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No evidence of SARS-CoV-2 in hospitalized patients with severe acute respiratory syndrome in five Italian hospitals from 1st November 2019 to 29th February 2020 --Manuscript Draft--

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Keywords:	SARS-CoV-2 in Italy, SARI hospitalized patients, phase 1 of pandemic, influenza season 2019-2020.
Abstract:	<p>Background On 9th January 2020, China CDC reported a novel coronavirus (later named SARS-CoV-2) as the causative agent of the coronavirus disease 2019 (COVID-19). Identifying the first virus introduction is of epidemiological interest for tracking and mapping SARS-CoV-2 spread in a country. In this view, a retrospective observational study was performed to detect SARS-CoV-2 in oropharyngeal samples collected from hospitalized patients with a Severe Acute Respiratory Infection (SARI) enrolled in DRIVE (Development of Robust and Innovative Vaccine Effectiveness) study in five Italian hospitals (CIRI-IT BIVE hospitals network) (1st November 2019 – 29th February 2020).</p> <p>Objectives To acquire new information on SARS-CoV-2 infection real trend during phase I pandemic and determine the possible virus early appearance in the Italian epidemiological scenario.</p> <p>Materials and Methods The samples were tested for SARS-CoV-2 [one-step real-time multiplex retro-transcription (RT) PCR] and for influenza [RT-PCR assay (A/H1N1, A/H3N2, B/Yam, B/Vic)] according to DRIVE study aim.</p> <p>Results Overall, 1683 samples were collected, and no evidence of SARS-CoV-2 was observed. Moreover, 28.3% (477/1683) swabs were positive for influenza. The majority were influenza type A (358 vs 119 type B). A/H3N2 was predominant among influenza A viruses (55%), and among influenza B viruses, B/Victoria was prevalent. The highest influenza incidence rate was reported in 0-17y patients (40.3%) followed by the 18-64y (24.4%) and by the ≥65y (14.8%).</p> <p>Conclusions In Italy, some studies have shown SARS-CoV-2 early circulation in northern regions, the most affected by the pandemic during phase I. On the contrary, in central and southern regions, no early circulation of the virus was registered. These results are aligned with ours. These findings enhance the need to understand the epidemiology of SARS-CoV-2 and the clinical characteristics of COVID-19 compared to other acute respiratory illnesses and evaluate the burden on the healthcare system.</p>
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No evidence of SARS-CoV-2 in hospitalized patients with severe acute respiratory syndrome in five Italian hospitals from 1st November 2019 to 29th February 2020

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Abstract

Background

On 9th January 2020, China CDC reported a novel coronavirus (later named SARS-CoV-2) as the causative agent of the coronavirus disease 2019 (COVID-19).

Identifying the first virus introduction is of epidemiological interest for tracking and mapping SARS-CoV-2 spread in a country. In this view, a retrospective observational study was performed to detect SARS-CoV-2 in oropharyngeal samples collected from hospitalized patients with a Severe Acute Respiratory Infection (SARI) enrolled in DRIVE (Development of Robust and Innovative Vaccine Effectiveness) study in five Italian hospitals (CIRI-IT BIVE hospitals network) (1st November 2019 – 29th February 2020).

Objectives

To acquire new information on SARS-CoV-2 infection real trend during phase I pandemic and determine the possible virus early appearance in the Italian epidemiological scenario.

Materials and Methods

The samples were tested for SARS-CoV-2 [one-step real-time multiplex retro-transcription (RT) PCR] and for influenza [RT-PCR assay (A/H1N1, A/H3N2, B/Yam, B/Vic)] according to DRIVE study aim.

Results

Overall, 1683 samples were collected, and no evidence of SARS-CoV-2 was observed. Moreover, 28.3% (477/1683) swabs were positive for influenza. The majority were influenza type A (358 vs 119 type B). A/H3N2 was predominant among influenza A viruses (55%), and among influenza B viruses, B/Victoria was prevalent. The highest influenza incidence rate was reported in 0-17y patients (40.3%) followed by the 18-64y (24.4%) and by the ≥ 65 y (14.8%).

Conclusions

In Italy, some studies have shown SARS-CoV-2 early circulation in northern regions, the most affected by the pandemic during phase I. On the contrary, in central and southern regions, no early circulation of the virus was registered. These results are aligned with ours. These findings enhance the need to understand the epidemiology of SARS-CoV-2 and the clinical characteristics of COVID-19 compared to other acute respiratory illnesses and evaluate the burden on the healthcare system.

Introduction

On December 31, 2019, the Wuhan Municipal Health Commission in Wuhan City, Hubei province, China, reported a cluster of 27 pneumonia cases of unknown aetiology [1]. On January 9, 2020, the China CDC said that a novel coronavirus (later named SARS-CoV-2, the virus causing COVID-19) had been detected as the causative agent for 15 of the 59 cases of pneumonia [2,3].

The first European case was officially reported from France on January 24, 2020 [4, 5]. One week later, in Italy, the first cases were described. Two Chinese tourists from Wuhan landed in Milan and then fell ill in Rome on January 30, 2020 [6]. The first autochthonous patient, a 38-year-old man, was diagnosed only one month later in Codogno (Lombardy), on February 21. It was believed to be the "patient zero", but the first introduction of the virus into Italy remains unclear.

Many European countries have been trying to ascertain whether SARS-CoV-2 infections had occurred before the official 1st case reported by health authorities [7-13]. In this regard, we conducted a retrospective observational study to detect SARS-CoV-2 in oropharyngeal samples collected from hospitalized patients with severe acute respiratory infection (SARI) [14] aged ≥ 6 months in five hospitals in four Italian cities (Genoa, Rome, Bari, Siena) in the period 1st November 2019 – 29th February 2020. Our intention was to acquire new information on the real trend of the infection during phase I of the epidemic, and to determine the possible early appearance of the virus in Italy.

Materials and Methods

Study population and period

Samples were collected during the 2019-2020 influenza season by hospitalized individuals with SARI enrolled in the European study DRIVE (Development of Robust and Innovative Vaccine Effectiveness) [15] between 1st November 2019 and 29th February 2020.

SARI and COVID-19 definition

According to the ECDC case definition, a case of SARI is defined as a hospitalized patient of any age with at least one respiratory sign or symptom (cough, sore throat, breathing difficulties) and at least one systemic sign or symptom (fever or low-grade fever, headache, myalgia, generalized malaise) or deterioration in general condition (asthenia, weight loss, anorexia or confusion and dizziness) [15].

A case of COVID-19 is considered any person with at least one symptom such as cough, fever, shortness of breath, sudden onset of anosmia, ageusia or dysgeusia. Additional less specific symptoms may include headache, chills, muscle pain, fatigue, vomiting and/or diarrhoea [15].

Molecular analysis for SARS-CoV-2 detection

Total RNA was extracted and tested for the identification of SARS-COV-2 by means of a one-step real-time multiplex retro-transcription (RT) PCR assay targeting the nucleoprotein region (N), RNA-dependent RNA-polymerase region (RdRp) and the envelope region (E), in accordance with international protocols [16].

Molecular analysis for influenza detection

Total RNA was extracted and tested for the identification of influenza viruses (A/H1N1, A/H3N2, B/Yamagata, B/Victoria) by means of a one-step real-time multiplex retro-transcription (RT) PCR assay, in accordance with international protocols [16].

Results

Overall, 1,683 samples were collected at different times during the study period (Figure 1).

Fig.1 Number of enrollments (= swabs) distribution by week, during the study period.

Data on demographic characteristics, chronic conditions, risk factors and influenza vaccination status were available for every patient (Table 1). The patients' mean age was 38.2 years and about 35.7% (600/1,683) were ≥ 65 years old.

Table 1. Patients' characteristics stratified by age.

	<18 y	18-64 y	≥65 y
Total	780 (100%)	303 (100%)	600 (100%)
Sex=male	422 (54.1%)	175 (57.8%)	331 (55.2%)
Any chronic condition*			
No (0)	666 (85.4%)	174 (57.4%)	95 (15.8%)
Yes (≥1)	114 (14.6%)	129 (42.6%)	505 (84.2%)
Influenza vaccination status (2019-2020 season)			
No	751 (96.3%)	265 (87.5%)	345 (57.5%)
Yes	27 (3.5%)	37 (12.2%)	254 (42.3%)
N/A	2 (0.2%)	1 (0.3%)	1 (0.2%)

* Chronic respiratory diseases, Heart or cardiovascular disease, Diabetes, Renal disease, Anemia, Cancer, Chronic liver disease, Dementia, History of stroke, Obesity, Autoimmune disease, Rheumatological diseases.

Clinical manifestations were also recorded for every patient (Table 2). The most common symptoms were fever (81.1%, 1,365/1683) and cough (60.1%, 1,012/1683) (Table 2); these are generic symptoms that could hypothetically be related to SARS-CoV-2 infection.

Table 2. Patients' clinical manifestations.

Symptoms	
Fever	1,365 (81.1%)
Malaise	798 (47.4%)
Headache	196 (11.6%)
Myalgia	271 (16.1%)
Cough	1,012 (60.1%)
Sore throat	731 (43.4%)
Short breath	590 (35.1%)

No evidence of SARS-CoV-2 was found.

According to DRIVE objectives we tested all swabs for influenza viruses. Generally, 28.3% (477/1,683) of our swabs were positive for influenza: 358 (75%, 358/477) were type A and 119 (25%, 119/477) type B. The details are shown in Figure 2. Most influenza cases were of subtype A/H3N2 and mainly affected subjects

aged <18 years and the elderly. By contrast, subtype A/H1N1 was prevalent in adults (aged 18-64 years), followed by the elderly (Figure 2). Subjects vaccinated against influenza in the 2019-2020 season were 18.9% (318/1,683). In particular, 79.9% (254/318) were the elderly, 11.6% (37/318) aged 18-64 years and 8.5% (27/318) aged <18 years.

Fig.2 Sample distribution by influenza type/subtype or lineage and age during the study period.

Discussion

In Italy, the first autochthonous case was diagnosed one month later in Codogno (Lombardy), on February 21, 2020. Over the following days, cases were reported from several different areas of the country, with Northern Italy being most severely affected at the beginning of the COVID-19 pandemic [17]. Identifying the first introduction of the virus is of epidemiological interest to track and map the spread of SARS-CoV-2 in a country.

The clinical information we collected showed that the definition of COVID-19 overlaps with that of SARI, confirming that the clinical picture is insufficient to diagnose SARS-CoV-2 infection [18,19]. Moreover, considering the evidence in the literature [18,19], we analyzed samples collected from hospitalized patients with SARI, as it has been demonstrated that patients with COVID-19 are more likely to be admitted to hospital; therefore, these patients were best suited to the aim of the study.

No evidence of SARS-CoV-2 was found. Our results are in line with some Italian and European data. Capalbo et al. [13] evaluated the prevalence of SARS-CoV-2 infection among SARI patients in a hospital in Central Italy from November 1, 2019 to March 1, 2020. Like us, they confirmed that SARS-CoV-2 was not circulating at the time of their study and that the COVID-19 pandemic did not start before its official onset in Italy. Similarly, in Scotland, Tomb et al. detected no SARS-CoV-2 in the population prior to March 2020 [12].

By contrast, other studies revealed the presence of the virus prior to the first “official” case. In Italy, some retrospective studies conducted on samples of a different sort (serological and environmental samples) and from patients with different health conditions reported different results from ours [7-10]. In Northern Italy, environmental wastewater monitoring detected positive samples as early as December 2019 [9,10]. In Milan, Amendola et al. detected the RNA of the virus in early December 2019 in a swab sample from a child with suspected measles [8]. Finally, Apolone et al. found seroprevalence evidence of SARS-CoV-2 in asymptomatic

patients. These were lifelong smokers who were screened for the early detection of lung cancer (high-risk group) from September to October 2019 [7].

It should be noted that the published Italian studies reporting early SARS-CoV-2 circulation were conducted in geographic areas that were different from ours, and which were severely affected during the initial phase I. In France, Deslandes et al. retrospectively analyzed nasopharyngeal swabs collected from hospitalized patients; their results suggested that the epidemic had probably started in early December 2019 [11].

The COVID-19 pandemic impacted influenza circulation from week 13 of 2020, when countries implemented strict lockdowns and issued hygiene recommendations [20]. In line with the European trend, the 2019-2020 influenza season in Italy had a shorter overall duration than previous seasons.

Like all the literature studies considered, ours has some limitations. We used oropharyngeal swabs, while it has been shown that nasopharyngeal swabs are the most suitable for the molecular detection of SARS-CoV-2, as the quantity of virus is greater in the nose [21]. Moreover, we stocked the swab aliquots at -20°C and later processed them for SARS-CoV-2 molecular research, and it is known that the defrosting step can affect the results of the extraction and real-time steps.

Conclusions

The results published in the literature show that it is very difficult to establish the exact time and place of the initial SARS-CoV-2 outbreak in Italy and Europe. These findings highlight the need to continue to carry out retrospective studies in order to understand the epidemiology of the novel coronavirus, to identify the clinical characteristics of COVID-19 in comparison with other acute respiratory illnesses (ARI), and to evaluate the burden of COVID-19 on the healthcare system.

In sum, it is crucial to strengthen routine monitoring (both epidemiological and laboratory) of the causative agents of SARI, in order to support preventive strategies for all respiratory pathogens and promote integrated strategies for influenza and COVID-19 vaccination.

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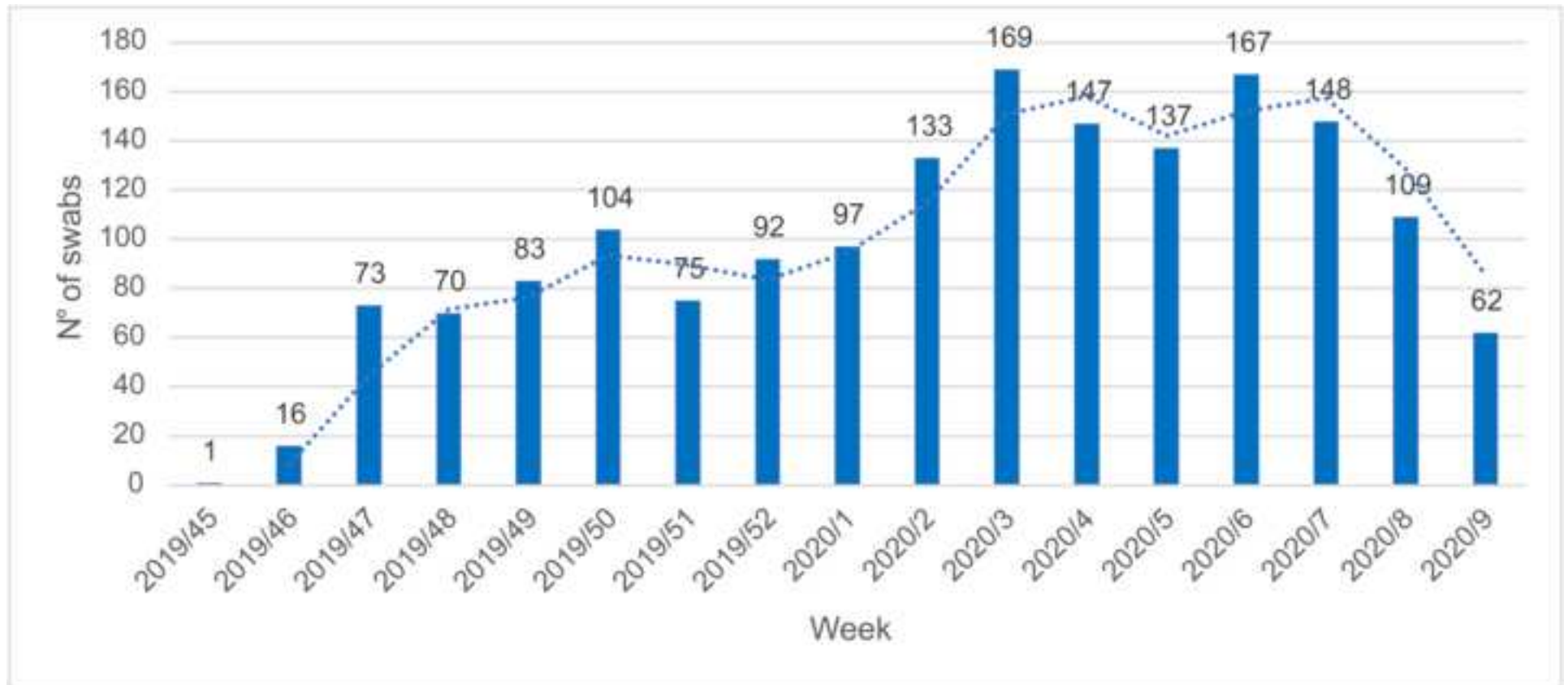


Figure 2

