoms for each group were 2 days (range 1-4) among early acute, 4.4 days (1-7) among late acute and 8 days
7 **67** (2-12) among late convalescent. Confirmed infection was associated with loss of taste. Acute infection was
8 **68** associated with loss of taste, nausea/vomiting, breathlessness, sore throat and myalgia; but not anosmia, fever or
9 **69** cough. Transmission clusters of three viral clades (G, GR and L) were identified.

10 **70 Conclusions:** RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people
11 **71** with flu-like illness in a heterogenous viral outbreak. Targeted testing in primary care can support national sentinel
12 **72** surveillance of coronavirus.
13

14 **73**

15 **74 Strengths and limitations of this study**

- 16 **75** • Our study was conducted in a state-of-the-art sentinel surveillance practice, participating in the Austrian
17 **76** National Influenza Screening Programme, covering the entire period of a regional COVID-19 outbreak.
- 18 **77** • Symptomatic patients received same-day appointments with a clinician for nasopharyngeal swabs, and people
19 **78** testing RT-qPCR reactive were notified within 24 hours.
- 20 **79** • Cases were confirmed using a combination of five different ELISA platforms and neutralising antibody assay.
- 21 **80** • The relatively small patient cohort from a single testing site limits conclusion on causality and generalisability.
- 22 **81** • Any difference in symptoms observed between study cohorts may be due to recall bias occurred, particularly
23 **82** among those people presenting late.

24 **83**

25 **84 INTRODUCTION**

26 **85** The coronavirus 2019 disease (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus
27 **86** 2 (SARS-CoV-2), continues to spread globally with more than 96 million cases, and over two million deaths
28 **87** reported as of January 22, 2021. Undetected infection and delays in implementing an effective test-trace-isolate
29 **88** (TTI) strategy have contributed to the spread of the virus becoming a pandemic. SARS-CoV-2 virus has a wide
30 **89** spectrum of manifestations including no symptoms (asymptomatic infection), mild to moderate to severe flu-like
31 **90** illness, loss of taste or smell, pneumonia and acute respiratory distress syndrome (ARDS), sepsis, multi-organ
32 **91** failure and death.¹ In studies to date, the reported time for the infection to become symptomatic (incubation period)
33 **92** varies among different cohorts and settings, with a median incubation period around 5.1 days,² infectivity starting
34 **93** 2.3 days before symptom onset, peaking 1-2 days before that, and gradually declining over 7-10 days.³⁻⁶

35 **94**

36 **95** SARS-CoV-2 has the potential for 'superspreading' events, resulting in clusters of disease outbreaks among a
37 **96** large number of people. Most infections remain isolated cases, but a small number of individuals (10%) may
38 **97** cause up to 80% of secondary transmissions.⁷ Although symptomatic infection is common (17 %, range 4-41%),
39 **98** the relative risk for symptomatic transmission may be up to six times higher than for asymptomatic infection.⁸⁻¹⁰
40 **99** Undocumented infection may constitute the majority of cases (86%), causing more than half (55%) of all
41 **100** documented infections.¹¹ Superspreading events have been reported from across the globe, and countries

1
2
3 101 achieving early viral suppression took rapid and decisive action to implement comprehensive case identification
4 102 and testing, combined with contact tracing and isolation.^{12,13} For epidemic control of COVID-19, the effective
5
6 103 reproduction number, R_e , needs to be less than 1; the presence of undetected and persistent infection within the
7
8 104 population, even if very small, can increase R_e and induce a secondary peak of infections. Therefore, rapid
9
10 105 identification and containment of infection is a key factor for the prevention of onward transmission and
11
12 106 controlling the virus to protect the public.¹⁴

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15 108 In Austria, the first two COVID-19 cases were reported among travelers from Italy in the city of Innsbruck on
16 109 February 25, 2020.¹⁵ Multiple superspreading events then occurred among tourists visiting Austrian ski-resorts,
17 110 including the town of Ischgl, that are believed to have led to further outbreaks in the tourists' home countries,
18 111 including Germany, Denmark and Sweden.^{15,16} Austria was one of the first countries to adopt comprehensive
19 112 lockdown measures on March 16, 2020, including protection of vulnerable groups, penalty fees for breaching
20 113 self-isolation, and a national health hotline to facilitate testing at acute care settings and *via* mobile units.¹⁷ The
21 114 first death from COVID-19 associated complications occurred on March 12, 2020, and as of January 21, 403.512
22 115 cases and 7.389 COVID-19 related deaths have been reported.

23
24 116

25
26 117 General practice (GP) is considered a key partner in case recording, managing high-risk groups and delivery of
27 118 equitable care.¹⁸⁻²⁰ The European Centre for Disease Prevention and Control (ECDC) recommended integration
28 119 of "COVID-19 surveillance with sentinel surveillance of influenza-like illness or acute respiratory infection."²¹
29 120 However, in some countries, like the UK and the USA, primary care has been largely excluded from the national
30 121 TTI strategy.^{22,23} In contrast, Austria additionally offered SARS-CoV-2 real-time reverse transcriptase-
31 122 polymerase chain reaction (RT-qPCR) testing to people presenting with mild to moderate flu-like symptoms to
32 123 any of the 92 sentinel surveillance sites (GPs and paediatric practices) beginning February 24, 2020.²⁴ The new
33 124 service supplemented the existing national health hotline for people at risk of COVID-19.²⁵ RT-qPCR is an
34 125 established technique to detect viral RNA from nasopharyngeal sampling used to diagnose COVID-19.²⁶ Early
35 126 detection of SARS-CoV-2 is essential for effective contact tracing,²⁷ and whole genome sequencing may provide
36 127 data on dynamics of transmission.²⁸

37
38 128

39 129 The overall aim of this work is to test whether rapid early RT-qPCR testing in primary care can accurately and
40 130 timely detect SARS-CoV-2, and inform outbreak surveillance. To attest this, we report the outcomes of SARS-
41 131 CoV-2 RT-qPCR testing at a sentinel GP in the ski-resort of Schladming-Dachstein, Austria. We report a) the
42 132 accuracy (via sensitivity and specificity) of rapidly deployed RT-qPCR testing in patients presenting with acute
43 133 infection by comparing it to anti-SARS-CoV-2 antibody status during convalescence in the same geographically
44 134 defined study cohort; b) the earliness of viral RNA detection by comparing the duration, number and type of
45 135 symptoms among patients presenting during the first half (early presenters) and the second half (late presenters)
46 136 of the outbreak, measured by the number of days from the first to the last case detected and dividing that period
47 137 by two; c) the identification of key clinical symptoms of acute and convalescent disease and determine a
48 138 correlation between these; and d) the number of SARS-CoV-2 clades implicated in the outbreak.

139

140 METHODS

141 Setting

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

151

152 Design

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

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

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

161

162 Intervention

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

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

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3 174 Since the winter season 2000/2001, the National Influenza Screening Network has conducted influenza screening
4 175 for patients attending sentinel GPs and paediatric practices. Between November and March of each year,
5 176 participating practices routinely collect nasopharyngeal swabs from patients presenting with flu-like symptoms.
6 177 Specimens are sent to the Center for Virology, Medical University of Vienna, Austria, for virus isolation on tissue
7 178 cultures and PCR detection. This surveillance programme allows for near real-time recording of seasonal
8 179 influenza virus activity in the country.

12 180 **Clinical data**

14 181 We obtained anonymous patient data held within the GP computer system. The practice lead clinician (OL)
15 182 generated a clinical master case report form before extracting pseudonymised patient records into an Excel
16 183 spreadsheet. EMH and CH verified the accuracy of the data extraction for all patients. Data were stored on a
17 184 secure computer at the Institute of General Practice and Evidence-based Health Services Research, University of
18 185 Graz, Austria, before sharing it with the study statistician (JPG) using encrypted email and secure storage at the
19 186 University of Oxford, UK.

23 187

25 188 **Testing**

27 189 RT-qPCR

28 190 SARS-CoV-2 RT-qPCR was performed in scope of the routine surveillance at the Center for Virology, Medical
29 191 University of Vienna on a Roche LightCycler (<http://www.roche.com>; Switzerland) using a primer-set provided
30 192 by TIB MOLBIOL (<https://www.tib-molbiol.com/>; Germany).²⁶ RT-qPCR targeting the E-gene was considered
31 193 reactive at a cycle threshold (Ct) value of less than 40, and Ct values above 32 were confirmed by RNA-dependent
32 194 RNA polymerase (RdRP) gene detection.

36 195 Enzyme linked immune assays (ELISA)

37 196 IgG serostatus assays were performed according to the manufacturers' protocol using five different commercial
38 197 test kits of Anti-SARS-CoV-2 IgG enzyme immune linked assays (ELISA) provided by the following companies:
39 198 EUROIMMUN (EUROIMMUN Medizinische Labordiagnostika AG, www.euroimmun.com),²⁹ and EPITOPE
40 199 DIAGNOSTICS (Immunodiagnostik AG www.euroimmun.com) respectively.³⁰ Reagent wells of the Anti-
41 200 SARS-CoV-2 IgG ELISA are coated with recombinant antigen derived from the spike protein (S1 domain) of
42 201 SARS-CoV-2. Reagent wells of the EDITM Novel Coronavirus COVID-19 IgG ELISA are coated with COVID-
43 202 19 recombinant full length nucleocapsid protein. ABBOTT performed on the Architect platform (ABBOTT
44 203 LABORATORIES INC., www.abbott.com), DIASORIN (DIASORIN S.p.A, <https://www.diasorin.com/home>)
45 204 performed on the LIAISON[®] platform and ROCHE performed on the cobas e 801 analyzer. The Abbott SARS-
46 205 CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG
47 206 against a recombinant SARS-CoV-2 nucleoprotein. Results are reported in form of an index value (S/C).
48 207 LIAISON[®] SARS-CoV-2 S1/S2 IgG assay is a chemiluminescence immunoassay (CLIA) for the quantitative
49 208 detection of IgG against the recombinant S1 and S2 domain of the spike protein. Results are reported in arbitrary
50 209 units (AU/mL). Elecsys[®] Anti-SARS-CoV-2 assay (Roche Diagnostics) is a electrochemiluminescence
51 210 immunoassay (ECLIA) for qualitative detection of SARS-CoV-2 antibodies in human serum against a
52 211 recombinant nucleocapsid protein of SARS-CoV-2. It is a total antibody assay not differentiating between IgA,

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

214 Neutralising antibody assay

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

222

223 **Outcome measures and statistical analysis**

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

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

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

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

249 positive) and all patients with confirmed infection by calculating the correlation coefficient r , and estimating the
250 95% CI.

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

258 RESULTS

259 Overall testing results

260 Baseline characteristics for confirmed cases were similar for sex, age, and ethnic origin (Table 1). All patients
261 were local residents and no endemic cases were documented among tourists. Figure 1 shows the flow-chart for
262 the patient cohorts of this study. 73 patients presented with mild to moderate flu-like illness, all of whom received
263 SARS-CoV-2 RT-qPCR (and influenza qPCR). Of those, 16 (21.9%) tested RT-qPCR reactive and 57 (78.1%)
264 tested non-reactive, including four that tested influenza PCR reactive. Due to lack of venous blood sampling
265 (obtained 3-6 weeks after initial presentation), antibody data was not available for 7 patients (1 RT-qPCR reactive
266 vs. 6 non-reactive) that were excluded from this analysis. Therefore, of the 66 patients included in this analysis,
267 22 patients (33.3%) had SARS-CoV-2 infection confirmed by antibody testing and 44 (66.7%) patients were
268 confirmed seronegative. Of the former, eight patients (early acute presenters) presented in the first half of the
269 outbreak (12 days from March 11 to 22, 2020) and 14 patients presented in the second half (March 23 to April
270 03, 2020); of the latter, seven patients were late acute and seven late convalescent (Figure 2A). Alternative
271 diagnoses of the 44 patients who tested SARS-CoV-2 negative included: influenza and infectious mononucleosis
272 (N=2, each); bacterial tonsillitis, bacterial pneumonia, bronchitis and exacerbation of chronic obstructive
273 pulmonary disease (COPD) (N=1, each) (see flow-chart, Figure 1). No hospital admissions or deaths were
274 reported.

275 **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%)

276

277 **Specificity and sensitivity of RT-qPCR**

278 In the absence of a gold standard, we used a consensus statement on serostatus, irrespective of RT-qPCR
 279 outcomes, to establish whether an infection had occurred. We considered an infection as confirmed in any patient
 280 who tested IgG ELISA positive on all five screening platforms (concordant results) or in any patient with
 281 mismatch between ELISA test results (discordant results) but positive neutralising antibody assay (see flow-chart,
 282 Figure 1). Of the 15 patients with reactive RT-qPCR, sera from nine patients were concordant positive and six
 283 were discordant; and of the 53 patients with non-reactive RT-qPCR, sera from 41 patients were concordant
 284 negative, 5 were concordant positive, and three were discordant. Sera from two patients diagnosed with influenza
 285 who tested RT-qPCR non-reactive were concordant negative and included in this analysis. For the nine patients
 286 with discordant results, we used neutralising antibody assay to confirm infection status. All patients (N=6) with
 287 reactive RT-qPCR were neutralising antibody positive; and of the three patients with non-reactive RT-qPCR, two
 288 were neutralising antibody positive, and one was negative. Therefore, overall, when combining ELISA and
 289 neutralising antibody assay, 22 patients had confirmed infection, of whom 15 patients were RT-qPCR reactive
 290 (true reactive) and seven were non-reactive (false non-reactive). There were no false reactive RT-qPCR results.
 291 Therefore, RT-qPCR correctly identified infection in 15/22 patients (overall sensitivity of 68.1%). Sensitivity of
 292 RT-qPCR among all acute (early and late) presenters and during the first half of the outbreak was high (100%),
 293 but dropped to 50% in the second half of the outbreak. RT-qPCR correctly identified absence of infection for all
 294 44 patients testing antibody negative (true non-reactive) indicating specificity of 100%.

295

296 **Earliness of RT-qPCR testing**

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

302

303 **Regression analysis on confirmed infection**

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

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

308 **Table 2: Regression analysis on symptoms reported by patients diagnosed with COVID-19.**

Clinical symptom	People with confirmed infection (seropositive, any RT-qPCR result) (N=22)			People with acute disease (RT-qPCR reactive and seropositive) (N=15)		
	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

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

312

313 **Regression analysis on acute disease**

314 All 15 patients with acute disease reported fatigue and therefore this covariate was removed from the analysis;
 315 and observations from two patients with non-reactive RT-qPCR, who did not report fatigue, were also removed
 316 (Table 2). The multivariate logistic regression on the remaining 66 patients showed that the following covariates
 317 were associated with acute disease: loss of taste (OR=571.72; p=0.029), nausea and vomiting (OR=370.11;
 318 p=0.018), breathlessness (OR=134.46; p=0.049), myalgia (OR=121.82; p=0.032) and sore throat (OR=0.002,
 319 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,
 320 p=0.069).

321

322 **Correlation between acute and confirmed infection**

323 Testing RT-qPCR reactive was correlated with testing seropositive for COVID-19 infection ($r=0.77$, 95%CI
 324 0.65~0.89). Among early and acute presenters, the correlation between the two tests was perfect (green and amber
 325 in Figure 2D), irrespective of the stage of the outbreak; whereas in the second half of the outbreak, RT-qPCR did
 326 not detect any case with convalescent infection (red curve on Figure 2D).

327

328 **Viral clade analysis**

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

335 **Table 3: Genomic sequences accessed via GISAID listing key amino acid locations used for SARS-CoV-2**
 336 **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

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

347

348 **DISCUSSION**

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

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3 351 patients presenting to the sentinel GP, 22 were diagnosed with COVID-19, including 15 patients with acute
4 352 disease and seven with late convalescent infection respectively. The sensitivity and specificity of RT-qPCR were
5 353 68.1% and 100%, but testing RT-qPCR reactive showed perfect correlation with seropositivity during the first
6 354 half of the outbreak and among early acute (N=8 patients) and late acute presenters (N=7). Strikingly, the mean
7 355 duration of symptoms of early presenters (2 days) was less than half of late acute presenters (4.4 days) and a
8 356 quarter of late convalescent presenters (8 days). These findings highlight the need to undertake RT-qPCR testing
9 357 rapidly and early as soon as symptoms occur. Acute infection was strongly associated with multiple symptoms,
10 358 including loss of taste, nausea and vomiting, breathlessness, myalgia and sore throat; but loss of smell, fever and
11 359 cough were not. Surprisingly, loss of taste, but not any other clinical symptom, was significantly associated with
12 360 convalescent infection. Finally, viral genome analysis demonstrated the presence of three major SARS-CoV-2
13 361 clades during the outbreak, suggesting that the outbreak was the result of independent transmission chains.
14 362

15 363 Overall our findings help untangle COVID-19 infection during an outbreak in a ski-resort in Austria. Our results
16 364 suggest that acute COVID-19 may be associated with a spectrum of symptoms and presence of multiple strains
17 365 within one setting. This highlights the heterogeneity of coronavirus and the importance in containing outbreaks
18 366 early before spread. While effective test-trace-isolate (TTI) strategies have been suggested as the key to containing
19 367 the outbreak without intermittent lockdowns,³⁵ we suggest that systemic changes may also be needed. For
20 368 example, behavioral changes, such as large-scale gathering of people in closed spaces has to be avoided as they
21 369 may trigger emergence of individual clusters to form a superspreading event. Keeping a level of compliance to
22 370 social distancing and reduced physical contacts is necessary to prevent any future wave. Enhanced testing is an
23 371 important factor, and our study suggests that testing in primary care at symptom onset is highly accurate and
24 372 should be something that governments should consider as an additional strategy.
25 373

26 374 Loss of taste of smell has been recognised as an important marker of COVID-19;^{36,37} however, more than half of
27 375 patients reported olfactory dysfunction after the onset of other symptoms when sensitivity of RT-qPCR may be
28 376 reduced.³⁸ Furthermore, loss of taste could not be objectively confirmed in one third of people³⁸ suggesting self-
29 377 assessment using a mobile phone application may not be as accurate as clinician-initiated RT-qPCR testing of
30 378 people presenting with acute disease.³⁹ Timely and accurate testing is also a prerequisite for effective contact
31 379 tracing.²⁷
32 380

33 381 The outbreak we explored occurred after a three-day party (March 13-15) just before the skiing season was
34 382 brought to a premature end due to the Austrian national lockdown measures on March 16. The index case was
35 383 diagnosed on March 11 and the first secondary cases were reported two days after the celebrations. Therefore, it
36 384 is possible that the outbreak we are describing here could be a possible superspreading event. Superspreading
37 385 events have been associated with high intensity aerosol producing activities (shouting, singing) in confined spaces
38 386 and potentially, the lockdown party might have triggered the local outbreak. The two acute disease clusters
39 387 observed in this study may represent different types of viral exposure. First, inhalation of high-density aerosols at
40 388 the party causing acute illness among early presenters and second, low level home transmission of party goers to
41 389 (late presenting) friends and family during the lockdown. In our study, no COVID-19 cases were observed among
42 390 children (persons <18 years of age), suggesting that any infected children may have remained asymptomatic or

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3 391 did not attend the practice because of mild disease.⁴⁰ No further endemic cases were detected after the outbreak.
4 392 This suggests that combination prevention including rapid testing and case notification in primary care, contact
5 393 tracing and isolation, and lockdown measures can effectively terminate an outbreak. To our knowledge, our study
6 394 is the first to demonstrate that the ECDC policy of additional COVID-19 screening at national influenza screening
7 395 sites can effectively detect and control a regional outbreak.²¹
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12 397 Our study has many strengths. Our study was enabled by data from a well-established sentinel GP, participating
13 398 in the National Influenza Screening Programme, covering the entire area of the outbreak. Importantly, national
14 399 SARS-CoV-2 screening was adopted early, starting the day before the first two cases were reported in Austria;
15 400 and 16 of 29 cases documented in the Schladming-Dachstein region, including the first and the last case, were
16 401 detected at the sentinel GP. RT-qPCR testing was rapidly deployed by offering same day GP appointments, and
17 402 result reporting and case notification within 24 hours. Rapid adoption of new commercial antibody platforms (Lab
18 403 Mustafa, Salzburg) and in-house neutralising antibody testing assay (Medical University of Vienna) enabled
19 404 accurate interpretation of RT-qPCR results.
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25 405
26 406 There are some limitations of our study. We used a relatively small patient cohort from a single sentinel GP,
27 407 potentially limiting conclusions on causality and generalisability of our finding to other areas excluding seven
28 408 patients for whom COVID-19 serostatus were not available. Lack of association with high fever and cough in our
29 409 COVID-19 cohort may be due to the national health hotline directing patients with more severe disease to attend
30 410 emergency service. Therefore, people with these symptoms might have preferred to attend acute services rather
31 411 than the GP. Although we collected data prospectively, recall bias cannot be excluded. This could be suggested
32 412 by the lack of association of symptoms of acute infection (nausea and vomiting, breathless and myalgia) among
33 413 all people confirmed with infection (when including those with negative RT-qPCR), compared to those people
34 414 presenting early (reactive RT-qPCR). Specific recall bias of taste is less likely, as it featured in both groups and
35 415 data collection was completed prior to publication of the first systematic review of altered taste and smell in the
36 416 media.⁴¹ However, change or loss in smell/taste were not quantified using an established tool such as the visual
37 417 analogue scale (VAS),^{42,43} but rather assessed by simple “yes” and “no” answers using a standard clinical
38 418 questionnaire, potentially leading to response style bias. Although asymptomatic infection is common,⁹
39 419 asymptomatic people were excluded from this study as we were focusing on symptom-driven presentation. This
40 420 potentially excludes an important segment of the infected population and future studies will focus on exploring
41 421 this further. The presence of three viral clades within the outbreak suggests heterogeneity of the virus, but we
42 422 have not explored this aspect in great details in this study, as this was beyond the scope of this work. In fact, the
43 423 data presented here is part of the ongoing work untangling the phylogeny of SARS-CoV-2 clades in Austria and
44 424 their worldwide spread.²⁸
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56 426 To our knowledge, this is the first study to show that primary care can contribute to early case detection and
57 427 termination of a SARS-CoV-2 outbreak in the community. Our study has important implications for patients,
58 428 public health, and health systems; nationally and internationally for outbreak epidemiology and control. As
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3 429 countries enter the viral suppression phase, early detection will be crucial in the prevention and control of the
4 430 disease. Early testing at onset of disease, followed by timely contact tracing and case isolation of secondary cases
5 431 should prevent onward transmission and reduce the reproduction number R_e below 1. Austria has increased the
6 432 number of its sentinel sites from 91 to 231 due to COVID-19, indicating that primary care has become an essential
7 433 partner in a comprehensive surveillance strategy for disease prevention and control. Clade analysis could greatly
8 434 enhance public health surveillance in the UK where only three quarters of contact tracing is being completed.⁴⁴
9 435 Key priorities for future research include systematic prospective quantitative and qualitative evaluation of the
10 436 Austrian National SARS-CoV-2 screening programme during the seasonal influenza season, and generalisability
11 437 of the intervention in multi-ethnic inner-city settings including genomic analysis using deep viral genome
12 438 sequencing to support complex contact tracing, and adaption of the REAP-1 protocol to include SARS-CoV-2
13 439 lateral flow antigen testing.

19 440

21 441 CONCLUSIONS

23 442 RT-qPCR testing in primary care can rapidly and accurately detect SARS-CoV-2 among people presenting with
24 443 mild-to-moderate illness in a heterogeneous viral community outbreak. This study demonstrates high rates of
25 444 accurate and early viral detection associated with symptomatic testing in primary care during a COVID-19
26 445 outbreak, which is required for an effective TTI strategy. Targeted testing in primary care can support national
27 446 sentinel surveillance of coronavirus.

30 447

32 448 **Authors' Contributions:** WL, OL, MRF, MEMK, EMH, CH and JPG contributed to the design of the study.
33 449 OL and EMH took nasopharyngeal swabs. OL, EMH and CH maintained the clinical data base. AS and RG
34 450 submitted the ethics application. MRF provided RT-qPCR data; BA, AL, AMP, JWG, TP, SA, CB and AB; and
35 451 JVC conducted clade analysis, MEMK produced ELISA data, KS performed the neutralising antibody assay.
36 452 JPG and WL conducted the statistical analysis. WL and JPG wrote the manuscript with contributions from OL,
37 453 MRF, MEMK, RCG, JVC, CB, AB, KS, EMH, CH, AS and CG. All authors read and approved the final
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55 463 19/20) approved collection of anonymised RT-PCR and antibody status data, and the Medical University of
56 464 Vienna Research Ethics Committee (reference number: EK1339/2017) additionally approved usage of
57 465 anonymised RT-PCR data collected as part of the National Influenza Surveillance Network including generation

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

468

469 **Patient and public involvement:** No patient involvement.

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

472 **Competing Interests:** None declared.

473

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591 **FIGURE LEGENDS**

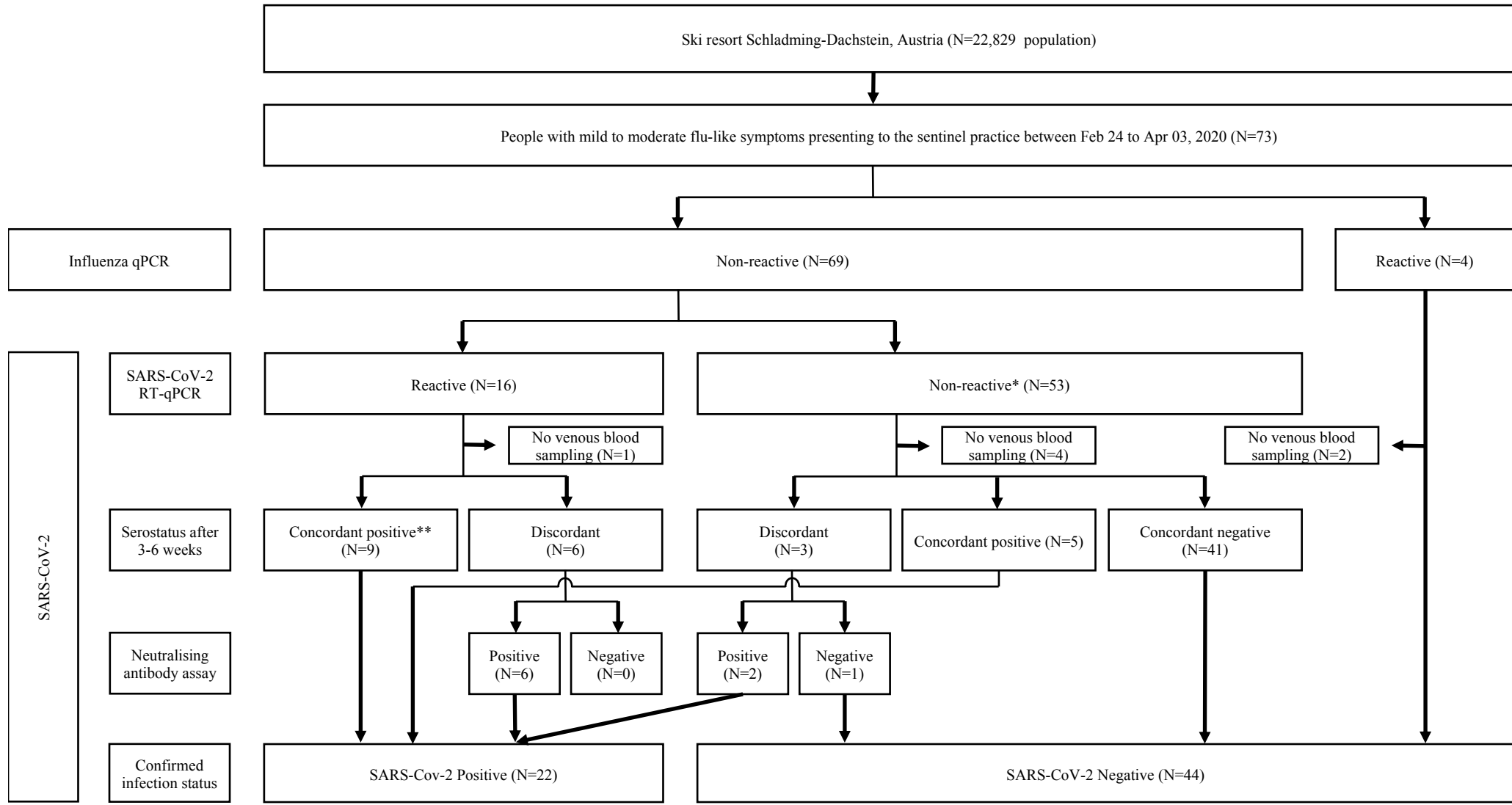
592 Figure 1: Flow-chart. Twenty-two patients had COVID-19 infection confirmed by antibody testing, including 15
593 patients diagnosed with acute disease (reactive RT-qPCR) and 7 with convalescent disease (non-reactive RT-
594 qPCR); among the former, 9 patients tested concordant antibody positive and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

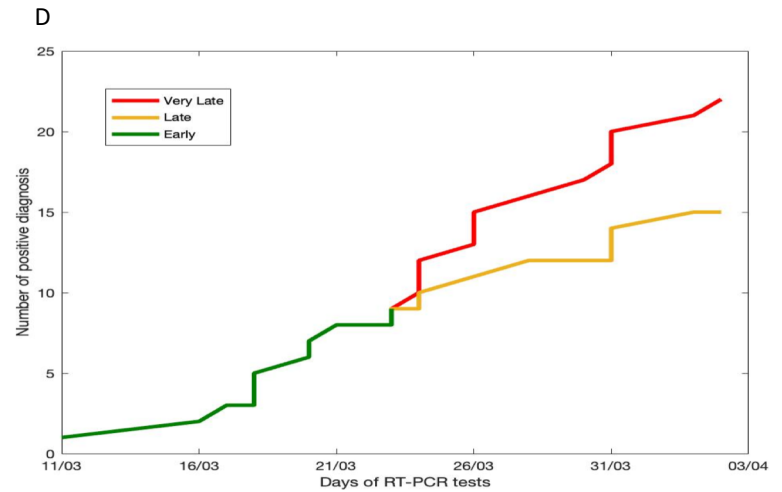
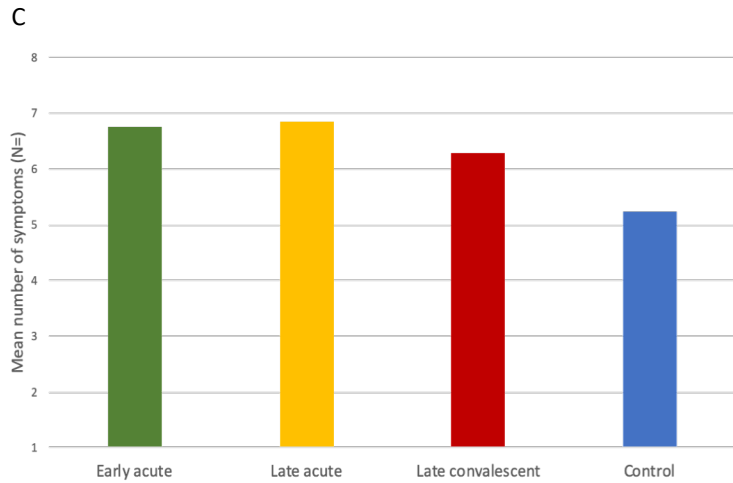
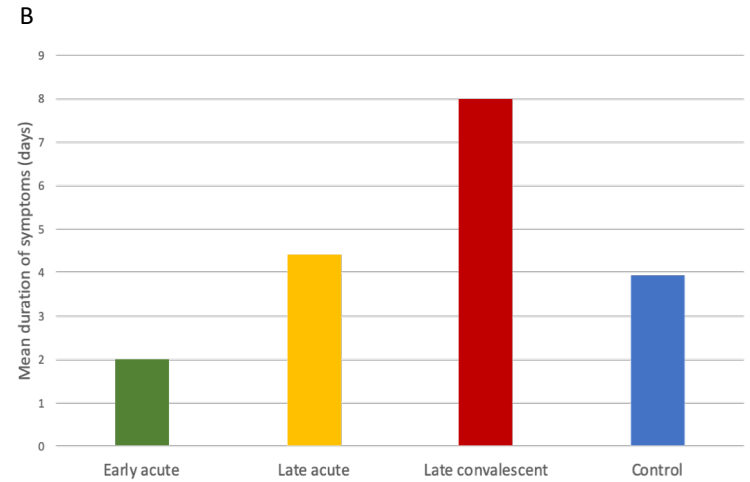
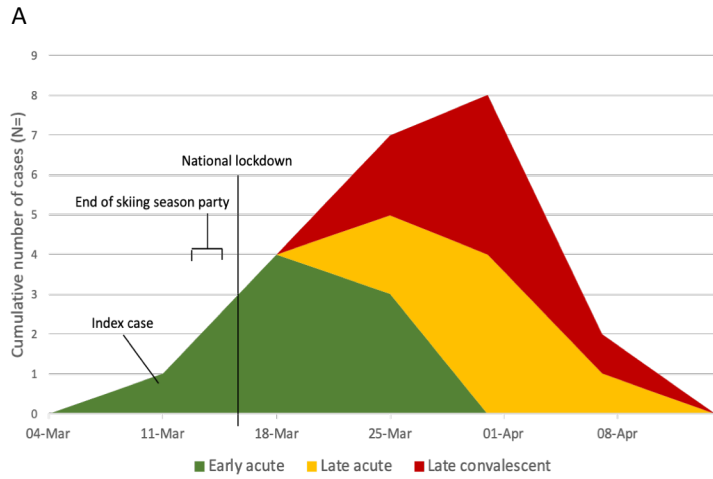
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3 603 Figure 2: (A) Cumulative COVID-19 diagnosis in the ski-resort Schladming-Dachstein over time. The main
4 604 outbreak occurred after a three-day party event (March 13 to 15) celebrating the early termination of the skiing
5 605 season due to National lockdown commencing on March 16. Between March 11 (index case) and April 03 (last
6 606 endemic case), 8 people were diagnosed with acute infection (RT-qPCR-reactive, confirmed antibody positive)
7 607 in the first half (12 days from March 11 to 22, 2020) of the outbreak (green colour), and 7 people with late acute
8 608 infection (amber) and 7 people with convalescent infection (red) were detected during the second half; (B)
9 609 Cumulative weekly numbers of confirmed COVID-19 cases during the outbreak. RT-qPCR was 100% sensitive
10 610 among all early acute and late acute presenters. RT-qPCR did not detect any of the late convalescent presenters;
11 611 (C) Mean duration of symptoms; and (D): Mean number of symptoms.

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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.