BMJ Open

Characterization of asymptomatic patients and efficacy of preventive measures of SARS-Cov-2 infection in a large population of the Southern Italy, a cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-043112
Article Type:	Original research
Date Submitted by the Author:	24-Jul-2020
Complete List of Authors:	Masarone, Mario; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Vaccaro, Emilia; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Molecolar Biology Unit Sciorio, Roberta; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Torre, Pietro; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Della Vecchia, Antonio; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Hospital Health Direction Aglitti, Andrea; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Caliulo, Rita; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Molecolar Biology Unit Borrelli, Anna; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Hospital Health Direction Persico, M. ; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit
Keywords:	COVID-19, Epidemiology < INFECTIOUS DISEASES, VIROLOGY

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Title: Characterization of asymptomatic patients and efficacy of preventive measures of SARS-Cov-2 infection in a large population of the Southern Italy, a cohort study.

Running title: SARS-Cov-2 in Southern Italy, a cohort study

Authors: Mario Masarone MD PhD[1], Emilia Vaccaro[2] PhD, Roberta Sciorio MD [1], Pietro Torre MD[1], Antonio Della Vecchia MD[3], Andrea Aglitti MD[1], Rita Caliulo PhD[2], Anna Borrelli MD[3], Marcello Persico MD[1]

Affiliations:

[1]Internal Medicine and Hepatology Unit; [2] Molecolar Biology Unit; [3] Hospital Health Direction, University Hospital San Giovanni di Dio e Ruggi d'Aragona, University of Salerno, Salerno. Italy.

Electronic Word Count: 3168

Number of tables: 3

Number of figures: 2

Corresponding Author:

Marcello Persico M.D.

Internal Medicine and Hepatology Unit,

PO G. DaProcida, AOU-San Giovanni e RuggiD'Aragona, Department of Medicine and Surgery

University of Salerno, Italy.

Via Salvatore Calenda 162

84126 Salerno, Italy

E-mail: mpersico@unisa.it

Tel: +39 089 674331

Financial support: this study didn't receive any specific funding

Keywords: SARS CoV2; COVID-19 Pandemic; Screening program, Nasopharingeal Swabs

List of abbreviations: NS: Nasopharingeal swabs; COVID-19: Coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; RT PCR: real-time Polymerase Chain Reaction.

Conflict of interests: the authors declare no conflict of interests for the present work

Summary

What is already known?

SARS-CoV2 pandemic has infected millions of people and caused more than 600.000 deaths worldwide, to date. Even if "lockdown measures" have been taken worldwide, there are several doubts about the prevalence and the importance of asymptomatic carriers in the spreading of the infection, as well as the efficacy of such measures to prevent it.

What are the new findings? Here **we** report the results of the SARS-Cov2 screening activities of a Province of the Southern Italy, to provide data on the COVID-19 epidemic and the burden of asymptomatic subjects.

What do the new findings imply? The combination of social distancing together with the systematic screening of close contacts of COVID-19 positive symptomatic subjects seemed to be an efficacious approach to limit the epidemic spreading.

Abstract

Background: SARS-CoV2 pandemic has infected millions of people and caused more than 600.000 deaths worldwide, to date. Several doubts arise about the importance of asymptomatic carriers for the virus diffusion. During the epidemic outbreak in Italy a large screening with nasopharyngeal swabs (NS) was performed in those who were "suspect" to be infected. **Aims:** To report the results of the SARS-Cov2 screening in a Province of the Southern Italy, to provide data on the COVID-19 epidemic and the burden of asymptomatic subjects.

Patients and methods: A retrospective cohort study was set up in all the Province healthcare facilities (12 hospitals and 13 sanitary districts – primary, secondary and tertiary centers) to analyze the results of NS made to all the subjects suspected to be infected with SARS-Cov2, either because presented suggestive symptoms, or "contacts" of positive subjects, or coming from an high prevalence area, or healthcare workers. NS were performed and managed as indicated by international guidelines. The specimens were processed for SARS-CoV2 detection by RT PCR.

Results: A total of 20.789 NS were performed from March 13 to May 15, 2020. Of these, 638(3.14%) resulted positive. Asymptomatic subjects were 75.3% of the positive persons. They were mostly among "contacts" of symptomatic cases (91.3%) and in domiciliary isolation. SARS-Cov2 three genes expressions did not differ between asymptomatic and symptomatic subjects. The strict measures of social distancing led to a continuous decrease of the cases during phase 1.

Conclusions: In a large area of the Southern Italy, 3.14% of the total subjects tested were positive for SARS CoV2. Most of them were asymptomatic (75.3%) and, of these, 91%were "close contacts" of symptomatic subjects. The combination of social distancing together with the systematic screening of close contacts of COVID-19 positive symptomatic subjects seems to be an efficacious approach to limit the epidemic spreading.

Strengths and Limitations

The present study is a retrospective cohort study providing a full picture of the screening activities and finding on the SARS-CoV2 infection during the "lockdown phase" in a province of Southern Italy.

It reports on the prevalence of COVID-19 in the screened population of this province, as well as the clinical and virological findings in the positive subjects, symptomatic and asymptomatic.

The main limitations are represented by its retrospective nature and by the fact that the data presented are on the cohort in study (the subjects undergone to the screening) and not on the whole population of this geographical area.

Introduction

The Coronavirus disease 2019 (COVID-19) is the clinical manifestation of an airborne infection by a *Coronavirus spp virus* which has been named "severe acute respiratory syndrome coronavirus-2" (SARS-CoV2) and was declared a pandemic by the World Health organization (WHO) on march 11 2020 [1]. In Italy, the epidemic outbreak led to adopt a strict lockdown of all the non-essential activities as soon as March 9, 2020. The lockdown "phase 1", which took place from March 9 to May 3, was intended to reduce the spreading of the infection which has caused, to date (June 13, 2020) 236.651 cases, with 34.301 deaths in Italy. From May 3, 2020 the restrictions of Italian citizens circulation were reduced, thanks to the reduction of the epidemic spreading, especially in southern regions, leading to the "phase 2" of the lockdown, even if the "social distancing measures" are still maintained[2].

SARS-Cov2 has infected millions of people and caused more than 500.000 deaths worldwide at the time of the writing. Its epidemiology has been largely investigated in the past few months, with increasing evidences that demonstrated as its clinical manifestations can vary from an asymptomatic upper-respiratory tract infection to a severe acute respiratory syndrome leading to the necessity of intensive care, with an high risk of death from respiratory failure[3]. Many speculations have been made on the epidemiology of the pandemic and, in particular, on the number of the asymptomatic cases and its importance on its spreading across the globe [4-6]. Nevertheless, there are very limited evidences on the burden of asymptomatic carriers, their number and their capacities to spread the infection. For these reasons, here we report the results of the SARS-Cov2 screening activities of a large single Province of a region of Southern Italy (Campania) during the phase 1 and phase 2 lockdown in Italy, with the aims of provide information on the coronavirus epidemic and the burden of asymptomatic infections.

Patients and methods

The present cohort study reports on the SARS-Cov2 infection screening program set-up in the Salerno province of Southern Italy during the so-called "phase 1" of the lockdown period, that was decided by Italian government from march 09 to may 3 2020 in Italy, due to the spreading of the COVID-19 epidemic in Italy. Salerno province is located in Campania region, Southern Italy, and it happens to be the largest Italian province by extension (4 952 km²) and number of municipalities (158), with a total population of about 1 million of inhabitants. As soon as the lockdown was

decided in Italy, the regional government decided for a profound reorganization of the healthcare system to face the emergency [7]. Among all, also a diagnostic service for SARS-Cov2 by mean of nasopharyngeal swabs (NS) analysis was set-up at the main University Hospital, the San Giovanni di Dio e Ruggi d'Aragona Hospital. All the National Healthcare system facilities belonging to the Local Health Company of Salerno (comprehending 12 hospitals, 13 healthcare districts and several territorial services facilities, spanning all over the Salerno territory) performed the NS on a daily basis, sending overnight the stabilized specimens (in universal transport media -UTM) that were collected on-site, to be centrally evaluated [8]

During the lockdown phase , an universal screening was not provided to the entire population and the NS for Covid19 were only performed, as mandated by the central and regional government, for these reasons: symptoms suggestive for an upper respiratory tract syndrome and/or cough and/or fever without any other cause and/or a contact (family members, cohabitants and/or co-workers and/or caregivers) with an infected subject and/or a person coming from high prevalence of infection geographical areas. Moreover, NS were also performed in all the inpatients admitted to hospital (with and without an upper respiratory tract syndrome) and all the healthcare workers of the province.

OS were performed by healthcare professionals (doctors or nurses) that were preliminarily trained to perform the specimen collection with the best procedures, as indicated by WHO [9 10]

All the subjects undergoing the OS were asked to sign an informed consent and to respond a brief questionnaire on their age, sex, address, provenience and the eventual symptoms they had (no symptoms, mild symptoms: flu-like or mild fever or cough or sneezing, Symptoms: a clinical presentation compatible with one of the five clinical presentations of a COVID-19 disease[11] . In case of a patient that was in hospital or in emergency room, the operator who carried out the OS compiled the form with all the necessary data (including symptoms).

Nasopharingeal Swabs analysis for SARS-Cov2 detection

Nasopharyngeal swabs (NS) were collected from all the subjects, and specimens sampled were transferred on universal transport media (UTM, Copan, Mylan, Italy) and managed as indicated by CDC guidelines [12]. The Allplex 2019-nCoV assay (Seegene Inc., Seoul, South Korea) that was demonstrated to be accurate for the confirmation diagnosis of SARS-CoV-2 infection, was used on a Nimbus IVD and Cfx-96™ Real Time PCR automatic extractor (Seegene Inc. Seoul, South Korea) for amplifying three viral targets: the E gene (specific of the subgenus Sarbecovirus), the N and the

RdRP genes (both specifics of the SARS-CoV-2) [13]. Sample displaying at least 2 viral targets for RT-PCR viral targets were considered positive, as previously indicated[13].

The present study was approved by Our local ethics committee (Ethics Committee of Campania Sud: cometicocampaniasud@aslnapoli3sud.it) and conducted in conformity with the ethical guidelines of the 1975 Declaration of Helsinki.

Patients and Public involvement

Due to its retrospective nature, this cohort study did not involve patients and public in the design, or conduct, or reporting, or dissemination plans.

Statistical analysis

All the data collected were analyzed by means of IBM SPSS Statistics for MacIntosh, version 26 (IBM Corp., Armonk, N.Y., USA). The continuous variables were analyzed with parametric or non-parametric tests, when appropriated. In particular, student's t-test and Mann-Whitney were performed for continuous variables, and chi-square test with Yates correction or Fisher-exact tests were used to compare frequencies and categorical variables. Before applying the correct analysis, a Kolgoromov-Smirnov K-S "Goodness of fit" test for normality was performed to assess if there was a normal or not-normal distribution of the continuous variables. Statistical significance was defined when "p<0,05" in a two-tailed test with a 95% confidence interval.

Results

From March 13, 2020 to May 15, 2020, a total of 20789 nasopharyngeal swabs were performed and analyzed. A total of 1097 swabs resulted positive for SARS-Cov2 RNA presence. Of these, 638 out of 20325 (3,14%) referred to newly diagnosed cases of COVID-19 disease, and 464 were secondary samples collected during the follow-up of the firstly positive subjects. Of the 638 firstly positive cases, 624 were those found in the phase 1 (up to May 3, 2020), 4.64% of the 13448 swabs performed in that period of time. Therefore, In the first 11 days of phase 2 (from May 4to may 15, 2020) 14 were the newly positive subjects screened for SARS-Cov2, 0.18% of the 7431swabs performed (Phase 1 vs Phase 2: p<0.0001 OR 25.827 - 95% CI 15.195-43.614).

The main characteristics of the positive patients vs the negative ones are reported in table 1.

The distribution of the positive patients, on the basis of their symptoms during phase 1 is reported in table 2. From these two tables we can find that the so called “contacts” [persons in whom the NS was primarily performed because of their contact with a positive subject (family member, co-worker, caregivers etc.)] were 556. Of them, 428 (76.98%) were asymptomatic, and 128 (23.02%) had symptoms that were not enough to require hospitalization, and therefore were posed in domiciliary isolation. They represented the 68.59% of the total positive patients screened in Salerno province.

As far as symptoms are concerned, all SARS Cov2 positive subjects with symptoms were more often of male gender in respect of asymptomatic ones, but this finding didn’t reach statistical significance (Male sex prevalence: 62.00% vs 53.94%; p:0.084 OR:1.393 – 0.959-2.030 95%CI) and they were significantly older (mean age 54.20 ±20.78 vs 49.99±16.82, p<0.0001). Moreover, in figure 1 is reported the prevalence of symptomatic and asymptomatic carriers of SARS Cov-2 by 20 years age classes. There was a statistical difference in <20 and 21-40 years age classes in which there were more asymptomatic positive subjects (p: 0.022 and 0.048, respectively), and in 41-60 years in which there were more symptomatic patients (p: 0.038).

The total number of NS specimens collected and processed “per-day” are reported in figure 2 (first panel). The highest number of tests performed was on May 04, 2020 (n: 903). In phase 1 the daily mean number of performed tests was 263 per day, however it was lower in the first weeks and higher in the last, because of the efforts in potentiating the service. The total number of positive NS “per day” are reported in the second panel. The highest number of positive NS was on March 26 (57/363 performed, 15,7%), then there was a constant decrease of the number of positive

subjects, reaching 0 on the last four days of the phase 1, despite the total number of performed tests in those day was of 1037.

Of notice, there were 918 patients (6.83% of the total) that, even if repeatedly resulted negative to NS for SARS Cov2, presented themselves with symptoms that were compatible for any of the clinical presentations of COVID 19. These subjects were "contacts" in the majority of cases (68.6%) and the others were either admitted to the hospital ward (15.6%), emergency department (11.9%), or intensive care unit (1.3%) (Table 2).

Finally, we report in table 3, the mean expression [displayed as "cycle threshold" to obtain positive fluorescence in RT-PCR -C(t)] of the three genes of SARS Cov-2 assays (N, E and RdRP) in subjects defined as positive. There was no statistically significant difference between asymptomatic and symptomatic subjects in mean genes expression.

Discussion

Hereby we report on the experience of the healthcare system of a large area of the Southern Italy in facing the Coronavirus Outbreak. As soon as the COVID-19 became epidemic in Italy, and briefly thereafter declared pandemic by WHO, several measures were set up by regional and central government to fight the spreading of it[7]. A profound and quick reorganization of the healthcare system was provided that reshaped the clinical activities with an increase of the capacity of Intensive Care Units, the creation of the so-called "COVID-hospitals", the re-allocation of healthcare professionals to face the emergency and the interruption of all the non-urgent or necessary activities of the healthcare system. Even if the major outbreak interested the northern Italy, the central and southern country were also subjected to the same rules and precautions [14] [15]. Screening for the COVID-19 infection was provided for those who were either symptomatic (with symptoms that were suggestive of the Coronavirus infection) or at high risk of infection (healthcare workers or "contacts" of infected persons). Our University Hospital, a tertiary structure covering the needs of all Salerno province, was identified as one of the centralized Center to perform all the Covid19 related biological tests. In Italy, during the phase 1 of the lockdown (March 9 to May 3 2020), all the commercial and working activities that were considered not necessary (mostly alimentary, pharmaceutics and logistics) were shut down, with the warning to not leave the home without a valid reason. Over a population of almost one million of inhabitants, during phase 1 of lockdown, we registered a total number of cases of SARS-Cov2

positive subjects of 624.Those 624 subjects were the 4.64% of the 13448 OS performed during phase 1. Nevertheless, it has to be pointed out that an universal screening of the population was not provided in Italy at the time, and it is still to be implemented. This is because of a series of factors: first of all, the availability of the commercial tests worldwide and the difficulties in organizing in a short period of time a very large screening activity. Therefore, this fact raised, in the scientific community and also in the public opinion, several concerns, particularly regarding the possibility of transmission of the disease from asymptomatic subjects, as it has been reported since the very beginning of the outbreak[4-6]. In fact, in absence of a systematic screening of the entire population, several doubts can arise on the safety of the relaxation of the lockdown measures, because of the lack of information on the prevalence of asymptomatic infection, as well as on its importance on the diffusion of the pandemic. At the time of the writing, there are no robust data on these points, even if limited evidences seem to indicate that it is crucial to screen not the entire population but rather the high-risk populations and the close contacts of the identified cases [6]. In this way, our data may represent an useful tool for the attempts to outline the epidemiology of such pandemic. In fact, as mandated by Italian regulation, the screening we performed was exactly that suggested by literature: apart from those who were admitted to hospitals' emergency departments with a syndrome that was suggestive for COVID-19, all the other OS were performed precisely on close contacts of positive cases (family member, co-habitants, co-workers, caregivers etc.) and in the highest risk population of the lockdown phase 1, represented by the healthcare workers for obvious reasons. As it can be deduced from the results of the present screening, the majority of SARS-Cov2 positive subjects found during phase were asymptomatic (470/624, 75.3%) and, of these, the vast majority was represented by close contacts of symptomatic cases (428/470, 91.3%) representing the 3.2% of the 13448 OS performed (see also table 1 and 2). If this finding may be, at first sight, interpreted as an alarming point, it is also to be noticed that the vast majority of positive cases were found precisely only on close contacts of the symptomatic cases (556/624, 89.1%). In fact, only 5 subjects (0.4%) resulted positive among the 1239 healthcare workers weekly screened in the same period of time, demonstrating that, among such an high risk population, the infection was marginal. This difference may be accounted by for the correct use of the personal protection equipment (PPE) by the healthcare workers, confirming, in particular, the usefulness of facemasks as recently reported [16 17]. However, the significant difference between the high prevalence of asymptomatic SARS Cov2 positive subjects between "contacts" of infected cases in respect of the low one in healthcare workers (and

occasionally voluntaries)suggests that the possibility of getting incidentally infected among the general population has to be rare if (and when)correct and rapid measures are applied in order to discover and appropriately follow-up, with containment measures, positive subjects and their contacts.

The fact that the policy to primarily screen the close contacts of the positive subjects has been a successful approach may be demonstrated by the rapid decline of the number of positive subjects found over time, as depicted in figure 2, in which the total number of NS performed (panel A) and the positive ones (panel B) are reported. In fact, even if every day there was an increasing number of NS performed, there was also a constant decrease of the number of infected subjects, with only 14 cases out of 7341 NS found after the phase 1 ending. This in our opinion demonstrates that social distancing measures (in particular the mandatory use of face masks in all the population)and the correct use of PPE by healthcare professionals, together with the systematic screening of close contacts, represented good measures to reduce the spreading of the infection in the province of Salerno.

Another critical point, that animated the scientific debate on SARS CoV2 epidemics in the last times, is the importance of viral load on defining symptoms and infectiousness of the subjects[18-21].In fact, on this point there are conflicting data, with some of the evidences pointing out as the high viral load of asymptomatic subjects may be accounted for their infectiousness, and therefore their dangerousness[21 22]. On the contrary other documents reported that in asymptomatic infected subjects there were lower viral loads, and this fact may account for the lack of symptoms [23-28]. On the other hand, the World Health Organization pointed out, in a recent “situation report” on the pandemic, that there are few evidences of asymptomatic transmission (Covid-19 Situation Report #79)[29] and that: “Available evidence from contact tracing reported by countries suggests that asymptomatically infected individuals are much less likely to transmit the virus than those who develop symptoms” [30]. Our findings revealed that there were no statistically significant differences between symptomatic and asymptomatic SARS CoV2 virus carriers on regards of viral load (table 3). The only statistically significant difference was in age distribution, being the asymptomatic subjects significantly younger, and the symptomatic subjects particularly represented in age class 41-60 years (Figure 1). Moreover, symptomatic subjects were more frequently of male sex. Our data confirmed some of the characteristics of asymptomatic carriers reported by others , but showed no relation with viral load[21-25 27] . Likely, the lack of symptoms (no cough, no sneezing, no dyspnea) together with a “normal” health condition in these subjects who

do not require any form of “caregiving” (by familiars, cohabitants or healthcare personnel) and then avoiding any closer contact together with the correct measures of social distancing, could be “responsible” for the lower infectiousness.

Finally, it has to be discussed another interesting point raising from our data, the prevalence of hospitalized subjects that underwent to NS because of symptoms that were suggestive of a COVID-19 and were found negative. As per protocol, a patient who presented himself with symptoms strongly suggestive for COVID-19 and found negative at the first NS, was isolated in a “grey zone” prepared on purpose to accommodate unconfirmed cases then subjected to further confirmation tests, to overcome the risk of false negatives. Nevertheless, in our cohort there were 918 subjects that had symptoms compatible with COVID-19 and resulted “confirmed negative”. They represented the 6.82% of all NS and were mostly “contacts” (68.6%) or hospitalized patients (28.75%). In particular, the last category is the most interesting: among those who were hospitalized and had symptoms suggestive of COVID-19, is very likely that an infectious disease other than SARS COv2 was in place. This finding has to be taken into account in the future when, hopefully, a vaccine will be available. In fact, it should be useful of “taking the chance” to perform other vaccinations together with that for COVID-19, particularly for airborne infections (as influenza viruses).

There are some limitations to this study. First of all, as already mentioned above, this was not an universal screening of the population and, therefore, we cannot affirm without any doubt that those that were screened were the only infected in the geographical area tested. Nevertheless, it has to be noticed that also the symptomatic patients reduced drastically their presence over time, being this an indirect marker of the slowing of the epidemic

Conclusions

The combination of social distancing together with the systematic screening of close contacts of COVID-19 positive symptomatic subjects seems to be an efficacious approach to contain SARS-Cov2 epidemic spreading suggesting then the rare eventuality of being occasionally infected by positive asymptomatic subjects.

Tables

	SARS-CoV2 Positive	SARS-CoV2 Negative	p
n (%)	624 (4.64%)	12824 (95.36%)	-
AGE (mean ± SD)	51.93 (±20.33)	50.44 (±17.1)	**0.016**
SEX (M/F) %	55.8/44.2	55.9/44.1	0.946 [OR 0.994 (0.846-1.169)]
Symptoms			
Asymptomatic	470 (75.3%)	11651 (90.9%)	**0.0001**
Symptomatic	150 (24.0%)	918 (7.2%)	
Mild Symptoms	4 (0.6%)	255 (2.0%)	
Placing			
Contacts	556 (89.1%)	9944 (77.5%)	**0.028**
Inpatients	25 (4.0%)	1126 (8.8%)	
ED patients	36 (5.8%)	469 (3.7%)	
ICU Patients	2 (0.3%)	46 (0.4%)	
Occupational Health surveillance	5 (0.8%)	1239 (9.7%)	

Table 1: Characteristics of "lockdown phase 1" patients undergone to SARS-CoV2 screening with Naso-pharingeal swabs

	SARS-Cov2 Positive	SARS-Cov2 Negative	p
Asymptomatic	**470/624 (75.3%)**	**11651/12824**(90.9%)	**<0.0001**
Contacts (domiciliary isolation)	428 (91.3%)	9236 (79.3%)	
Inpatients	14 (3.0%)	850 (7.3%)	
ED patients	21 (4.5%)	321 (2.8%)	
ICU Patients	1 (0.2%)	31 (0.3%)	
Occupational Health surveillance	5 (1.1)	1214 (10.4%)	
Symptomatic	**150/624 (24.0%)**	**918/12824**(7.2%)	**<0.0001**
Contacts (domiciliary isolation)	126 (84.0%)	630 (68.6%)	
Inpatients	9 (6.0%)	143 (15.6%)	
ED	14 (9.3%)	109 (11.9%)	
ICU	1 (0.7%)	12 (1.3%)	
Occupational Health surveillance	0	24 (2.6%)	
Mild Symptoms	**4/624 (0.6%)**	**255/12824**(2.0%)	**0.853**
Contacts (domiciliary isolation)	2 (50.0%)	79 (30.9%)	
Inpatients	1 (25.0%)	134 (52.3%)	
ED patients	1 (25.0%)	39 (15.2%)	
ICU patients	0	3 (1.2%)	
Occupational Health surveillance	0	1 (0.4%)	

Table 2: Prevalence of Symptomatic and asymptomatic subjects divided by provenience of NS.

	Overall	Symptomatic	Asymptomatic	p
E gene Ct	23.9040 (±4.28123)	22.2785 (±4.97021)	22.8605 (±4.88053)	0.486
RdRP Ct	25.1360 (±4.42040)	23.5495 (±4.75518)	24.1136 (±4.69109)	0.583
N Ct	26.4580 (±4.15840)	24.7405 (±4.55574)	25.4944 (±4.72231)	0.448

Table 3: overall gene expression [presented as C(t)] of the three genes of SARS-CoV2 as detected by RT-PCR in positive patients and difference between Symptomatic and Asymptomatic subjects (mean ± SD).

Legend to figures:

Figure 1: prevalence of symptomatic and asymptomatic subjects among SARS Cov2 positive subjects, by age classes.

Figure 2: total number of executed and positive Swabs per day in the Salerno Province from March 03 to May 15, 2020

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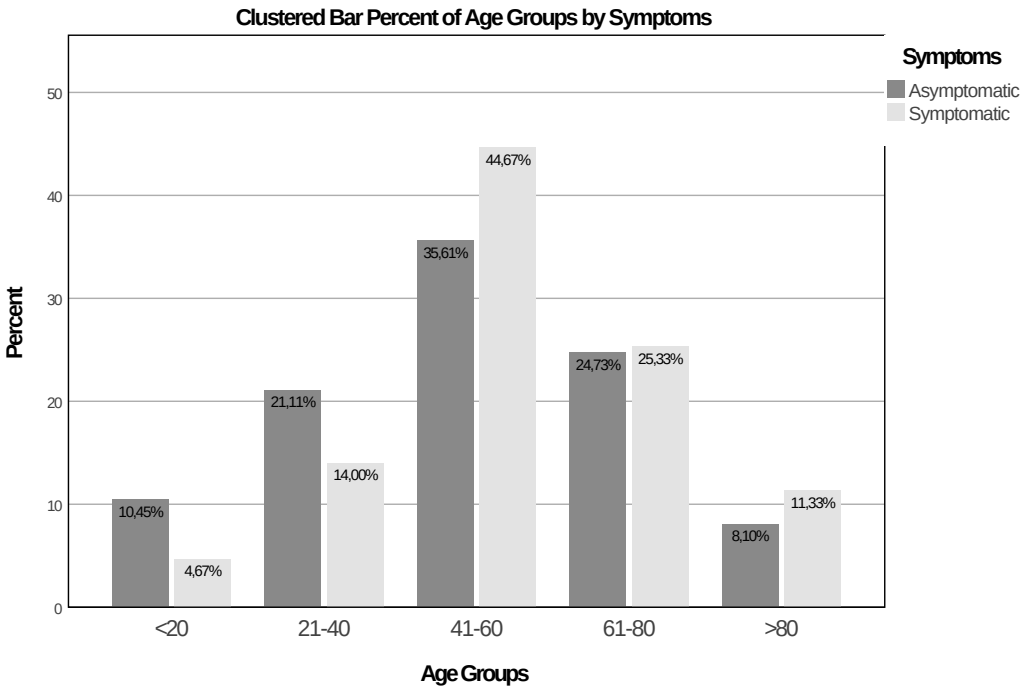

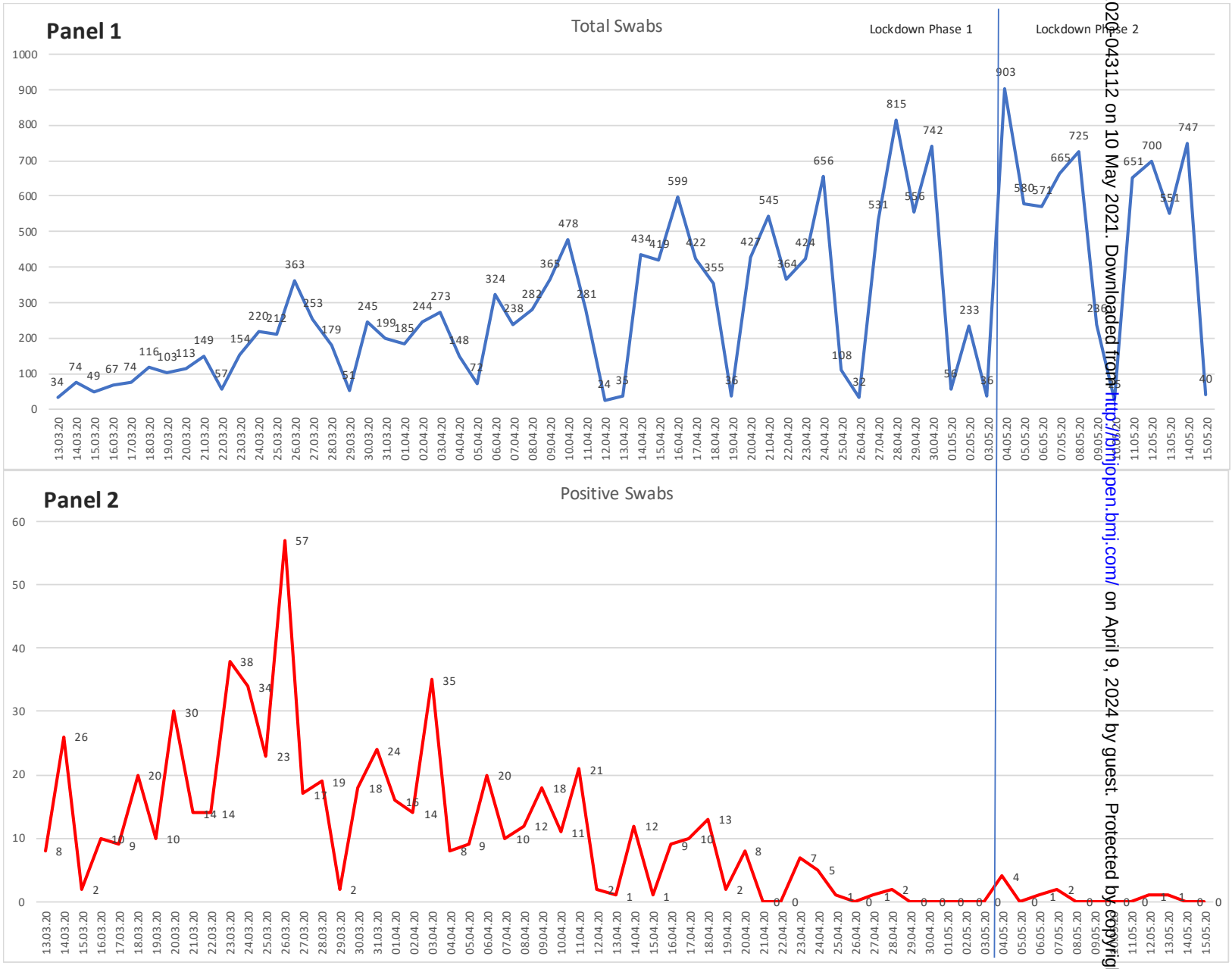

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	na
		(*e*) Describe any sensitivity analyses	na
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	na
		(c) Consider use of a flow diagram	na
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	na
		(c) Summarise follow-up time (eg, average and total amount)	na
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8 8 na
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for exposed and unexposed groups.

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

BMJ Open

Characterization of asymptomatic patients and efficacy of preventive measures of SARS-Cov-2 infection in a large population of the Southern Italy, a cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-043112.R1
Article Type:	Original research
Date Submitted by the Author:	13-Feb-2021
Complete List of Authors:	Masarone, Mario; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Vaccaro, Emilia; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Molecolar Biology Unit Sciorio, Roberta; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Torre, Pietro; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Della Vecchia, Antonio; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Hospital Health Direction Aglitti, Andrea; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Caliulo, Rita; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Molecolar Biology Unit Borrelli, Anna; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Hospital Health Direction Persico, M. ; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Health policy, Infectious diseases
Keywords:	COVID-19, Epidemiology < INFECTIOUS DISEASES, VIROLOGY

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Title: Characterization of asymptomatic patients and efficacy of preventive measures of SARS-Cov-2 infection in a large population of the Southern Italy, a cohort study.

Running title: SARS-Cov-2 in Southern Italy, a cohort study

Authors: Mario Masarone MD PhD[1], Emilia Vaccaro[2] PhD, Roberta Sciorio MD [1], Pietro Torre MD[1], Antonio Della Vecchia MD[3], Andrea Aglitti MD[1], Rita Caliulo PhD[2], Anna Borrelli MD[3], Marcello Persico MD[1]

Affiliations:

[1]Internal Medicine and Hepatology Unit; [2] Molecolar Biology Unit; [3] Hospital Health Direction, University Hospital San Giovanni di Dio e Ruggi d'Aragona, University of Salerno, Salerno. Italy.

Electronic Word Count: 3560

Number of tables: 3

Number of figures: 3

Corresponding Author:
Marcello Persico M.D.
Internal Medicine and Hepatology Unit,
AOU-San Giovanni e Ruggi D'Aragona, Department of Medicine and Surgery
University of Salerno, Italy.
Via Salvador Allende
84081, Baronissi (Salerno), Italy
E-mail: mpersico@unisa.it
Tel: +39 338 3338695

Keywords: SARS CoV2; COVID-19 Pandemic; Screening program, Nasopharingeal Swabs

List of abbreviations: NS: Nasopharingeal swabs; COVID-19: Coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; RT PCR: real-time Polymerase Chain Reaction.

Abstract

Background: SARS-CoV2 pandemic has infected millions of people and caused more than 2.30 millions deaths worldwide, to date. Several doubts arise about the importance of asymptomatic carriers for the virus diffusion. During the first epidemic outbreak in Italy a large screening with nasopharyngeal swabs (NS) was performed in those who were "suspect" to be infected. **Aims:** To report the results of the SARS-Cov2 screening in a Province of the Southern Italy, to provide data on the COVID-19 epidemic and the burden of asymptomatic subjects.

Patients and methods: A retrospective cohort study was set up in all the Province healthcare facilities (12 hospitals and 13 sanitary districts – primary, secondary and tertiary centers) to analyze the results of NS made to all the subjects suspected to be infected with SARS-Cov2, either because presented suggestive symptoms, or "contacts" of positive subjects, or coming from an high prevalence area, or healthcare workers. NS were performed and managed as indicated by international guidelines. The specimens were processed for SARS-CoV2 detection by RT PCR.

Results: A total of 20.789 NS were performed from March 13 to May 15, 2020. Of these, 638(3.14%) resulted positive. Asymptomatic subjects were 75.3% of the positive persons. They were mostly among "contacts" of symptomatic cases (91.3%) and in domiciliary isolation. SARS-Cov2 three genes expressions did not differ between asymptomatic and symptomatic subjects. The strict measures of social distancing led to a continuous decrease of the cases during phase 1.

Conclusions: In a large area of the Southern Italy, 3.14% of the total subjects tested were positive for SARS CoV2. Most of them were asymptomatic (75.3%) and, of these, 91%were "close contacts" of symptomatic subjects. The combination of social distancing together with the systematic screening of close contacts of COVID-19 positive symptomatic subjects seems to be an efficacious approach to limit the epidemic spreading.

Strengths and Limitations

The study here reports on a retrospective cohort of SARS-Cov2 screened patients during the "first wave" of pandemic in Southern Italy.

A total of 20.789 naso-pharyngeal swabs for SARS-Cov2 RT-PCR detection was analyzed.

The cohort was composed by symptomatic subjects, asymptomatic contacts of positive subjects, subjects coming from high prevalence areas and healthcare workers.

Also age, sex and clinical presentations were collected among the whole cohort.

The main limitations of the paper are that the data presented are on the study cohort and not on the whole population and its retrospective nature.

Introduction

The Coronavirus disease 2019 (COVID-19) is the clinical manifestation of an airborne infection by a *Coronavirus spp virus* which has been named "severe acute respiratory syndrome coronavirus-2" (SARS-CoV2) and was declared a pandemic by the World Health organization (WHO) on march 11 2020 [1]. In Italy, the epidemic outbreak led to adopt a strict lockdown of all the non-essential activities as soon as March 9, 2020. The lockdown "phase 1", which took place from March 9 to May 3 2020, was intended to reduce the spreading of the infection which has caused, to date (February, 2021) 2,650 million of cases, with 92.001 deaths in Italy. From May 3, 2020 the restrictions of Italian citizens circulation were reduced, thanks to the reduction of the epidemic spreading, especially in southern regions, leading to the "phase 2" of the lockdown, even if the "social distancing measures" were still maintained[2].

SARS-Cov2 has infected millions of people and caused more than 2,34 millions of deaths worldwide at the time of the writing. Its epidemiology has been largely investigated, with increasing evidences that demonstrated as its clinical manifestations can vary from an asymptomatic upper-respiratory tract infection to a severe acute respiratory syndrome leading to the necessity of intensive care, with an high risk of death from respiratory failure[3]. Many speculations have been made on the epidemiology of the pandemic and, in particular, on the number of the asymptomatic cases and its importance on its spreading across the globe [4-6]. Nevertheless, there are limited data on the burden of asymptomatic carriers, their number and their capacity to spread the infection. For these reasons, here we report the results of the SARS-Cov2 screening activities of a large single Province of a region of Southern Italy (Campania) during the phase 1 and phase 2 lockdown in Italy, with the aim of providing information on the coronavirus epidemic and the burden of asymptomatic infections.

Patients and methods

The present cohort study reports on the SARS-Cov2 infection screening program set-up in the Salerno province of Southern Italy during the so-called "phase 1" of the lockdown period, that was decided by Italian government from march 09 to may 3 2020 in Italy, due to the spreading of the COVID-19 epidemic in Italy. Salerno province is located in Campania region, Southern Italy, and it happens to be the largest Italian province by extension (4 952 km²) and number of municipalities (158), with a total population of about 1 million of inhabitants. As soon as the lockdown was

instituted in Italy, the regional government decided for a profound reorganization of the healthcare system to face the emergency [7]. Among all, a diagnostic service for SARS-Cov2 by mean of nasopharyngeal swabs (NS) analysis was set-up at the main University Hospital, the San Giovanni di Dio e Ruggi d'Aragona Hospital. All the National Healthcare system facilities belonging to the Local Health Company of Salerno (comprehending 12 hospitals, 13 healthcare districts and several territorial services facilities, spanning all over the Salerno territory) performed the NS on a daily basis, sending overnight the stabilized specimens (in universal transport media -UTM) that were collected on-site, to be centrally evaluated [8]

During this phase , an universal screening was not provided to the entire population and the NS for Covid19 were only performed, as mandated by the central and regional government, for these reasons: symptoms suggestive for an upper respiratory tract syndrome and/or cough and/or fever without any other cause and/or a contact (family members, cohabitants and/or co-workers and/or caregivers) with an infected subject and/or a person coming from geographical areas with high prevalence of infection (i.e. northern Italy regions). Moreover, NS were also performed in all the inpatients admitted to hospital (with and without an upper respiratory tract syndrome) and all the healthcare workers of the province.

OS were performed by healthcare professionals (doctors or nurses) that were preliminarily trained to perform the specimen collection with the best procedures, as indicated by WHO [9 10].

All the subjects undergoing the OS were asked to sign an informed consent and to respond a brief questionnaire on their age, sex, address, provenience and the eventual symptoms they had (no symptoms; mild symptoms: flu-like or mild fever or cough or sneezing; symptoms: a clinical presentation compatible with one of the five clinical presentations of a COVID-19 disease)[11] . In case of a patient that was in hospital or in emergency room, the operator who carried out the OS compiled the form with all the necessary data (including symptoms).

Nasopharyngeal Swabs analysis for SARS-Cov2 detection

Nasopharyngeal swabs (NS) were collected from all the subjects, the sampled specimens were transferred on universal transport media (UTM, Copan, Mylan, Italy) and managed as indicated by CDC guidelines [12]. The Allplex 2019-nCoV assay (Seegene Inc., Seoul, South Korea) that was demonstrated to be accurate for the confirmation diagnosis of SARS-CoV-2 infection, was used on a Nimbus IVD and Cfx-96™ Real Time PCR automatic extractor (Seegene Inc. Seoul, South Korea) for amplifying three viral targets: the E gene (specific of the subgenus Sarbecovirus), the N and the

RdRP genes (both specifics of the SARS-CoV-2) [13]. Samples displaying at least 2 viral targets for RT-PCR viral targets were considered positive, as previously indicated[13].

The present study was approved by our local ethics committee (Ethics Committee of Campania Sud: cometicocampaniasud@aslnapoli3sud.it) and conducted in conformity with the ethical guidelines of the 1975 Declaration of Helsinki.

Patients and Public involvement

Due to its retrospective nature, this cohort study did not involve patients and public in the design, or conduct, or reporting, or dissemination plans.

Statistical analysis

All the data collected were analyzed by means of IBM SPSS Statistics for MacIntosh, version 26 (IBM Corp., Armonk, N.Y., USA). The continuous variables were analyzed with parametric or non-parametric tests, when appropriated. In particular, student's t-test and Mann-Whitney were performed for continuous variables, and chi-square test with Yates correction or Fisher-exact tests were used to compare frequencies and categorical variables. Before applying the correct analysis, a Kolgoromov-Smirnov K-S "Goodness of fit" test for normality was performed to assess if there was a normal or not-normal distribution of the continuous variables. Statistical significance was defined when "p<0,05" in a two-tailed test with a 95% confidence interval.

Results

From March 13, 2020 to May 15, 2020, a total of 20789 nasopharyngeal swabs were performed and analyzed. A total of 1097 swabs resulted positive for SARS-Cov2 RNA presence. Of these, 638 out of 20325 (3.14%) referred to newly diagnosed cases of COVID-19 disease, and 464 were secondary samples collected during the follow-up of the firstly positive subjects. Of the 638 firstly positive cases, 624 were those found in the phase 1 (up to May 3, 2020), 4.64% of the 13448 swabs performed in that period of time. Therefore, In the first 11 days of phase 2 (from May 4to may 15, 2020) the newly positive subjects screened for SARS-Cov2 were 14, 0.18% of the 7431 swabs performed (Phase 1 vs Phase 2: p<0.0001 OR 25.827 - 95% CI 15.195-43.614).

The main characteristics of the positive patients vs the negative ones are reported in table 1.

The distribution of the patients found to be positive during phase 1, on the basis of their symptoms, is reported in table 2. From these two tables we can find that the so called “contacts” [persons in whom the NS was primarily performed because of their contact with a positive subject (family member, co-worker, caregivers etc.)] were 556. Of them, 428 (76.98%) were asymptomatic, and 128 (23.02%) had symptoms that were not enough to require hospitalization, and therefore were posed in domiciliary isolation. They represented the 68.59% of the total positive patients screened in Salerno province.

As far as symptoms are concerned, all SARS Cov2 positive subjects with symptoms were more often of male gender in respect of asymptomatic ones, but this finding didn’t reach statistical significance (Male sex prevalence: 62.00% vs 53.94%; p:0.084 OR:1.393 – 0.959-2.030 95%CI) and they were significantly older (mean age 54.20 ±20.78 vs 49.99±16.82, p<0.0001). Moreover, in figure 1 is reported the prevalence of symptomatic and asymptomatic carriers of SARS-CoV2 by age classes. There was a statistical difference in <20 and 21-40 years age classes in which there were more asymptomatic positive subjects (p: 0.022 and 0.048, respectively), and in 41-60 years in which there were more symptomatic patients (p: 0.038). Finally, when analyzing the prevalence of SARS-CoV2 positivity among symptomatic and asymptomatic subjects we found that the prevalence of positive NS among symptomatic patients was of 14.04% (150/1068), whereas it was of 3.82% (470/12275) in asymptomatic subjects [RR: 3.668 (3.070-4.372 95% CI) p<0.0001].

The total number of NS specimens collected and processed “per-day” are reported in figure 2 (Panel A). The highest number of tests performed was on May 04, 2020 (n: 903). In phase 1 the daily mean number of performed tests was 263 per day, however it was lower in the first weeks

and higher in the last, because of the efforts in potentiating the service. The percentage of positive NS "per day" are reported in panel B of figure 2. The highest number of positive NS was on March 26 (57/363 performed, 15,7%), then there was a constant decrease of the total of positive subjects, reaching 0 on the last four days of the phase 1, despite the number of performed tests in those day was of 1037. Moreover, as it can be seen from panel B of figure 2, the percentage of positive patients decreased constantly over time, in an inverse trend in respect of the increase of the total number of performed tests. Finally, in panel C of the same figure is reported the daily percentage of symptomatic positive patients that showed a decreasing trend over time as well.

Of notice, there were 918 patients (6.83% of the total) that, even if repeatedly resulted negative to NS for SARS-CoV2, presented themselves with symptoms that were compatible for any of the clinical presentations of COVID 19. These subjects were "contacts" in the majority of cases (68.6%) and the others were either admitted to the hospital ward (15.6%), emergency department (11.9%), or intensive care unit (1.3%) (Table 2).

Finally, we report the mean expression [displayed as "cycle threshold" to obtain positive fluorescence in RT-PCR -C(t)] of the three genes of SARS Cov-2 assays (N, E and RdRP) in subjects defined as positive. There was no statistically significant difference in mean genes expressions between asymptomatic and symptomatic subjects (table 3). However, when grouped in four age classes (<25; 26-50; 51-75 and >75 years of age), the positive subjects showed a statistically higher expression for RdRP and N genes, in >75 years patients in respect of <25 years subjects (p:0.036 and 0.032, respectively), see Figure 3.

Discussion

Hereby we report on the experience of the healthcare system of a large area of the Southern Italy in facing the Coronavirus Outbreak. As soon as the COVID-19 became epidemic in Italy, several measures were set up to fight the spreading of it[7]. A profound and quick reorganization of the healthcare system was provided that reshaped the clinical activities with an increase of the capacity of Intensive Care Units, the creation of the so-called "COVID-hospitals", the re-allocation of healthcare professionals to face the emergency and the interruption of all the non-urgent or necessary activities of the healthcare system. Even if the major outbreak interested the northern Italy, the central and southern regions of the country were also subjected to the same rules and precautions [14 15]. A screening for the COVID-19 infection was provided for those who were either

symptomatic or at high risk of infection (see methods). Our University Hospital was identified as one of the centralized centers dedicated to the execution of the Covid19 related biological tests. Moreover, during the phase 1 of the lockdown, also all the commercial and working activities that were considered not necessary (mostly alimentary, pharmaceutics and logistics) were shut down in Italy, with the warning to not leave the home without a valid reason. During phase 1 of the lockdown, over a population of almost one million of inhabitants of our province, we registered a total number of cases of SARS-Cov2 positive subjects of 624, the 4.64% of the 13448 OS performed. However, to correctly interpret this data, it has to be pointed out that an universal screening of the population was not provided in Italy at the time, and it is still to be implemented. This was because of a series of factors, such as the scarce availability of commercial tests worldwide and the difficulties in organizing in a short period of time a very large screening activity. Therefore, this fact raised several concerns in the scientific community and in the public opinion about the COVID 19 epidemiology, particularly regarding the possibility of transmission of the disease from asymptomatic subjects, as it has been reported since the very beginning of the outbreak[4-6]. In fact, in absence of a systematic screening of the entire population, several doubts can arise on the safety of the relaxation of the lockdown measures, because of the lack of information on the prevalence of asymptomatic infection, as well as on its importance on the diffusion of the pandemic. At the time of the writing, there are no robust data on these points, even if limited evidences seem to indicate that it is crucial to screen not the entire population but rather the high-risk populations and the close contacts of the identified cases [6]. In this way, our data can represent an useful tool for the stakeholders and the medical governances, in Italy and Europe, that are attempting to outline the epidemiology of such pandemic, in order to find a correct way to balance the needs of the recovery of normal social and commercial activities with the safety of the population. This is particularly crucial in the actual phase, when the vaccine programs are beginning, in order to avoid excessive measures in both directions and give the time for carrying out the vaccination campaigns. In fact, as mandated by Italian regulation, our screening was performed exactly as suggested by literature: apart from those who were admitted to hospitals' emergency departments with a syndrome that was suspect for COVID-19, all the other NS were performed precisely on close contacts of positive cases (family member, co-habitants, co-workers, caregivers etc.) and in the highest risk population of the lockdown phase 1, represented by the healthcare workers for obvious reasons. As it can be deduced from the results of the present screening, the majority of SARS-Cov2 positive subjects found during phase were

asymptomatic (470/624, 75.3%) and, of these, the vast majority was represented by close contacts of symptomatic cases (428/470, 91.3%) representing the 3.2% of the 13448 OS performed (see also table 1 and 2). If this finding may be, at first sight, interpreted as an alarming point, it is also to be noticed that the vast majority of positive cases were found precisely only on close contacts of the symptomatic cases (556/624, 89.1%). Moreover, when analyzing the prevalence of positivity among symptomatic and asymptomatic people, we found that it was significantly higher in symptomatic patients in respect to asymptomatic ones (14.04% vs 3.82%), with a more than three times higher relative risk ratio of having the infection. This may be in part accounted for the higher number of asymptomatic subjects screened [RR: 3.668 (3.070-4.372 95% CI) p<0.0001]. Another point of interest is that only 5 subjects (0.4%) resulted positive among the 1239 healthcare workers weekly screened in the same period of time, demonstrating that, among such a high risk population, the infection was marginal during that particular phase of epidemic in the Salerno province. This finding may be accounted by for the correct use of the personal protection equipment (PPE) by the healthcare workers, confirming, in particular, the usefulness of facemasks as frequently reported [16 17]. However, the significant difference between the high prevalence of asymptomatic SARS-CoV2 positive subjects between "contacts" of infected cases in respect of the low one in healthcare workers (and occasionally voluntaries) suggests that the possibility of getting incidentally infected among the general population has to be rare if (and when) correct and rapid measures are applied in order to discover and appropriately follow-up, with containment measures, positive subjects and their contacts.

The fact that the policy to primarily screen the close contacts of the positive subjects has been a successful approach may be demonstrated by the rapid decline of the number of positive subjects found over time, as depicted in figure 2, in which the total number of NS performed (panel A) , the percentage of total positive (panel B) and symptomatic positive (panel C) ones are reported. In fact, even if every day there was an increasing number of NS performed, there was also a constant decrease in the total number and in the percentage of symptomatic infected subjects, with only 14 cases out of 7341 NS found after the phase 1 ending. This, in our opinion, demonstrates that the "social distancing" (in particular the lockdown of unnecessary activities and the mandatory use of face masks in all the population) and the correct use of PPE by healthcare professionals, together with the systematic screening of close contacts, represented good measures to reduce the spreading of the infection in the province of Salerno in that phase.

Another critical point, that animated the scientific debate on SARS CoV2 epidemics in the last times, is the importance of viral load on defining symptoms and infectiousness of the subjects[18-21].In fact, on this point there are conflicting data, with some of the evidences pointing out as the high viral load of asymptomatic subjects may be accounted for their infectiousness, and therefore their dangerousness[21 22]. On the contrary other documents reported that in asymptomatic infected subjects there were lower viral loads, and this fact may account for the lack of symptoms [23-28]. On the other hand, the World Health Organization pointed out, in a "situation report" on the pandemic, that there are few evidences of asymptomatic transmission (Covid-19 Situation Report #79)[29] and that: "Available evidence from contact tracing reported by countries suggests that asymptomatically infected individuals are much less likely to transmit the virus than those who develop symptoms" [30]. Our findings revealed that there were no statistically significant differences between symptomatic and asymptomatic SARS CoV2 virus carriers on regards of viral load (table 3). However, when analyzed by age classes, the SARS-CoV2 gene expression showed a statistically significant difference for RdRP and N genes that were more expressed in older age (>75 years) than in younger age (<25 years) patients, as showed in Figure 3, possibly meaning that viral load may have had a marginal impact on the development of symptoms. In fact, the asymptomatic subjects were also significantly younger, and the symptomatic subjects particularly represented in age class 41-60 years (Figure 1). Moreover, symptomatic subjects were more frequently of male sex. In this way, our data confirmed some of the characteristics of asymptomatic carriers reported by others, but showed also a certain correlation of age with symptoms and viral load[21-25 27] . However, very likely, the lack of symptoms (no cough, no sneezing, no dyspnea) together with a "normal" health condition, and a younger age, in subjects who therefore did not require any form of "caregiving" (by familiars, cohabitants or healthcare personnel) which might have required a "close contact", together with the correct measures of social distancing, could be "responsible" for the lower infectiousness of asymptomatic subjects rather than just the lower viral load itself.

Finally, it has to be discussed another interesting point raising from our data, the prevalence of hospitalized subjects that underwent to NS because of symptoms that were suggestive of a COVID-19 and were found negative. As per protocol, a patient who presented himself with symptoms strongly suggestive for COVID-19 and found negative at the first NS, was isolated in a "grey zone", prepared on purpose to accommodate unconfirmed cases, then subjected to further confirmation tests, to overcome the risk of false negatives. Nevertheless, in our cohort there were

918 subjects that had symptoms compatible with COVID-19 and resulted “confirmed negative”. They represented the 6.82% of all NS and were mostly “contacts” (68.6%) or hospitalized patients (28.75%). In particular, the last category is the most interesting: among those who were hospitalized and had symptoms suggestive of COVID-19, is very likely that an infectious disease other than SARS-CoV2 was in place. This finding has to be taken into account in the present phase in which the vaccine campaign has been started. In fact, it should be useful to consider “taking the chance” to perform other vaccinations together with that for COVID-19, particularly for airborne infections (as influenza viruses).

There are some limitations to this study. First of all, as already mentioned above, this was not an universal screening of the population and, therefore, we cannot affirm without any doubt that those that were screened were the only infected in the geographical area tested. Nevertheless, it has to be noticed that also the symptomatic patients reduced drastically their presence over time, being this an indirect marker of the slowing of the epidemic.

Conclusions

The combination of social distancing together with the systematic screening of close contacts of COVID-19 positive symptomatic subjects seems to be an efficacious approach to contain SARS-Cov2 epidemic spreading suggesting then the rare eventuality of being occasionally infected by positive asymptomatic subjects.

Tables

	Overall	SARS-CoV2 Positive	SARS-CoV2 Negative	p
n (%)	13448	624 (4.64%)	12824 (95.36%)	-
AGE (mean ± SD)	50.75 (±17.0)	51.93 (±20.3)	50.44 (±17.1)	**0.016**
SEX (M/F) %	55.89/44.11	55.8/44.2	55.9/44.1	0.946 [OR 0.994 (0.846-1.169)]
Symptoms				
Asymptomatic	12591 (93.6%)	470 (75.3%)	11651 (90.9%)	**0.0001**
Symptomatic	1068 (7.9%)	150 (24.0%)	918 (7.2%)	
Mild Symptoms	259 (1.9%)	4 (0.6%)	255 (1.9%)	
Placing				
Contacts	10500 (78.1%)	556 (89.1%)	9944 (77.5%)	**0.028**
Inpatients	1151 (8.5%)	25 (4.0%)	1126 (8.8%)	
ED patients	505 (3.7%)	36 (5.8%)	469 (3.7%)	
ICU Patients	48 (0.3%)	2 (0.3%)	46 (0.3%)	
Occupational Health surveillance	1244 (9.2%)	5 (0.8%)	1239 (9.7%)	

Table 1: Characteristics of "lockdown phase 1" patients undergone to SARS-CoV2 screening with Naso-pharingeal swabs

	Overall	SARS-Cov2 Positive	SARS-Cov2 Negative	p
Total	**13448**	**624**	**12824**	
Total Asymptomatic	**12275 (91.3%)**	**470 (75.3%)**	**11651** (90.8%)	**<0.0001**
Contacts (domiciliary isolation)	9664 (74.1%)	428 (68.6%)	9236 (72.0%)	
Inpatients	864 (2.5%)	14 (2.2%)	850 (6.6%)	
ED patients	342 (2.5%)	21 (3.4%)	321 (2.5%)	
ICU Patients	32 (0.2%)	1 (0.2%)	31 (0.2%)	
Occupational Health surveillance	1219 (9.1%)	5 (0.8%)	1214 (9.4%)	
Total Symptomatic	**1068 (7.9%)**	**150 (24.0%)**	**918** (7.1%)	**<0.0001**
Contacts (domiciliary isolation)	756 (5.6%)	126 (20.2%)	630 (4.9%)	
Inpatients	152 (1.1%)	9 (1.4%)	143 (1.1%)	
ED	123 (0.9%)	14 (2.2%)	109 (0.8%)	
ICU	13 (0.1%)	1 (0.2%)	12 (0.1%)	
Occupational Health surveillance	24 (0.2%)	0	24 (0.2%)	
Total Mild Symptoms	**259 (1.9%)**	**4 (0.6%)**	**255** (1.9%)	**0.853**
Contacts (domiciliary isolation)	81 (0.6%)	2 (0.3%)	79 (0.6%)	
Inpatients	1 (0.01%)	1 (0.2%)	134 (1.0%)	
ED patients	1 (0.01%)	1 (0.2%)	39 (0.3%)	
ICU patients	0	0	3 (0.02%)	
Occupational Health surveillance	0	0	1 (0.01%)	

Table 2: Prevalence of Symptomatic and asymptomatic subjects divided by provenience of NS.

	Overall	Symptomatic	Asymptomatic	p
E gene Ct	23.9040 (±4.28123)	22.2785 (±4.97021)	22.8605 (±4.88053)	0.486
RdRP Ct	25.1360 (±4.42040)	23.5495 (±4.75518)	24.1136 (±4.69109)	0.583
N Ct	26.4580 (±4.15840)	24.7405 (±4.55574)	25.4944 (±4.72231)	0.448

Table 3: overall gene expression [presented as C(t)] of the three genes of SARS-CoV2 as detected by RT-PCR in positive patients and difference between Symptomatic and Asymptomatic subjects (mean ± SD).

Legend to figures:

Figure 1: prevalence of symptomatic and asymptomatic subjects among SARS-CoV2 positive subjects, by age classes.

Figure 2. Panel A. total number of executed SARS-Cov2 Swabs per day in the Salerno Province from March 03 to May 15, 2020. **Panel B.** Percentage of Swabs resulted positive among the total executed per day. **Panel C.** Percentage of symptomatic patients among positive subjects per day.

Figure 3. Expression [presented as C(t)] of the three genes of SARS-CoV2 as detected by RT-PCR in positive patients divided by 4 age classes (<25, 26-50, 51-75, >76 years). The only statistical differences were found in the mean expressions of RdRP and N between <25 and >75 age classes in which significant lower expressions [higher C(t) cycles] were found in the older ones (p:0.036 and 0.032 respectively, with a Bonferroni corrected Kruskal-Wallis pairwise comparison for independent samples).

a. Contributorship statement: Marcello Persico is the guarantor of the manuscript. MM, AB and MP conceived and designed the experimental study; MM, EV, RS, PT, AA, ADV RC and AB performed the epidemiological evaluations and the data' collection. MM and PTperformed statistical analysis. MP and MM wrote the manuscript. MP and AB edited the manuscript. All the authors approved the final version of the manuscript.

b. Competing interests: the authors declare no conflict of interests for the present work.

c. Funding: this study didn't receive any specific funding.

d. Data Sharing statement: Data will available upon reasonable request of the corresponding author (M. Persico).

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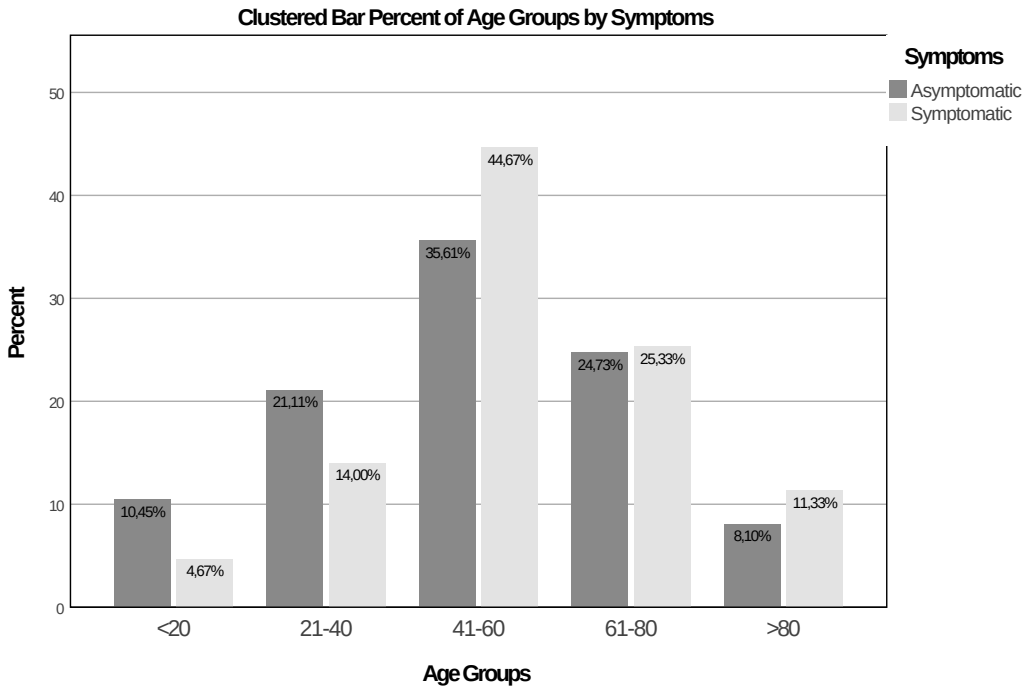

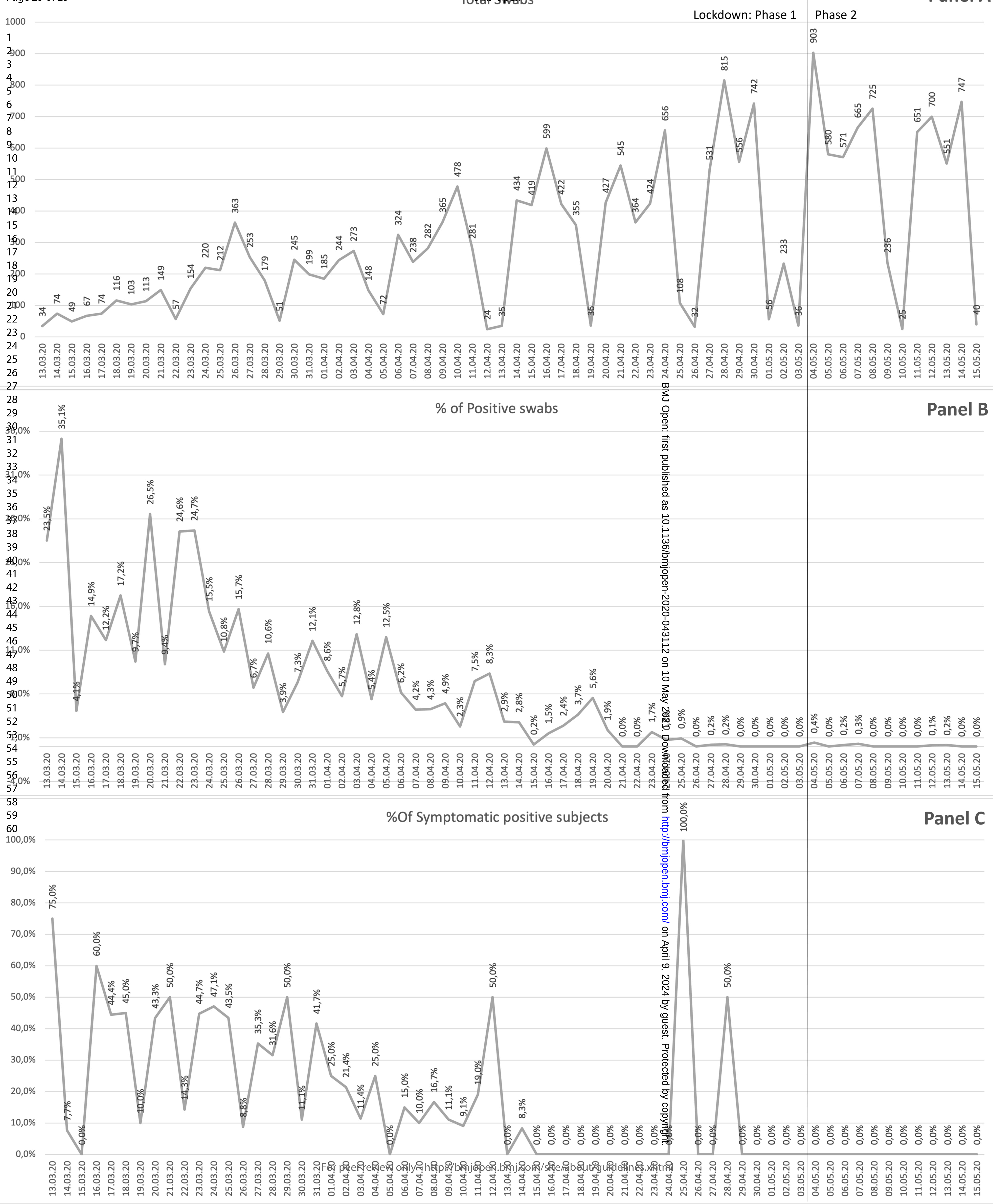

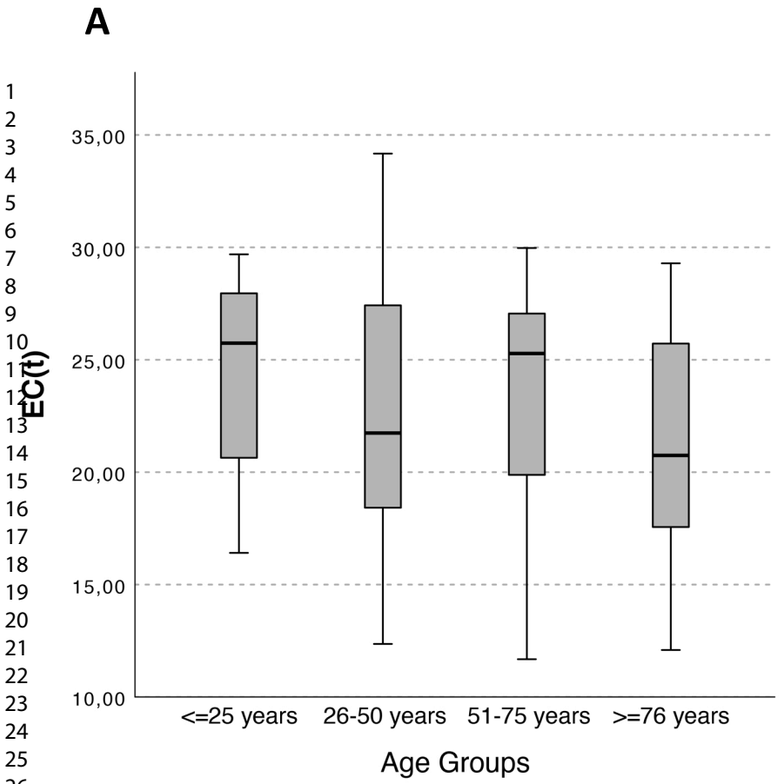

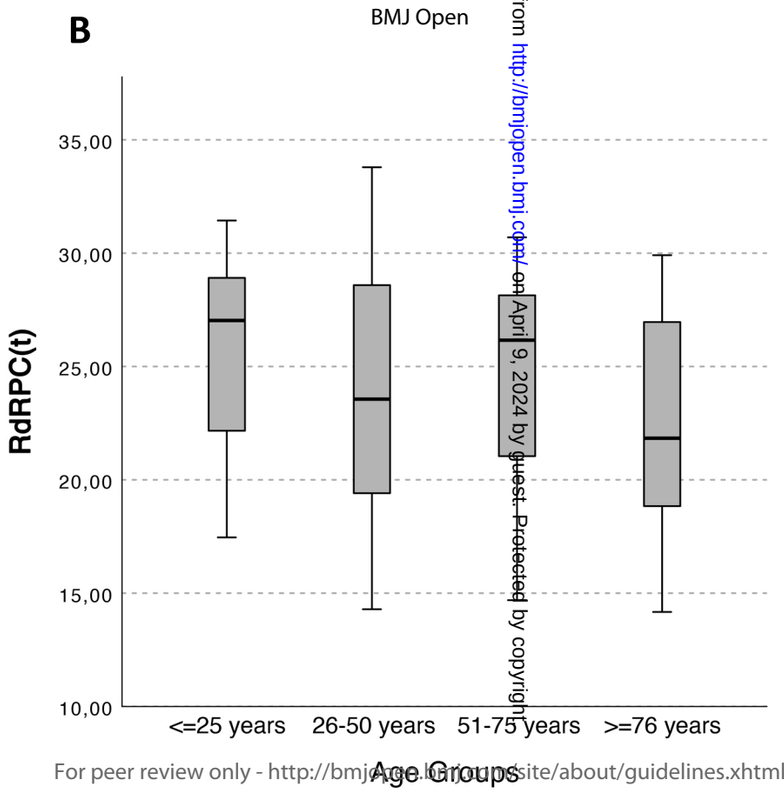

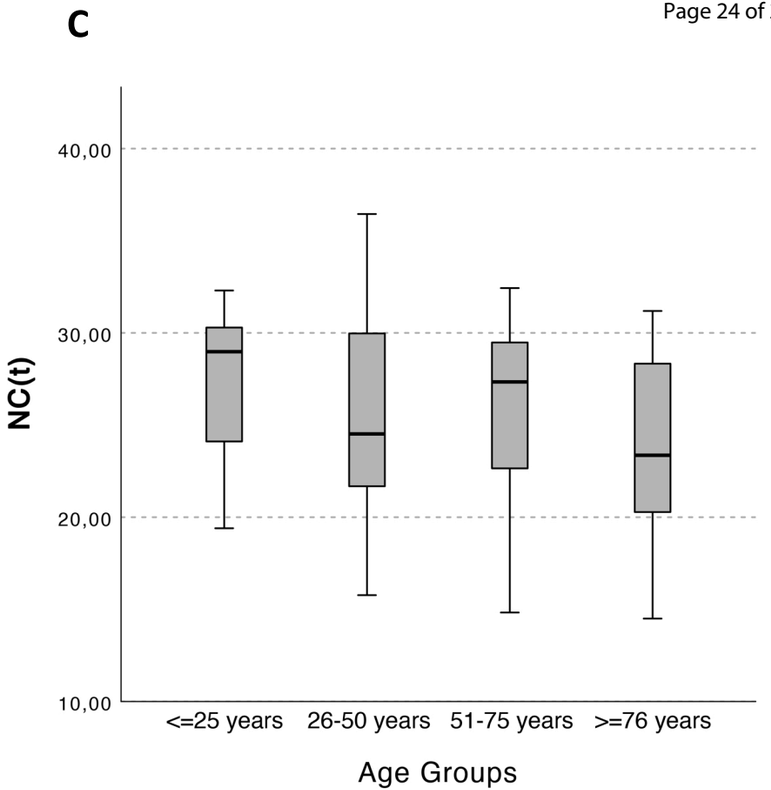

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	na
		(*e*) Describe any sensitivity analyses	na
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	na
		(c) Consider use of a flow diagram	na
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	na
		(c) Summarise follow-up time (eg, average and total amount)	na
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8 8 na
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for exposed and unexposed groups.

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

BMJ Open

Characterization of asymptomatic patients and efficacy of preventive measures of SARS-Cov-2 infection in a large population of the Southern Italy, a cohort study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-043112.R2
Article Type:	Original research
Date Submitted by the Author:	26-Mar-2021
Complete List of Authors:	Masarone, Mario; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Vaccaro, Emilia; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Molecolar Biology Unit Sciorio, Roberta; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Torre, Pietro; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Della Vecchia, Antonio; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Hospital Health Direction Aglitti, Andrea; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit Caliulo, Rita; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Molecolar Biology Unit Borrelli, Anna; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Hospital Health Direction Persico, M. ; Azienda Ospedaliera Universitaria 'San Giovanni di Dio e Ruggi d'Aragona', Internal Medicine and Hepatology Unit
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Health policy, Infectious diseases
Keywords:	COVID-19, Epidemiology < INFECTIOUS DISEASES, VIROLOGY

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Title: Characterization of asymptomatic patients and efficacy of preventive measures of SARS-Cov-2 infection in a large population of the Southern Italy, a cohort study.

Running title: SARS-Cov-2 in Southern Italy, a cohort study

Authors: Mario Masarone MD PhD[1], Emilia Vaccaro[2] PhD, Roberta Sciorio MD [1], Pietro Torre MD[1], Antonio Della Vecchia MD[3], Andrea Aglitti MD[1], Rita Caliulo PhD[2], Anna Borrelli MD[3], Marcello Persico MD[1]

Affiliations:

[1]Internal Medicine and Hepatology Unit; [2] Molecolar Biology Unit; [3] Hospital Health Direction, University Hospital San Giovanni di Dio e Ruggi d'Aragona, University of Salerno, Salerno. Italy.

Electronic Word Count: 3560

Number of tables: 3

Number of figures: 3

Corresponding Author:
Marcello Persico M.D.
Internal Medicine and Hepatology Unit,
AOU-San Giovanni e Ruggi D'Aragona, Department of Medicine and Surgery
University of Salerno, Italy.
Via Salvador Allende
84081, Baronissi (Salerno), Italy
E-mail: mpersico@unisa.it
Tel: +39 338 3338695

Keywords: SARS CoV2; COVID-19 Pandemic; Screening program, Nasopharingeal Swabs

List of abbreviations: NS: Nasopharingeal swabs; COVID-19: Coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus-2; RT PCR: real-time Polymerase Chain Reaction.

Abstract

Background: SARS-CoV2 pandemic has infected millions of people and caused more than 2.30 millions deaths worldwide, to date. Several doubts arise about the importance of asymptomatic carriers for the virus diffusion. During the first epidemic outbreak in Italy a large screening with nasopharyngeal swabs (NS) was performed in those who were "suspect" to be infected. **Aims:** To report the results of the SARS-Cov2 screening in a Province of the Southern Italy, to provide data on the COVID-19 epidemic and the burden of asymptomatic subjects.

Patients and methods: A retrospective cohort study was set up in all the Province healthcare facilities (12 hospitals and 13 sanitary districts – primary, secondary and tertiary centers) to analyze the results of NS made to all the subjects suspected to be infected with SARS-Cov2, either because presented suggestive symptoms, or "contacts" of positive subjects, or coming from an high prevalence area, or healthcare workers. NS were performed and managed as indicated by international guidelines. The specimens were processed for SARS-CoV2 detection by RT PCR.

Results: A total of 20325 NS were performed from March 13 to May 9, 2020. Of these, 638(3.14%) resulted positive. Asymptomatic subjects were 470, 75.3% of the positive persons. They were mostly among "contacts" of symptomatic cases (428/470, 91%) and in domiciliary isolation. SARS-Cov2 three genes expressions did not differ between asymptomatic and symptomatic subjects. The strict measures of social distancing led to a continuous decrease of the cases during phase 1.

Conclusions: In a large area of the Southern Italy, 3.14%(638/20325) of the total subjects tested were positive for SARS CoV2. Most of them were asymptomatic (470/624,75.3%) and, of these, 91% (428/470) were "close contacts" of symptomatic subjects. The combination of social distancing together with the systematic screening of close contacts of COVID-19 positive symptomatic subjects seems to be an efficacious approach to limit the epidemic spreading.

Strengths and Limitations

The study here reports on a retrospective cohort of SARS-Cov2 screened patients during the "first wave" of pandemic in Southern Italy.

The screening was performed with naso-pharyngeal swabs for SARS-Cov2 RT-PCR detection from March 09 to May 3, 2020.

It was performed in symptomatic subjects, asymptomatic contacts of positive subjects, subjects coming from high prevalence areas and healthcare workers.

The study reports on prevalence, demographical and clinical characteristics of SARS-Cov2 infected patients during that period.

The main limitations of the paper are that the data presented are on the study cohort and not on the whole population and its retrospective nature.

Introduction

The Coronavirus disease 2019 (COVID-19) is the clinical manifestation of an airborne infection by a *Coronavirus spp virus* which has been named "severe acute respiratory syndrome coronavirus-2" (SARS-CoV2) and was declared a pandemic by the World Health organization (WHO) on march 11 2020 [1]. In Italy, the epidemic outbreak led to adopt a strict lockdown of all the non-essential activities as soon as March 9, 2020. The lockdown "phase 1", which took place from March 9 to May 3 2020, was intended to reduce the spreading of the infection which has caused, to date (February, 2021) 2,650 million of cases, with 92.001 deaths in Italy. From May 3, 2020 the restrictions of Italian citizens circulation were reduced, thanks to the reduction of the epidemic spreading, especially in southern regions, leading to the "phase 2" of the lockdown, even if the "social distancing measures" were still maintained[2].

SARS-Cov2 has infected millions of people and caused more than 2,34 millions of deaths worldwide at the time of the writing. Its epidemiology has been largely investigated, with increasing evidences that demonstrated as its clinical manifestations can vary from an asymptomatic upper-respiratory tract infection to a severe acute respiratory syndrome leading to the necessity of intensive care, with an high risk of death from respiratory failure[3]. Many speculations have been made on the epidemiology of the pandemic and, in particular, on the number of the asymptomatic cases and its importance on its spreading across the globe [4-6]. Nevertheless, there are limited data on the burden of asymptomatic carriers, their number and their capacity to spread the infection. For these reasons, here we report the results of the SARS-Cov2 screening activities of a large single Province of a region of Southern Italy (Campania) during the phase 1 and phase 2 lockdown in Italy, with the aim of providing information on the coronavirus epidemic and the burden of asymptomatic infections.

Patients and methods

The present cohort study reports on the SARS-Cov2 infection screening program set-up in the Salerno province of Southern Italy during the so-called "phase 1" of the lockdown period, that was decided by Italian government from March 09 to May 3 2020 in Italy, due to the spreading of the COVID-19 epidemic in Italy. Salerno province is located in Campania region, Southern Italy, and it happens to be the largest Italian province by extension (4 952 km²) and number of municipalities (158), with a total population of about 1 million of inhabitants. As soon as the lockdown was

instituted in Italy, the regional government decided for a profound reorganization of the healthcare system to face the emergency [7]. Among all, a diagnostic service for SARS-Cov2 by mean of nasopharyngeal swabs (NS) analysis was set-up at the main University Hospital, the San Giovanni di Dio e Ruggi d'Aragona Hospital. All the National Healthcare system facilities belonging to the Local Health Company of Salerno (comprehending 12 hospitals, 13 healthcare districts and several territorial services facilities, spanning all over the Salerno territory) performed the NS on a daily basis, sending overnight the stabilized specimens (in universal transport media -UTM) that were collected on-site, to be centrally evaluated [8]

During this phase , an universal screening was not provided to the entire population and the NS for Covid19 were only performed, as mandated by the central and regional government, for these reasons: symptoms suggestive for an upper respiratory tract syndrome and/or cough and/or fever without any other cause and/or a contact (family members, cohabitants and/or co-workers and/or caregivers) with an infected subject and/or a person coming from geographical areas with high prevalence of infection (i.e. northern Italy regions). Moreover, NS were also performed in all the inpatients admitted to hospital (with and without an upper respiratory tract syndrome) and all the healthcare workers of the province.

OS were performed by healthcare professionals (doctors or nurses) that were preliminarily trained to perform the specimen collection with the best procedures, as indicated by WHO [9 10].

All the subjects undergoing the OS were asked to sign an informed consent and to respond a brief questionnaire on their age, sex, address, provenience and the eventual symptoms they had (no symptoms; mild symptoms: flu-like or mild fever or cough or sneezing; symptoms: a clinical presentation compatible with one of the five clinical presentations of a COVID-19 disease)[11] . In case of a patient that was in hospital or in emergency room, the operator who carried out the OS compiled the form with all the necessary data (including symptoms).

Nasopharyngeal Swabs analysis for SARS-Cov2 detection

Nasopharyngeal swabs (NS) were collected from all the subjects, the sampled specimens were transferred on universal transport media (UTM, Copan, Mylan, Italy) and managed as indicated by CDC guidelines [12]. The Allplex 2019-nCoV assay (Seegene Inc., Seoul, South Korea) that was demonstrated to be accurate for the confirmation diagnosis of SARS-CoV-2 infection, was used on a Nimbus IVD and Cfx-96™ Real Time PCR automatic extractor (Seegene Inc. Seoul, South Korea) for amplifying three viral targets: the E gene (specific of the subgenus Sarbecovirus), the N and the

RdRP genes (both specifics of the SARS-CoV-2) [13]. Samples displaying at least 2 viral targets for RT-PCR viral targets were considered positive, as previously indicated[13].

The present study was approved by our local ethics committee (Ethics Committee of Campania Sud: cometicocampaniasud@aslnapoli3sud.it) and conducted in conformity with the ethical guidelines of the 1975 Declaration of Helsinki.

Patients and Public involvement

Due to its retrospective nature, this cohort study did not involve patients and public in the design, or conduct, or reporting, or dissemination plans.

Statistical analysis

All the data collected were analyzed by means of IBM SPSS Statistics for MacIntosh, version 26 (IBM Corp., Armonk, N.Y., USA). The continuous variables were analyzed with parametric or non-parametric tests, when appropriated. In particular, student's t-test and Mann-Whitney were performed for continuous variables, and chi-square test with Yates correction or Fisher-exact tests were used to compare frequencies and categorical variables. Before applying the correct analysis, a Kolgoromov-Smirnov K-S "Goodness of fit" test for normality was performed to assess if there was a normal or not-normal distribution of the continuous variables. Statistical significance was defined when "p<0,05" in a two-tailed test with a 95% confidence interval.

Results

From March 13, 2020 to May 15, 2020, a total of 20789 nasopharyngeal swabs were performed and analyzed. A total of 1097 swabs resulted positive for SARS-Cov2 RNA presence. Of these, 638 out of 20325 (3.14%) referred to newly diagnosed cases of COVID-19 disease, and 464 were secondary samples collected during the follow-up of the firstly positive subjects. Of the 638 firstly positive cases, 624 were those found in the phase 1 (up to May 3, 2020), 4.64% of the 13448 swabs performed in that period of time. Therefore, In the first 11 days of phase 2 (from May 4to may 15, 2020) the newly positive subjects screened for SARS-Cov2 were 14, 0.18% of the 7431 swabs performed (Phase 1 vs Phase 2: p<0.0001 OR 25.827 - 95% CI 15.195-43.614).

The main characteristics of the positive patients vs the negative ones are reported in table 1.

The distribution of the patients found to be positive during phase 1, on the basis of their symptoms, is reported in table 2. From these two tables we can find that the so called “contacts” [persons in whom the NS was primarily performed because of their contact with a positive subject (family member, co-worker, caregivers etc.)] were 556. Of them, 428 (76.98%) were asymptomatic, and 128 (23.02%) had symptoms that were not enough to require hospitalization, and therefore were posed in domiciliary isolation. They represented the 68.59% of the total positive patients screened in Salerno province.

As far as symptoms are concerned, all SARS Cov2 positive subjects with symptoms were more often of male gender in respect of asymptomatic ones, but this finding didn’t reach statistical significance (Male sex prevalence: 62.00% vs 53.94%; p:0.084 OR:1.393 – 0.959-2.030 95%CI) and they were significantly older (mean age 54.20 ±20.78 vs 49.99±16.82, p<0.0001). Moreover, in figure 1 is reported the prevalence of symptomatic and asymptomatic carriers of SARS-CoV2 by age classes. There was a statistical difference in <20 and 21-40 years age classes in which there were more asymptomatic positive subjects (p: 0.022 and 0.048, respectively), and in 41-60 years in which there were more symptomatic patients (p: 0.038). Finally, when analyzing the prevalence of SARS-CoV2 positivity among symptomatic and asymptomatic subjects we found that the prevalence of positive NS among symptomatic patients was of 14.04% (150/1068), whereas it was of 3.82% (470/12275) in asymptomatic subjects [RR: 3.668 (3.070-4.372 95% CI) p<0.0001].

The total number of NS specimens collected and processed “per-day” are reported in figure 2 (Panel A). The highest number of tests performed was on May 04, 2020 (n: 903). In phase 1 the daily mean number of performed tests was 263 per day, however it was lower in the first weeks

and higher in the last, because of the efforts in potentiating the service. The percentage of positive NS "per day" are reported in panel B of figure 2. The highest number of positive NS was on March 26 (57/363 performed, 15,7%), then there was a constant decrease of the total of positive subjects, reaching 0 on the last four days of the phase 1, despite the number of performed tests in those day was of 1037. Moreover, as it can be seen from panel B of figure 2, the percentage of positive patients decreased constantly over time, in an inverse trend in respect of the increase of the total number of performed tests. Finally, in panel C of the same figure is reported the daily percentage of symptomatic positive patients that showed a decreasing trend over time as well.

Of notice, there were 918 patients (6.83% of the total) that, even if repeatedly resulted negative to NS for SARS-CoV2, presented themselves with symptoms that were compatible for any of the clinical presentations of COVID 19. These subjects were "contacts" in the majority of cases (68.6%) and the others were either admitted to the hospital ward (15.6%), emergency department (11.9%), or intensive care unit (1.3%) (Table 2).

Finally, we report the mean expression [displayed as "cycle threshold" to obtain positive fluorescence in RT-PCR -C(t)] of the three genes of SARS Cov-2 assays (N, E and RdRP) in subjects defined as positive. There was no statistically significant difference in mean genes expressions between asymptomatic and symptomatic subjects (table 3). However, when grouped in four age classes (<25; 26-50; 51-75 and >75 years of age), the positive subjects showed a statistically higher expression for RdRP and N genes, in >75 years patients in respect of <25 years subjects (p:0.036 and 0.032, respectively), see Figure 3.

Discussion

Hereby we report on the experience of the healthcare system of a large area of the Southern Italy in facing the Coronavirus Outbreak. As soon as the COVID-19 became epidemic in Italy, several measures were set up to fight the spreading of it[7]. A profound and quick reorganization of the healthcare system was provided that reshaped the clinical activities with an increase of the capacity of Intensive Care Units, the creation of the so-called "COVID-hospitals", the re-allocation of healthcare professionals to face the emergency and the interruption of all the non-urgent or necessary activities of the healthcare system. Even if the major outbreak interested the northern Italy, the central and southern regions of the country were also subjected to the same rules and precautions [14 15]. A screening for the COVID-19 infection was provided for those who were either

symptomatic or at high risk of infection (see methods). Our University Hospital was identified as one of the centralized centers dedicated to the execution of the Covid19 related biological tests. Moreover, during the phase 1 of the lockdown, also all the commercial and working activities that were considered not necessary (mostly alimentary, pharmaceutics and logistics) were shut down in Italy, with the warning to not leave the home without a valid reason. During phase 1 of the lockdown, over a population of almost one million of inhabitants of our province, we registered a total number of cases of SARS-Cov2 positive subjects of 624, the 4.64% of the 13448 OS performed. However, to correctly interpret this data, it has to be pointed out that an universal screening of the population was not provided in Italy at the time, and it is still to be implemented. This was because of a series of factors, such as the scarce availability of commercial tests worldwide and the difficulties in organizing in a short period of time a very large screening activity. Therefore, this fact raised several concerns in the scientific community and in the public opinion about the COVID 19 epidemiology, particularly regarding the possibility of transmission of the disease from asymptomatic subjects, as it has been reported since the very beginning of the outbreak[4-6]. In fact, in absence of a systematic screening of the entire population, several doubts can arise on the safety of the relaxation of the lockdown measures, because of the lack of information on the prevalence of asymptomatic infection, as well as on its importance on the diffusion of the pandemic. At the time of the writing, there are no robust data on these points, even if limited evidences seem to indicate that it is crucial to screen not the entire population but rather the high-risk populations and the close contacts of the identified cases [6]. In this way, our data can represent an useful tool for the stakeholders and the medical governances, in Italy and Europe, that are attempting to outline the epidemiology of such pandemic, in order to find a correct way to balance the needs of the recovery of normal social and commercial activities with the safety of the population. This is particularly crucial in the actual phase, when the vaccine programs are beginning, in order to avoid excessive measures in both directions and give the time for carrying out the vaccination campaigns. In fact, as mandated by Italian regulation, our screening was performed exactly as suggested by literature: apart from those who were admitted to hospitals' emergency departments with a syndrome that was suspect for COVID-19, all the other NS were performed precisely on close contacts of positive cases (family member, co-habitants, co-workers, caregivers etc.) and in the highest risk population of the lockdown phase 1, represented by the healthcare workers for obvious reasons. As it can be deduced from the results of the present screening, the majority of SARS-Cov2 positive subjects found during phase were

asymptomatic (470/624, 75.3%) and, of these, the vast majority was represented by close contacts of symptomatic cases (428/470, 91.3%) representing the 3.2% of the 13448 OS performed (see also table 1 and 2). If this finding may be, at first sight, interpreted as an alarming point, it is also to be noticed that the vast majority of positive cases were found precisely only on close contacts of the symptomatic cases (556/624, 89.1%). Moreover, when analyzing the prevalence of positivity among symptomatic and asymptomatic people, we found that it was significantly higher in symptomatic patients in respect to asymptomatic ones (14.04% vs 3.82%), with a more than three times higher relative risk ratio of having the infection. This may be in part accounted for the higher number of asymptomatic subjects screened [RR: 3.668 (3.070-4.372 95% CI) p<0.0001]. Another point of interest is that only 5 subjects (0.4%) resulted positive among the 1239 healthcare workers weekly screened in the same period of time, demonstrating that, among such a high-risk population, the infection was marginal during that particular phase of epidemic in the Salerno province. This finding may be accounted by for the correct use of the personal protection equipment (PPE) by the healthcare workers, confirming, in particular, the usefulness of facemasks as frequently reported [16 17]. However, the significant difference between the high prevalence of asymptomatic SARS-CoV2 positive subjects between "contacts" of infected cases in respect of the low one in healthcare workers (and occasionally voluntaries) suggests that the possibility of getting incidentally infected among the general population has to be rare if (and when) correct and rapid measures are applied in order to discover and appropriately follow-up, with containment measures, positive subjects and their contacts.

The fact that the policy to primarily screen the close contacts of the positive subjects has been a successful approach may be demonstrated by the rapid decline of the number of positive subjects found over time, as depicted in figure 2, in which the total number of NS performed (panel A) , the percentage of total positive (panel B) and symptomatic positive (panel C) ones are reported. In fact, even if every day there was an increasing number of NS performed, there was also a constant decrease in the total number and in the percentage of symptomatic infected subjects, with only 14 cases out of 7341 NS found after the phase 1 ending. This, in our opinion, demonstrates that the "social distancing" (in particular the lockdown of unnecessary activities and the mandatory use of face masks in all the population) and the correct use of PPE by healthcare professionals, together with the systematic screening of close contacts, represented good measures to reduce the spreading of the infection in the province of Salerno in that phase.

Another critical point, that animated the scientific debate on SARS CoV2 epidemics in the last times, is the importance of viral load on defining symptoms and infectiousness of the subjects[18-21].In fact, on this point there are conflicting data, with some of the evidences pointing out as the high viral load of asymptomatic subjects may be accounted for their infectiousness, and therefore their dangerousness[21 22]. On the contrary other documents reported that in asymptomatic infected subjects there were lower viral loads, and this fact may account for the lack of symptoms [23-28]. On the other hand, the World Health Organization pointed out, in a "situation report" on the pandemic, that there are few evidences of asymptomatic transmission (Covid-19 Situation Report #79)[29] and that: "Available evidence from contact tracing reported by countries suggests that asymptomatically infected individuals are much less likely to transmit the virus than those who develop symptoms" [30]. Our findings revealed that there were no statistically significant differences between symptomatic and asymptomatic SARS CoV2 virus carriers on regards of viral load (table 3). However, when analyzed by age classes, the SARS-CoV2 gene expression showed a statistically significant difference for RdRP and N genes that were more expressed in older age (>75 years) than in younger age (<25 years) patients, as showed in Figure 3, possibly meaning that viral load may have had a marginal impact on the development of symptoms. In fact, the asymptomatic subjects were also significantly younger, and the symptomatic subjects particularly represented in age class 41-60 years (Figure 1). Moreover, symptomatic subjects were more frequently of male sex. In this way, our data confirmed some of the characteristics of asymptomatic carriers reported by others but showed also a certain correlation of age with symptoms and viral load[21-25 27] . However, very likely, the lack of symptoms (no cough, no sneezing, no dyspnea) together with a "normal" health condition, and a younger age, in subjects who therefore did not require any form of "caregiving" (by familiars, cohabitants or healthcare personnel) which might have required a "close contact", together with the correct measures of social distancing, could be "responsible" for the lower infectiousness of asymptomatic subjects rather than just the lower viral load itself.

Finally, it has to be discussed another interesting point raising from our data, the prevalence of hospitalized subjects that underwent to NS because of symptoms that were suggestive of a COVID-19 and were found negative. As per protocol, a patient who presented himself with symptoms strongly suggestive for COVID-19 and found negative at the first NS, was isolated in a "grey zone", prepared on purpose to accommodate unconfirmed cases, then subjected to further confirmation tests, to overcome the risk of false negatives. Nevertheless, in our cohort there were

918 subjects that had symptoms compatible with COVID-19 and resulted “confirmed negative”. They represented the 6.82% of all NS and were mostly “contacts” (68.6%) or hospitalized patients (28.75%). In particular, the last category is the most interesting: among those who were hospitalized and had symptoms suggestive of COVID-19, is very likely that an infectious disease other than SARS-CoV2 was in place. This finding has to be taken into account in the present phase in which the vaccine campaign has been started. In fact, it should be useful to consider “taking the chance” to perform other vaccinations together with that for COVID-19, particularly for airborne infections (as influenza viruses).

There are some limitations to this study. First of all, as already mentioned above, this was not an universal screening of the population and, therefore, we cannot affirm without any doubt that those that were screened were the only infected in the geographical area tested. Nevertheless, it has to be noticed that also the symptomatic patients reduced drastically their presence over time, being this an indirect marker of the slowing of the epidemic.

Conclusions

The combination of social distancing together with the systematic screening of close contacts of COVID-19 positive symptomatic subjects seems to be an efficacious approach to contain SARS-Cov2 epidemic spreading suggesting then the rare eventuality of being occasionally infected by positive asymptomatic subjects.

Tables

	Overall	SARS-CoV2 Positive	SARS-CoV2 Negative	p
n (%)	13448	624 (4.64%)	12824 (95.36%)	-
AGE (mean ± SD)	50.75 (±17.0)	51.93 (±20.3)	50.44 (±17.1)	**0.016**
SEX (M/F) %	55.89/44.11	55.8/44.2	55.9/44.1	0.946 [OR 0.994 (0.846-1.169)]
Symptoms				
Asymptomatic	12591 (93.6%)	470 (75.3%)	11651 (90.9%)	**0.0001**
Symptomatic	1068 (7.9%)	150 (24.0%)	918 (7.2%)	
Mild Symptoms	259 (1.9%)	4 (0.6%)	255 (1.9%)	
Placing				
Contacts	10500 (78.1%)	556 (89.1%)	9944 (77.5%)	**0.028**
Inpatients	1151 (8.5%)	25 (4.0%)	1126 (8.8%)	
ED patients	505 (3.7%)	36 (5.8%)	469 (3.7%)	
ICU Patients	48 (0.3%)	2 (0.3%)	46 (0.3%)	
Occupational Health surveillance	1244 (9.2%)	5 (0.8%)	1239 (9.7%)	

Table 1: Characteristics of "lockdown phase 1" patients undergone to SARS-CoV2 screening with Naso-pharingeal swabs

	Overall	SARS-Cov2 Positive	SARS-Cov2 Negative	p
Total	**13448**	**624**	**12824**	
Total Asymptomatic	**12275 (91.3%)**	**470 (75.3%)**	**11651** (90.8%)	**<0.0001**
Contacts (domiciliary isolation)	9664 (74.1%)	428 (68.6%)	9236 (72.0%)	
Inpatients	864 (2.5%)	14 (2.2%)	850 (6.6%)	
ED patients	342 (2.5%)	21 (3.4%)	321 (2.5%)	
ICU Patients	32 (0.2%)	1 (0.2%)	31 (0.2%)	
Occupational Health surveillance	1219 (9.1%)	5 (0.8%)	1214 (9.4%)	
Total Symptomatic	**1068 (7.9%)**	**150 (24.0%)**	**918** (7.1%)	**<0.0001**
Contacts (domiciliary isolation)	756 (5.6%)	126 (20.2%)	630 (4.9%)	
Inpatients	152 (1.1%)	9 (1.4%)	143 (1.1%)	
ED	123 (0.9%)	14 (2.2%)	109 (0.8%)	
ICU	13 (0.1%)	1 (0.2%)	12 (0.1%)	
Occupational Health surveillance	24 (0.2%)	0	24 (0.2%)	
Total Mild Symptoms	**259 (1.9%)**	**4 (0.6%)**	**255** (1.9%)	**0.853**
Contacts (domiciliary isolation)	81 (0.6%)	2 (0.3%)	79 (0.6%)	
Inpatients	1 (0.01%)	1 (0.2%)	134 (1.0%)	
ED patients	1 (0.01%)	1 (0.2%)	39 (0.3%)	
ICU patients	0	0	3 (0.02%)	
Occupational Health surveillance	0	0	1 (0.01%)	

Table 2: Prevalence of Symptomatic and asymptomatic subjects divided by provenience of NS.

	Overall	Symptomatic	Asymptomatic	p
E gene Ct	23.9040 (±4.28123)	22.2785 (±4.97021)	22.8605 (±4.88053)	0.486
RdRP Ct	25.1360 (±4.42040)	23.5495 (±4.75518)	24.1136 (±4.69109)	0.583
N Ct	26.4580 (±4.15840)	24.7405 (±4.55574)	25.4944 (±4.72231)	0.448

Table 3: overall gene expression [presented as C(t)] of the three genes of SARS-CoV2 as detected by RT-PCR in positive patients and difference between Symptomatic and Asymptomatic subjects (mean ± SD).

Legend to figures:

Figure 1: prevalence of symptomatic and asymptomatic subjects among SARS-CoV2 positive subjects, by age classes.

Figure 2. Panel A. total number of executed SARS-Cov2 Swabs per day in the Salerno Province from March 03 to May 15, 2020. **Panel B.** Percentage of Swabs resulted positive among the total executed per day. **Panel C.** Percentage of symptomatic patients among positive subjects per day.

Figure 3. Expression [presented as C(t)] of the three genes of SARS-CoV2 as detected by RT-PCR in positive patients divided by 4 age classes (<25, 26-50, 51-75, >76 years). The only statistical differences were found in the mean expressions of RdRP and N between <25 and >75 age classes in which significant lower expressions [higher C(t) cycles] were found in the older ones (p:0.036 and 0.032 respectively, with a Bonferroni corrected Kruskal-Wallis pairwise comparison for independent samples).

a. Contributorship statement: Marcello Persico is the guarantor of the manuscript. MM, AB and MP conceived and designed the experimental study; MM, EV, RS, PT, AA, ADV RC and AB performed the epidemiological evaluations and the data' collection. MM and PTperformed statistical analysis. MP and MM wrote the manuscript. MP and AB edited the manuscript. All the authors approved the final version of the manuscript.

b. Competing interests: the authors declare no conflict of interests for the present work.

c. Funding: this study didn't receive any specific funding.

d. Data Sharing statement: Data will available upon reasonable request of the corresponding author (M. Persico).

e. Ethical approvement statement: The present study was approved by our local ethics committee (Ethics Committee of Campania Sud: cometicocampaniasud@aslnapoli3sud.it - number of protocol 102 of July 03, 2020) and conducted in conformity with the ethical guidelines of the 1975 Declaration of Helsinki.

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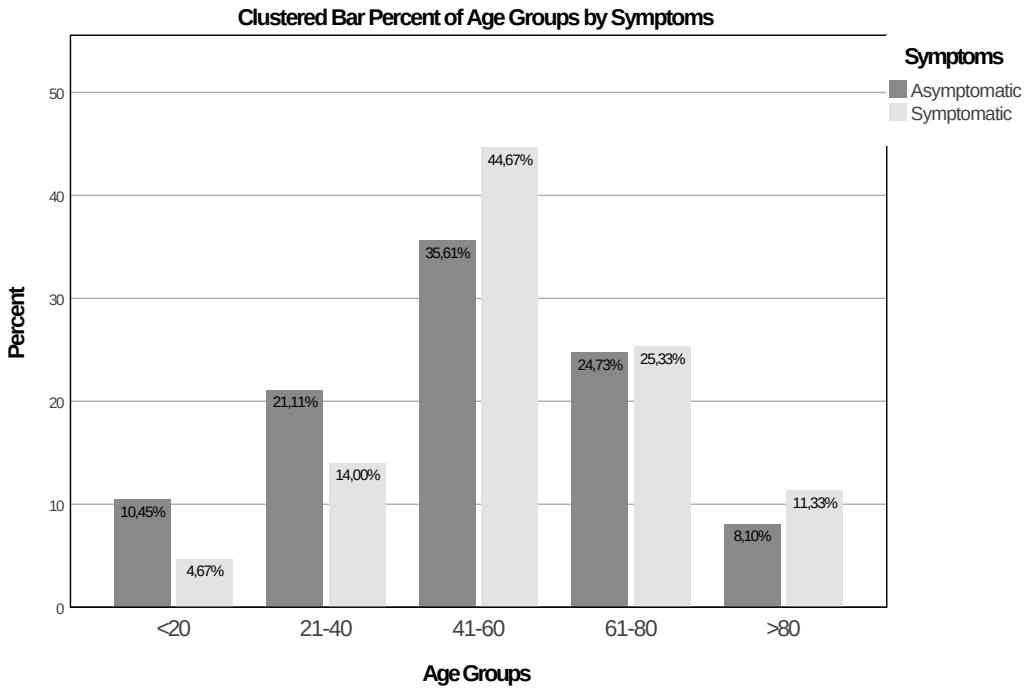

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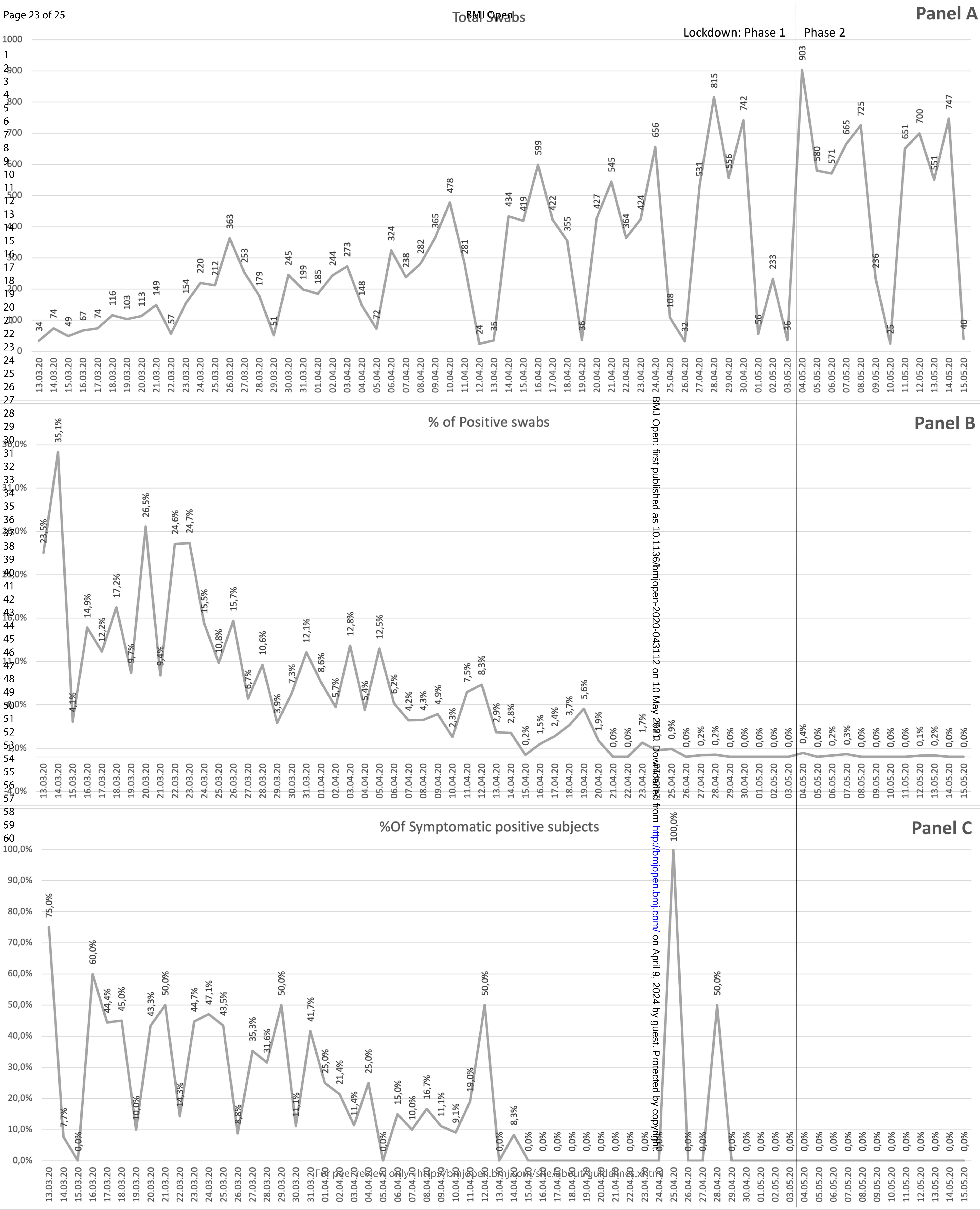

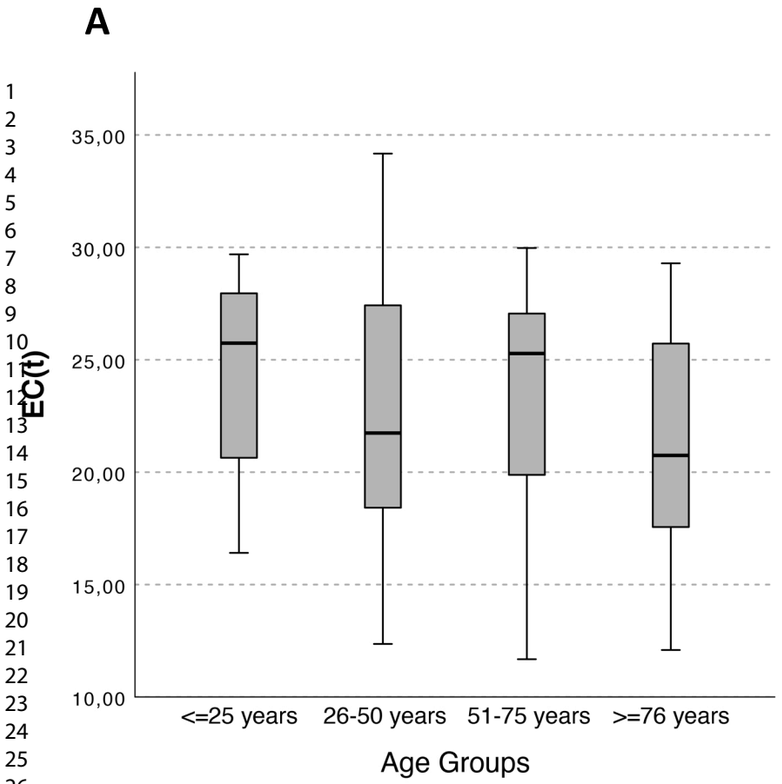

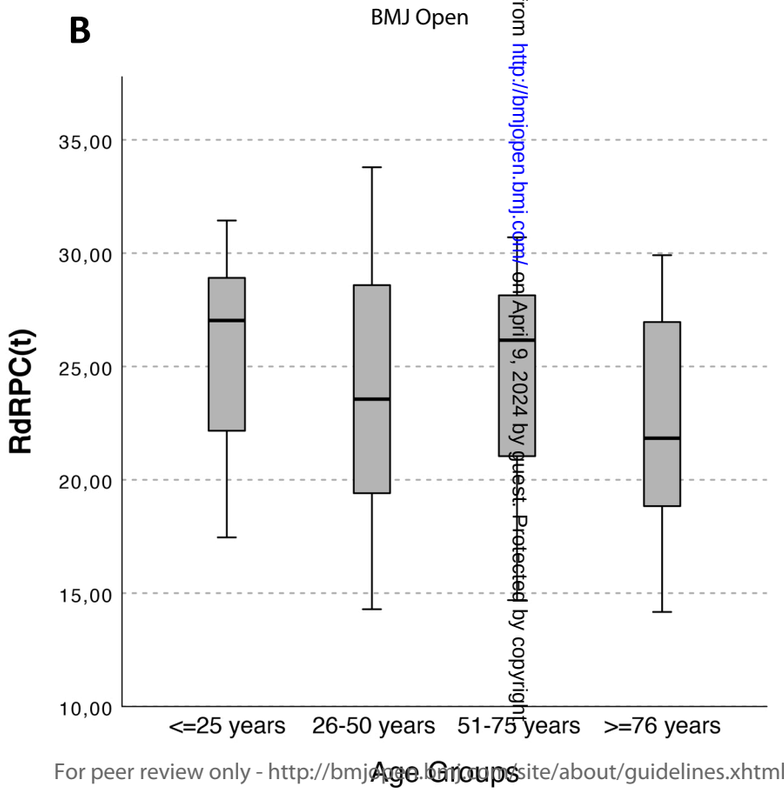

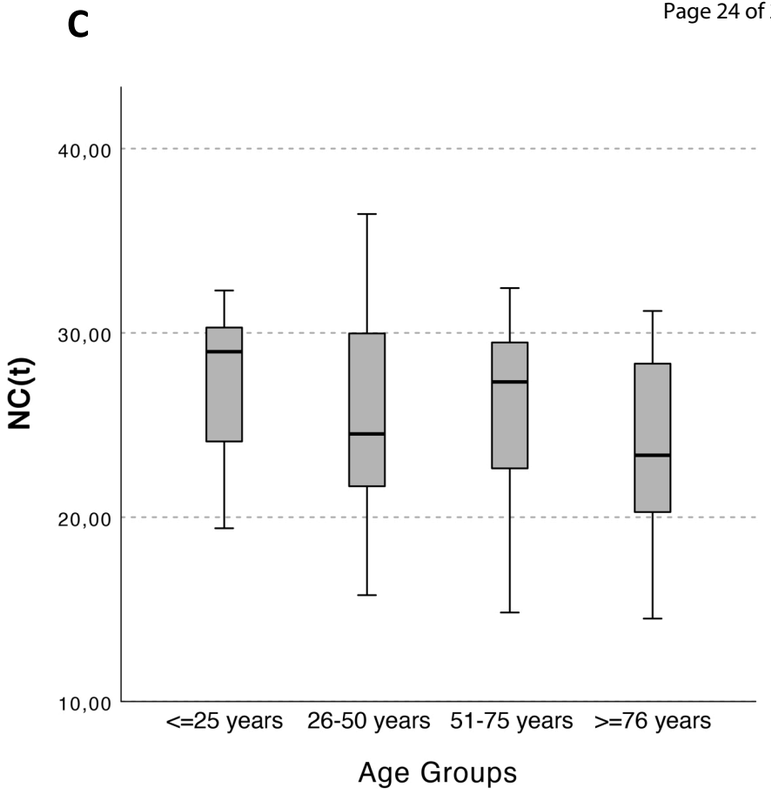

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	na
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	7
		(*c*) Explain how missing data were addressed	7
		(*d*) If applicable, explain how loss to follow-up was addressed	na
		(*e*) Describe any sensitivity analyses	na
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	na
		(c) Consider use of a flow diagram	na
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8
		(b) Indicate number of participants with missing data for each variable of interest	na
		(c) Summarise follow-up time (eg, average and total amount)	na
Outcome data	15*	Report numbers of outcome events or summary measures over time	8

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8 8 na
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for exposed and unexposed groups.

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