Current smoking and SARS-CoV-2 infection: findings from the Italian cross-sectional EPICOVID19 internet-based survey.

Federica Prinelli, Fabrizio Bianchi, Gaspare Drago, Silvia Ruggieri, Aleksandra Sojic, Nithiya Jesuthasan, Sabrina Molinaro, Luca Bastiani, Stefania Maggi, Marianna Noale, Massimo Galli, Andrea Giacomelli, Raffaele Antonelli Incalzi, Fulvio Adorni, Fabio Cibella

Submitted to: JMIR Public Health and Surveillance
on: January 11, 2021

Table of Contents

Current smoking and SARS-CoV-2 infection: findings from the Italian cross-sectional EPICOVID19 internet-based survey.

Federica Prinelli[1] PhD; Fabrizio Bianchi[2] PhD; Gaspare Drago[3] PhD; Silvia Ruggieri[3] PhD; Aleksandra Sojic[1] PhD; Nithiya Jesuthasan[1] MPH; Sabrina Molinaro[2] PhD; Luca Bastiani[2] PhD; Stefania Maggi[4] MD, PhD; Marianna Noale[4] MSc; Massimo Galli[5] MD; Andrea Giacomelli[5] MD; Raffaele Antonelli Incalzi[6] MD; Fulvio Adorni[1*] MPH; Fabio Cibella[3*] MD

[1]Institute of Biomedical Technologies-National Research Council Segrate (MI) IT
[2]Institue of Clinical Physiology-National Research Council Pisa IT
[3]Institute for Biomedical Research and Innovation -National Research Council Palermo IT
[4]Institute of Neuroscience-National Research Council Padova IT
[5]University of Milan Milano IT
[6]Campus Biomedico Roma IT
[*]these authors contributed equally

Corresponding Author:
Federica Prinelli PhD
Institute of Biomedical Technologies-National Research Council
Via Fratelli Cervi 93
Segrate (MI)
IT

Abstract

Background: Several studies reported a low prevalence of current smoking among hospitalized COVID-19 cases however, no definitive conclusions can be drawn.

Objective: We investigated the association of tobacco smoke exposure with the nasopharyngeal swab (NPS) test result for SARS-CoV-2 infection and the disease severity accounting for possible confounders.

Methods: The cross-sectional EPICOVID19 web-based survey was performed in an Italian population of 198,822 adults who filled in an online questionnaire between April 13 and June 2, 2020. For the present study we analyzed 6857 individuals with known NPS test result. The association of smoking status and the dose-response relationship with the positivity to NPS test and infection severity were analyzed using logistic and multinomial regression models adjusting for socio-demographic, clinical and behavioral characteristics.

Results: Out of the 6857 individuals, 63.2% had never smoked, 21.3% were former and 15.5% were current smokers. Compared to non-smokers, current smokers were younger, more educated, less affected by chronic diseases, reported less frequently COVID-like symptoms, were less hospitalized and tested for COVID-19. In multivariate analysis current smokers had almost halved odds of a positive NPS test (OR 0.54, 95% CI 0.45-0.65) compared to non-smokers. We also found a dose-dependent relationship with tobacco smoke: mild smokers (OR 0.76, 95% CI 0.55-1.05), moderate (OR 0.56, 95% CI 0.42-0.73) and heavy smokers (OR 0.38, 95% CI 0.27-0.53). This inverse association persisted also when considering the severity of the infection.

Conclusions: Current smoking was negatively associated with SARS-CoV-2 infection with a dose-dependent relation. Ad-hoc experimental studies are needed to elucidate the mechanisms underlying this association. Clinical Trial: ClinicalTrials.gov NCT04471701

(JMIR Preprints 11/01/2021:27091)
DOI: https://doi.org/10.2196/preprints.27091

Preprint Settings

1) Would you like to publish your submitted manuscript as preprint?
✓ **Please make my preprint PDF available to anyone at any time (recommended).**

Please make my preprint PDF available only to logged-in users; I understand that my title and abstract will remain visible to all users.
Only make the preprint title and abstract visible.
No, I do not wish to publish my submitted manuscript as a preprint.

2) If accepted for publication in a JMIR journal, would you like the PDF to be visible to the public?

✔ **Yes, please make my accepted manuscript PDF available to anyone at any time (Recommended).**
Yes, but please make my accepted manuscript PDF available only to logged-in users; I understand that the title and abstract will remain v
Yes, but only make the title and abstract visible (see Important note, above). I understand that if I later pay to participate in <a href="http

Original Manuscript

Current smoking and SARS-CoV-2 infection: findings from the Italian cross-sectional EPICOVID19 internet-based survey.

Federica Prinelli[1] PhD, Fabrizio Bianchi[2] PhD, Gaspare Drago[3] PhD, Silvia Ruggieri[3] PhD, Aleksandra Sojic[1] PhD, Nithiya Jesuthasan[1] MPH, Sabrina Molinaro[2] PhD, Luca Bastiani[2] PhD, Stefania Maggi[4] PhD, Marianna Noale[4] MSc, Massimo Galli[5] MD, Andrea Giacomelli[5] MD, Raffaele Antonelli Incalzi[6] MD, Fulvio Adorni[1]* MPH, and Fabio Cibella[3*] MD on behalf of the EPICOVID19 Working Group°.

[1]Institute of Biomedical Technologies - National Research Council, Via Fratelli Cervi 93, 20090 Segrate (MI), Italy

[2]Institute of Clinical Physiology - National Research Council, Via G. Moruzzi 1, 56124 Pisa (PI), Italy

[3]Institute for Biomedical Research and Innovation - National Research Council, Via Ugo La Malfa 153, 90146 Palermo, Italia

[4]Institute of Neuroscience - National Research Council, Aging Branch, Via Vincenzo Maria Gallucci 16, 35128 Padova, Italy.

[5]Infectious Diseases Unit, Department of Biomedical and Clinical Sciences L. Sacco, Università di Milano, ASST Fatebenefratelli Sacco, 20157 Milan, Italy

[6]Unit of Geriatrics, Department of Medicine, Biomedical Campus of Rome, via Alvaro del Portillo, 21, 00128 Rome, Italy

*These authors contributed equally as last authors

Corresponding author:

Federica Prinelli, PhD

Epidemiology Unit, Institute of Biomedical Technologies-National Research Council

Via Fratelli Cervi 93, 20090 Segrate (MI), Italy

Phone +39 0226422629

E-mail: federica.prinelli@itb.cnr.it

ABSTRACT

Background

Several studies reported a low prevalence of current smoking among hospitalized COVID-19 cases however, no definitive conclusions can be drawn.

Objective

We investigated the association of tobacco smoke exposure with the nasopharyngeal swab (NPS) test result for SARS-CoV-2 infection and the disease severity accounting for possible confounders.

Methods

The nationwide self-administered cross-sectional EPICOVID19 web-based survey was performed in an Italian population of 198,822 voluntary adults who filled in an online questionnaire between April 13 and June 2, 2020. For the present study, we analyzed 6857 individuals with known NPS test result. The associations of smoking status and the dose-response relationship with the positivity to NPS test and infection severity were calculated as odds ratios with 95% Confidence Intervals (OR, 95%CI) by means of ~~analyzed using~~ logistic and multinomial regression models adjusting for socio-demographic, clinical, and behavioral characteristics.

Results

Out of the 6857 individuals (mean age 47.9 years, 65.9% females), 63.2% had never smoked, 21.3% were former and 15.5% were current smokers. Compared to non-smokers, current smokers were younger, more educated, less affected by chronic diseases, reported less frequently COVID-like symptoms, were less hospitalized and tested for COVID-19. In

multivariate analysis current smokers had almost halved odds of a positive NPS test (OR 0.54, 95% CI 0.45-0.65) compared to non-smokers. We also found a dose-dependent relationship with tobacco smoke: mild smokers (OR 0.76, 95% CI 0.55-1.05), moderate (OR 0.56, 95% CI 0.42-0.73) and heavy smokers (OR 0.38, 95% CI 0.27-0.53). This inverse association persisted also when considering the severity of the infection. Current smokers had a statistically significant lower probability of having asymptomatic (OR 0.50 95%CI 0.27-0.92), mild (OR 0.65 95%CI 0.53-0.81), and severe infection (OR 0.27 95%CI 0.17-0.42) compared to never smokers.

Conclusions

Current smoking was negatively associated with SARS-CoV-2 infection with a dose-dependent relation. Ad-hoc experimental studies are needed to elucidate the mechanisms underlying this association.

Trial Registration: ClinicalTrials.gov NCT04471701

Keywords

SARS-CoV-2; COVID-19; smoking habit; dose-response relationship; nasopharyngeal swab testing; infection severity; web-based survey; self-reported; cross-sectional design.

INTRODUCTION

In June 2020 the WHO released a report warning that smoking habits could be associated with adverse coronavirus disease (COVID-19) prognosis[CITATION Wor \l 1040]. Based on extensive evidence, the report highlighted the negative impact of tobacco use on lung health and its causal association with both viral and bacterial respiratory infections[CITATION Wor \l 1040]. In humans, the spike protein (S-protein) – Angiotensin-converting enzyme 2 (ACE2) binding pathway constitutes a cell-binding site for the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) S-protein[CITATION XuX \l 1040]. ACE2 was found upregulated in the small airway epithelia of smokers [CITATION Exp1 \l 1040], which partially explain the increased risk of severe COVID-19 in this sub-population [CITATION Iva \l 1040].

However, studies from several European and non-European countries including China[CITATION Gua \l 1040], USA[CITATION Cum \l 1040], Mexico[CITATION Par \l 1040], Israel[CITATION Yan \l 1040], France [CITATION Miy \l 1040], UK[CITATION Ham \l 1040], and Italy [CITATION Gai \l 1040] [CITATION Lod \l 1040][CITATION Inc \l 1040] have shown an unusual low proportion of active smokers among hospitalized patients with respect to the general population. Moreover, a negative association between current smoking prevalence and COVID-19 occurrence at the population level was found in an ecological study performed in 38 European countries[CITATION Tsi \l 1040] and in a few non-hospitalized populations[CITATION Gab \l 1040][CITATION Smo \l 1040][CITATION Ren \l 1040]. Possible biological mechanisms have been proposed to explain the counterintuitive underrepresentation of smokers among COVID-19 patients [CITATION Rus \l 1040] [CITATION Far \l 1040] strengthening the concept of “smoker’s paradox” [20-21]

Nevertheless, possible explanations for these findings could be due to biases in the available data. In fact, considering the emergency of the epidemic, it has been suggested

that the smoking status and smoking history (included the duration, the quantity or the time from possible smoking cessation) of patients could have not been accurately recorded or some patients were unable to report their smoking habits, leading to a misclassification of smoking status. Moreover, the ascertainment of smoking exposure has not been supported by the use of objective biomarkers [19-20], or again, smokers may be taking medications or having behaviors, inducing some protection against COVID-19 [CITATION Leu \l 1040]. Finally, the majority of the studies conducted to date, were performed in clinical settings without a detailed evaluation of possible confounders (comorbidities, area of residence socio-economic factors) and, in meta-analyses, heterogeneous studies were pooled together [CITATION Gru \l 1040].

Bearing these considerations in mind, in the present study we postulated that smoking habit was associated with both SARS-CoV-2 infection and disease severity in a general population, with a dose-response relationship independently from confounding factors not considered in previous studies. To verify this hypothesis, we used data from the self-administered EPICOVID19 web-based survey with the aims: 1) to evaluate the frequency distribution of socio-demographic, clinical and behavioural characteristics among participants according to smoking status; and 2) to investigate the cross-sectional association of smoking patterns (intensity and duration) with SARS-CoV-2 nasopharyngeal swab (NPS) test result and infection severity, taking into account for a wide number of potential confounding factors.

METHODS

Study design, setting and population

The study population was derived from the EPICOVID19 national internet-based survey [24] conducted using a cross-sectional research design in a self-selected sample of adult volunteers living in Italy during the lockdown in March-May 2020 (during this same period

the total confirmed COVID-19 infected cases in Italy were 233,515 [25]). The study procedures were described elsewhere [24]. Briefly, the link to the web-based survey was implemented using the EUSurvey management tool, uploaded, and shared from April 13 to June 2, 2020 via several channels: e-mails, social media platforms (Facebook, Twitter, Instagram, Whatsapp), press releases, internet pages, local radio and TV stations, institutional websites, mailing lists, and the study website. The inclusion criteria to take part in the survey were age ≥18 years; access to a mobile phone, computer, or tablet with internet connectivity; and provision of online consent to participate in the study. Out of the 198,822 participants who provided the consent to participate and completed the on-line survey, 254 had missing data about smoking duration,191,250 did not perform the NPS test, and 461 did not yet know the NPS test result leading to a final sample of 6,857 (3.4%) participants for the present analysis (**Figure 1).**

Compared to people excluded (N=191,250) for not having performed the NPS test, those included (N=6857) in the analysis were more likely to be females, less educated, more employed, white collars, healthcare professionals, residents in Northern regions, more affected by chronic diseases, more frequently vaccinated for flu and pneumococcal, more likely to report symptoms, more frequently hospitalized, never smokers, living in big suburbs/cities and crowded houses, more frequently reported contact with COVID-19 cases and called the emergency numbers, and had a lower self-perceived health status (**Supplementary Table 1**).

The Ethics Committee of the Istituto Nazionale per le Malattie Infettive I.R.C.C.S. Lazzaro Spallanzani (Protocol No. 70, 12/4/2020) approved the EPICOVID19 study protocol. When participants first accessed the web-based platform, they were informed about the study and filled in the informed consent form. Participation was voluntary and no compensation was expected for respondents. Data were handled and stored in accordance with the European

Union General Data Protection Regulation (EU GDPR) 2016/679, and data transfer was safeguarded by encrypting/decrypting and password protection. The study was registered (ClinicalTrials.gov NCT04471701).

Data Collection and variables definition

The EPICOVID19 study was established as a collaborative project of a working group including epidemiologists, physicians expert in infectious diseases, biostatisticians, and public health professionals with the aim to improve SARS-CoV-2-related knowledge. To guarantee the maximal comparability with other studies, several questions were defined based on standardized and validated questionnaires, as elsewhere described in details[CITATION Sel \l 1040][CITATION EPI \l 1040][CITATION Noa \l 1040]. The participants were asked to complete an anonymous 38-item questionnaire (**Annex 1**), which mainly contained mandatory and closed questions divided into six sections: socio-demographic, clinical features, personal characteristics, behaviours before the lockdown, lifestyles, and behaviours following the lockdown (**Supplementary file**).

Smoke exposure

A number of questions on present and past smoking habit were asked in the questionnaire. These included smoking status defined as never smoked (persons who had never smoked regularly or had smoked less than 100 cigarettes), former smokers (regular smokers who have smoked at least 100 cigarettes during the lifetime and did not smoke at the time of the survey), and current smokers [CITATION htt6 \l 1040]. In order to explore the dose-response effect, we created a variable by collapsing data on smoking status and number of years of smoke as follows: former smokers (categorized for smoking duration ≤ 10 years or > 10 years) and current smokers grouped in mild smoker (<10 cigarettes/day for <15 years), moderate smokers (< 10 cigarettes/day for more than 15 years or >10 cigarettes/day for less than 15 years), and heavy smoker (>10 cigarettes/day for more than

15 years).

Main outcomes

We investigated two different outcomes: 1) positive result to the NPS molecular test; and 2) SARS-CoV-2 infection severity by combining information on NPS test, symptoms and hospitalization for COVID-19 defined as follows:

- *No infection*: negative NPS test
- *Asymptomatic infection*: positive NPS test without symptoms
- *Mild infection*: positive NPS test with at least one symptom
- *Severe infection*: positive NPS test and pneumonia or hospitalization for COVID-19.

Statistical analysis

The continuous variables were represented as mean and standard deviation (SD) and the categorical variables as counts and percentages. Continuous and categorical data according to smoking status were compared using one-way analysis of variance (ANOVA) and chi-square test respectively. To explore the association between smoking habit and positive versus negative NPS test results and 4-level infection severity dependent variable (no infection, asymptomatic infection, mild infection, and severe infection), logistic-regression and multinomial-regression models were used to estimate the odds ratios (ORs) and 95% Confidence Intervals (CIs). A first model (model 1) was only adjusted for age and sex. In the fully adjusted model (model 2) we further controlled for variables considered potential confounders as education, occupation, area of residence, heart diseases, lung diseases, hypertension, metabolic diseases, contact with suspected/confirmed COVID-19 cases, living area, crowding index, and living with at risk co-habitants. Models were applied considering separately the smoking status and the dose-response relationship as exposures. We explored our data for potential effect modification by sex, age, and education by adding cross-product terms of these variables to the regression models. When

heterogeneity was present, stratum-specific estimates were evaluated. Three sensitivity analyses were performed in order to evaluate whether the effect of smoking on SARS-CoV-2 infection was primarily due to the current amount of cigarettes smoked and/or to the smoking history during lifespan. In the first sensitivity analysis, we categorized current smokers based on years of smoking classified as ≤15, 15-30, and >30 years. Second sensitivity analysis explored the association between the number of cigarettes smoked categorized as less than 10 cigarettes/day or more than 10 cigarettes/day and NPT test. Third sensitivity analysis repeated the analysis by calculating the pack-years of smoking. We assigned a median number of cigarettes/day to each current smoking category (5 for 'less than 10 cig/day'; 15 for '10 to 20 cig/day'; 25 for 'more than 20 cig/day'), then we multiplied the number of packs per day (1 pack=20 cigarettes) by the number of years the person had smoked, and finally we tertilized the variable**.** All statistical analyses were performed using Stata 15.0 version (StataCorp LP, College station, Texas, USA), and a two-sided P-value <.05 was considered statistically significant.

RESULTS

Characteristics of participants

The participants' characteristics according to smoking status are summarized in **Table 1, 2 and 3**. The mean age of the whole sample was 47.9±14.1 years, 65.9% were females and 70.5% had university or higher degree. Out of the 6857 individuals, 63.2% (N=4334) had never smoked, 21.3% (N=1463) were former and 15.5% (N=1060) were current smokers. The 24.7% (N=1691) had a positive NPS test, among them 9.2% (N=156) were asymptomatic, 62.0% (N=1049) had mild infection, and 28.7% (N=486) reported conditions compatible with a severe infection. Compared with those who never smoked, current smokers were younger, with higher educational level, more frequently working as employers, healthcare professionals, and were frequently residents in Central and Southern

regions (**Table 1**).

Table 1. Socio-demographic characteristics of study participants with known molecular test results by smoking status (N=6,857), Italy, from April 13 to June 2, 2020.

SOCIO-DEMOGRAPHIC CHARACTERISTICS	Never smoked N=4,334 (63.2%)		Former smoker N=1,463 (21.3%)		Current smoker N=1,060 (15.5%)		*P* value *(current vs never)*	*P* value (overall)	All N=6,857	
Sex, female	2,991	69.0	807	55.2	420	70.1	*.42*	<.001	4,516	65.9
Age, years (mean, SD)	47.7	14.7	50.5	13.1	45.0	12.4	*>.001*	<.001	47.9	14.1
European ethnicity	4,281	98.8	1,458	99.7	1,052	99.3	*.20*	.01	6,791	99.0
Education							*.01*	.01		
Illiterate or primary school	359	8.3	96	6.6	30	5.0			525	7.7
Middle or high school	893	20.6	347	23.7	126	21.0			1,498	21.9
University or post-graduate	3,082	71.1	1,020	69.7	443	74.0			4,834	70.5
Employment status							*<.001*	<.001		
Employed	3,626	83.7	1,223	83.6	548	91.5			5,811	84.8
Student	125	2.9	18	1.2	19	3.2			172	2.5
Unemployed	63	1.5	28	1.9	8	1.3			106	1.6
Retired	291	6.7	144	9.8	8	1.3			459	6.7
Other	229	5.3	50	3.4	16	2.7			309	4.5
Occupational cluster*							*0.07*	<.001		
White collar	3,428	79.1	1,173	80.2	804	75.9			5,405	78.8
Blue collar	58	1.3	37	2.5	17	1.6			112	1.6
Others	848	19.6	253	17.3	239	22.6			1,340	19.5
Health professionals	2164	49.9	680	46.5	628	59.3	*<.001*	<.001	3472	50.6
Italian area of residence							*.01*	.01		
Northern	3,318	76.6	1,084	74.1	755	71.2			5157	75.2
Central	686	15.8	231	15.8	204	19.3			1121	16.4
Southern	320	7.8	144	9.8	98	9.3			562	8.2
Other	10	0.2	4	0.3	3	0.3			17	0.3

*White collar including legislators, senior officials and managers, professionals, technicians, associate professionals, clerks and service workers and shop and market sales workers: blue collar including skilled agricultural and fishery workers and craft and related trades workers, plant and machine operators and assemblers and elementary occupations, others including armed forces and unspecified occupations.

Current smokers were less affected by heart diseases (CVD), hypertension, oncological diseases (1.5% vs 3.2%), and allergies. They were less dependent in daily activities; were less vaccinated for flu and for pneumococcal infections, assumed less frequently thyroid drugs and supplements, and more frequently anti-inflammatory drugs. Smokers reported less frequently COVID-19-like symptoms as fever olfactory and taste disorders, shortness of breath, cough, and pneumonia; they were less hospitalized for COVID-19, had less frequently a NPS positive test, and in case of infection, they were less likely to be asymptomatic, mild or severe symptomatic (**Table 2**).

Table 2. Clinical features of study participants with known molecular test results by smoking status (N=6,857), Italy, from April 13 to June 2, 2020.

CLINICAL FEATURES	Never smoked N=4,334 (63.2%)		Former smoker N=1,463 (21.3%)		Current smoker N=1,060 (15.5%)		*P* value *(current vs never)*	*P* value (overall)	All N=6,857	
SELF-REPORTED DISEASES										
Lung diseases	340	7.8	130	8.9	77	7.3	*.53*	.29	547	8.0
Heart disease	196	4.5	76	5.2	26	2.5	*.01*	.01	298	4.4
Hypertension and/or medications	723	16.7	326	22.3	143	13.5	*.01*	<.001	1,192	17.4
Oncological diseases	138	3.2	67	4.6	16	1.5	*.01*	.001	221	3.2
Liver diseases	39	0.9	14	1.0	6	0.6	*.28*	.52	59	0.9
Renal diseases	52	1.2	14	1.0	10	0.9	*.48*	.64	76	1.1
Metabolic diseases and/or medications	238	5.5	86	5.9	47	4.4	*.17*	.27	371	5.4
Depression/anxiety and/or medications	505	11.7	167	11.4	122	11.5	*.90*	.97	794	11.6
Immune system diseases	431	9.9	146	10.0	88	8.3	*.10*	.25	665	9.7
Surgical procedures last year	168	3.9	82	5.6	38	3.6	*.66*	.01	288	4.2
Transplants	12	0.3	6	0.4	0	0	*.09*	.13	18	0.3
Allergies	786	18.1	223	15.2	163	15.4	*.04*	.01	1,172	17.1
Dependency in daily activities	209	4.8	15	1.0	9	0.9	*<.001*	<.001	233	3.4
Flu shot during last autumn	1,542	35.6	489	33.4	273	25.8	*<.001*	<.001	2,304	33.6
Anti-pneumococcal in the last 12	219	5.1	73	5.0	37	3.5	*.03*	0.10	329	4.8
SELF-REPORTED MEDICATIONS										
Aspirin	192	4.4	131	9.0	46	4.3	*.10*	<.001	369	5.4
Cholesterol treatment drugs	252	5.8	161	11.0	69	6.5	*.39*	<.001	482	7.0
Oncological drugs	42	1.0	23	1.6	6	0.6	*.21*	.04	71	1.0
Chorticosteroids	95	2.2	39	2.7	24	2.3	*.89*	.58	158	2.3
Thyroid drugs	369	8.5	131	9.0	64	6.0	*.01*	.02	564	8.2
Anti-inflammatory drugs	222	5.1	108	7.4	101	9.5	*.00*	<.001	431	6.3
Supplements/vitamins	928	21.4	304	20.8	190	17.9	*.01*	.04	1,422	20.7
SELF-REPORTED SYMPTOMS										
Fever	1,221	28.2	491	33.6	184	17.4	*.00*	<.001	1,896	27.7
Headache	1,594	36.8	570	39.0	397	37.5	*.68*	0.33	2,561	37.4
Muscle/bone pain	1,476	34.1	563	38.5	340	32.1	*.22*	<.001	2,379	34.7

CLINICAL FEATURES	Never smoked N=4,334 (63.2%)		Former smoker N=1,463 (21.3%)		Current smoker N=1,060 (15.5%)		*P* value *(current vs never)*	*P* value (overall)	All N=6,857	
Olfactory and taste disorders	903	20.8	365	25.0	180	17.0	*.01*	<.001	1,448	21.1
Shortness of breath	643	14.8	264	18.1	127	12.0	*.02*	<.001	1,034	15.1
Chest pain	596	13.8	224	15.3	144	13.6	*.89*	.30	964	14.1
Heart palpitations	572	13.2	185	12.7	118	11.1	*.07*	.19	875	12.8
Gastrointestinal disturbances	1,210	27.9	441	30.1	275	25.9	*.20*	.06	1,926	28.1
Conjunctivities	527	12.2	174	11.9	117	11.0	*.31*	.60	818	11.9
Sore throat/rhinorrhea	1,579	36.4	558	38.1	392	37.0	*.74*	.50	2,529	36.9
Cough	1,537	35.5	536	36.6	294	27.7	*<.001*	<.001	2,367	34.5
Pneumonia	354	8.2	170	11.6	32	3.0	*<.001*	<.001	556	8.1
No symptoms	1,154	26.6	321	21.9	308	29.1	*.11*	<.001	1,783	26.0
Hospitalized for COVID-19	319	7.4	175	12.0	33	3.1	*<.001*	<.001	527	7.7
NPS test positive result	1,124	25.9	407	27.8	160	15.1	*<.001*	<.001	1,691	24.7
Infection severity*							-	<.001		
No infection	*3,210*	*74.1*	*1,056*	*72.2*	*900*	*84.9*			*5,166*	*75.3*
Asymptomatic	*117*	*2.7*	*27*	*1.9*	*12*	*1.1*			*156*	*2.3*
Mild	*697*	*16.1*	*225*	*15.4*	*127*	*12.0*			*1,049*	*15.3*
Severe	*310*	*7.2*	*155*	*10.6*	*21*	*2.0*			*486*	*7.1*

* No infection (negative NPS test), asymptomatic infection (positive NPS test without symptoms), mild infection (positive NPS test with at least one symptom), and severe infection (positive NPS test and pneumonia or hospitalizing for COVID-19).

They lived less frequently with co-habitants at risk, after the lockdown more frequently went out and used public transport, contacted less frequently the emergency number and were more afraid about the infection for themselves and for family members than no smokers (**Table 3**).

Table 3. Behavioural characteristics of study participants with known molecular test results by smoking status (N=6,857), Italy, from April 13 to June 2, 2020.

BEHAVIOURAL CHARACTERISTICS	Never smoked N=4,334 (63.2%)		Former smoker N=1,463 (21.3%)		Current smoker N=1,060 (15.5%)		*P* value *(current vs never)*	*P* value (overall)	All N=6,857	
HOUSING CONDITIONS										
Traffic near house							*.25*	.17		
Low	1,880	43.4	655	44.8	461	43.5			2,996	43.7
Moderate	1,499	34.6	525	35.9	388	36.6			2,412	35.2
High	955	22.0	283	19.3	211	19.9			1,449	21.1
Co-habitants at risk°	934	21.6	250	17.1	182	17.2	*.01*	<.001	1,366	19.9
Residence area							*.32*	.25		
Countryside	492	11.4	164	11.2	136	12.8			792	11.6
Small town	1,797	41.5	609	41.6	411	38.8			2,817	41.1
Suburbs > 100.000 inhabitants	772	17.8	233	15.9	198	18.7			1,203	17.5
City town > 100.000 inhabitants	1,273	29.4	457	31.2	315	29.7			2,045	29.8
Household crowding index#							*.33*	.10		
Low	3,941	90.9	1,354	92.6	974	91.9			6,269	91.4
Middle	387	8.9	105	7.2	86	8.1			578	8.4
High	6	0.1	4	0.3	0	0			10	0.2
BEHAVIOURS BEFORE THE LOCKDOWN										
Number of daily contacts							*.17*	.01		
Less than 10	738	17.0	293	20.0	162	15.3			1,193	17.4
10 or more	3,596	83.0	1,170	80.0	898	84.7			5,664	82.6
Physical activity							*.88*	<.001		
> 2.5 h/week	1,099	25.4	449	30.7	275	25.9			1,826	26.6
10 minutes to 2.5 h/week	1,870	43.1	631	43.1	449	42.4			2,950	43.0
< 10 minutes/week	1,363	31.5	383	26.2	336	31.7			2,082	30.4
BEHAVIOURS AFTER THE LOCKDOWN										

BEHAVIOURAL CHARACTERISTICS	Never smoked N=4,334 (63.2%)		Former smoker N=1,463 (21.3%)		Current smoker N=1,060 (15.5%)		*P* value *(current vs never)*	*P* value (overall)	All N=6,857	
Contact COVID-19 cases§	3,118	71.9	989	67.6	754	71.1	*.60*	.01	4,861	70.9
Weekly outing							*<.001*	<.001		
Never	1,043	24.1	364	24.9	140	13.2			1,547	22.6
1-3 times	1,083	25.0	417	28.5	290	27.4			1,790	26.1
4 times or more	2,208	51.0	682	46.6	630	59.4			3,520	51.3
Use of public transport							*.05*	.05		
Never	4,186	96.6	1,423	97.3	1,008	95.1			6,617	96.5
1-3 times a week	62	1.4	18	1.2	25	2.4			105	1.5
4 times a week or more	86	2.0	22	1.5	27	2.6			135	2.0
PERSONAL CHARACTERISTICS										
Emergency number contact							*<.001*	<.001		
No	2,323	53.6	722	49.4	676	63.8			3,721	54.3
No, but I went to a hospital on my own	103	2.4	47	3.2	18	1.7			168	2.5
Yes, and they did not suggest to me self-	235	5.4	88	6.0	72	6.8			395	5.8
Yes, and they suggested to me self-	1,361	31.4	448	30.6	239	22.6			2,048	29.9
Yes, I was sent to a hospital	312	7.2	158	10.8	55	5.2			525	7.7
Self-perceived health status							*.81*	.59		
Good	3,493	80.6	1,155	79.0	863	81.4			5,511	80.4
Adequate	769	17.7	282	19.3	179	16.9			1,230	17.9
Bad	72	1.7	26	1.8	18	1.7			116	1.7
Afraid to be infected							*.02*	.01		
Not	1,556	35.9	521	35.6	401	37.8			2,478	36.1
Neutral	918	21.2	253	17.3	184	17.4			1,355	19.8
Yes	1,860	42.9	689	47.1	475	44.8			3,024	44.1
Afraid for family members							*<.001*	<.001		
Not	669	15.4	251	17.2	179	16.9			1,099	16.0
Neutral	514	11.9	118	8.1	64	6.0			696	10.2
Yes	3,151	72.7	1,094	74.8	817	77.1			5,062	73.8

In bold variables with a *P*-value <0.05.
°Elderly persons or anyone with immunocompromising or chronic disease conditions
#Number of co-habitants/number of rooms. §Suspected/confirmed

In comparison with never- and current smokers, former smokers were significantly older, retired, more affected by chronic conditions as heart diseases and hypertension, assumed more frequently aspirin, drugs for lowering cholesterol, oncological and thyroid drugs. They reported less frequently COVID-19 like symptoms and were more likely to be hospitalized for COVID-19.

Association analyses

In Supplementary table 2 and **Figure 2** are shown the logistic regression results for positive NPS test. In the age- and sex-adjusted model, current smoking was significantly inversely associated with positive NPS test (OR 0.54 95%CI 0.45-0.65), with never smoker as reference category. Results did not change when potential confounders were accounted for in the fully adjusted model (aOR 0.54 95%CI 0.44-0.65). Being former smokers was not associated with positive NPS (aOR 1.03 95%CI 0.90-1.19) even when we considered the dose-response relationship, and the lifetime smoking duration (≤ 10 years and > 10 years). Adjusted odds ratio for testing positive was 0.76 in mild smokers (95%CI 0.55-1.05) although not statistically significant, 0.56 in moderate (95%CI 0.42-0.73), and 0.38 in heavy smokers (95%CI 0.27-0.53) suggesting a dose-response relation between smoking habit and NPS test.

Table 4 reports the association between smoking status and infection severity. Current smokers had a statistically significant lower probability of having asymptomatic (aOR 0.50 95%CI 0.27-0.92), mild (aOR 0.65 95%CI 0.53-0.81), and severe infection (aOR 0.27 95%CI 0.17-0.42) compared to never smokers. The inverse dose-dependent relationship persisted also when considering the gravity of the infection, showing a gradient of association across smoking patterns. Since we found a significant interaction between smoking status and age (P=0.001), we created a six-level indicator variable by combining age, dichotomized in ≤48 and >48 years (median), and smoking status. Compared to the never-smokers aged ≤ 48, never- or former smokers aged > 48 had

1.5 folds and 1.7 folds higher probability of a positive NPS test, respectively.

Table 4. Odds ratios* of SARS-CoV-2 severity° by smoking habit (N=6,857)

Smoking habit	No infection N=5,166 (75.3)	Asymptomatic infection N=156 (2.3)			Mild infection N=1,049 (15.3)			Severe infection N=486 (7.1)		
Smoking status	**N (%)**	**OR**	**(95% CI)**	**N (%)**	**OR**	**(95% CI)**	**N (%)**	**OR**	**(95% CI)**	**N (%)**
Never smoked	3210 (62.1)	1 (ref.)	-	117 (75.0)	1 (ref.)	-	697 (66.4)	1 (ref.)	-	310 (63.8)
Former smokers	1056 (20.4)	0.78	0.50-1.21	27 (17.3)	0.99	0.84-1.18	225 (21.5)	1.20	0.97-1.50	155 (31.9)
Current smokers	900 (17.4)	0.50	0.27-0.92	12 (7.7)	0.65	0.53-0.81	127 (12.1)	0.27	0.17-0.42	21 (4.3)
Dose-response relationship										
Never smoked	3210 (62.1)	1 (ref.)	-	117 (75.0)	1 (ref.)	-	697 (66.4)	1 (ref.)	-	310 (63.8)
Former smokers (≤ 10	487 (9.4)	0.84	0.42-1.71	9 (5.8)	1.00	0.79-1.27	99 (9.4)	1.22	0.88-1.69	50 (10.3)
Former smokers (>10 yrs)	569 (11.0)	0.74	0.44-1.27	18 (11.5)	0.98	0.79-1.22	126 (12.0)	1.20	0.92-1.55	105 (21.6)
Mild smokers	249 (4.8)	1.16	0.41-3.29	4 (2.6)	0.84	0.59-1.18	42 (4.0)	0.23	0.07-0.73	3 (0.6)
Moderate smokers	365 (7.1)	0.42	0.15-1.15	4 (2.6)	0.67	0.49-0.91	52 (5.0)	0.35	0.19-0.66	11 (2.3)
Heavy smokers	286 (5.5)	0.36	0.13-0.99	4 (2.6)	0.50	0.34-0.72	33 (3.2)	0.20	0.09-0.43	7 (1.4)

°No infection (negative NPS test) – reference category, asymptomatic infection (positive NPS test without symptoms), mild infection (positive NPS test with at least one symptom), and severe infection (positive NPS test and pneumonia or hospitalizing for COVID-19).
*Adjusted for age, sex, education, occupation, area of residence, heart diseases, lung diseases, hypertension, metabolic and oncological diseases, contact with COVID-19 cases, living area, crowding index, and living with at risk co-habitants..
Mild smokers (<10 cigarettes/day and <15 yrs); Moderate smokers (< 10 cigarettes/day for more than 15 years or >10 cigarettes/day for less than 15 years); Heavy smokers (>10 cigarettes/day for more than 5 yrs).

The odds were reduced by 33% and 42% in current smokers aged ≤ 48 and > 48, respectively (**Supplementary table 3)**. In sensitivity analyses, considering never smoker as reference category, we found that the inverse relationship between smoking and positive NPS test was stronger in heavy smokers (>10 cigarettes/day, aOR 0.42 95%CI 0.31-0.56), in long-term smokers (>30 years, aOR 0.40 95%CI 0.26-0.61), and in those in the highest pack-years categories (11.3-65, aOR 0.43 95%CI 0.32-0.58). In moderate smokers (≤10 cigarettes/day aOR 0.64 95%CI 0.51-0.81), more recent current smokers (≤ 15 years, aOR 0.70 95%CI 0.53-0.92, and among those in the lowest category of pack-years of smoking (0.5-4.9 aOR 0.73 95%CI 0.54-1.00), the odds reduction was lower (**Figure 3, Supplementary table 4**).

DISCUSSION

Principal findings

The present study evaluated the association between smoking habit and the odds for positive SARS-CoV-2 molecular test and infection severity in an Italian adult population recruited on-line during the first national lockdown. We found that current smoking was associated with a significant risk reduction of having a positive SARS-CoV-2 test and with a severe infection in a dose-response relationship even after taking into account for all the available confounding factors.

In our sample, the percentage of positive tests was 24.7% (1,691/6,857 participants), close to the positive test ratio shown by Romagnani and colleagues who reported for Italy, at the beginning of April 2020, an overall percentage of positive tests of 18.6%, with a marked regional difference ranging from 38.5% in Lombardy to 7.5% in Lazio[CITATION Rom \l 1040]. The relatively high percentage of positive tests reflects the initial phase of the pandemic spread, during which, in Italy, molecular tests were reserved to clinically relevant cases. This is in keeping with the low percentage of asymptomatic subjects in our sample: 2.3% of the overall evaluated sample and 9.2%

among confirmed SARS-CoV-2 infection cases. Although the 70.5% of participants had a university or higher degree and the female gender was predominant (65.9%), the prevalence of smoking habits in our sample was quite similar to that known for the Italian general population. Indeed, we found that 63.2% of the included participants had never smoked, 21.3% were former smokers and 15.5% were current smokers. In Italy, the prevalence of former smokers is 23.0%, while active smokers represent the 18.4% of the population and those who never smoked are the 57.4%[CITATION IST \l 1040].

When compared to never smokers, current smokers had a lower prevalence of chronic conditions (50.8% vs 55.4%) including those known to be influenced by smoking habits, such as CVD and hypertension (2.5% vs 4.5% and 13.5% 16.7%, respectively). Former smokers were older and more frequently retired compared to never- and current smokers were and, as expected, more affected by chronic diseases as CVD and hypertension (5.2% and 22.3%, respectively). This finding is consistent with the successful smoking cessation obtained by subjects affected by hypertension and myocardial infarction[CITATION 40 \l 1040]. Current smokers had significantly fewer COVID-19-like symptoms and were less hospitalized for COVID-19 than never and former smokers and this is in agreement with a previous meta-analysis study showing a lower prevalence of current smokers among hospitalized COVID-19 patients [19].

We found that current smoking was associated with reduced odds of NPS positive by 46%. Analogously, Israel and colleagues [CITATION Smo \l 1040] found reduced odds by 53% for the association between current smoking and fatal or severe disease in a population-based study among over 3,000,000 adults in Israel. Similar results were observed in a study on middle-aged veterans in US in which smokers were less likely to test positive (OR 0.43) although there was no significant difference in hospitalization[CITATION Ren \l 1040]. A large cohort study of 17,278,392 adults from the general population in the UK found that current smoking was associated with

an increased risk of COVID-19-related death controlling for age and sex. However, after adjustment for multiple adjusted covariates (e.g. chronic respiratory diseases) the study found that smoking was associated with a risk lowered by 11% [32]. A negative association between smoking prevalence and COVID-19 occurrence at the population level was also found in an ecological study conducted in 38 European countries, although the authors cautioned that this association may not imply a causal relationship [CITATION Tsi \l 1040].

In our study, we also observed a significant dose-response relationship between smoking habit and NPS test results. In the full-adjusted logistic model, mild smokers had 24% lower probability of positive NPS test, whereas moderate smokers and heavy smokers had, respectively, 44% and 62% lower probability with the respect to never smokers. Conversely, among former smokers we did not find a significant effect of the time interval (more or less than 10 years) since they quitted on the risk for SARS-CoV-2 infection. A French study evaluating the smoking habits among symptomatic COVID-19 inpatients and outpatients showed that, in both groups active smokers were less infected by COVID-19 when compared with the general population [33].

When we analysed the association between smoking habits and SARS-CoV-2 infection severity, we found that active smokers were less likely to develop severe infection. Furthermore, by evaluating NPS positive participants in relation to their reported infection severity, i.e., asymptomatic, mild, or severe infection, being a current smoker reduced the odds by 50%, 35% and 73%, respectively. Likewise, the dose-response effect found for positive NPS result, heavy smokers showed a lower risk of developing different levels of gravity of SARS-CoV-2 infection, in particular severe COVID-19 (80% odds reduction). The link between smoking and infection severity is highly controversial in literature. For example, in the previously cited meta-analysis, Farsalinos and colleagues reported that, although the risk for current smokers to be hospitalized was

lower than among non-smokers, current smokers were more likely to have an adverse outcome during the hospital admission [19]. In a population of over 2.4 million UK users of the Zoe COVID-19 Symptom Study app, Hopkinson found for current smokers a statistically significant OR of 1.14 of self-reporting a triad of three symptoms (fever, persistent cough, and shortness of breath) that, although to some extent attributable also to constipation or normal flu, the authors identified as suggestive of COVID-19. On the contrary, when analysing the stronger endpoint of positive SARS-CoV-2 test, they observed a lower smoking rate (7.4% among positive vs 9.3% among negative) leading to a reduced adjusted OR of 0.7 that they considered not generalizable to their general population, due to the physiological difference between tested and non-tested individuals [34]. In their systematic review, Vardavas and Nikitara concluded that smoking was associated with disease progression and increased adverse outcomes in COVID-19 positive patients [35], even though, in both meta-analyses, the authors acknowledged that their studies were conducted with limited availability of data, the included studies came mostly from hospital contexts, and their analyses were not adjusted for confounding factors. Similar methodological limitations have been reported in the meta-analysis conducted by Patanavanich who found smoking as a risk factor for progression of COVID-19 [36] conversely, Lippi and Henry did not observe any association [37].

Our findings, which highlight the existence of a negative association of current smoking with SARS-CoV-2 infection and its severity, drive the focus to possible suggestive explanations. Since ACE2 is necessary for infection of cells by SARS-CoV-2 [38], the risk of contracting a SARS-CoV-2 severe infection, as well as the risk of a disadvantageous clinical outcome, could be influenced by the number of available ACE2 receptors and by the receptor-ligand interaction of ACE2 and SARS-CoV-2 S protein [39]. As concerns the number of ACE2 receptors, nicotine would seem to have a

controversial role. Recent evidence indicates that a higher number of receptors are expressed in smoker's lung tissues [40]. On the other hand, it has been suggested that nicotine downregulates the expression and/or the activity of ACE2 [41]. However, a better disease outcome was associated to an overexpression of ACE2, able to compensate the negative effects of the ACE2 downregulation induced by the cell entry of SARS-CoV-2 [42]. Moreover, a direct role of nicotine on disrupting spike protein glycosylation, could in turn directly affect the ability of SARS-CoV-2 to infect [43]. A recent study performed on a mice model proposes the modulation of the renin-angiotensin pathways as therapeutic target to protect individuals with SARS from developing acute severe lung failure and acute respiratory distress syndrome [44]. In addition to that, nicotine might exert an anti-inflammatory effect by protecting against the 'cytokine-storm syndrome' responsible of severe SARS-CoV-2 infections [45] [21]. It has been also hypothesized that the cytokine storm, with excessive production of pro-inflammatory molecules, could possibly more easily be triggered in individual who never smoked rather than in smokers, whose immune system is more tolerant and less reactive [46].

Another potential mechanism of action involves nitric oxide (NO) produced during smoking that, due to its reported antiviral effect, might inhibit the virus replication/entry in the cells [21] [47].

Alternatively, from the behavioural perspective, we cannot exclude that smokers, considering themselves at higher risk of developing the disease, were more careful than never smokers in adopting preventative measures, such as physical distancing, hand hygiene, covering coughs, wearing masks when appropriate, having fewer social-relationship, etc. [48].

4.1 Limitations and Strengths

The present study has some limitations. Firstly, because of the observational nature of

the study and the cross-sectional design, we cannot infer any causal relationship between smoking habit and COVID-19. In addition, a misclassification of the outcome of severity may exist, since some cases (although numerically limited) might have worsened their conditions a few days after the survey with a subsequent potential distortion of measures of association. Secondly, smoking habit was self-reported therefore recall bias might have led to misclassification of the exposure. Thirdly, the sample was self-selected and not entirely representative of the Italian population because restricted to relatively younger, females, highly educated, and relatively healthy participants, therefore results should be treated with caution when generalized to different populations [49]. Moreover, the low percentage of asymptomatic subjects in our sample may have influenced the evaluation of smoking habit effect on asymptomatic NPS positive subjects. Nevertheless, in a previous study smokers were proportionally represented in asymptomatic patients [50]. Lastly, although we controlled for several potential confounders, we cannot completely rule out the possibility of residual confounding due to unmeasured factors (e.g. passive smoking). Our study has also several strengths. The first one was that evaluating the effect of smoking was the primary goal of the work. The presence in our study sample of subjects from a general population with negative NPS test allows an internal control group (NPS negative individuals). The web-survey reached a large sample of adults with an acceptable geographical coverage reflecting the distribution of SARS-CoV-2 infection in the study period[CITATION Sel \l 1040] and a proportion of smokers that is almost overlapped with the prevalence of current smoking in the Italian population. Finall, and differently from previous published works, we recorded factors not easy to obtain from medical records of inpatients such as the exhaustive details regarding smoking habits (distinguishing between former, active, or never-smokers) and those suspected to play a role of confounders in the observed association, i.e. the socioeconomic status, clinical,

behavioural, and environmental characteristics.

Conclusions

In summary, we are aware that our findings must be carefully evaluated. This article takes as its premise the need to strengthen prevention actions of the most powerful human carcinogen known, which is also a heavy risk factor for many non-communicable diseases [51] and for disease progression in COVID-19 patients. However, we are now facing a second pandemic wave requiring to consider each issue still unresolved on possible role played by smoking in COVID-19 disease. Further researches on the mechanisms of interaction between tobacco smoke exposure and SARS-CoV-2 infection are warrants to fill this knowledge gap.

Abbreviations

Coronavirus disease - COVID-19

Angiotensin-converting enzyme 2 - ACE2

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

Nasopharyngeal swab - NPS

European Union General Data Protection Regulation - EU GDPR

Standard deviations - SD

Analysis of variance - ANOVA

Odds Ratios - OR

95% Confidence Intervals - 95%CI

Cardiovascular disease - CVD

Nitric oxide - NO

Captions

Title: Figure 1. Flow-chart of the study population. EPICOVID19: Italian National Epidemiological Survey on COVID-19.

Title: Figure 2. Adjusted odds ratios° and relative 95%CI for smoking status and intensity and duration (N=6,857).

Legend: °Age, sex, education, occupation, area of residence, heart diseases, lung diseases, hypertension, metabolic and oncological diseases, contact with confirmed or suspected COVID-19 cases, living area, crowding index, and living with at risk co-habitants. Mild smokers (<10 cig/day and <15 yrs); Moderate smokers (< 10 cigarettes/ day for more than 15 years or >10 cigarettes/day for less than 15 years); Heavy smokers (>10 cigarettes/day for more than 5 yrs). Dots and vertical lines indicate ORs and 95%CI.

Title: Figure 3. Adjusted odds ratios* for positive SARS-CoV-2 test by smoke-related variables (intensity, duration, and pack-years of smoking) (N=6,857)
Legend: °Age, sex, education, occupation, area of residence, heart diseases, lung diseases, hypertension, metabolic and oncological diseases, contact with COVID-19 cases, living area, crowding index, and living with at risk co-habitants.

References

[1] World Health Organization, Smoking and COVID-19. https://www.who.int/publications/i/item/WHO-2019-nCoV-Sci_Brief-Smoking-2020.2.

[2] Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, et al. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci. 2020;63(3):457-460. doi:10.1007/s11427-020-1637-5.

[3] Zhang H, Rostami MR, Leopold PL, Mezey JG, O'Beirne SL, Strulovici-Barel, et al. Expression of the SARS-CoV-2 ACE2 Receptor in the Human Airway Epithelium. Am J Respir Crit Care Med. 2020 Jul 15; 202(2):219-229. doi:10.1164/rccm.202003-0541OC.

[4] Berlin I, Thomas D, Le Faou AL, Cornuz J. COVID-19 and Smoking, Nicotine & Tobacco Research, 2020, 24;22(9):1650-1652 doi:10.1093/ntr/ntaa059.

[5] Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. China Medical Treatment Expert Group for Covid-19. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020;382(18):1708-1720. doi:10.1056/NEJMoa2002032.

[6] Cummings MJ, Baldwin MR, Abrams D, Jacobson SD, Meyer BJ, Balough EM, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: a prospective cohort study. Lancet. 2020 Jun 6;395(10239):1763-1770. doi: 10.1016/S0140-6736(20)31189-2..

[7] Parra-Bracamonte GM, Lopez-Villalobos N and Parra-Bracamonte FE, Clinical characteristics and risk factors for mortality of patients with COVID-19 in a large data set from Mexico. Ann Epidemiol. 2020 Aug 14:S1047-2797(20)30286-6. doi: 10.1016/j.annepidem.2020.08.005..

[8] Yanover C, Mizrahi B, Kalkstein N, Marcus K, Akiva P, Barer Y, et al. What Factors Increase the Risk of Complications in SARS-CoV-2 Positive Patients? A Cohort Study in a Nationwide Israeli Health Organization. JMIR Public Health Surveill. 2020 Aug 25;6(3):e20872. doi: 10.2196/20872..

[9] Miyara M, Tubach F, Martinez V, Morelot-Panzini C, Pernet J, Haroche J, et al. Low Rate of Daily Smokers in Patients with Symptomatic COVID-19.medRxiv 2020.06.10.20127514; doi: https://doi.org/10.1101/2020.06.10.20127514.

[10] Hamer M, Kivimäki M, Gale CR and Batty GD. Lifestyle risk factors, inflammatory mechanisms, and COVID-19 hospitalization: A community-based cohort study of 387,109 adults in UK. Brain Behav Immun. 2020 Jul;87:184-187. doi:10.1016/j.bbi.2020.05.059..

[11] Gaibazzi D, Tuttolomondo A, Guidorossi A, Botti A, Tedeschi A, Martini C, et al. Smoking prevalence in low in symptomatic patients admitted for COVID-19, MedRxiv (2020), https://doi.org/10.1101/2020.05.05.20092015..

[12] Lodigiani C, Iapichino G, Carenzo L, Cecconi M, Ferrazzi P, Sebastian T, et al. Humanitas COVID-19 Task Force. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Thromb Res. 2020 Jul;191:9-14. doi:10.1016/j.thromres.2020.04.024.

[13] Inciardi RM, Adamo M, Lupi L, Cani DS, Di Pasquale M, Tomasoni D, et al. Characteristics and outcomes of patients hospitalized for COVID-19 and cardiac disease in Northern Italy. Eur Heart J. 2020 May 14;41(19):1821-1829. doi: 10.1093/eurheartj/ehaa388..

[14] Tsigaris P and Teixeira da Silva JA, Smoking Prevalence and COVID-19 in Europe.

Nicotine Tob Res. 2020 Aug 24;22(9):1646-1649. doi: 10.1093/ntr/ntaa121..

[15] Pagani G, Conti F, Giacomelli A, Bernacchia D, Rondanin R, Prina A, et al. Seroprevalence of SARS-CoV-2 significantly varies with age: Preliminary results from a mass population screening. J Infect 2020 Dec;81(6):e10-e12. doi: 10.1016/j.jinf.2020.09.021.

[16] Israel A, Feldhamer I, Lahad A, Levin-Zamir D and Lavie G, Smoking and the risk of COVID-19 in a large observational population study. medRxiv 2020.06.01.20118877; doi: https://doi.org/10.1101/2020.06.01.20118877..

[17] Rentsch CT, Kidwai-Khan F, Tate JP, Park LS, King JT, Skanderson M, et al. Covid-19 Testing, Hospital Admission, and Intensive Care Among 2,026,227 United States Veterans Aged 54-75 Years. medRxiv [Preprint]. 2020 Apr 14:2020.04.09.20059964. doi: 10.1101/2020.04.09.20059964..

[18] Russo P, Bonassi S, Giacconi R, Malavolta M, Tomino C and Maggi F, COVID-19 and smoking: is nicotine the hidden link? Eur Respir J. 2020 Jun 4;55(6):2001116. doi: 10.1183/13993003.01116-2020..

[19] Farsalinos K, Barbouni A, Poulas K, Polosa R, Caponnetto P and Niaura R, Current smoking, former smoking, and adverse outcome among hospitalized COVID-19 patients: a systematic review and meta-analysis. Ther Adv Chronic Dis. 2020 Jun 25;11:2040622320935765. doi: 10.1177/2040622320935765..

[20] Usman MS, Siddiqi TJ, Khan MS, Patel UK, Shahid I, Ahmed J, et al. Is there a smoker's paradox in COVID-19? BMJ Evid Based Med. 2020 Aug 11:bmjebm-2020-111492. doi: 10.1136/bmjebm-2020-111492..

[21] Grines CL, Topol EJ, O'Neill WW, et al. Effect of cigarette smoking on outcome after thrombolytic therapy for myocardial infarction. Circulation 1995;91:298–303.

[22] Leung JM, Yang CX, Tam A, Shaipanich T, Hackett TL, Singhera GK, et al. ACE-2 expression in the small airway epithelia of smokers and COPD patients: implications for COVID-19. Eur Respir J. 2020 May 14;55(5):2000688. doi: 10.1183/13993003.00688-2020..

[23] Grundy EJ, Suddek T, Filippidis FT, Majeed A and Coronini-Cronberg S, Smoking, SARS-CoV-2 and COVID-19: A review of reviews considering implications for public health policy and practice. Tob Induc Dis. 2020 Jul 3;18:58. doi: 10.18332/tid/124788..

[24] Adorni F, Prinelli F, Bianchi F, Giacomelli A, Pagani G, Bernacchia D, et al. Self-Reported Symptoms of SARS-CoV-2 Infection in a Nonhospitalized Population in Italy: Cross-Sectional Study of the EPICOVID19 Web-Based Survey. JMIR Public Health Surveill. 2020 Sep 18;6(3):e21866. doi: 10.2196/21866..

[25] COVID-19 repository- Dipartimento di Protezione Civile, Webpage in Italian. GitHub. URL: https://github.com/pcm-dpc/.

[26] Bastiani L, Fortunato L, Pieroni S, Bianchi F, Adorni F, Prinelli F, et al. Rapid COVID-19 Screening Based on Self-Reported Symptoms: Psychometric Assessment and Validation of the EPICOVID19 Short Diagnostic Scale. J Med Internet Res 2021;23(1):e23897 DOI: 10.2196/23897.

[27] Noale M, Trevisan C, Maggi S, Antonelli Incalzi R, Pedone C, Di Bari M, et al. The Association between Influenza and Pneumococcal Vaccinations and SARS-Cov-2 Infection: Data from the EPICOVID19 Web-Based Survey. Vaccines (Basel). 2020 Aug 23;8(3):471. doi: 10.3390/vaccines8030471..

[28] Centers for Disease Prevention and Control. National Health Interview Survey – Adult Tobacco Use Information. Available at:

https://www.cdc.gov/nchs/nhis/tobacco/tobacco_glossary.htm.

[29] Romagnani P, Gnone G, Guzzi F, Negrini S, Guastalla A, Annunziato F, et al. The COVID-19 infection: lessons from the Italian experience. J Public Health Policy. 2020 Sep;41(3):238-244. doi: 10.1057/s41271-020-00229-y.

[30] ISTAT, http://dati.istat.it/Index.aspx?lang=en&SubSessionId=a1f09136-11d9-4642-9d92-f80514384c37, [Online].

[31] Yang JJ, Song M, Yoon HS, Lee HW, Lee Y, Lee SA, et al. What Are the Major Determinants in the Success of Smoking Cessation: Results from the Health Examinees Study. PLoS One. 2015 Dec 3;10(12):e0143303. doi: 10.1371/journal.pone.0143303..

[32] Williamson EJ, Walker AJ, Golfacre B, et al.–Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020 Aug;584(7821):430-436. doi: 10.1038/s41586-020-2521-4. Epub 2020 Jul 8.

[33] Miyara M, Tubach F, Pourcher V and Morelot-Panzini C, Low rate of daily active tobacco smoking in patients with symptomatic COVID-19. Qeios. Published online May 9, 2020. doi:10.32388/WPP19W.4.

[34] **Hopkinson NS, Rossi N, El-Sayed_Moustafa J, Laverty AA, Quint JK, Freidin M, Visconti A, et al. Current smoking and COVID-19 risk: results from a population symptom app in over 2.4 million people. Thorax Published Online First: 05 January 2021. doi: 10.1136/thoraxjnl-2020-216422.**

[35] Vardavas CI and Nikitara K, COVID-19 and smoking: A systematic review of the evidence. Tob Induc Dis. 2020;18:20. Published 2020 Mar 20. doi:10.18332/tid/119324.

[36] ~~S. A. G.~~ Roengrudee Patanavanich and Glantz SA. Smoking Is Associated With COVID-19 Progression: A Meta-analysis, Nicotine & Tobacco Research, 2020, 1653–1656. doi:10.1093/ntr/ntaa082.

[37] Lippi G and Henry BM, Active smoking is not associated with severity of coronavirus disease 2019 (COVID-19). Eur J Intern Med. 2020;75:107-108. doi:10.1016/j.ejim.2020.03.014.

[38] Zhou P; Yang XL; Wang XG; Hu B; Zhang L; Zhang W; et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579, 270–273 (2020). https://doi.org/10.1038/s41586-020-2012-7.

[39] Alifano M, Alifano P, Forgez P and Iannelli A, Renin-angiotensin system at the heart of COVID-19 pandemic. Biochimie. 2020 Jul;174:30-33. doi:10.1016/j.biochi.2020.04.008.

[40] Brake SJ, Barnsley K, Lu W, McAlinden KD, Eapen MS and Sohal SS, A Potential Adhesion Site for Novel Coronavirus SARS-CoV-2 (Covid-19). JCM. 2020 Mar 20;9(3):841. doi:10.3390/jcm9030841.

[41] Oakes JM, Fuchs RM, Gardner JD, Lazartigues E and Yue X, Nicotine and the renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol. 2018 Nov 1;315(5):R895-R906. doi: 10.1152/ajpregu.00099.2018.

[42] Verdecchia P, Cavallini C, Spanevello A and Angeli F, The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. Eur J Intern Med. 2020 Jun;76:14-20. doi: 10.1016/j.ejim.2020.04.037. Epub 2020 Apr 20..

[43] Engin AB, Engin ED and Engin A, Two important controversial risk factors in SARS-CoV-2 infection: Obesity and smoking. Environ Toxicol Pharmacol. 2020 Aug;78:103411. doi: 10.1016/j.etap.2020.103411. Epub 2020 May 15..

[44] Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al.–A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med.

2005 Aug;11(8):875-9. doi: 10.1038/nm1267. Epub 2005 Jul 10..
[45] McGonagle D, Sharif K, O'Regan A and Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. Autoimmun Rev. 2020 Jun;19(6):102537. doi: 10.1016/j.autrev.2020.102537. Epub 2020 Apr 3..
[46] Garufi G, Carbognin L, Orlandi A, Tortora G, Bria E et al. Smoking habit and hospitalization for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-related pneumonia: The unsolved paradox behind the evidence., Eur J Intern Med. 2020 Jul; 77: 121–122. doi: 10.1016/j.ejim.2020.04.042.
[47] Hedenstierna G, Chen L, Hedenstierna M, Lieberman R and Fine DH, Nitric oxide dosed in short bursts at high concentrations may protect against Covid 19. Nitric Oxide. 2020 Oct 1;103:1-3. doi: 10.1016/j.niox.2020.06.005. Epub 2020 Jun 23..
[48] Alla F, Berlin I, Nguyen-Thanh V, Guignard R, Pasquereau A, et al. Tobacco and COVID-19: a crisis within a crisis? Canadian Journal of Public Health. Dec;111(6):995-999 https://doi.org/10.17269/s41997-020-00427-x.
[49] Griffith GJ, Morris TT, Tudball MJ, Herbert A, Mancano G, Pike L, et al. Collider bias undermines our understanding of COVID-19 disease risk and severity. Nature Communication (2020) 11:5749
[50] Balabanski LL. An International Review of Tobacco Use and the COVID-19 Pandemic: Examining Hospitalization, Asymptomatic Cases, and Severity. Epidemiology; 2020. medRxiv preprint doi:10.1101/2020.06.12.20129478
[51] Italian Ministry of Health, 2018. Activities for smoking prevention, Report Available on: http://www.salute.gov.it/imgs/C_17_pubblicazioni_2851_allegato.pdf

Acknowledgments
The authors would like to thank all the participants who took part in this study and made it possible and all the collaborators of the EPICOVID19 Working Group.

Contributors

F.P., F.B., F.A., F.C. conceived, designed, and planned the study. F.P. and F.A. are responsible for the study procedures. F.P. did the statistical analysis and prepared a first draft of the manuscript; F.B., G.D., S.R., F.A., F.C. contributed to draft the manuscript; F.B., F.A. and F.C. supervised the study; G.D., S.R, A.S, N.J., M.G., A.G., S.M., L.B., S.M., M.N., and R.A.I. critically edited and revised the manuscript for important intellectual content. All authors have approved the submitted version (and any substantially modified version that involves the author's contribution to the study). The corresponding author, F.P., attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest

All authors declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval

The Ethics Committee of the Istituto Nazionale per le Malattie Infettive I.R.C.C.S. Lazzaro Spallanzani (Protocol No. 70, 12/4/2020) approved the EPICOVID19 study protocol. When they first accessed the on-line platform, the participants were informed of the purpose of the study, the data to be collected, and the methods of storage and filled in the informed consent. Participation was voluntary and no compensation was expected for respondents. The planning conduct and reporting of studies was in line with the Declaration of Helsinki, as revised in 2013. Data were handled and stored in accordance with the European Union General Data Protection Regulation (EU GDPR) 2016/679, and data transfer was safeguarded by encrypting/decrypting and password protection.

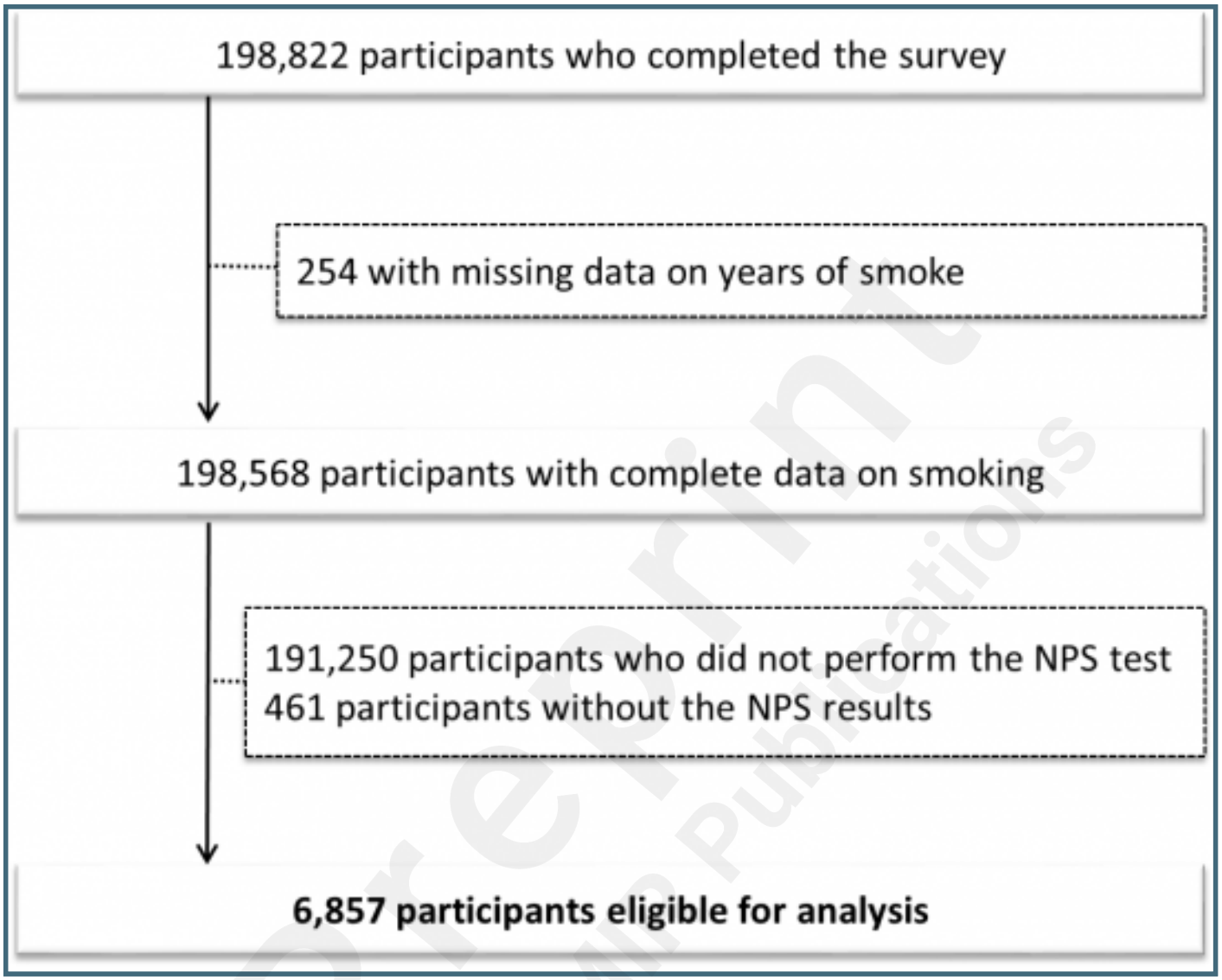
Members of the EPICOVID19 Working Group° (in alphabetical order):
Adorni Fulvio, National Research Council, Institute of Biomedical Technologies, Epidemiology Unit, Via Fratelli Cervi 93, 20090 Segrate (MI), Italy. fulvio.adorni@itb.cnr.it
Andreoni Massimo, Infectious Diseases Clinic, Department of System Medicine, Tor Vergata University of Rome, 00133 Rome, Italy. andreoni@uniroma2.it
Antonelli Incalzi Raffaele, Unit of Geriatrics, Department of Medicine, Biomedical Campus of Rome, via Alvaro del Portillo, 21, 00128 Rome, Italy. r.antonelli@unicampus.it
Bastiani Luca, National Research Council, Institute of Clinical Physiology, Via G. Moruzzi 1, 56124 Pisa (PI), Italy. luca.bastiani@ifc.cnr.it
Bianchi Fabrizio, National Research Council, Institute of Clinical Physiology, Via G. Moruzzi 1, 56124 Pisa (PI), Italy. fabriepi@ifc.cnr.it
Di Bari Mauro, Geriatric Intensive Care Medicine, University of Florence and Azienda Ospedaliero-Universitaria Careggi, Viale Peraccini 18, 50139 Florence, Italy. mauro.dibari@unifi.it
Fortunato Loredana, National Research Council, Institute of Clinical Physiology, Via G. Moruzzi 1, 56124 Pisa (PI), Italy. loredana.fortunato@ifc.cnr.it
Galli Massimo, Infectious Diseases Unit, Department of Biomedical and Clinical Sciences L. Sacco, Università di Milano, ASST Fatebenefratelli Sacco, 20157 Milan, Italy. massimo.galli@unimi.it
Giacomelli Andrea, Infectious Diseases Unit, Department of Biomedical and Clinical Sciences L. Sacco, Università di Milano, ASST Fatebenefratelli Sacco, 20157 Milan, Italy. andrea.giacomelli@unimi.it
Jesuthasan Nithiya, National Research Council, Institute of Biomedical Technologies, Epidemiology Unit, Via Fratelli Cervi 93, 20090 Segrate (MI), Italy. nithiya.jesuthasan@itb.cnr.it
Maggi Stefania, National Research Council-Neuroscience Institute, Aging Branch, Via Vincenzo Maria Gallucci 16, 35128 Padova, Italy. stefania.maggi@in.cnr.it
Mastroianni Claudio, Public Health and Infectious Disease Department, "Sapienza" University, Piazzale Aldo Moro 1, 00185, Rome, Italy. claudio.mastroianni@uniroma1.it
Molinaro Sabrina, National Research Council, Institute of Clinical Physiology, Via G. Moruzzi 1, 56124 Pisa (PI), Italy. sabrina.molinaro@ifc.cnr.it
Noale Marianna, National Research Council-Neuroscience Institute, Aging Branch, Via Vincenzo Maria Gallucci 16, 35128 Padova, Italy. marianna.noale@in.cnr.it
Pagani Gabriele, Infectious Diseases Unit, Department of Biomedical and Clinical Sciences L. Sacco, Università di Milano, ASST Fatebenefratelli Sacco, 20157 Milan, Italy. gabriele.pagani@unimi.it
Pedone Claudio, Unit of Geriatrics, Department of Medicine, Biomedical Campus of Rome, via Alvaro del Portillo, 21, 00128 Rome, Italy. claudio.pedone@gmail.com
Pettenati Carla, National Research Council, Institute of Biomedical Technologies, Via Fratelli Cervi 93, 20090 Segrate (MI), Italy. cpettenati@me.com
Prinelli Federica, National Research Council, Institute of Biomedical Technologies, Epidemiology Unit, Via Fratelli Cervi 93, 20090 Segrate (MI), Italy. federica.prinelli@itb.cnr.it
Rusconi Stefano, Infectious Diseases Unit, Department of Biomedical and Clinical Sciences L. Sacco, Università di Milano, ASST Fatebenefratelli Sacco, 20157 Milan, Italy. stefano.rusconi@unimi.it
Sojic Aleksandra, National Research Council, Institute of Biomedical Technologies, Epidemiology Unit, Via Fratelli Cervi 93, 20090 Segrate (MI), Italy. aleksandra.sojic@itb.cnr.it

Tavio Marcello, Division of Infectious Diseases, Azienda Ospedaliero Universitaria Ospedali Riuniti, Via Conca 71, Torrette, Ancona, Italy. marcello.tavio@ospedaliriuniti.marche.it
Trevisan Caterina, Geriatric Unit, Department of Medicine (DIMED), University of Padova, Via
Giustiniani 2, 35128 Padova, Italy; National Research Council-Neuroscience Institute, Aging Branch, Via Vincenzo Maria Gallucci 16, 35128 Padova, Italy. caterina.trevisan.5@studenti.unipd.it

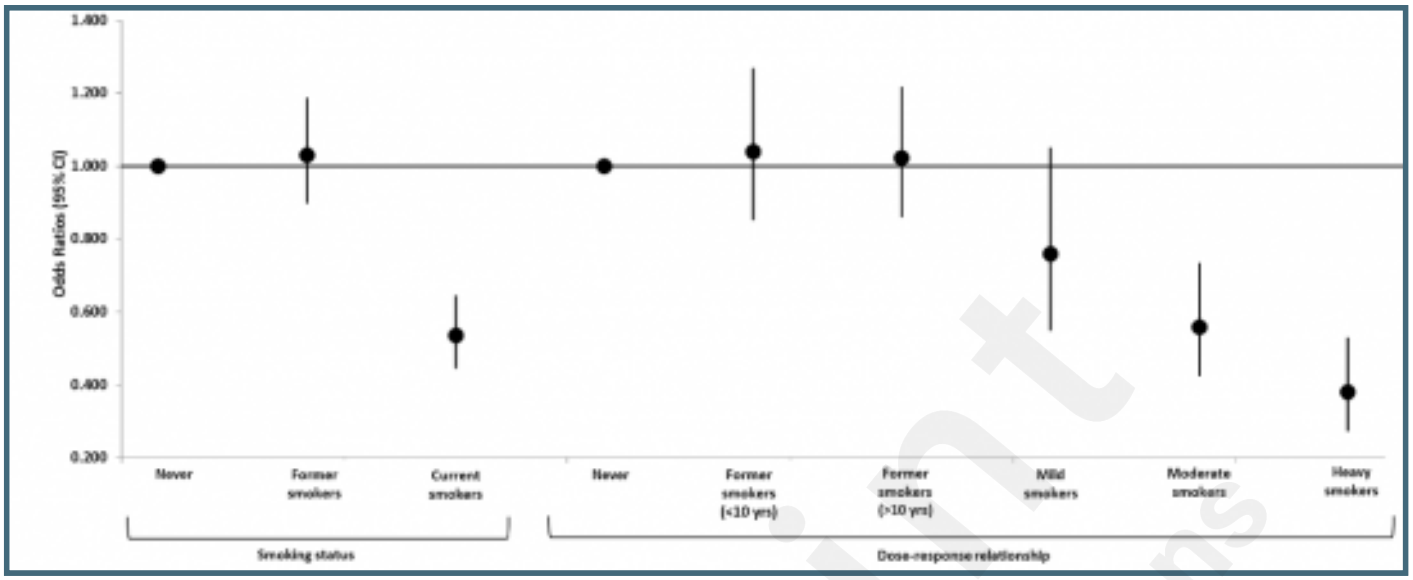
Supplementary Files

Figures

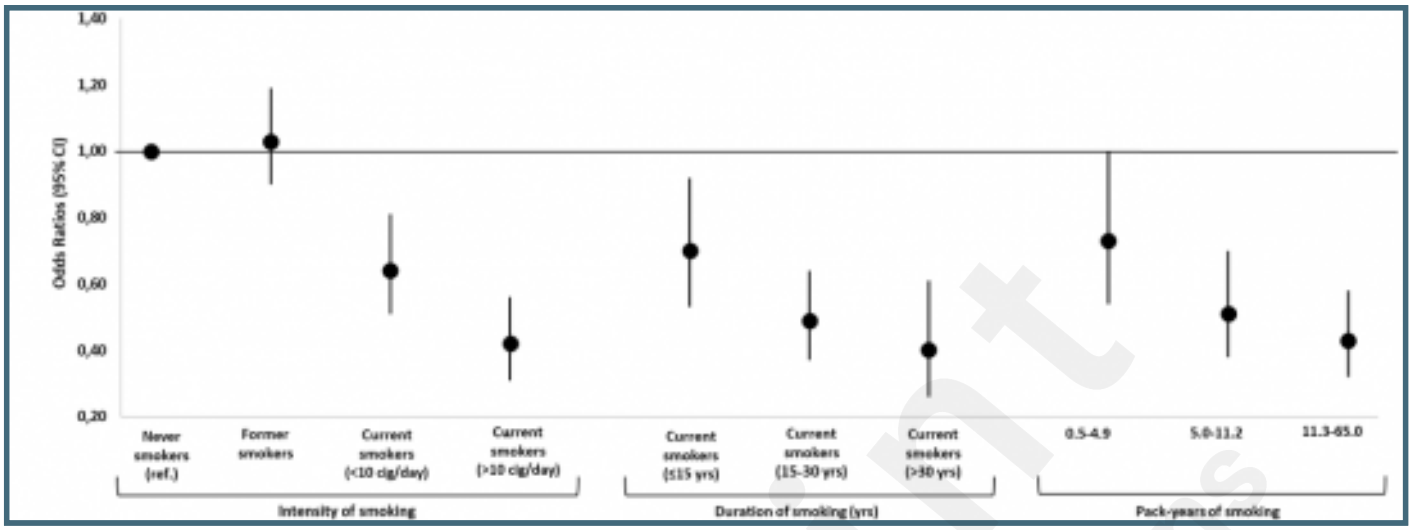
Flow-chart of the study population.



Adjusted odds ratios° and relative 95%CI for smoking status and intensity and duration (N=6,857).



Adjusted odds ratios* for positive SARS-CoV-2 test by smoke-related variables (intensity, duration, and pack-years of smoking) (N=6,857).



Multimedia Appendixes

Annex 1.
URL: https://asset.jmir.pub/assets/d058dd662634915635a6bc28084a24b9.docx

Supplementary file_cleaned.
URL: https://asset.jmir.pub/assets/a304ae236627bb5f78d7e647131cf544.docx

CONSORT (or other) checklists

STROBEchecklist_27091.
URL: https://asset.jmir.pub/assets/6883aa8b839e4a312369b021fbe30dd7.pdf