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Rapid viral diagnosis and ambulatory management of suspected COVID-19 cases presenting at the infectious diseases referral hospital in Marseille, France, - January 31st to March 1st, 2020: A respiratory virus snapshot.

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ABSTRACT:

Background: Rapid virological diagnosis is needed to limit the length of isolation for suspected COVID-19 cases.

Method: We managed the first 280 patients suspected to have COVID-19 through a rapid care circuit and virological diagnosis in our infectious disease reference hospital in Marseille, France. Rapid viral detection was performed on sputum and nasopharyngeal samples.

Results: Over our study period, no SARS-CoV-2 was detected. Results were obtained within approximately three hours of the arrival of patient samples at the laboratory. Other viral infections were identified in 49% of the patients, with most common pathogens being influenza A and B viruses, rhinovirus, metapneumovirus and common coronaviruses, notably HKU1 and NL63.

Conclusion: Early recognition of COVID-19 is critical to isolate confirmed cases and prevent further transmission. Early rule-out of COVID-19 allows public health containment measures to be adjusted by reducing the time spent in isolation.

INTRODUCTION:

In December 2019, a novel coronavirus (now named SARS-CoV-2) was identified as a cause of a cluster of pneumonia cases in Wuhan, a city in the Hubei province of China [1]. The number of cases rapidly rose. COVID-19 has spread globally within 60 days with focus areas in Asia, Europe and the Middle East [2]. Epidemiological data indicate that human-to-human transmission is occurring [3,4]. As of 1 March 2020, 130 confirmed COVID-19 cases had been reported in France with three deaths [5]. In this context, rapid virological diagnosis is valuable to reduce the duration of isolation for suspected cases and to reduce fear among individuals who have potentially ben in contact with them. It also allows for optimal management of any COVID-19 cases that are diagnosed. We aim to share our initial experiences in managing patients suspected of having COVID-19 through a rapid ambulatory care circuit and virological diagnosis in our infectious disease reference hospital in Marseille, France.

METHODS:

Definition of suspected cases:

In France, the definition of a possible COVID-19 case changed during the study period [5] (Figure 1):

- Between 30 January 2020 and 3 February 2020, COVID-19 cases were suspected on the basis of the association of an acute respiratory presentation, whatever the severity, with a body temperature ≥38°C within 14 days of return from Hubei Province in the Peoples' Republic of China, and/or in the event of close contact with a confirmed case.
- Between 4 and 20 February 2020, the definition was extended to include the association of respiratory symptoms with a body temperature ≥38°C or subjective

fever, within 14 days of return from anywhere on the continental Peoples' Republic of China.

- From 21 to 23 February 2020, the definition was extended to include any respiratory symptoms (including isolated upper respiratory tract symptoms) and the risk zone was enlarged to the whole of China (including Hong Kong and Macao) and to Singapore.
- From 24 to 26 February 2020, South Korea and two regions of north Italy (Lombardy and Venetia) were added to the risk zone.
- On 27 February 2020, Iran, and another region of Italy (Emilia Romagna) were added to the risk zone. Additionally, any acute respiratory distress syndrome regardless of travel or close contact with a confirmed case was considered as a suspected case.
- From 28 February to 1 March 2020, the definition was enlarged to any unexplained pneumonia regardless of travel or close contact with a confirmed case.

Clinical management:

The Mediterranean Infection University Hospital Institute is a 27,000m² building that includes 75 hospital beds (single rooms), 25 of which are likely to be converted into three NSB3 modules with negative pressure, as well as a day-care hospital, an outpatient department with 14 consultation rooms and a travel clinic. Part of the outpatient department (five single rooms) can be isolated from the rest of the department and kept under negative pressure. If needed, this can be extended to the entire consultation ward. This Institute also includes five large NSB3 laboratories with a total area of 1,200 m², on four floors with diagnostic laboratories, research laboratories, and technological platforms [6].

An infectious disease specialist was available 24 hours a day and seven days a week for telephonic counselling for emergency units and helped with screening cases in collaboration with regional public health services. This clinician referred suspected patients for SARS-

CoV-2 testing. In our specific outpatient unit, a nurse and an infectious disease specialist managed patients who did not present severe symptoms and who arrived between 8am and 6.30pm. Others were hospitalised in the high-level isolation unit. Naso and oro-pharyngeal swab and/or sputum were obtained from each patient.

Laboratory tests:

Viral RNA was extracted from 200 µL of naso- and oro-pharyngeal swab fluid and/or sputum, using the EZ1 Virus Mini Kit v2.0 (Qiagen[®], Courtaboeuf, France). For the detection of SARS-CoV-2 RNA we used two different RT-PCR systems with a hydrolysis probe and the LightCycler Multiplex RNA Virus Master kit (Roche Diagnostics[®], Mannheim, Germany). The first system targets the envelope protein (E)-encoding gene and was previously described [7] and uses a synthetic RNA positive control (supplied by the Charité virology institute - Universitätsmedizin Berlin, Berlin, Germany [8]). The second system was designed in-house, targets the spike protein-encoding gene (forward primer: 5'-AAACTTGTGCCCTTTTGGTG-3'; reverse primer: 5'-TGCTGATTCTCTTCCTGTTCC-3'; probe: 5'-

CGCCACCAGATTTGCATCTG-3'), and uses a synthetic RNA positive control ordered from Eurogentec® (Seraing, Belgium). In some cases, a real-time RT-PCR was carried out with the QuantiNova SYBR Green RT-PCR kit (Qiagen®) that targeted either the E gene with the same primers as above or previously described primers targeting the RNA-dependent RNA polymerase (RdRp)-encoding gene [7] with a synthetic RNA positive control (Eurogentec). A phage RNA internal control [9] was added to each clinical sample before extraction to ensure RNA extraction and PCR amplification were accurate. All experiments were performed on a LightCycler 480 instrument (Roche Diagnostics) by trained qualified technicians available who were available around the clock, either during routine working days and hours, or on an on-call basis. Concurrently, a multiplex molecular assay that detects respiratory pathogens

was performed at the point-of-care laboratory [10] with the Biofire FilmArray Respiratory panel 2 test (Biomérieux, Marcy-l'Etoile, France) or the FTD Respiratory pathogens 21 kit (Fast Track Diagnosis, Luxembourg).

RESULTS:

Between 31 January and 1 March 2020, 283 patients were admitted in our centre with suspected COVID-19. Three patients with missing travel data were excluded from our analysis. A marked increase in the daily number of patients was observed from 24 February 2020 (Figure 2). Of these 280 patients, 60 (21.4%) had returned from Asia (including 31 returning from China), 210 (75%) had returned from Italy and 10 patients hadn't travelled outside France but were considered as contact cases. The M/F sex ratio was 1/1.2, and the mean age was 21 years (ranging from 1-84 years), including 42 children under the age of 18, of whom seven were under the age of three. 28 patients were hospitalised and 252 were treated as outpatients (Table 1).

Two-thirds of patients were febrile and most of their respiratory symptoms were suggestive of upper respiratory tract infections (Table 1).

SARS-CoV-2 PCR assays were negative for all samples from all patients. As measured for the 22 first tests, results were obtained within an average of 175 minutes (ranging from 150-195 minutes) of patients' samples arriving at the laboratory. Thereafter, results continued to be available in approximately three hours. A viral diagnosis was obtained in 137/280 patients (48.9%) (Table 2). The most frequent viruses were influenza A virus (12.1% of all patients), rhinovirus (11.8%), influenza B virus (7.9%) and metapneumovirus (7.1%). Common coronaviruses (E229, OC43, NL63 and HKU1) were detected in 12.9% of patients. Twelve (4.3%) patients had viral co-infections (Table 2). Patterns of viral infection slightly differed between patients returning from Italy and those returning from China. Notably, patients

returning from Italy were more likely to be infected with influenza B virus, metapneumovirus and coronavirus HKU1 compared to those returning from Asia (Figure 3). By contrast, patients returning from Asia were more likely to be infected with rhinovirus/enterovirus and respiratory syncytial virus.

DISCUSSION:

Because COVID-19 infection symptoms range from unspecific mild respiratory symptoms to acute respiratory distress [3] and because these symptoms are very similar to those of many seasonal viruses [11–13], we implemented an outpatient rapid diagnosis for mildly affected patients which operated ten hours a day, seven days a week. This involved testing a broad spectrum of clinical presentations in potentially exposed persons [14]. As in Italy, an infectious diseases clinician was key to patient triage [15]. The genome sequence of SARS-CoV-2 enabled the rapid design and implementation of dedicated point-of-care real-time RT-PCR diagnostic tests [16] in addition to other respiratory pathogens testing. The Mediterranean Infection University Hospital Institute is one of the nine French metropolitan hospital centres habilitated by the French Ministry of Health (MoH) to manage highly contagious patients. These centres were designated by the MoH to test patients suspected of COVID-19 at the early start of the epidemic (corresponding to the present study). Among these nine hospitals, our institute is the only one organized to manage large populations of out-patients in a dedicated isolated area, under negative pressure with laboratory facilities on site. The number of French centres designated for testing was then extended to 33 hospitals [5]. Finally, on March 6th 2020, the French MoH authorized private medical laboratories to perform nasopharyngeal sampling and testing for SARS-CoV-2. In France, such laboratories are managed by medical doctors or pharmacists. The clinical features of our 255 outpatients were compatible with the COVID-19 prodromal phase, as previously reported [3,17]. Similar to an earlier study of suspected cases of MERS-CoV [18], no cases of SARS-CoV-2 was detected, whereas other common viral pathogens were detected in 49% of the patients. Our results in patients returning from Italy corroborate those of an Italian study conducted among 126 patients between 21 January and 7 February 2020 with regards to the predominance of influenza viruses among positive samples followed by rhinovirus [11]. Timely identification of influenza virus infections allows for early treatment with antiviral therapy. Respiratory virus acquisition following travel is common. In previous studies conducted in pilgrims returning from the Grand Magal (Senegal) or from the Hajj (Kingdom of Saudi Arabia) acquisition of rhinovirus and common coronaviruses was frequently observed [19,20]. In addition, we revealed evidence that metapneumovirus accounted also for a significant proportion of positive cases and found a higher proportion of common coronaviruses, notably HKU1 and NL63. This high proportion of common coronaviruses reflects the circulation of these viruses in the community, which are mainly responsible for mild upper respiratory tracts infections [21]. Interestingly, we found several seasonal coronaviruses co-infections with influenza A or B viruses and with rhino/enteroviruses. Although such results cannot be extrapolated to SARS-CoV-2, this is indeed a question in healthcare setting where SARS-CoV-2 diagnosis is not available, whether the diagnosis of influenza can rule out the diagnosis of COVID-19. No cross-reaction between common coronaviruses or other common viruses and SARS-CoV-2 has been yet described, to our knowledge and the role of viral co-infections in the disease severity is still unknown. SARS CoV and metapneumovirus co-infections were documented in 2003 but did not correlate with disease severity [22].

CONCLUSION:

Early recognition of COVID-19 is critical to isolate confirmed cases and prevent further transmission. In the United States, the CDC has decided to mobilise state and local

departments in order to obtain collections of clinical and epidemiologic data that describe the characteristics of persons tested for COVID-19 and to decrease the risk of global dissemination [12].

We were able to make a prompt medical assessment and rapid biological diagnosis of COVID-19 suspected patients, leading to a much easier and fast management of patients. The short turnaround time for results and outpatient management makes it possible to reduce the time needed to introduce a specific treatment if necessary. Early rule-out of COVID-19 allows public health containment measures to be adjusted by reducing the time spent in isolation.

NB. During the study period seven tests returned positive for SARS-CoV-2 from patients hospitalised in other regions of France whose samples were tested in our laboratory. Between 2 and 12 March, 41 new patients tested positive for SARS-CoV-2 in our hospital.

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ADDITIONNAL INFORMATIONS:

Competing interest: None to declare.

Funding: None to declare.

1 Table 1: Characteristics of patients

| | All patients | Patients returning from Italy | Patients returning from Asia |
|-----------------------------------------|-----------------------|---------------------------------------|-------------------------------------|
| | n=280 | n=210 | n=60 |
| Demographic data | | | |
| Mean age (years) | 21 (1-84) | 41 (1-84) | 36 (1-63) |
| Sex ratio (M/F) | 1/1.2 | 1/1.3 | 1/1 |
| Place of exposure | Asia n=60 (21.4%) | Lombardy, n=78 (37%) | Wuhan, n=11 (18%) |
| | Italy, n=210 (75%) | Veneto, n=114 (54%) | Other part of China n=20 (33%) |
| | France, n=10 (3.6%) | Emilia Romagna, n=8 (4%) | Other Asia, n=29 (49%) ^b |
| | | Other regions, n=10 (5%) ^a | |
| Dates of inclusion | 24/02/2020-01/03/2020 | 24/02/2020-01/03/2020 | 31/01/2020-01/03/2020 |
| Symptoms and in/out-patient status | | | |
| Fever | 168 (60%) | 130 (61.9%) | 34 (56.7%) |
| Cough | 189 (67.5%) | 146 (69.5%) | 37 (61.7%) |
| Rhinitis | 151 (53.9%) | 122 (58.1%) | 27 (45%) |
| Pharyngitis | 93 (33.2%) | 77 (36.7%) | 15 (25%) |
| Asthenia and/or myalgia and/or headache | 52 (18.6%) | 35 (16.7%) | 14 (23.3%) |
| Chest pain | 17 (6.1%) | 14 (6.7%) | 2 (3.3%) |
| Pneumonia | 3 (1.1%) | 1 (0.5%) | 2 (3.3%) |
| Out-patient | 252 (90%) | 201 (95.7%) | 42 (70%) |
| In-patient | 28 (10%) | 9 (4.3%) | 18 (30%) |
| | | | |

^aLiguria (n=5), Tuscany (n=3), Friuli Venezia Giulia (n=2), ^bSingapore (n=24), South Korea (n=1), Thailand (n=2), Taiwan (n=2).

| | All patients | Patients returning from Italy | Patients returning from Asia |
|----------------------------------------------------------------|--------------|-------------------------------|------------------------------|
| | n=280 | n=210 | n=60 |
| Influenza A virus | 34 (12.1%) | 28 (13.3%) | 5 (8.3%) |
| Influenza B virus | 22 (7.9%) | 19 (9.1%) | 1 (1.7%) |
| Rhinovirus/Enterovirus | 33 (11.8%) | 25 (11.9%) | 8 (13.3%) |
| Metapneumovirus | 20 (7.1%) | 19 (9.1%) | 1 (1.7%) |
| Coronavirus HKU1 | 19 (6.8%) | 18 (8.6%) | 1 (1.8%) |
| Coronavirus NL63 | 12 (4.3%) | 10 (4.8%) | 2 (3.3%) |
| Coronavirus OC43 | 3 (1.1%) | 2 (1%) | 1 (1.7%) |
| Coronavirus E229 | 2 (0.7%) | 2 (1%) | 0 (0%) |
| SARS-CoV-2 | 0 (0%) | 0 (0%) | 0 (0%) |
| Respiratory syncytial virus (RSV) | 6 (2.1%) | 3 (1.4%) | 3 (5%) |
| Adenovirus (ADV) | 3 (1.1%) | 2 (1%) | 1 (1.7%) |
| Bocavirus | 0 (0%) | 0 (0%) | 0 (0%) |
| Parainfluenza viruses | 0 (0%) | 0 (0%) | 0 (0%) |
| Negative for any viruses | 143 (51.1%) | 98 (46.7%) | 38 (63.3%) |
| Mixed infections | n=12 | n=11 | n=1 |
| Influenza A virus + Adenovirus | 1 | 1 | 1 |
| Influenza A virus + Coronavirus NL63 | 1 | 1 | 0 |
| Influenza A virus + Influenza B virus + Rhinovirus/Enterovirus | 1 | 1 | 0 |

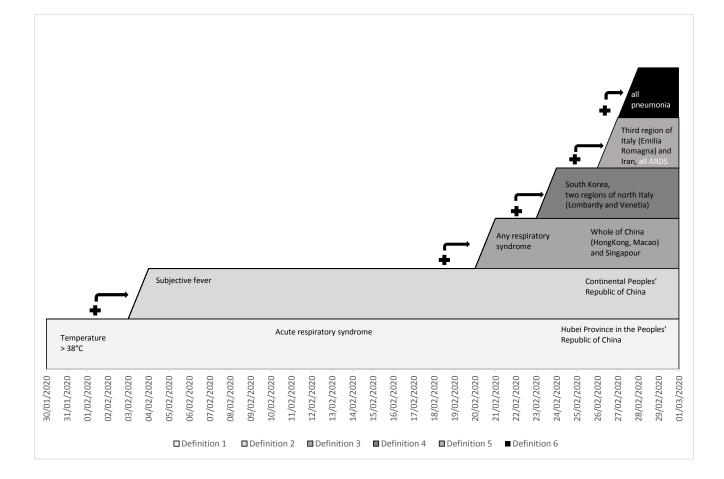
Table 2: Virological data for patients with suspected COVID-19.

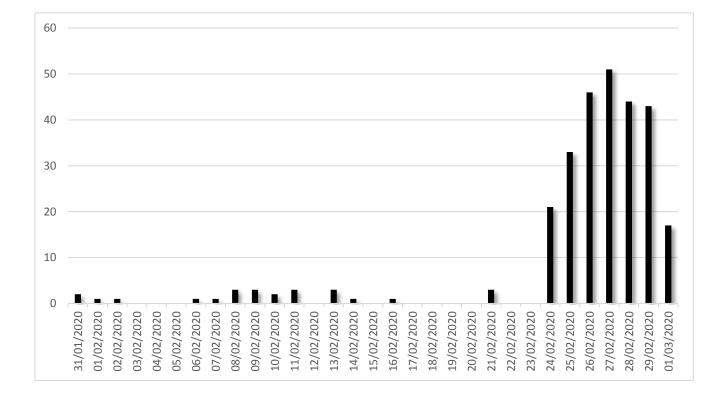
| Influenza B virus + Respiratory syncytial virus | 1 | 1 | 0 |
|-------------------------------------------------|---|---|---|
| Influenza B virus + Coronavirus HKU1 | 2 | 2 | 0 |
| Rhinovirus/Enterovirus + Metapneumovirus | 4 | 3 | 0 |
| Rhinovirus/Enterovirus + Coronavirus HKU1 | 2 | 2 | 0 |

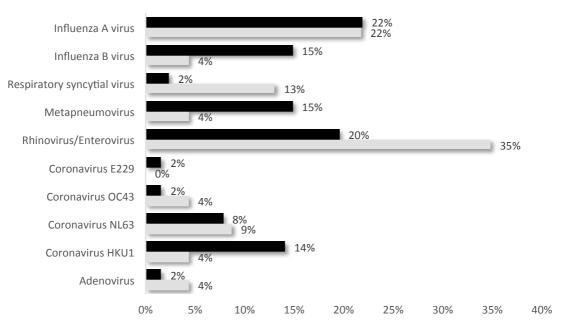
Figure 1: Definition of suspected COVID-19 cases overtime in France

Figure 2: Numbers of patients by date of consultation.

Figure 3. Proportion of each virus among patients testing positive according to region of exposure.







Italy Asia