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Correspondence

No evidence of SARS-CoV-2 circulation in the framework of influenza surveillance between October 2019 and February 2020 in Lombardy, Italy

ARTICLE INFO

Keywords

SARS-CoV-2

COVID-19

Molecular epidemiology

Pandemic virus

Respiratory samples

Real-time RT-PCR

Dear Editor,

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) global pandemic spread from China in a very short period of time. Undetected infectious individuals have significantly contributed to its dissemination. In our region, Lombardy, Italy, the first laboratory-confirmed COVID-19 case was identified on February 20, 2020 and this was one of the earliest and most serious cluster during the first wave of COVID-19. Thus, respiratory samples, stored at -80°C , which were collected from October 1st, 2019 to February 20th, 2020, in the framework of the regional influenza surveillance system in the Lombardy region (nearly 10 million inhabitants) were retrospectively analyzed to evaluate the possible presence of SARS-CoV-2 RNA in a period preceding the first recorded COVID-19 case in Lombardy (February 20th, 2020). Respiratory samples were collected from patients with severe acute respiratory infection (SARI) or acute respiratory distress syndrome (ARDS) requiring admission to intensive care unit (ICU) and from outpatients with influenza-like illness (ILI) by sentinel paediatricians and general practitioners and analyzed in the two regional reference laboratories (Molecular Virology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, and Department of Biomedical Sciences for Health, University of Milan, Milan). [Table 1](#) presents the characteristics of samples/patients included in this retrospective study according to their temporal (by month of collection) and geographical (by local health authority, ATS: Agenzia di Tutela della Salute) distribution. A total of 1581 respiratory samples from 1224 patients (554 females and 670 males) were included in the study. Nearly 35% ($n = 431$) of patients were ≤ 14 years, 46% ($n = 562$) were aged between 15 and 64 years, and 19% ($n = 231$) were ≥ 65 years. 1395 (88.2%) samples were collected from upper respiratory tract and 186 (11.8%) from lower respiratory tract. Nearly 40% ($n = 601$) of samples were collected in the period October–December 2019, and the remaining samples were evenly distributed between January ($n = 502$) and February ($n = 478$) 2020. Total RNA was extracted using the QIA Symphony® instrument with QIA Symphony® DSP Virus/Pathogen Midi Kit (Complex 400 protocol (QIAGEN) or QIAamp Viral RNA Mini kit (QIAGEN) by means of an

automated extractor (QIAcube, QIAGEN) according to the manufacturer's instructions. According to World Health Organization (WHO) guidelines [1], specific real-time reverse transcriptase–polymerase chain reactions (RT-PCR) targeting the RdRp (RNA-dependent RNA polymerase) and E (envelope) genes or two different portions of the N (nucleocapsid) gene were used to detect the presence of SARS-CoV-2 RNA [1].

Of the 1581 samples tested, none (0%) resulted positive for the presence of SARS-CoV-2 RNA. The results of our retrospective analysis in respiratory samples collected from patients with respiratory symptoms are in keeping with those of others [2] and do not support evidence of widespread circulation for SARS-CoV-2 from October 2019 to January 2020 in Lombardy. This conclusion is also in agreement with data from a serologic analysis of the first SARS-CoV-2 outbreak (the Lodi Red Zone) [3] as well as a phylodynamic evaluation of SARS-CoV-2 genomes detected in the first epidemic wave, which both estimate (using different experimental approaches) the seeding of SARS-CoV-2 in Lombardy in early January 2020 [4]. In contrast, a study on stored sera from oncologic patients dated back early antibody seropositivity to SARS-CoV-2 to September 2019 [5]. This study reported that several individuals across Italy had antibodies against SARS-CoV-2 weeks or months before the first outbreak in Lombardy, while it remains unexplained why the epidemic did not evolve simultaneously in multiple regions [5]. Another study detected SARS-CoV-2 RNA in a stored oropharyngeal swab collected in early December 2019 from a child among 39 samples tested for suspected measles infection [6]. The child lived in Milan and not in one of the two major and earliest outbreak epicentres (Lodi and Bergamo), and the Authors concluded that this positivity could be occasional to a small self-limiting outbreak [6]. Our study has strengths and limitations. The major strengths are: i) the large number of patients analyzed, ii) the high quality of samples (from both upper and lower respiratory tract) from surveillance networks of respiratory syndromes (ARDS, SARI and ILI) as well as the correct specimens and storage condition, and iii) an overall good temporal and geographical distribution of samples. A major limitation of this study, however, is the small number of specimens available from the areas that were involved in the

<https://doi.org/10.1016/j.tmaid.2021.102002>

Received 11 February 2021; Received in revised form 20 February 2021; Accepted 22 February 2021

Available online 26 February 2021

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Table 1
Summary of samples and patients included in the study.

Categories		Total (n = 1581)	Fondazione IRCCS Policlinico San Matteo (n = 1104)	University of Milan (n = 477)
Gender (n = 1224)	Female	315 (41.8%)	239 (50.1%)	239 (50.1%)
	Male	432 (58.2%)	238 (49.9%)	238 (49.9%)
Age (n = 1224)	0–4 years	230 (18.8%)	138 (18.4%)	92 (19.3%)
	5–14 years	201 (16.4%)	76 (10.2%)	125 (26.2%)
	15–64 years	562 (45.9%)	333 (44.6%)	229 (48.0%)
	≥65 years	231 (18.9%)	200 (26.8%)	31 (6.5%)
ATS ^a	Città Metropolitana di Milano	245 (15.5%)*	113 (10.2%)	132 (27.7%)
	Insubria	66 (4.2%)	44 (4.0%)	22 (4.6%)
	Brianza	39 (2.5%)	9 (0.8%)	30 (6.3%)
	Bergamo	78 (4.9%)	8 (0.7%)	70 (14.7%)
	Brescia	92 (5.8%)	2 (0.2%)	90 (18.9%)
	Pavia	912 (57.7%)	912 (82.6%)	0 (0%)
	Val Padana	123 (7.8%)	7 (0.6%)	116 (24.3%)
	Montagna	26 (1.6%)	9 (0.8%)	17 (3.5%)
	Period	October 2019	168 (10.6%)	168 (15.2%)
November 2019	202 (12.8%)	147 (13.3%)	55 (11.5%)	
December 2019	231 (14.6%)	161 (14.6%)	70 (14.7%)	
January 2020	502 (31.8%)	317 (28.7%)	185 (38.8%)	
February 2020	478 (30.2%)	311 (28.2%)	167 (35.0%)	
Samples	Nasopharyngeal swab	1382 (87.4%)	905 (82.0%)	477 (100.0%)
	Pharyngeal swab	13 (0.9%)	13 (1.2%)	0 (0%)
	Bronco aspirates	7 (0.4%)	7 (0.6%)	0 (0%)
	Bronchoalveolar lavage	179 (11.3%)	179 (16.2%)	0 (0%)

ATS, local health authorities ^ahttp://www.cartografia.regione.lombardia.it/metadata/ats/doc/Nuovo_Sistema_Socio_Sanitario_Lombardo.pdf. *16/245 (6.5%) samples were from Lodi.

two initial and major outbreaks (Lodi and Bergamo). Determining the circulation of SARS-CoV-2 in Italy in the pre-pandemic period, is of paramount importance to increase our current knowledge on SARS-CoV-2 epidemiology and its massive spread in our region. Further studies combining molecular and serological investigations are needed to clarify the introduction and circulation of SARS-CoV-2 in Italy prior to its first identification in February 2020.

Funding

This study was supported by Ricerca Finalizzata from Ministry of Health, Italy (grants no. GR-2013-02358399 and COVID-2020-12371817).

Author contributions

Conceived and designed the study: EP, AP, FB. Designed and/or performed experiments: FG, CG, LP, SP, MT. Analyzed and interpreted data: AP, FG, CG, EP. Wrote the manuscript: AP, FB, EP. All authors provided substantial scientific input to the manuscript. All authors revised and agreed upon the final version of the manuscript.

Declaration of competing interest

All authors have no conflicts of interest to disclose.

Acknowledgements

We thank Daniela Sartori for manuscript editing. We thank prof. Alessandro R. Zanetti for his critical revision.

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