

1 **Title**

2 What was behind the first recognition and characterization of autochthonous SARS-CoV-2
3 transmission in Italy: who opened Pandora's Box in Europe?

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28 **Abstract**

29 An Italian male with no link to China SARS-CoV-2 epidemic presented at Emergency Room with
30 severe respiratory impairment. The RT-PCR on 20th February, 2020, nasopharyngeal swab revealed
31 SARS-CoV-2 infection, confirmed with viral culture and sequencing. This was the first identified
32 autochthonous SARS-CoV-2 transmission in Italy, that unveiled global pathogen diffusion.

33 **Keywords**

34 SARS-CoV-2; Pandemic; RT-PCR; viral culture; serology.

35 **Key Clinical Message**

36 This clinical case underlines an underestimation of SARS-CoV-2 circulation, making initial
37 containment measures unfit to face the real situation and delaying the management of potentially
38 affected SARS-CoV-2 patients.

39 **Introduction**

40 The pandemic of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) originated in
41 Wuhan, China, in December 2019 [1]. Chinese health authorities put in place containment
42 measures, apparently limiting diffusion inside national borders and occasionally involving travelers
43 abroad [2]: in Italy, on February 5th, 2020, only three cases were reported coming from Hubei
44 province, two Chinese tourists and an Italian repatriate [3]. However, the first identification of local
45 transmission in Italy here reported revealed that global diffusion was a fact and no more a
46 hypothesis.

47 **Case presentaion**

48 A 38 years old male presented at Codogno Hospital Emergency Room (ER) on February 18th, 2020,
49 with fever and nonproductive cough which had arisen on February 10th, 2020; however, despite
50 pneumonia diagnosis, no epidemiological link with Coronavirus Disease 2109 (COVID-19)
51 Chinese epidemic was found and the patient was discharged with prescription of levofloxacin
52 therapy. On February 19th, 2020, he presented at ER again due to worsened conditions and the

53 situation negatively evolved in few hours: the patient was dysphonic, with a body temperature of
54 40.8° C, a P/F = 80, normal WBC, negative PCT and CRP 80, while the chest CT showed multiple
55 foci bilateral pneumonia and ground glass areas (Figure 1); considering the clinical picture, the
56 subject was moved to Intensive Care Unit where, after CPAP trial failure, he was intubated and
57 pronated. During anesthetist visit, the patient's wife disclosed a possible exposure to the new
58 Coronavirus: in the previous days they found out that a patient's colleague had fever and respiratory
59 symptoms after a business meeting with Chinese co-workers.

60 A nasopharyngeal swab, collected on 20th February, 2020, was sent to Sacco Hospital Laboratory of
61 Clinical Microbiology, Virology and Bioemergencies (CLIMVIB) for SARS-CoV-2 diagnosis.
62 According to internal procedure, the presence of respiratory pathogens was investigated using the
63 multiplex BioFire® FilmArray® Respiratory 2 (RP2) Panel (Biomerieux, France) assay: all targets
64 resulted negative, excluding infection with most common bacteria and viruses and confirming the
65 negative results of legionella and pneumococcal urine antigen tests. At the same time, a sample
66 aliquot of 500 µL was used for RNA extraction by means of NUCLISENS® easyMAG®
67 (Biomerieux, France) instrument; RNA was then processed using the SARS-CoV-2 Hong-Kong
68 University (HKU) detection protocol [4], including SARS-CoV RNA from laboratory biological
69 repository as positive control, as indicated in the HKU protocol: both Nucleoprotein (N) and Orf-
70 1ab targets (for screening and confirmation, respectively) were detected, putting the last piece into
71 the diagnostic puzzle. According to Italian guidelines, a swab aliquot was immediately delivered to
72 the Istituto Superiore di Sanità (ISS) Department of Infectious Diseases for diagnosis confirmation.
73 However, considering that no other cases had been previously found, in order to further support
74 these preliminary data the sample was analyzed using reagents provided as trial kits by different
75 companies: Allplex™ 2019-nCoV Assay (Seegene Inc., Republic of Korea), Liferiver Novel
76 Coronavirus (2019-nCoV) Real Time Multiplex RT-PCR Kit (Liferiver™, Shanghai ZJ Bio-Tech
77 Co., Ltd., China), and TaqMan 2019-nCoV Assay Kit v1 (Applied Biosystems, Thermo Fisher

78 Scientific Inc., USA). All commercial assays returned positive results for all the gene targets, as
79 confirmed in the following days also by ISS.

80 Further investigations were conducted during the following months to better depict the case. A
81 plasma sample collected on February 21st, 2020, was tested for SARS-CoV-2 specific antibodies,
82 using anti-SARS-CoV-2 IgG and IgM chemiluminescence immunoassay (CLIA) kits on fully
83 automated iFlash1800 analyzer (Shenzhen YHLO Biotech Co., Ltd., Shenzhen, China) [5]: both IgM
84 (0.30 AU/mL, cut-off = 10.00 AU/mL) and IgG (0.57 AU/mL, cut-off = 10.00 AU/mL) resulted
85 negative, in accordance with the time spanning between symptoms onset and blood collection (10
86 days). Given the patient's disease severity and considering that the presence of viral genome in
87 blood was recognized as a negative prognostic factor [6-8], a RT-PCR was performed on the same
88 sample using ELITe InGenius[®] system and the GeneFinder[™] COVID-19 Plus RealAmp Kit assay
89 (ELITechGroup, France): not surprisingly, a positivity to N gene (Ct value = 33) was found. Viral
90 culture on VERO E6 cells (ATCC[®] CRL-1586[™]) was also performed from both nasopharyngeal
91 swab and Bronchoalveolar Lavage Fluid (BALF). The first one permitted whole genome
92 sequencing by means of Illumina Miseq Reagent Nano kit: the sequence was deposited in the
93 Global Initiative on Sharing All Influenza Data (GISAID; accession number EPI_ISL_412973) and
94 phylogenetic analysis placed the viral strain in a cluster close to the German isolates one, but distant
95 from the three first imported Italian cases [9]. Interestingly, a cytopathic effect was observed after
96 only 24h of incubation of BALF sample, suggesting a high viral concentration in the patient's low
97 respiratory tract, likely linked to severe clinical conditions.

98 After the notification of positivity, Codogno Hospital promptly activated isolation and containment
99 measures and the case was notified to Italian Ministry of Health and competing authorities.

100 Patient's close contacts were tested for SARS-CoV-2: several individuals resulted positive,
101 including his pregnant wife; on the contrary, both nasopharyngeal swab and serum sample from the
102 putative transmitting subject were negative, thus increasing uncertainty on the possible infection

103 acquisition. The patient was moved to San Matteo Hospital in Pavia on February 22nd, 2020, and he
104 completely recovered after several weeks of hospitalization.

105 **Discussion**

106 The identification of this first local transmission paved the way to pandemic recognition. CLIMVIB
107 had processed 68 samples in the period January 22nd - February 20th: on February 21st, 2020 only,
108 the received nasopharyngeal swabs were more than 50, becoming thousands in few days in all
109 regional referral centers, with plenty of symptomatic individuals, probable contacts and frightened
110 people rushing to ERs. In only two weeks SARS-CoV-2 epidemiology radically changed, as
111 depicted by the European situation: accordingly to WHO reports, on February 20th, 2020 confirmed
112 cases were 3, 12, 16, 2 and 9 in Italy (IT), France (FR), Germany (DE), Spain (SP) and the United
113 Kingdom (UK), respectively, rising on March 4th, 2020 to IT = 2502, FR = 212, DE = 196, FR =
114 151 and UK = 51 [10, 11]; these data, however, were just the tip of the iceberg of the real situation,
115 as demonstrated by more than 3 million infections and 217 thousand deaths by the end of April,
116 2020 worldwide [12].

117 This first unexpected autochthonous case increased incertitude on when and how the virus started
118 its global circulation. Zhou and colleagues stated that the initial SARS-CoV-2 outbreak started on
119 December 12th, 2019 in Wuhan, based on the admission date of seven patients with severe
120 pneumonia to ICU of Jin Yin-Tan Hospital [1]: therefore, it is reasonable that these subjects
121 acquired the infection at least in late November from an unknown source. Considering that Chinese
122 authorities declared the Wuhan quarantine on January 22nd, 2020, a virus unrestrained circulation of
123 approximately two months can be hypothesized, during which SARS-CoV-2 spread due to human
124 mobility, also reaching travelers moving around the world: the World Bank estimated more than
125 611 millions passengers in China airports for 2018, each of them representing a potential virus
126 carrier during an epidemic [13]. It is relevant that the first local transmission in Europe was detected
127 in January 2020: a Chinese woman from Shanghai, who had attended a business meeting in Munich
128 on January 20th and 21st, with mild symptoms wrongly attributed to jetlag, fell ill on the flight back

129 home and tested positive on January 26th; the company as well as German health authorities were
130 immediately informed and the contact tracing was started: a total of 20 cases out of more than 200
131 contacts were included in this epidemic cluster (4 as first generation cases, the other 16 belonging to
132 a subsequent group) and the outbreak was considered contained, with no further expansion in the
133 country; notably only two patients (n 1 and n 2) had direct contact with the index patient (the
134 Chinese woman), the others had direct or secondary contact only with German patient n 1 [14,15].
135 Aside from the outbreak description, the German studies made evident that SARS-CoV-2 can be
136 transmitted by asymptomatic individuals too: the Chinese woman developed symptoms only after
137 contact with her colleague, who then infected relatives and co-workers before becoming sick. The
138 keystone of the unrestricted circulation of SARS-CoV-2 at least at the beginning of the outbreak is
139 that asymptomatic people tended not to get tested despite their capacity to spread the infection.
140 Interestingly, as above mentioned, phylogenetic studies revealed that SARS-CoV-2 likely entered
141 Italy with at least two transmission events, probably in late January, and that the patient 1 virus
142 mapped in a cluster containing also German sequences, but not the other Italian ones [9, 16]. Such
143 evidences suggest that SARS-CoV-2 was spreading undisturbed everywhere via asymptomatic
144 subjects, thus making epidemic estimations and containment measures unfit to face the real
145 situation.
146 Following Italian Patient 1 identification and the increase in confirmed cases, on March 9th, the
147 Italian Government declared the “lockdown” status, limiting citizens’ activities and transfers, in
148 order to control the epidemic spread; with the same purpose, other countries prohibited access to
149 Italian travelers, even in absence of local containment measures or lockdown policies.
150 But it was too late: Pandora’s box was already wide open.

151

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157 **CRedit Author statement**

158 Conceptualization, M.V., M.A., M.A.; Investigation, M.A., V.P.G., P.C., A.O.; Data curation,
159 M.V., M.A., M.D., R.A.; Writing - Original Draft, M.A., M.D., R.A.; Writing - Review & Editing,
160 M.V., M.A.; Supervision, M.V., G.M.R.

161 **Conflicts of Interest**

162 The authors declare no conflicts of interest.

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168 [anno=2020&codLeg=73195&parte=1+&serie=null](http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2020&codLeg=73195&parte=1+&serie=null). Accessed: 24 June 2020.

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210 PMC7461481.

211 **Figures Captions**

212 Figure 1. Chest Computer Tomography Scan. CT scan that shows some areas of ground-glass and
213 consolidations in the lower lobes.