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Clinical and virological data of the first cases of COVID-19 in Europe: a case series.

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SUMMARY

Background

On December 31, 2019, China reported a cluster of cases of pneumonia in people at Wuhan, Hubei Province. The responsible pathogen is a novel coronavirus, named SARS-CoV-2. We report the relevant features of the first cases in Europe of confirmed infection, named COVID-19, with the first patient diagnosed with the disease on Jan 24, 2020.

Methods

We followed five patients admitted at Bichat-Claude-Bernard University Hospital (Paris) and Pellegrin University Hospital (Bordeaux) and diagnosed with SARS-CoV-2 by semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) on nasopharyngeal swabs. We assessed patterns of clinical disease and viral load from different samples (nasopharyngeal and other body fluids).

Findings

The patients were three men (aged 31 years, 48 years, and 80 years) and two women (aged 30 years and 46 years), all of Chinese origin, who had recently travelled to France from China. Three different clinical evolutions are described: (1) two paucisymptomatic women diagnosed very quickly over their disease course, with high nasopharyngeal titers of SARS-CoV-2 within the first 24 hours of the illness onset (5·2 and 7·4 $\log_{10}$ copies/1000 cells, respectively) and viral RNA detection in stool; (2) a two-step disease progression in two young men, with a secondary worsening around 10 days after disease onset despite a decreasing viral load in nasopharyngeal samples; and (3) an 80-year-old man with a rapid evolution towards multi-organ failures and a persistent high viral load in lower and upper respiratory tract with systemic virus dissemination and virus detection in plasma. Four of the cases have recovered and been discharged, but the 80-year old patient died on day 14 of illness.

Interpretation

We illustrated 3 different clinical and biological types of evolution in 5 patients infected with SARS-CoV-2 with detailed and comprehensive viral sampling strategy. We believe that these findings will contribute to better understanding of the disease natural history and will contribute to advances in the implementation of more efficient infection control strategies.

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INTRODUCTION

The COVID-19 epidemic spread within China, and secondarily also outside China, with a basic reproductive number estimated to be from 2·2 (1) to 3·3 (2) and a mortality rate around 2·3% (3). In the EU/EEA and the UK, as of 6 March, 5 544 cases have been reported (France: n=423), including 159 deaths (France: n=7)(4).

To date multiple studies have described demographic, clinical, and biological characteristics of, and radiological or pathological findings associated with COVID-19. More specifically, they reported most common symptoms, incubation periods, biological, radiographic and CT abnormalities, and treatment data. In addition, they described varying degrees of illness and their proportion: mild, severe or critical. They reported proportion of complications, including acute respiratory distress syndrome, or case fatality rates, and variables associated with these complications and death (1, 5-9).

In this paper, through a detailed and comprehensive sampling strategy, we report the clinical and biological features of the first five cases of confirmed COVID-19 in Europe, that occurred in France, and their dynamics in parallel with changes in their viral load, based on SARS-CoV-2 RNA detection. These patients, at different stages of infection, include two patients with a mild disease at admission and a secondary worsening triggering their admission to Intensive Care Unit (ICU), one initially severely ill patient directly admitted to ICU for an acute respiratory failure, and two patients with a mild disease diagnosed very early after infection.

METHODS

Participants and source of data

All patients at French hospitals who were diagnosed with COVID-19 according to the French National Health Agency criteria, were enrolled in this study from January 24 to 29, 2020 (5).

With the French National Regulatory authorities and French Ministry of Health, on January 25, we expectantly determined the indication criteria for compassionate use of an investigational antiviral treatment (remdesivir): signs of severe illness at diagnosis or secondary clinical aggravation (respiratory symptoms or general signs) (WHO/nCoV/Clinical/2020.2 (10) used to define illness severity, see in Appendix). Based on expert opinion and available data in January 2020, we considered that remdesivir may be the best potential drug for treatment of COVID-19, although restricted to patients with severe disease (intravenous route; a loading dose of 200 mg, then maintenance daily dose of 100 mg for a total duration of 10 days). Criteria for discharge with total recovery were from ECDC guidelines: asymptomatic patients having two RT-PCR negative nasopharyngeal samples performed at least at 48 hours apart (11).

Procedures

Samples Collection

Clinical samples for SARS-CoV-2 diagnostic testing were obtained according to WHO guidelines. For each patient, a sampling strategy was implemented combining once daily for 3 days, and subsequently once every two/three days until patient discharge, with upper and lower (when possible) respiratory tract samples, and also blood, urine and stool samples/rectal swabs if appropriate (see Appendix). Upper respiratory samples were either nasopharyngeal aspirates or nasopharyngeal swabs (Sigma Virocult®, Medical Wire Instrument, UK); stool samples were either fecal swabs or stools; blood samples were EDTA tube adapted for RT-PCR. All samples were

refrigerated and shipped to the laboratories of the National Reference Center for Respiratory Viruses (Institut Pasteur and Hospices Civils de Lyon). Details for procedures about RNA extraction, real-time RT-PCR (rtRT-PCR), and virus isolation and titration are presented in Appendix.

Ethics Approval and Funding

The study is part of an overall French clinical cohort assessing patients with COVID-19 and registered in clinicaltrials.gov (NCT04262921). It was approved by the French Ethics Committee, and written informed consent was obtained from each patient involved, or his next of kin. We used the open-source 'Clinical Characterization Protocol for Severe Emerging Infections' of the International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC), supported by the World Health Organization (WHO)(12), which has been updated in response to COVID-19. The study is funded by REACTing (REsearch & ACtion emergING infectious diseases), INSERM, Paris, France, who had no role in study design, data collection and analysis, and report/manuscript writing. The corresponding author had full access to study data and final responsibility for publication submission.

RESULTS

The main clinical and biological characteristics of the five patients at admission are presented in Table 1. All patients but case #3 were diagnosed with COVID-19 the day of their hospital admission. Case #3 was diagnosed three days after because he did not fulfill the National Health Agency case definition at admission (no history of travel to or residence in the city of Wuhan). On February 19, 2020, all patients but #3 had fully recovered and were discharged. Patient #3 died on February 14.

Clinical data: 3 types of presentation.

Schematic presentation of major events for each case are presented in Figure 1. Clinical and biological characteristics day by day for each case are presented in Appendix, Table S3.

Case #1 - Case #2: mild disease at admission and secondarily severe (9)

Case #1, a 31-year-old man, Paris

A 31-year Chinese male tourist originated from Wuhan was diagnosed with COVID-19 on January 24 (illness day 6), 5 days after his arrival in Paris with his wife (case #4). He visited a hospital in Wuhan on January 16 for a gout episode. He had flu-like symptoms (Table 1) since January 19 (illness day 1). He was admitted at hospital on diagnosis day, with mild lymphopenia, thrombopenia, and no abnormalities on the chest X-ray. On illness day 10, he was transferred in ICU due to oxygen saturation worsening (PO_2=58mmHg; flow nasal cannula 4l/min), and bilateral lung abnormalities including ground glass, reticulo-nodular syndrome and alveolar opacities on chest CT-scan (Appendix, Figure S1). A loading dose of remdesivir was administered on January 29, 2020 (illness day 11), followed by maintenance treatment. On January 31, he was discharged back to Infectious Diseases ward. On illness day 15, remdesivir treatment was discontinued, because of ALT elevation (levels three times normal) and a maculopapular rash, without any anaphylaxis, eosinophilia, or systemic symptoms. The patient was screened for hepatitis B and C, Cytomegalovirus, Epstein-Barr Virus, and Herpes Simplex virus, and no active infections was detected. Skin and liver abnormalities decreased within three days. The patient became asymptomatic the day after. He was discharged on February 12.

Case #2: a 48-year old man, Bordeaux

A 48-year old man of Chinese origin, based in France, travelled for business in China and flew back from Shanghai to France on January 22. He did not report any specific exposures within the 14 days prior to symptom onset but a 3-day stay at Wuhan. Arterial hypertension was his only underlying disease. Flu-like symptoms started on January 16, and he was diagnosed on January 24 (illness day 9). On illness day 11, he was transferred in ICU with fever above 38°5C and skin mottling suggesting a sepsis. On January 29, a CT-scan showed bilateral lung abnormalities including ground glass, reticulo-nodular syndrome and scarce alveolar opacities. In ICU, a loading dose of remdesivir was administered on January 30 (illness day 15), followed by maintenance treatment until February 8. After full recovery, he was discharged on February 14.

Case #3, a rapidly progressive disease classified critical at diagnosis (9)

An 80-year-old Chinese male tourist originated from Yichang, Hubei Province, was diagnosed with COVID-19 on January 28 (illness day 7), 11 days after his arrival in Europe with his daughter (case #5). He did not report any specific exposure within the 14 days prior to symptoms onset. He had a thyroid cancer removed 10 years ago. He got fever and diarrhea from January 22, and went to Emergency Room on January 25, where the chest X-ray showed bilateral alveolar opacities. He did not fulfill the COVID-19 case definition. However, airborne and contact precautions were observed during his stay before COVID-19 diagnosis. On January 26, an acute respiratory failure triggered his ICU admission. He subsequently developed a multiple organ failure with acute respiratory distress syndrome, acute kidney injury, liver failure and sepsis-like shock. Since the COVID-19 diagnosis was confirmed on January 28, he was transferred to the referent ICU at Bichat-Claude-Bernard Hospital in Paris, where broad-spectrum antibacterials were started for a possible superinfection. Remdesivir was started with a loading dose. As two pathogens were identified: a susceptible *Acinetobacter baumannii* (multiplex PCR, confirmed by tracheal aspirates culture) and an *Aspergillus flavus* (tracheal aspirates culture), the anti-infective treatment was adapted. Remdesivir was discontinued on January 30 because the patient needed renal replacement therapy. CT-scan on January 31 showed a bilateral pleuropneumopathy associating pleural effusion, alveolar condensations, ground glass and pulmonary cysts. On February 5, because of the severity of the disease and persistence of viral detection, and as the risk-benefit assessment was considered favorable, remdesivir was re-initiated. Multiple organ failures persisted despite an appropriate treatment against *Acinetobacter baumannii* and *Aspergillus flavus* and no other superinfection identified. He died on February 14 (illness day 24).

Case #4 – case #5: early hospital admission after the first symptoms onset, and classified as mild disease (9).

Case #4: a 30-year-old woman, Paris

A 30-year-old Chinese woman (case #1's wife) was diagnosed with COVID-19 on January 24, 2020 (illness day 2), 5 days after her arrival in Paris. She stayed with Case #1. She had moderate flu-like symptoms since January 23 (illness day 1) with no abnormalities on the chest X-ray. After some days of persistent and incapacitating cough, her condition improved without any specific treatment, she became asymptomatic on February 2 (illness day 11) and was discharged on February 12.

Case #5: a 46-year-old woman, Paris

A 46-year-old Chinese healthy woman (case #3's daughter) was diagnosed with COVID-19 on January 29 (illness day 2), 2020. She stayed with Case #3 since their travel from China. She had mild symptoms, with sore throat and dry cough from January 28 (illness day 1), and a normal chest X-ray. Her cough initially mild increased transiently

over time; she was asymptomatic from February 4 (illness day 8) without any specific treatment, and was discharged on February 17th.

Virological data

Cases #4 and #5, identified early after disease symptoms onset, had nasopharyngeal samples collected within the first 24 hours of illness onset. These early specimens had a high viral load enabling whole genome virus sequencing and virus isolation (Table 2). The maximal normalized viral load obtained in their respiratory specimens were at 5·2 and 7·4 log_{10} copies/1000 cells for case #4 and #5, respectively (based on the RdRp quantified-rtRT-PCR). This viral load in respiratory samples decreased over time (Figure 2). SARS-CoV-2 detection by RT-PCR was negative in illness day 12 for case #4, and illness day 16 for case #5 (Figure 2). These patients had also a positive detection of SARS-CoV-2 in stools, with viral load as high as 6·8 and 8·1 log_{10} copies/g of stool for case #4 and #5, respectively. However, the virus was not detected in the serum or the urine samples.

Cases #1 and #2 had nasopharyngeal samples collected at illness day 6 and 9, respectively, which were positive by rtRT-PCR, with a SARS-CoV-2 viral load at 7·1 log_{10} copies/1000 cells, and detected but not quantifiable viral load, respectively. The whole genome virus sequence was obtained by direct sequencing for case #1 only; virus isolation was unsuccessful in both cases. The secondarily evolution to severe disease experienced by two patients (days 10 and 11) was not correlated to any viral load increase (Figure 2). Both received remdesivir IV at a time the viral load had already decreased below the detection threshold. During the whole course of the disease of these two cases, SARS-CoV-2 detection by RT-PCR was negative in stools, serum, and urine.

At illness day 6, Case #3 classified as critical was positive by RT-PCR in both a nasopharyngeal sample and bronchoalveolar lavage (BAL), with cycle threshold (Ct) values for the E gene target (Charité protocol) of 30·3 and 27·4, and comparable Ct values for the house-keeping gene GAPDH of 25·7 and 24·7, respectively (Table 2). The SARS-CoV-2 titers in the nasopharynx was rather stable (from 6.7 to 4.4 log_{10} copies/1000 cells) over time although with a trend toward decrease after the first unique remdesivir IV dose, and thereafter when remdesivir was reinitiated (Figure 2). This patient had a RNAemia on illness day 8 and subsequently, with a low viral load (detected but below the quantification limit). During the course of the disease, he developed a pleural exudative effusion, with SARS-CoV-2 detection positive in the pleural fluid, and negative bacterial cultures.

Figure 3 illustrates the kinetics of the viral load in nasopharyngeal samples of all patients after disease onset. The patient's viral load decreased over time and became negative between illness day 9 and 14 in four patients (case #1, 2, 4 and 5). In the most severely ill patient (case #3), nasopharyngeal virus detection persisted until death.

When available, the sequence analysis of the virus of these patients showed that cases #1 and #4 compared to case #5 correspond to two distinct events of importation. For cases #1 and #4, the virus was clustering with viruses from cases in Wuhan, Shenzhen, California, Australia and Taiwan, whereas for case #5 the virus was clustering with those from Chongqing and Singapore (https://nextstrain.org/ncov). Furthermore, the very high level of identity of the sequences from cases #1 and #4 supports the epidemiological link between these cases and the likelihood of transmission.

DISCUSSION

In this case series of 5 COVID-19 patients, we illustrated 3 different clinical and biological types of evolution: (1) mild cases through two pauci-symptomatic patients aged <50, diagnosed early, with high viral load

in nasopharyngeal samples, suggesting a significant shedding of SARS-CoV-2 reflected by virus detection by RT-PCR; (2) two young patients presenting with mild symptoms at admission and experiencing a secondary progression to pneumonia and severe disease by day 10-11; and (3) an elderly patient with a rapid evolution towards critical disease with multi-organ failures and a long and sustained persistence of SARS-CoV-2 nasopharyngeal detection associated to viral RNA detection in multiple sites, including blood.

Among the five cases investigated here, two were mild, diagnosed at an early stage of the disease because they had a contact with a confirmed case. Virus detection with high viral loads in upper respiratory tract samples are suggestive of potentially high risk of transmissibility during the very first days of symptoms. This is in line with data reported by Zou *et al* who analyzed viral load in upper respiratory tract from the 17 symptomatic patients in relation to day of onset of symptoms in whom higher viral loads were detected soon after symptom onset (13). This suggests that the virus shedding pattern of patients infected with SARS-CoV-2 is different from that seen with SARS-CoV, where virus load was very low at disease onset (14-16). These findings may influence the implementation of infection control measures. It implies that COVID-19 control measures should combine immediate isolation of cases together with a rapid screening and monitoring of the contacts of infected patients, to detect those with very mild symptoms. In two out of five patients reported here, SARS-CoV-2 RNA was detected in stool samples. This possible route of transmission must be investigated; detection of viral RNA does not necessarily imply that infectious particles are present and transmissible (17), particularly when patients, like in these two, have no diarrhea or other gastrointestinal symptoms.

In this case series, except for the patient classified as critical, the viral load decreased over time and became negative between illness day 9 to 14. Of note, the virus was also detected by rtRT-PCR at low levels in the upper respiratory tract, even after full symptoms resolution. Whether infectious virus might be still present despite symptoms resolution should require further attempts of virus isolation. This justifies the ECDC recommendation to obtain two RT-PCR negative nasopharyngeal samples prior to discharge asymptomatic patients (11). This conservative recommendation is however no more feasible in many European countries that are in an epidemic situation.

In this case series, three patients had a severe/critical disease with two different patterns. The first one, in those with severe diseases (cases #1 and #2), is characterized by a bi-phasic evolution starting with a mild presentation followed with a secondary respiratory worsening despite a decreasing viral load in the nasopharyngeal samples: SARS-CoV-2 was no longer detected in the upper respiratory tract in one patient and at very low levels in the other. In patients with this pattern, CT scan performed at the moment of the worsening showed ground-glass lung opacities, in line with those reported by others in patients with COVID-19 (8, 17). Time-to-worsening of respiratory symptoms was around 10 days after disease onset in these two cases, close to the median disease duration before worsening (8·0 days; IQR 5·0–13), previously reported by Huang and collaborators (18). In these patients, one may postulate that the lung damage is more related to immunopathological lesions, resulting from an over-exuberant pro-inflammatory host response, rather than to uncontrolled viral replication (18). Of note, we did not perform viral detection in low respiratory tract samples from these two patients.

The second pattern, observed in the patient classified as critical and who died (case #3), exhibits a persistent and high viral excretion in the upper respiratory tract samples combined with a positive virus detection by rtRT-PCR in other body fluids including blood. By contrast to the previous pattern, this persistent high viral load suggests the ability of the SARS-CoV-2 to evade from the immune response. Indeed, we can speculate that, as shown during

MERS and SARS coronaviruses infection, SARS-CoV-2 may be able to inhibit the interferon signaling pathways (19, 20), resulting in higher respiratory virus load, positive viremia and eventually poor prognosis as for MERS-CoV (21, 22). Indeed, the 80-year-old patient, unlike the other cases, had evidence of high viral replication in the respiratory tract and evidence for systemic virus dissemination beyond the respiratory tract, with virus detection in plasma and pleural effusion fluid. The impaired immune response may have facilitated the bacterial and fungal super-infections. Patients with similar very severe patterns (sustained viral RNA in the respiratory tract and detection of SARS-CoV-2 in the blood) have also been reported in China (18). As reported in previous studies (12, 23), severely ill patients are often elderly patient with comorbidities. Case #3 was 80-year old, and might have had an impaired interferon pathway.

These different patterns, and especially the fact that those with severe/critical may have different viral kinetics in the upper respiratory tract, may be of interest. It may suggest different therapeutic approaches, based on viral kinetics monitoring, in those in whom we observed a virus load decrease in the upper respiratory tract decrease versus those with high viral replication and systemic virus dissemination. Of course, we should be cautious when analyzing these data because of the small number of patients, but this should be considered in the future studies.

There is no currently validated antiviral treatment to control such SARS-CoV-2 infections. Among potential candidates, remdesivir is an antiviral pro-drug (nucleosidic analogs family) which has a broad-spectrum *in vitro* and *in vivo* activity against numerous RNA viruses, among which SARS-CoV-2. In animal models, compared to lopinavir/ritonavir combined with interferon-β, two other potential candidates, remdesivir reduced more significantly the virus titer of mice infected with the MERS-CoV and decreased the lung tissue damage (24). Remdesivir treatment improved disease outcomes and reduced viral loads in SARS-CoV-infected mice (25) and is inhibitory for SARS-CoV-2 *in vitro* (26). A phase III clinical trial assessed this drug for the treatment of Ebola virus infection; therefore, data exist for the safety of use in humans (27). Hence, based on expert opinion, we considered remdesivir use in the three patients with severe disease patterns. To the best of our knowledge, only one case of remdesivir use in COVID-19 has been reported so far (17). Two randomized clinical trials are enrolling patients in China to assess the clinical benefit of this treatment (NCT04257656; NCT04252664). Based on our data, we cannot draw any conclusions on the potential efficacy of remdesivir on COVID-19 infections. In two patients, the drug was initiated at the time of disease worsening, when the virus was already barely detectable in the clinical specimens. In one of them, remdesivir was discontinued after five days because of a combined ALT elevation and a rash, although it could not be confirmed that this adverse event was related to remdesivir. In the third patient (For Case #3), remdesivir was discontinued after a single dose because of renal replacement therapy to avoid risk of cyclodextrin accumulation. Remdesivir contains cyclodextrin, an excipient whose clearance is linearly related to creatinine clearance. Because patient's condition was worsening and viral load was not decreasing, we however reinitiated remdesivir.

Conclusion

In this paper, we report clinical and virological data on the first cases of COVID-19 in Europe. Although we acknowledge the fact that the results provided are based on a limited number of cases, a detailed and comprehensive sampling strategy enabled us to illustrate the different courses of the disease we observed, and provide some relevant criteria regarding the severity of disease. We believe that these findings will contribute to

better understanding of the natural history of the disease and will contribute to advances in the implementation of more efficient infection control strategies.

Contributions of each co-author:

XL, LB, DN, MP, PHW, AM, JCL, FM, XD, DM, JFT and YY collected the clinical and epidemiological data, and summarized all data.

SB, AG, MBD, FD, QLH, VE, NHF, MV, DD , BL and SvdW performed the virological assays.

SB, AG, MBD, FD, VE, MV, BL and SvdW set up and performed the rtRT-PCR assays.

Figures and tables were drafted by XL, LB, AG, MBD, and SvdW.

XL, LB, BL, SvdW and YY drafted the manuscript, and revised the final version.

All authors revised the final version.

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We dedicate this article to Mr. Z, who died in our hospital in Paris on February 14, 2020, and to his daughter.

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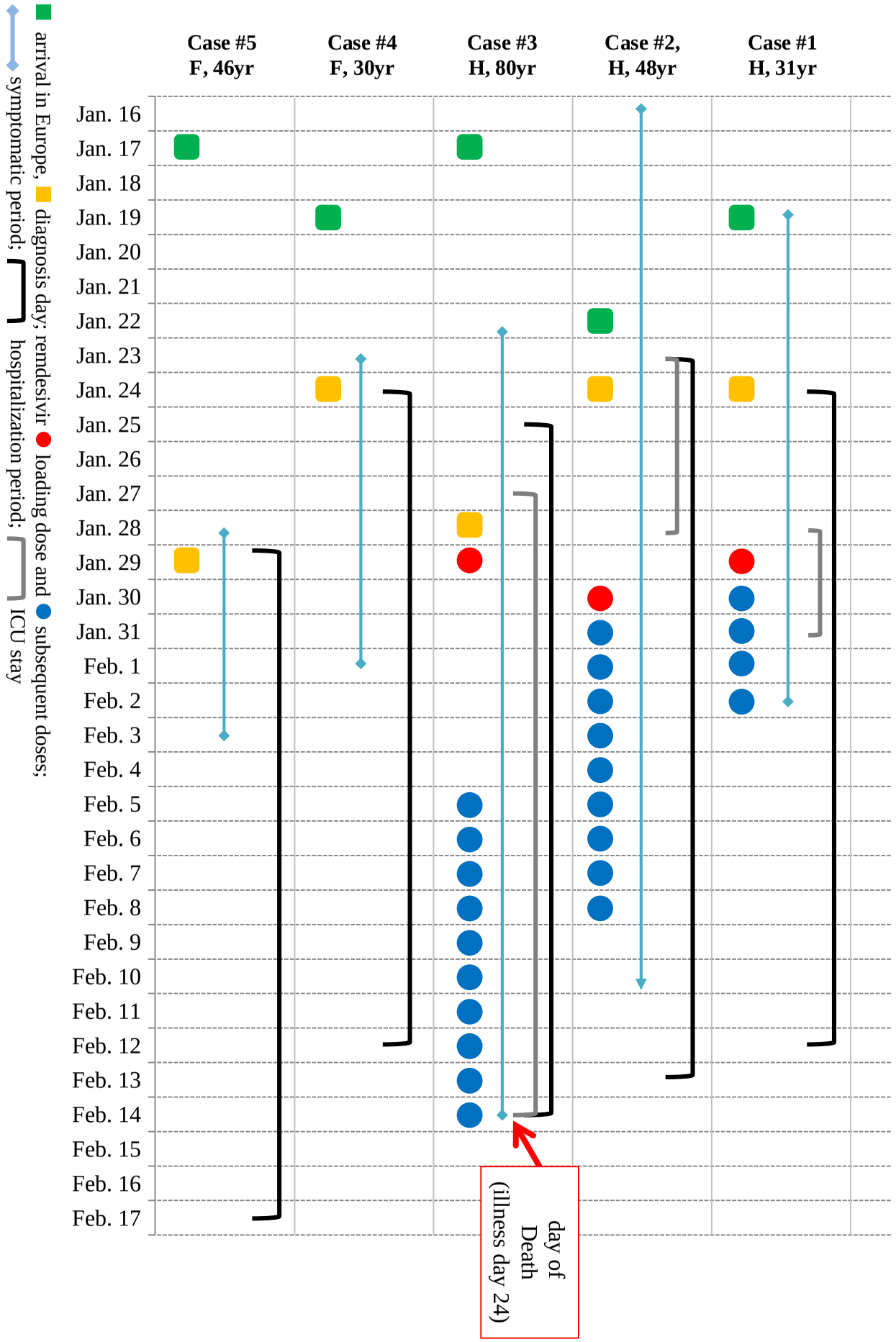
Legends of the Figures

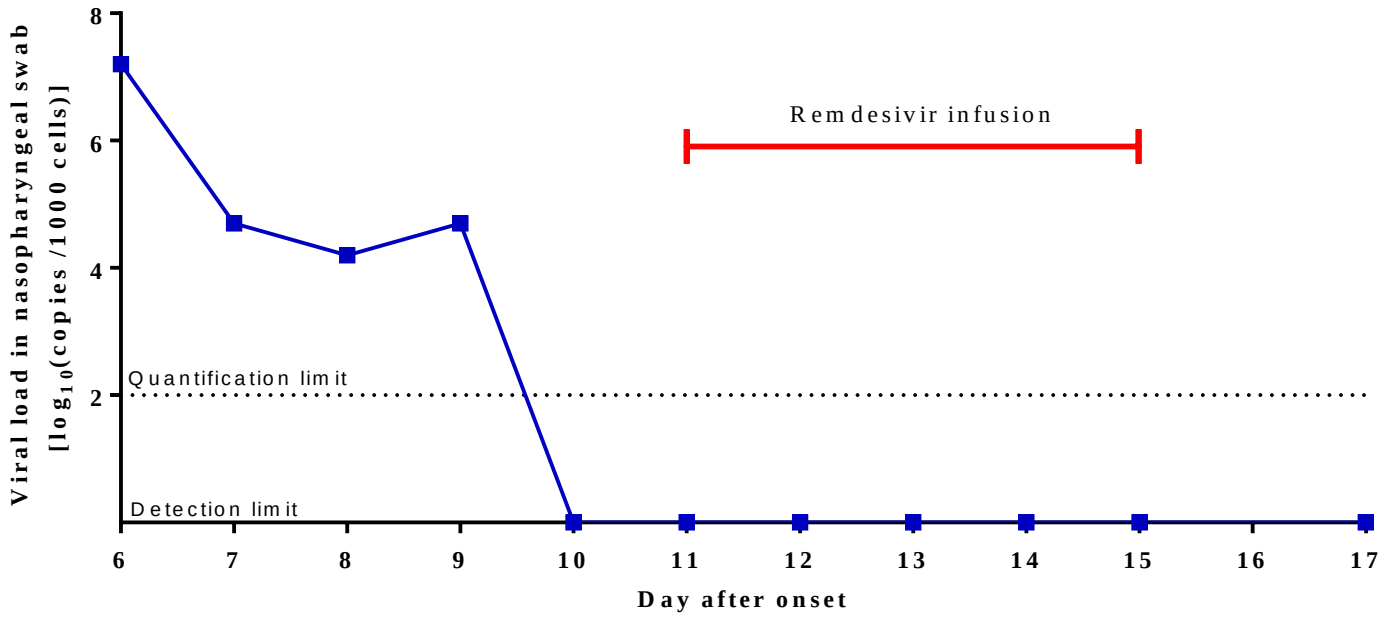
Fig. 1 – Schematic description of the French cases of COVID-19

Fig. 2 – Description of the individual dynamics of the nasopharyngeal viral load and virus detection in other body fluids in the 5 French COVID-19 cases (A: case #1; B: case #2; C: case #3; D: case #4; E: case#5). Blue lines represent the viral load in nasopharyngeal swab normalized using the cell quantification. All positive samples below the quantification limit were represented on the quantification limit line. For readability, all negative results were represented on the x axis which correspond to our detection limit. Red lines represent administration period for remdesivir. /: not done; -: negative results.

Fig. 3 - Overall dynamics of the nasopharyngeal viral load and virus detection in other body fluids in the 5 French COVID-19 cases


arrival in Europe, diagnosis day; remdesivir loading dose and subsequent doses;
symptomatic period; hospitalization period; ICU stay
Case #5
F, 46yr
Case #4
F, 30yr
Case #3
H, 80yr
Case #2,
H, 48yr
Case #1
H, 31yr
Jan. 16
Jan. 17
Jan. 18
Jan. 19
Jan. 20
Jan. 21
Jan. 22
Jan. 23
Jan. 24
Jan. 25
Jan. 26
Jan. 27
Jan. 28
Jan. 29
Jan. 30
Jan. 31
Feb. 1
Feb. 2
Feb. 3
Feb. 4
Feb. 5
Feb. 6
Feb. 7
Feb. 8
Feb. 9
Feb. 10
Feb. 11
Feb. 12
Feb. 13
Feb. 14
Feb. 15
Feb. 16
Feb. 17
day of
Death
(illness day 24)

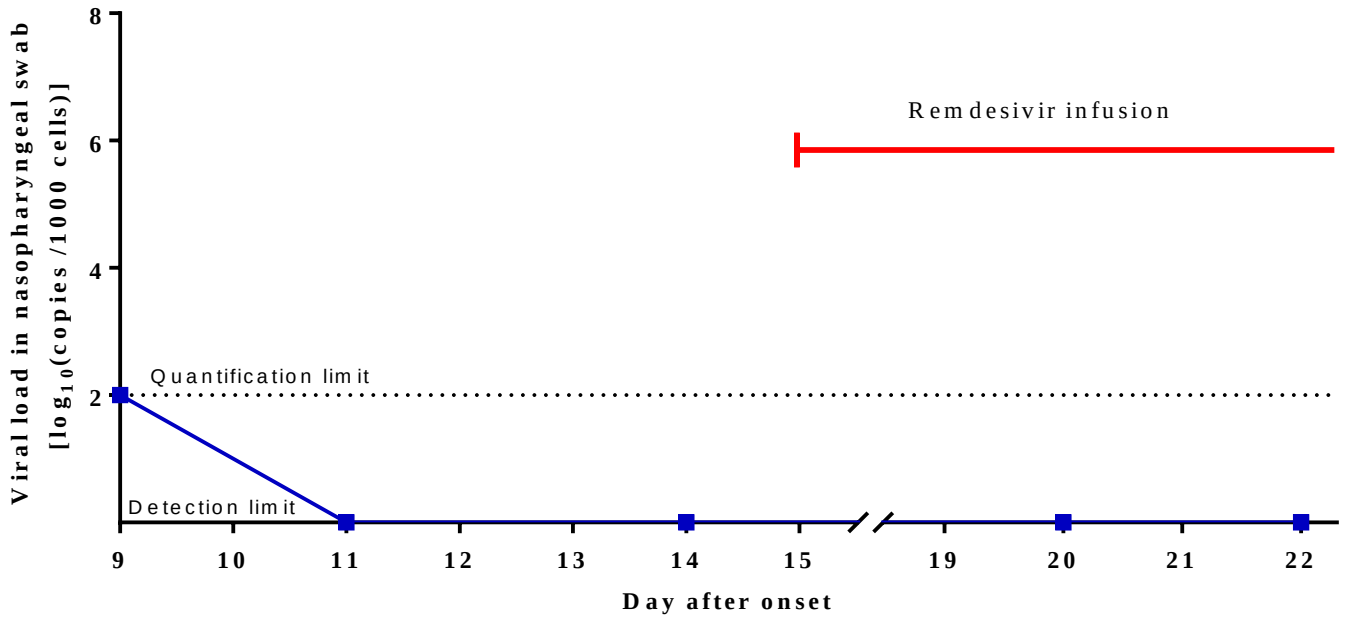
A - CASE #1 - 31-year-old man, Paris (husba/ of case #4)



	Jan. 24	Jan. 25	Jan. 26	Jan. 27	Jan. 28	Jan. 29	Jan. 30	Jan. 31	Feb. 1	Feb. 2	Feb. 3	Feb. 4
Virus detection in other samples												
Plasma	/	-	-	-	-	-	/	/	-	-	/	/
Urine	/	-	-	/	-	/	-	/	/	/	/	/
Stools	/	-	-	/	/	-	-	/	/	-	/	/
Conjonctiva	/	/	-	-	/	-	/	/	-	-	/	/

/ = not done; + = positive result; - = negative result

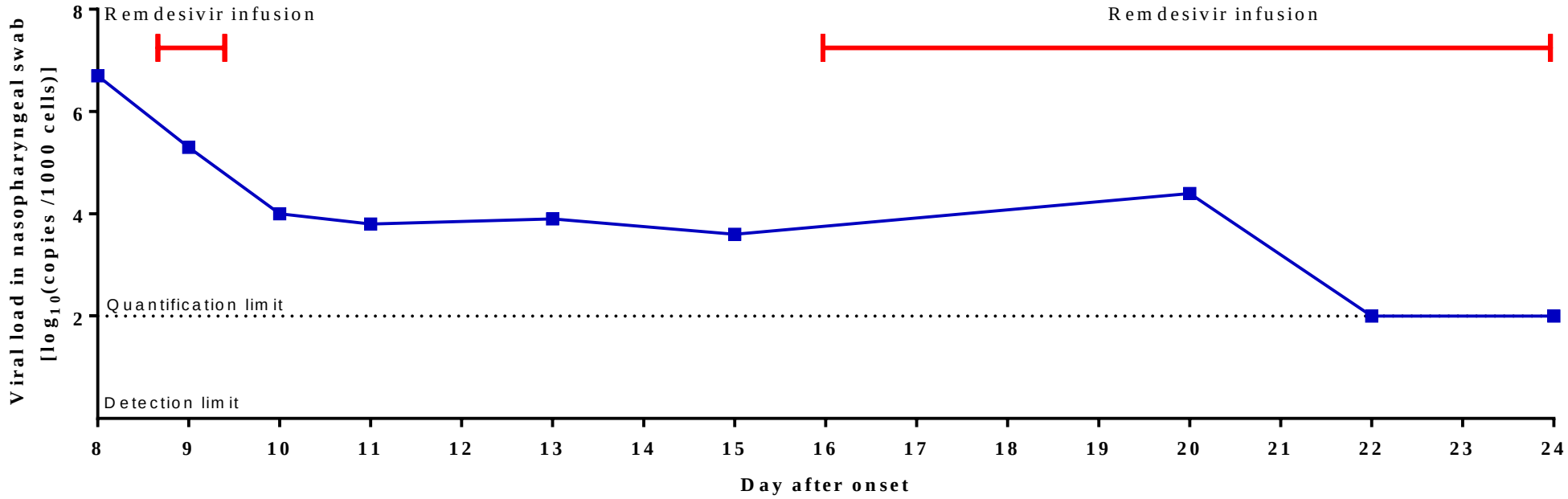
B - CASE #2 - 48-year old man, Bordeaux



	Jan. 24	Jan. 25	Jan. 26	Jan. 27	Jan. 28	Jan. 29	Jan. 30	Feb. 3	Feb. 4	Feb. 5	Feb. 6
Virus detection in other samples											
Plasma	/	/	-	/	/	/	/	/	/	/	/
Urine	/	/	/	/	/	/	/	/	/	/	/
Stools	/	/	-	/	/	/	/	/	/	/	/
Conjonctiva	/	/	/	/	/	/	/	/	/	/	/

/ = not done; + = positive result; - = negative result

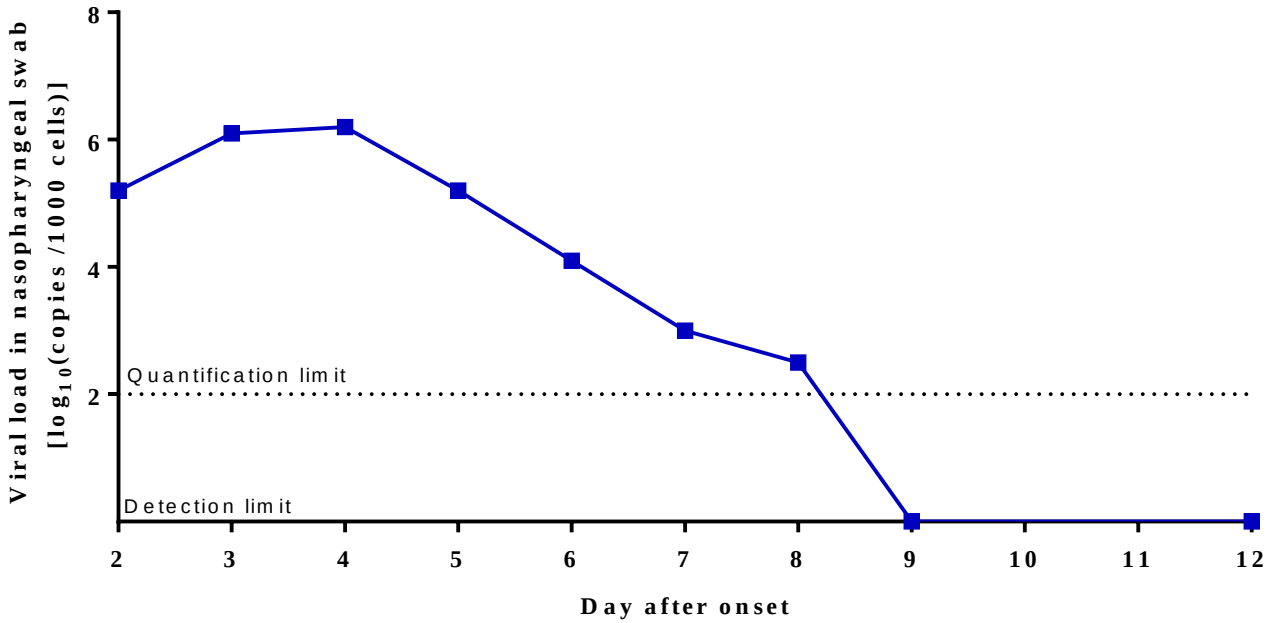
C - CASE #3 - 80-year-old man, Paris (father of case #5)



	Jan. 28	Jan. 29	Jan. 30	Jan. 31	Feb. 1	Feb. 2	Feb. 3	Feb. 4	Feb. 5	Feb. 6	Feb. 7	Feb. 8	Feb. 9	Feb. 10	Feb. 11	Feb. 12	Feb. 13	Feb. 14
Virus detection in other samples																		
Plasma (*RT-PCR CT*)	+ **(36,9)**	+ **(38,4)**	+ **(35,8)**	/	/	+ **(37,3)**	/	/	/	-	/	/	/	-	/	/	-	/
Urine	/	/	/	-	/	/	/	/	/	/	/	/	/	/	/	/	/	/
Stools	/	-	/	-	/	-	/	/	/	/	/	/	/	/	/	/	/	/
Conjonctiva	/	-	/	-	/	/	/	-	/	/	/	/	/	/	/	/	/	/

/ = not done; + = positive result; - = negative result

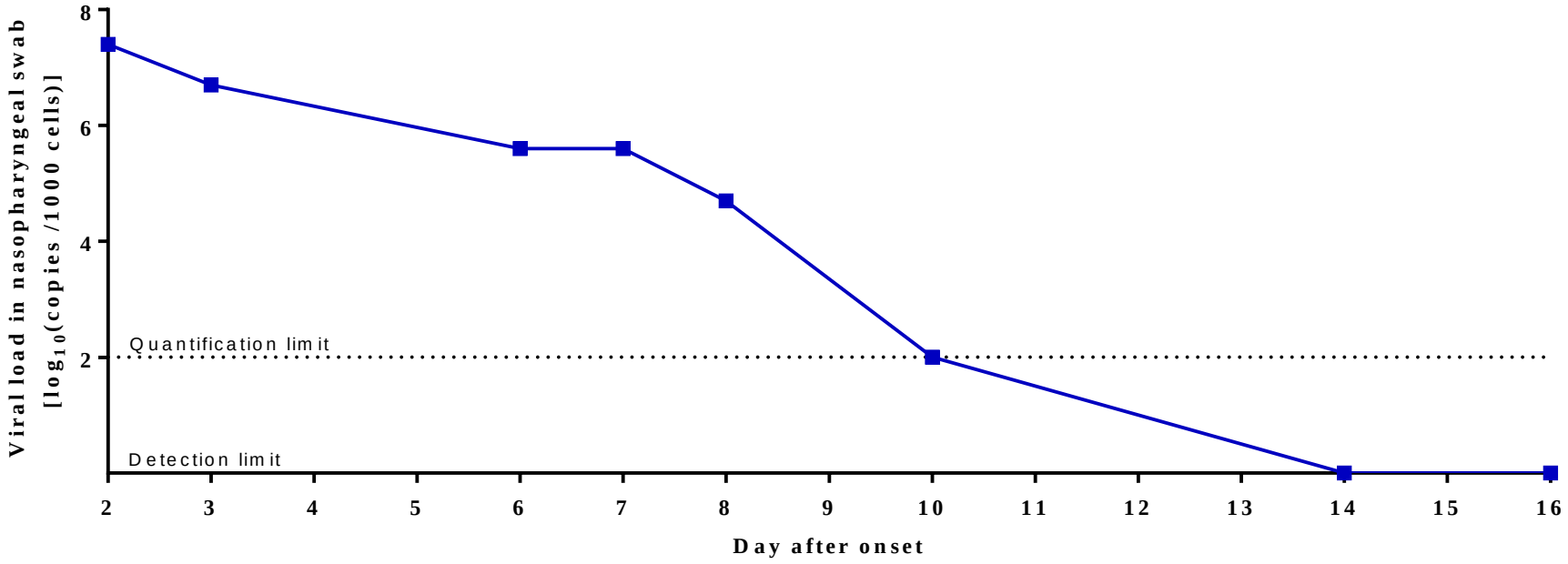
D - CASE #4 - 30-year-old woman, Paris (wife of case #1)



	Jan. 24	Jan. 25	Jan. 26	Jan. 27	Jan. 28	Jan. 29	Jan. 30	Jan. 31	Feb. 1	Feb. 2	Feb. 3
Virus detection in other samples											
Plasma	/	-	-	-	-	/	/	/	/	/	/
Urine	/	-	-	/	/	-	-	/	-	/	/
Stools (titer*)	/	+ **(6,8)**	+ **(6,2)**	/	/	+ **(6,2)**	/	/	+ **(NQ¤)**	/	/
Conjonctiva	/	/	-	-	-	/	-	/	-	/	-

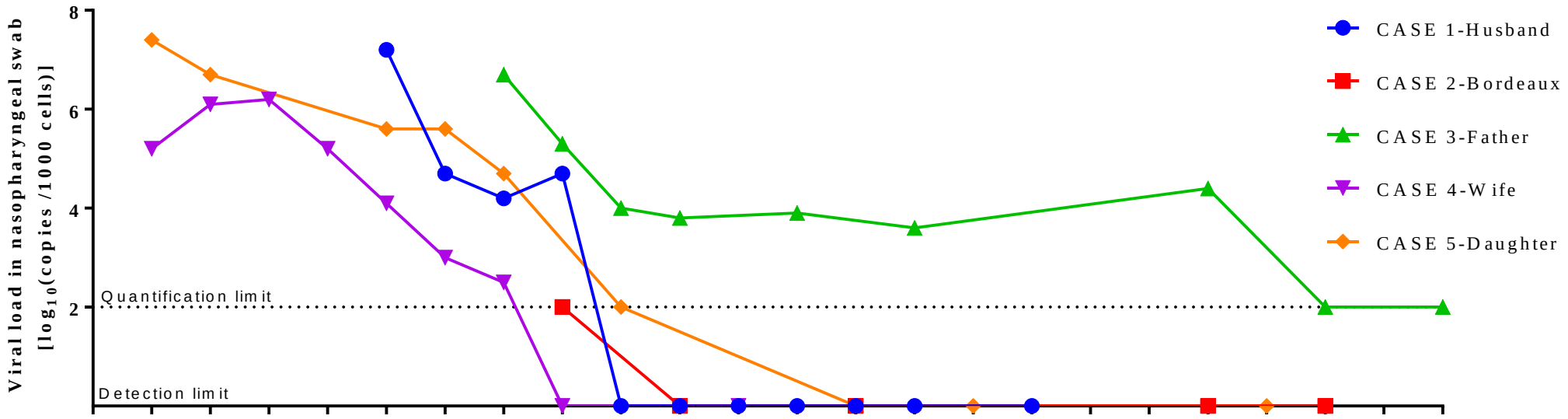
*: titer in log of copies/g of stools /: not done; + = positive result; - = negative result NQ: not quantifiable

E - CASE #5 - 46-year-old woman, Paris (daughter of case #3)



	Jan. 29	Jan. 30	Jan. 31	Feb. 1	Feb. 2	Feb. 3	Feb. 4	Feb. 5	Feb. 6	Feb. 7	Feb. 8	Feb. 9	Feb. 10	Feb. 11	Feb. 12
Virus detection in other samples															
Plasma	/	-	/	/	/	/	/	/	/	/	/	/	/	/	/
Urine	/	/	-	/	-	/	/	/	/	/	/	/	-	/	/
Stools (titer*)	/	/	+ **(7.5)**	/	+ **(8.1)**	/	/	/	+ **(7.5)**	/	/	+ **(7.8)**	+ **(7.4)**	/	/
Conjonctiva	/	/	-	/	-	/	/	/	/	/	/	/	/	/	/

*: titer in log of copies/g of stools /: not done; + = positive result; - = negative result



Other body fluids		Day after onset	1*	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
	Blood	case #1						/	-	-	-	-	-	/	/	-	-	/	/							
		case #2									/	/	-	/	/	/	/	/	/	/	/	/	/	/		
		case #3							+	+	+	/	/	+	/	/	/	-	/	/	/	-	/	/	-	/
		case #4		/	-	-	-	-	/	/	/	/	/	/	/	/	/	/	/	/	/	/					
		case #5		/	-	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/		
	Stools	case #1						/	-	-	/	-	-	-	/	/	-	/	/							
		case #2									/	/	-	/	/	/	/	/	/	/	/	/	/	/		
		case #3							/	-	/	-	/	-	/	/	/	/	/	/	/	/	/	/	/	/
		case #4		/	+	+	/	/	+	/	/	+	/	/	/	+	/	/	/	/	+					
		case #5		/	/	+	/	+	/	/	/	+	/	/	+	+	/	/	/	/	/	/	/		
	Conjunctiva	case #1						/	/	-	-	/	-	-	/	-	-	/	/							
		case #3							/	-	/	-	/	/	/	-	/	/	/	/	/	/	/	/	/	/
		case #4		/	/	-	-	-	-	-	/	-	/	/	/	/	/	/	/	/	/					
		case #5		/	/	-	/	-	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/		
	Pleural fluid	case #3							/	/	/	/	/	+	/	/	/	/	/	/	/	/	/	/	/	/

* COVID-19 symptoms onset / = not done; + = positive result; - = negative result

Table 1. Main characteristics of the patient at admission

	Case #1	**Case #2**	**Case #3**	**Case #4**	**Case #5**
Age/Sex	31 yr/M	48 yr/M	80 yr/M	30 yr/F	46 yr/F
Chronic medical illness/history	Gout	High blood pressure	Thyroid cancer	None	None
Exposure and setting	Wuhan (Hubei Province, China)	Wuhan, Ningbo, and Shanghai, China	Yichang (Hubei Province, China)	Wuhan (Hubei Province, China)	Yichang (Hubei Province, China)
Duration of illness	15	26	23	11	16
Diagnosis day	Jan. 24, 2020	Jan. 24, 2020	Jan. 28, 2020	Jan. 24, 2020	Jan. 29, 2020
Symptoms	Fever, cough, conjunctivitis	Fever, cough	Fever, diarrhea, shortness of breath	Cough	Cough
Tests results on admission					
White blood cell counts, 10^9/L	5·8	4	8·0	3·3	3·1
Neutrophil count, 10^9/L	4·7	1·81	ND	ND	1·7
Lymphocyte count, 10^9/L	1	1·64	ND	1·2	1·3
Hemoglobin, g/l	15·5	16·9	12·3	13	13·2
Platelet count, 10^9/L	148	182	134	195	184
Prothrombin time, s	17	10	ND	20	20
Albumin, g/L	37	ND	ND	37	40
Creatinine kinase, UI/L	122	147	ND	88	66
Alanine aminotransferase, UI/L	37	22	21	42	11
Aspartate aminotransferase, U/L	32	32	66	46	29
Total bilirubin, mmol/L	7	7	ND	9	10
Sodium, mmol/L	140	139	136	142	139
Potassium, mmol/L	4·3	3·7	3·2	4·5	4
Urea, mmol/L	2·8	4·4	8	2·9	3·3
Creatinine, µmol/L	44	68	92	38	66
C-reactive protein mg/L	7	ND	123	<5	<5
Lactate UI/L	ND	ND	ND	ND	ND
Chest X-ray	Bilateral pneumonia	No findings	Bilateral pneumonia	No findings	No findings
Admission to Intensive Care Unit	YES	YES	YES	NO	NO

ND: not determined

Table 2: Confirmation of COVID-19 cases by RT-PCR, whole genome sequencing, and virus isolation

	Samples		RT-PCR targets				Specimen	Virus Isolate
Case #	Day post-symptoms onset	Nature	RdRp gene Wuhan Charité (Ct)	E gene Charité (Ct)	RdRp IP1 (Ct)	GAPDH (Ct)	virus sequence	Titer (PFU/mL)
1	6	NP	28.5	27·3	26·7	27.4	EPI_ISL406597*	No
2	9	NP	Neg	34·7	33.0	27.1	No	No
3	7	NP	Neg	30·3	29·2	25.7	partial	No
3	7	BAL	Neg	27·4	27.3	24.7	partial	No
4	2	NP	23.6	22·8	23.0	26.5	EPI_ISL406596	$6{\cdot}25 \times 10^5$
5	2	NP	24.3	20·0	19·3	25.6	EPI_ISL408430	$3{\cdot}0 \times 10^7$

NP: nasopharyngeal swab; BAL: bronchoalveolar lavage; ND: not determined; Ct: cycle threshold; PFU: plaque forming unit;

* : sequence number in GISAID database