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Risk factors for SARS-CoV-2 infection: a test-negative case–control study with additional population controls in Norway

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ABSTRACT

Objectives This study aims to assess risk factors for SARS-CoV-2 infection by combined design; first comparing positive cases to negative controls as determined by PCR testing and then comparing these two groups to an additional prepandemic population control group.

Design and setting Test-negative design (TND), multicentre case–control study with additional population controls in South-Eastern Norway.

Participants Adults who underwent SARS-CoV-2 PCR testing between February and December 2020. PCR-positive cases, PCR-negative controls and additional age-matched population controls.

Primary outcome measures The associations between various risk factors based on self- reported questionnaire and SARS-CoV-2 infection comparing PCR-positive cases and PCR-negative controls. Using subgroup analysis, the risk factors for both PCR-positive and PCR-negative participants were compared with a population control group.

Results In total, 400 PCR-positive cases, 719 PCR-negative controls and 14 509 population controls were included. Male sex was associated with the risk of SARS-CoV-2 infection only in the TND study (OR 1.9, 95% CI 1.4 to 2.6), but not when PCR-positive cases were compared with population controls (OR 1.2, 95% CI 0.9. to 1.5). Some factors were positively (asthma, wood heating) or negatively (hypertension) associated with SARS-CoV-2 infection when PCR-positive cases were compared with population controls, but lacked convincing association in the TND study. Smoking was negatively associated with the risk of SARS-CoV-2 infection in both analyses (OR 0.5, 95% CI 0.3 to 0.8 and OR 0.6, 95% CI 0.4 to 0.8).

Conclusions Male sex was a possible risk factor for SARS-CoV-2 infection only in the TND study, whereas smoking was negatively associated with SARS-CoV-2 infection in both the TND study and when using population controls. Several factors were associated with SARS-CoV-2 infection when PCR-positive cases were compared with population controls, but not in the TND study, highlighting the strength of combining case–control study designs during the pandemic.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The test-negative design can reduce confounding from healthcare-seeking bias because PCR-negative (PCR–) controls are likely to have similar healthcare-seeking attitudes as PCR-positive (PCR+) cases.
⇒ This study mostly included non-hospitalised patients, and the findings can be generalisable to the general population.
⇒ The use of an additional control group from the general population for comparison with the PCR+ and PCR– participants (triangulation) strengthens the study inferences by adding two more dimensions of comparison.
⇒ In the subgroup analyses, PCR+ cases and PCR– controls were compared with the population controls to assess the risk factors for those aged 18–55 years. Hence, the results may not be generalisable to patients older than 55 years.
⇒ PCR test results, rather than symptoms, were used to categorise the participants into cases or controls, and therefore risk factors for SARS-CoV-2 infection and not COVID-19 disease were assessed.

INTRODUCTION

Understanding the risk factors for SARS-COV-2 infection is essential for the prevention of new waves of COVID-19, developing new vaccination strategies and in preparation for future pandemics. The clinical spectrum of COVID-19 varies from asymptomatic SARS-CoV-2 infection to mild pneumonia and may lead to serious respiratory illness and death. Various studies have explored the risk of COVID-19 severity and mortality, but only a few studies have assessed the risk of SARS-CoV-2 infection, which can be asymptomatic and mild. Although some risk factors for SARS-CoV-2 infection and COVID-19 have been identified, findings have been conflicting.[1–4] Particularly, the

association between smoking status, obstructive lung diseases, including chronic obstructive pulmonary disease (COPD) and asthma, and the risk of SARS-CoV-2 infection and development of severe COVID-19, has shown varying results in the studies.[3 5–12] Possible explanations for the conflicting results can be the different study designs, varying selection methods for cases and controls, different risk factors for severe disease and asymptomatic and mild infection, small sample size and geographical location for the studies.

Diabetes mellitus is an important risk factor for both disease severity and hospital mortality.[1 3 13] In a meta-analysis of adults hospitalised in 11 countries, overweight and patients with diabetes were more likely to require respiratory support.[13] Another meta-analysis showed that obesity was associated with both COVID-19 susceptibility and severity.[14] Among the 50 most affected countries, obesity increased both susceptibility for SARS-CoV-2 infection and mortality.[4] Hypertension has also been linked with COVID-19 severity, but a meta-analysis early in the pandemic showed no association of hypertension and susceptibility for SARS-CoV-2 infection.[2] In addition, older age and male sex were associated with increased mortality.[2 6] Air pollution can be a risk factor for upper and lower respiratory tract diseases. There are few previous studies that report association of concentrations of particulate pollutants in cities and COVID-19 incidence.[15] To our knowledge, no studies have investigated environmental factor, such as air pollution from wood-fired heating, as potential risk factor for SARS-CoV-2 infection.[15]

Most SARS-CoV-2-infected individuals have mild symptoms and are not hospitalised, but few studies assess these patients.[2 4 16] To date, most studies have been retrospective, designed as traditional case–control studies and cohort studies involving hospitalised patients with severe COVID-19, and have demonstrated substantial heterogeneity among findings.[1 5–7 10 17 18]

Therefore, this study aimed to determine the association between risk factors for SARS-CoV-2 PCR test positivity, by combination of three case–control study designs. We compared individuals with PCR positive (PCR+) and PCR negative (PCR−) tests as part of a test-negative design (TND) case–control study and each of them with additional population controls.[19–21]

METHODS

Study design and setting

We designed a TND case–control study with additional population controls. TND differs from the classical case–control study in that the controls are defined by a negative test result and not sampled from a wider source population.[19–21] The additional population control group makes it possible to assess risk factors for both the PCR test positivity and the PCR test negativity by a triangulation approach. PCR-negative participants have other infections than SARS-CoV-2. With this design, it is possible to distinguish between exposures that are combined risk factors for both SARS-CoV-2 infection and other respiratory infections, and risk factors that are specific for SARS-CoV-2 infection.[21] This study design can also reduce potential bias resulting from differences in health care-seeking attitude between cases and controls.[19] Participants were defined as 'cases' or 'controls' based on their PCR+ and PCR- test results, respectively. We used the first PCR test result of each participant. The participants were recruited from the counties of Agder and Telemark in South-Eastern Norway from February to December 2020 during the first and second waves of COVID-19 pandemic, and when the SARS-CoV-2 Alpha variant was dominant.

SARS-CoV-2 PCR+ and PCR– participants in our geographical area were first identified from results lists at the test centres and hospital laboratories. Eligible participants were then contacted by telephone by test centre or hospital, and invited to participate in the research project. On verbal agreement to participate, patients were invited to the hospital laboratory by the research team 3–5 months after PCR tests. At this appointment, written information about the project was provided. After the consent form was signed, the questionnaire was filled in. The population control group data were obtained from the pre-existing Telemark study dataset which included a random sample of 14 509 participants, aged 21–55 years, residing in Telemark, Norway in 2018.[22]

The inclusion criteria were as follows: (1) adults aged ≥18, (2) SARS-CoV-2 RT-PCR test result and (3) resident of South-Eastern Norway, specifically Agder and Telemark counties, during the inclusion period. Participants who were unable to answer the questionnaire, which was conducted in Norwegian, were excluded. PCR tests were used for inclusion as they are the gold standard for detection of SARS-CoV-2.[23]

Our first study is a TND study, including mainly non-hospitalised patients. Our second and third studies are case–control studies using additional population controls as a control group.

In the first study (hereafter study I), we compared risk factors for 400 SARS-CoV-2 PCR+ individuals (cases) with risk factors for 719 SARS-CoV-2 PCR– individuals (controls) in a classic TND. The controls in this design are 'other patient' controls who undergo the same PCR tests for same reasons and at the same healthcare facility as cases, but test negative.[20]

In the second traditional case–control study (hereafter study II), we compared risk factors for a subgroup of 286 SARS-CoV-2 PCR+ individuals (aged 18–55 years) with risk factors for the population control group (N=14 509, aged 21–55 years). Given that Telemark study dataset was collected more than 1 year before the pandemic, it was assumed to be PCR-negative for SARS-CoV-2.

In the third case–control study (hereafter study III), we compared risk factors for other infections than SARS-CoV-2 for a subgroup of 502 PCR- participants (aged 18–55 years) with the risk factors for the population study group from the Telemark study (N=14 509, aged 21–55

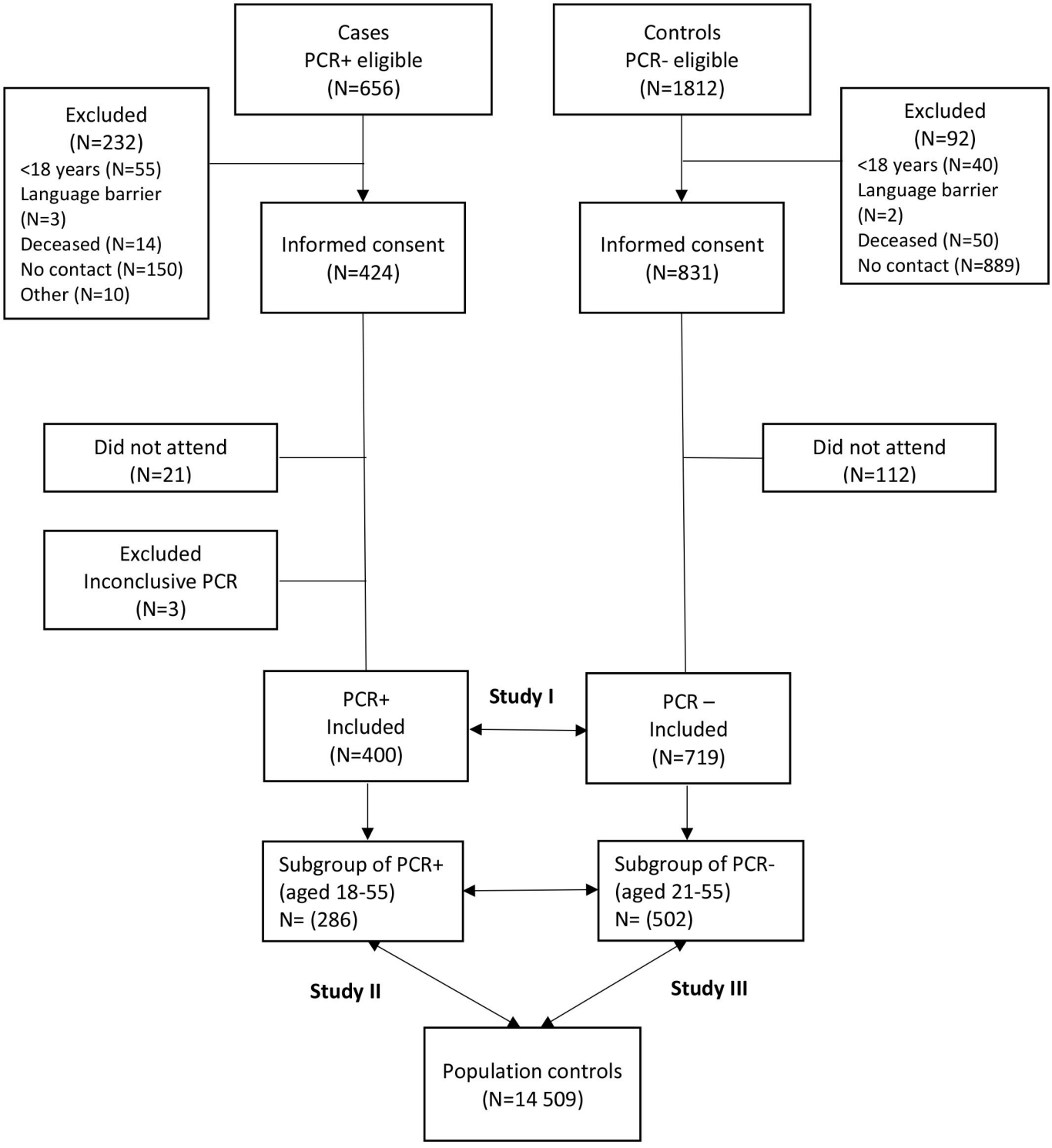

Figure 1 Flow chart for study inclusion.

years). Study II and III were restricted to these age groups because the Telemark study had participants up to 55 years.

The extensive restrictions for risk groups with non-pharmaceutical interventions during lockdowns in Norway did not differ from the restrictions for the general population in 2020. Norway locked down on 12 March 2020 until summer 2020. These restrictions were partly eased during the summer months, but reinstated for all residents from autumn 2020. Risk groups for more serious COVID-19 were defined as people aged >65 years, age <65 with comorbidities such as diabetes, overweight and heart disease. The official Norwegian testing criteria for SARS-CoV-2 changed over time but were the same for the PCR+ and PCR– participants in the study period. In the first wave of the pandemic, PCR testing was restricted to symptomatic patients. In the second wave, PCR testing was additionally applied to close contacts and asymptomatic individuals during the outbreaks.

Participants were included regardless of their symptoms. Only 58 participants (5%) in the PCR+ cases and PCR– controls were asymptomatic. Most PCR+ cases had mild symptoms, with only 22 (6%) participants hospitalised.[24] At the inclusion period, it was not possible to know how the pandemic waves would develop. We aimed to include 400 eligible PCR+ cases, and two PCR– controls per case matched for test time and geographical location to increase the power of the study.

We used strengthening the reporting of observational studies in epidemiology (STROBE) case–control reporting guidelines for our study.[25]

Questionnaire design

We used questions from the Norwegian Health Institute COVID-19 questionnaire and the questionnaire data from the Telemark study questionnaire,[22 26 27] in addition, a few questions were provided by the study group. The questionnaire consisted of questions related to (1) education status, (2) smoking habits, (3) respiratory symptoms and/or diseases, such as asthma or COPD, (4) comorbidities, (5) exercise and (6) environmental exposure to air pollution from traffic or wood-fired heating. Questions are shown in online supplemental table S1.

Statistical analysis

The mean, SD and median were reported for continuous variables, as appropriate. Categorical data were reported as frequencies and percentages. We used categorical variables for the adjustment. We followed the strategy proposed by Greenland *et al*[28] and adjusted for all potential confounders, while checking for multicollinearity. Since there was no evidence that this was occurring, all potential confounders were retained in the final model. Wood heating and diabetes status were considered to be potential confounders; wood heating is potentially associated with respiratory symptoms, and diabetes is potentially associated with healthcare seeking behaviour. Both education and income were included in the regression models, but income did not impact the estimation. Hence, we used only education as a predictor for the socioeconomic status. Logistic regression models were used to assess the possible risk factors. We used a one-step regression analysis for each variable. All the variables considered had some a priori evidence that they could be potential risk factors for COVID-19 infection. Thus, we considered that the problem of multiple comparisons did not apply. Therefore, we did not adjust for multiple comparisons.

To determine the association between risk factors and PCR test positivity, ORs were reported with 95% CIs. Statistical analyses were performed using R (V.4.2; R Core Team, Vienna, Austria).

Our questionnaire had a low rate of missing data, ranging from 0% to 6.2% for each question, except for those related to smoking habits, which had 11.2% (N=45) and 15.3% (N=110) missing data for PCR+ and PCR– participants, respectively. Due to the subsequent follow-up questions, the questionnaire used in the population control group had no missing data for questions related to smoking habits, asthma, COPD, diabetes, hypertension and wood heating. There were no missing for the question about wood heating in our study due to the subsequent follow-up questions. The remaining questions among the population controls had a low rate of missing data, ranging from 1.8% to 6.2%, except for exercise where 13.5% of data was missing. A sensitivity analysis was performed excluding participants with missing values for smoking and the results were not impacted. We did not perform data imputation.

Patient and public involvement

According to the Norwegian National Guidelines for User Involvement in Health Research in May 2018, two user representatives of SARS-CoV-2 PCR-positive patients were involved. They played an active role in all project phases, including the development and testing of questionnaires. The user representatives helped us understand the patient's perspective, gave feedback on our study protocol, study methods, information and consent forms, and questionnaires, and participated actively in the dissemination of results achieved until now. All study results are also communicated via www.sthf.no/helsefaglig/forskning-og-innovasjon/forskningsprosjekter/covita and www.sshf.no/helsefaglig/forskning-og-innovasjon/covita-studien.

RESULTS

Of 656 eligible PCR+ participants and 1812 eligible PCR– participants, 400 PCR+ cases and 719 PCR– controls were included. The study flow chart is shown in figure 1. The characteristics and comorbidities of the PCR+, PCR– and population controls are shown in table 1.

The PCR+ cases and PCR– controls had a mean age of 48±15 years and 47±14 years, respectively. Male participants represented 49% PCR+, 34% PCR– and 42% of the population controls. Asthma was present in 64 (16.0%) of the PCR+ group, 135 (18.8%) of the PCR– group and 1760 (12.1%) of the population controls.

In study I, risk factors for SARS-CoV-2 PCR-positive individuals (cases) were compared with risk factors for SARS-CoV-2 PCR-negative individuals (controls) in a classic TND. The results are presented in online supplemental table S2. Male sex was significantly associated with the risk for SARS-CoV-2 infection when comparing PCR+ cases and PCR– controls (OR 1.92, 95% CI 1.43 to 2.57). Age, body mass index (BMI), education level and comorbidities were not associated with SARS-CoV-2 infection. Daily or occasional smoking was negatively associated with SARS-CoV-2 infection (OR 0.50, 95% CI 0.31 to 0.81).

Characteristics and comorbidities for the subgroup of the PCR+ cases (18–55 years) and PCR– controls (18–55 years) and population control group (21–55 years) are shown in online supplemental table S3. In study II, risk factors for SARS-CoV-2 PCR+individuals (aged 18–55 years) were compared first with risk factors for PCR– controls (aged 18–55 years) in a TND and then with the population control group (aged 21–55 years) in a traditional case–control study. The results

Table 1 Characteristics of the PCR+ cases and PCR– controls 3–5 months after PCR test and the population control group data from the pre-existing Telemark study dataset

Characteristics	PCR+ cases N=400 (%)	PCR– controls N=719 (%)	Population controls N=14 509 (%)
Demographics			
Age in years, mean (SD), median	47.6 (15.1), 47.0	47.3 (14.3), 47.0	42.6 (9.7), 45.0
Age categories			
18–30	59 (14.8)	92 (12.8)	2392 (16.5)
31–40	78 (19.5)	175 (24.3)	2904 (20.0)
41–50	94 (23.5)	159 (22.1)	5360 (36.9)
51–60	85 (21.2)	146 (20.3)	3853 (26.6) *
>60	84 (21.0)	147 (20.4)	*
Sex, males	197 (49.3)	241 (33.5)	6142 (42.3)
females	203 (50.7)	478 (66.5)	8367 (57.7)
BMI in kg/m^2, mean (SD), median	26.4 (4.5), 25.6	26.7 (5.7), 25.6	26.3 (4.9), 25.5
BMI in category, kg/m^2			
18.5–24.9	162 (40.5)	276 (38.4)	6140 (42.3)
<18.5	5 (1.3)	13 (1.8)	161 (1.1)
25–29.9	149 (37.3)	262 (36.4)	5255 (36.2)
30–39.9	67 (16.8)	148 (20.6)	2623 (18.1)
Missing data	17 (4.2)	20 (2.8)	330 (2.3)
Education			
Primary+secondary school	39 (9.8)	77 (10.7)	1246 (8.6)
High school+certificate	151 (37.8)	221 (30.7)	5146 (35.5)
University	199 (49.8)	413 (57.4)	7851 (54.1)
Missing data	11 (2.8)	8 (1.1)	266 (1.8)
Smoking			
Never smoker	218 (54.5)	321 (44.6)	8359 (57.6)
Past smoker	106 (26.5)	204 (28.4)	3667 (25.3)
Occasional and daily smoker	31 (7.8)	84 (11.7)	2483 (17.1)
Missing data	45 (11.2)	110 (15.3)	0 (0)
Comorbidities			
Asthma			
Yes	64 (16.0)	135 (18.8)	1760 (12.1)
No	313 (78.3)	552 (76.8)	12 749 (87.9)
Missing data	23 (5.7)	32 (4.4)	0 (0)
COPD			
Yes	6 (1.5)	27 (3.8)	155 (1.1)
No	369 (92.3)	657 (91.4)	14 354 (98.9)
Missing data	25 (6.2)	35 (4.8)	0 (0)
Diabetes			
Yes	20 (5.0)	27 (3.8)	370 (2.6)
No	369 (92.3)	689 (95.8)	14 139 (97.4)
Missing data	11 (2.7)	3 (0.4)	0 (0)
Hypertension			
Yes	38 (9.5)	73 (10.2)	1369 (9.4)
No	351 (87.7)	643 (89.4)	13 140 (90.6)
Missing data	11 (2.8)	3 (0.4)	0 (0)

Continued

Table 1 Continued

Characteristics	PCR+ cases N=400 (%)	PCR– controls N=719 (%)	Population controls N=14 509 (%)
Exercise†			
<Once a week	60 (15.0)	126 (17.5)	2699 (18.6)
Once a week	87 (21.7)	128 (17.8)	2416 (16.7)
2–3 times a week	139 (34.8)	287 (40.0)	4993 (34.4)
4–7 times a week	93 (23.2)	152 (21.1)	2443 (16.8)
Missing data	21 (5.3)	26 (3.6)	1958 (13.5)
Bedroom window‡			
No	253 (63.2)	433 (60.2)	8226 (56.7)
Yes, little trafficked road	105 (26.3)	224 (31.2)	4443 (30.6)
Yes, moderate/ busy road	27 (6.7)	52 (7.2)	1121 (7.7)
Missing data	15 (3.8)	10 (1.4)	719 (5.0)
Wood heating§			
No use	154 (38.5)	236 (32.8)	7551 (52.0)
Seldom	101 (25.2)	181 (25.2)	1524 (10.5)
2–3 times a week	70 (17.5)	137 (19.1)	2943 (20.3)
Daily	75 (18.8)	165 (22.9)	2491 (17.2)
Missing data	0 (0)	0 (0)	0 (0)

*Population controls were aged 21–55 years.
†How often do you exercise?
‡Is your bedroom window closer than 20 m from a busy road?
§How often do you use wood heating in your house during the winter season?
BMI, body mass index; COPD, chronic obstructive pulmonary disease; SD, standard deviation.

are shown in online supplemental table S4. Comparison of PCR+ cases with population controls in study II revealed that exercising once a week (OR 2.02, 95% CI 1.35 to 3.05), 2–3 times a week (OR 1.47, 95% CI 1.01 to 2.19) and 4–7 days a week (OR 1.85, 95% CI 1.23 to 2.83), having asthma (OR 1.56, 95% CI 1.12 to 2.14) and using wood heating seldom (OR 4.25, 95% CI 3.07 to 5.86), 2–3 times a week (OR 1.65, 95% CI 1.17 to 2.30) and daily during the winter season (OR 2.13, 95% CI 1.50 to 2.99) were associated with SARS-CoV-2 infection. Comparison of PCR+ cases with PCR– controls or with the population controls revealed that daily or occasional smoking (OR 0.48, 95% CI 0.28 to 0.79) and (OR 0.55, 95% CI 0.35 to 0.82), respectively, was negatively associated with SARS-CoV-2 infection. Hypertension was negatively associated with SARS-CoV-2 infection when PCR+ cases were compared with population controls (OR 0.37, 95% CI 0.19 to 0.65). Age, BMI and comorbidities were not associated with SARS-CoV-2 infection when comparing PCR+and PCR– controls.

In study III, risk factors for other infections than SARS-CoV-2 for PCR– participants (aged 18–55 years) were compared with risk factors for the population study group from the Telemark study (aged 21–55 years). The outcome of interest was non-SARS-CoV-2 infections. More than 95% of the PCR-negative participants had symptoms similar to SARS-CoV-2 infection. The results are shown in online supplemental table S5. BMI>30 (OR 1.54, 95% CI 1.17 to 1.99), past smoking (OR 1.39, 95% CI 1.13 to 1.73) and asthma (OR 1.70, 95% CI 1.34 to 2.16) were associated with PCR negativity in the study III when comparing PCR-negative participants with the population control group. Daily wood heating in the winter season was also associated with PCR negativity (OR 3.42, 95% CI 2.66 to 4.44).

ORs from the three different case–control studies are summarised in table 2.

Male sex was associated with the risk of SARS-CoV-2 infection in study I (OR 1.92, 95% CI 1.43 to 2.57). Smoking was negatively associated with SARS-CoV-2 infection in study I (OR 0.50, 95% CI 0.31 to 0.81) and in study II (OR 0.55, 95% CI 0.35 to 0.82), respectively. BMI>30 (OR 1.54, 95% CI 1.17 to 1.99), past smoking (OR 1.39, 95% CI 1.13 to 1.73) and asthma (OR 1.70, 95% CI 1.34 to 2.16) were associated with PCR negativity in study III. Wood heating was associated with SARS-CoV-2 infection in study II (OR 2.13, CI 95% 1.50 to 2.99) for daily use in the winter season and it was associated with PCR negativity in study III (OR 3.42, 95% CI 2.66 to 4.44).

DISCUSSION

In study I (TND), we identified the male sex as a risk factor for SARS-CoV-2 infection. In addition, smoking was negatively associated with SARS-CoV-2 infection in both studies I and II (PCR+ vs population controls) analyses. No evidence of association was found between asthma

Table 2 Risk factors for SARS-CoV-2 infection and non-SARS-CoV-2 infection, adjusted OR* (OR_{adj}) (95% CI) from three case–control studies: study I (PCR+ cases vs PCR– controls), study II (PCR+ cases vs population controls), study III (PCR– vs population controls)

	Study I	Study II	Study III
Variables			
Age in years, category			
18–30 (reference)			
31–40	0.78 (0.48 to 1.25)	1.03 (0.71 to 1.50)	1.22 (0.91 to 1.63)
41–50	1.18 (0.84 to 1.91)	**0.67 (0.46 to 0.97)**	**0.49 (0.36 to 0.67)**
51–60	1.24 (0.75 to 2.03)	0.92 (0.63 to 1.35)†	0.66 (0.48 to 0.91)†
>60	1.16 (0.68 to 1.98)	†	†
Sex			
Female (reference)			
Male	**1.92 (1.43 to 2.57)**	1.20 (0.93 to 1.54)	**0.61 (0.50 to 0.76)**
BMI in kg/m^2 in category			
18.5–24.9 (reference)			
<18.5	0.51 (0.14 to 1.55)	1.65 (0.50 to 4.10)	2.08 (0.98 to 3.90)
25–29.9	0.92 (0.66 to 1.27)	1.06 (0.80 to 1.41)	1.16 (0.93 to 1.46)
> 30	0.67 (0.44 to 1.01)	1.12 (0.77 to 1.59)	**1.54 (1.17 to 1.99)**
Education			
Primary+secondary school (reference)			
High school+certificate	1.70 (0.99 to 2.98)	1.14 (0.69 to 1.20)	**0.60 (0.42 to 0.88)**
University	1.06 (0.63 to 1.83)	1.00 (0.60 to 1.70)	0.76 (0.54 to 1.08)
Smoking			
Never smoker (reference)			
Past smoker	0.74 (0.53 to 1.02)	0.88 (0.65 to 1.17)	**1.39 (1.13 to 1.73)**
Daily+occasional smoker	**0.50 (0.31 to 0.81)**	**0.55 (0.35 to 0.82)**	1.05 (0.79 to 1.38)
Comorbidities			
Asthma No (reference)			
Yes	0.83 (0.57 to 1.20)	**1.56 (1.12 to 2.14)**	**1.70 (1.34 to 2.16)**
Diabetes No (reference)			
Yes	1.05 (0.48 to 2.26)	1.28 (0.57 to 2.49)	1.28 (0.70 to 2.18)
COPD No (reference)			
Yes	0.41 (0.13 to 1.11)	0.77 (0.13 to 2.49)	1.46 (0.60 to 3.03)
Hypertension No (reference)			
Yes	0.75 (0.43 to 1.28)	**0.37 (0.19 to 0.65)**	**0.53 (0.34 to 0.79)**
Exercise‡			
<Once a week (reference)			
Once a week	1.46 (0.92 to 2.33)	**2.02 (1.35 to 3.05)**	1.15 (0.85 to 1.55)
2–3 times a week	0.97 (0.64 to 1.47)	**1.47 (1.01 to 2.19)**	1.13 (0.87 to 1.48)
4–7 times a week	1.16 (0.73–1.85)	**1.85 (1.23 to 2.83)**	1.34 (0.99 to 1.80)
Bedroom window§			
No (reference)			
Yes, little traffic	0.90 (0.65 to 1.24)	0.94 (0.72 to 1.23)	0.96 (0.79–1.19)

Continued

Table 2 Continued

	Study I	Study II	Study III
Yes, moderate/ busy road	1.06 (0.60 to 1.85)	0.77 (0.46 to 1.22)	0.80 (0.54–1.14)
Wood heating¶			
No (reference)			
Seldom	0.83 (0.57 to 1.21)	**4.25 (3.07 to 5.86)**	**5.00 (3.86 to 6.49)**
2–3 times a week	0.79 (0.53 to 1.18)	**1.65 (1.17 to 2.30)**	**1.95 (1.51 to 2.56)**
Daily	**0.60 (0.40 to 0.89)**	**2.13 (1.50 to 2.99)**	**3.42 (2.66 to 4.44)**

Statistically significant values are givend in bold.
* Age adjusted for sex, BMI, education, smoking, comorbidities, exercise, bedroom window and wood heating in season; sex adjusted for age, BMI, education, smoking, comorbidities, exercise, bedroom window and wood heating in season; other variables adjusted for age, sex, BMI, education, smoking, comorbidities, exercise, bedroom window and wood heating in season.
†. PCR– controls (aged 18–55 years) and population controls (aged 21–55 years).
‡ How often do you exercise?.
§Is your bedroom window closer than 20 m from a busy road?.
¶How often do you use wood heating in your house during the winter season?.
BMI, body mass index; COPD, chronic obstructive pulmonary disease; OR, Odds ratio.

and SARS-CoV-2 infection in study I, but there was a positive association in study II. COPD showed no association with SARS-CoV-2 infection in both studies I and II. While exercising and wood heating during the winter were highlighted as possible risk factors for SARS-CoV-2 infection in study II, this was not the case in study I.

Male sex was associated with SARS-CoV-2 infection, which is in line with previous studies.[29] In a systematic review and meta-analysis, a higher ratio of SARS-COV-2 infection in males than females (100:82.5) was reported.[16 29] A meta-analysis also showed a higher risk for SARS-CoV-2 infection in men than that in women, with a relative risk of 1.08.[16]

In our study I (TND) and study II (PCR+ cases vs population controls), current smoking status was negatively associated with SARS-CoV-2 infection. This paradoxical finding is reflected in the literature, with many studies reporting discordant results depending on disease severity and other comorbidities associated with smoking.[5 12 30 31] The lack of severe COVID-19 among our participants can have contributed to this result, with only 6% of our participants hospitalised. Moreover, the PCR– controls in our study had other common respiratory infections, which may be associated with smoking.[12] Previous studies have shown that when infected with COVID-19, current smokers have worse outcomes than non-smokers.[8 30 31] Given that we did not obtain data related to the pack-years or duration of smoking, our results should be interpreted with caution.

Asthma and COPD were not associated with SARS-CoV-2 infection in study I, which is comparable to studies from the early phase of the pandemic.[5 9] This may be due to the willingness of individuals with asthma to be tested whenever they develop respiratory symptoms that could indicate COVID-19. Additionally, in study I-III, the PCR– participants had signs and symptoms of respiratory tract infections other than SARS-CoV-2 infection. Thus, asthma may still be associated with COVID-19, as well as with other respiratory tract infections. Interestingly, asthma was associated with SARS-CoV-2 infection in our study II with subgroup analysis comparing PCR+ cases with population controls. The reason for this finding is not clear; however, we observed a relatively high prevalence of asthma (16%) among the PCR+ cases in our study, but not COPD (1.5%). In many COVID-19 studies, a low prevalence of asthma (1%) and varying prevalence of COPD (2%–14%) for SARS-CoV-2 infected patients have been reported.[5–7 9] Patients with chronic diseases may have been isolated more than others during lockdowns in some countries or regions. The potential protective immunity provided by therapies used to treat chronic respiratory diseases may also explain the low prevalence of COVID-19 among adults with asthma or COPD in some studies.[5 7 9] In a study by Lacedonia *et al*, the prevalence of COPD and current smokers was low for SARS-CoV-2 infection, but when infected, these groups had the highest all-cause mortality.[5] A nationwide Korean study showed that COPD was associated with an increased risk of COVID-19 susceptibility; however, the prevalence of COPD among severe COVID-19 patients or COVID-19 mortality did not increase, but smoking influenced COPD outcomes.[10] The heterogeneity of these findings may be attributed to differences in disease severity, study design, such as the selection of controls, sample size and geographical location.

Our study demonstrated no association between age, BMI, diabetes, having a bedroom window close to a trafficked road and SARS-CoV-2 infection. Hypertension was inversely associated with SARS-CoV-2 infection in study II, which contradicts other studies.[7] Obesity has been associated with COVID-19 susceptibility and severity,[17 32] and is thought to be an important prognostic factor.[4 14 17 32 33] Diabetes has also been proposed as a risk factor for developing severe COVID-19 and mortality.[1 3 13] Given that our study mostly included patients with mild COVID-19 symptoms and few hospitalised participants, this may have contributed to the finding of no associations between these factors and SARS-CoV-2 infection. In the subgroup analysis (study II) comparing PCR+ cases and population

controls, asthma, exercise and wood heating were possible risk factors for SARS-CoV-2 infection. However, given the possibility of selection bias due to differences in healthcare-seeking attitudes, these findings should be interpreted with caution. Analysing PCR+ cases with PCR– participants as controls in study I (TND) may have reduced this bias.

We obtained different results for asthma, exercise and wood heating in study I than in the study II, although findings for sex, age, smoking and COPD showed similar directions of association. However, the interpretation of how smoking habits affect the risk of SARS-CoV-2 infection requires further assessment owing to the limited study size.

This study had some limitations. First, the questionnaire was conducted in South-Eastern Norway during a period when the SARS-CoV-2 Alpha variant was dominant; therefore, these results may not be entirely representative of other countries or virus strains. However, Telemark and Agder have both rural and urban areas and are considered to represent Nordic populations. Second, we compared PCR+ cases and PCR– controls with population controls in study II and III to assess the risk factors for individuals aged 18–55 years; hence, the results of our subgroup analyses may not be generalisable among those >55 years. The data from the population controls were collected 2 years prior to the beginning of the pandemic. We cannot rule out the possibility that this has affected our results, although the time period is relatively short. Third, there is a possibility of recall bias due to the use of a self-reported questionnaire; however, questions included were comparable to other studies,[22 34] including studies assessing COVID-19.[26 27] Fourth, the study might not be generalisable to all migrant groups; still we included Norwegian-speaking migrants in the study. The Telemark study questionnaire from 2018 was also restricted to Norwegian-speaking participants in the same way. Fifth, confounding unknown factors are possible in all epidemiological studies. Theoretically, misclassification of controls in TND may be more likely than in classical case–control studies.[21] Misclassification of cases was considered less likely due to the high sensitivity of PCR tests.[35 36] We also confirmed a high specificity of the PCR tests with only few PCR– controls with positive antibodies in our previous study.[24] Due to time constraints during the pandemic, the study protocol was not published.

With the TND, which is often used for vaccine studies, it is possible to identify risk factors that are specific for COVID-19 by adding population controls.[21 37 38] Furthermore, in the traditional TND design, participants are included before the test results. In our study, all individuals who matched our inclusion criteria were recruited and defined as cases or controls regardless of symptoms and depending only on their PCR test results.[39 40] However, we did not consider this as a limitation because the majority of the participants in our study had symptoms.

Healthcare-seeking attitude as a possible source of selection bias may be reduced with TND, as both groups have the same reason for testing.[41 42] In contrast to traditional case–control studies, controls are tested for the disease under study and are those with negative test results without exception. Although the criteria for PCR testing changed, the differences in the groups due to the variation of testing strategies during the two pandemic phases were reduced because the PCR tests were matched for time and place. Population controls are useful to strengthen the study inferences by adding two more dimensions of comparison. As demonstrated in our study, the choice of test-negative controls or population controls can affect outcomes regarding risk factors for SARS-CoV-2 infection.

Overall, selecting appropriate study designs and combining all relevant information from studies assessing risk factors for SARS-CoV-2 infection and COVID-19 are vital for the prevention of new waves of COVID-19 and other pandemics in the future. In particular, findings from TND studies assessing risk factors may also contribute to the development of new vaccination strategies. Combined design with TND and additional population controls can be applied to future pandemics. However, further research is needed to address the evolution of virus variants, uptake of vaccination and differences in humoral and cellular protective immunity among risk groups.

CONCLUSION

Male sex was associated with the risk of SARS-CoV-2 infection only in the TND study, but not when PCR-positive cases were compared with population controls. Smoking was negatively associated with SARS-CoV-2 infection in both the TND study and when comparing PCR-positive cases to population controls. Several factors were associated with SARS-CoV-2 infection when PCR-positive cases were compared with population controls, but not in the TND study, highlighting the strength of combining different case–control study designs during the pandemic.

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Data availability statement Data are available on reasonable request. There are legal and ethical restrictions on sharing our dataset. Our dataset is not fully anonymised and has a relatively small sample size making identification possible. The potentially identifying patient information is age, birthdate, location and dates for PCR testes. However, data requests for the minimal dataset, which includes only the main variables of the final analyses, can be made to the Research department at the Telemark Hospital trust, Ulefossvegen 55, 3710 Skien, Norway email: fou@sthf.no.

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