



Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards

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Abstract

In a preliminary clinical study, we observed that the combination of hydroxychloroquine and azithromycin was effective against SARS-CoV-2 by shortening the duration of viral load in Covid-19 patients. It is of paramount importance to define when a treated patient can be considered as no longer contagious. Correlation between successful isolation of virus in cell culture and Ct value of quantitative RT-PCR targeting E gene suggests that patients with Ct above 33–34 using our RT-PCR system are not contagious and thus can be discharged from hospital care or strict confinement for non-hospitalized patients.

Keywords SARS-CoV2 · Covid-19 · RT-PCR · Co-culture · Viral load · Correlation

Introduction

An outbreak of an emerging disease (Covid-19) due to SARS-CoV-2 started in Wuhan, China, then rapidly spread in China, and was declared pandemic on March 12, 2020, by the WHO [1–3]. Currently, the overall case fatality rate is about 2.3% in China, which is likely an overestimate because most patients have mild symptoms and are thus not tested [4]. Because of a study showing that chloroquine and hydroxychloroquine inhibit SARS-CoV-2 in vitro, we tested hydroxychloroquine as a treatment in Covid-19 patients [5, 6]. Our results show that in treated patients, the nasopharyngeal viral load of SARS-CoV-2-infected patients was cleared in only 3 to 6 days. Our results also suggest a synergistic effect of the combination of hydroxychloroquine and azithromycin, two molecules previously demonstrated to be active in vitro against Zika and

Ebola viruses [7–10] and to prevent severe respiratory tract infections when administered to patients suffering viral infection [11]. These results are of great importance because a recent paper has shown that the median duration of viral RNA detection in patients suffering from Covid-19 in China was 20 days, with the longest duration being 37 days [12]. We are now facing a massive influx of patients in need of treatment and hospitalization, ideally in infectious disease wards equipped with NSB3 modules with negative pressure. Thus, in addition to obtaining complete disappearance of virus RNA in respiratory samples, having a PCR-based indicator of loss of contagiousness is a major priority for discharge from infectious diseases ward. Based on a set of 183 samples from 155 patients, we observed a significant relationship between viral RNA load and culture positivity.

The Méditerranée Infection University Hospital Institute in Marseille is the reference center for highly infectious diseases for Southeastern France. It was the only center in this region with diagnostic tests available during the first weeks of the epidemic in France and received patients' samples from this whole area. From the day of the first positive test, on February 27 until March 12, 4384 clinical samples were tested by RT-PCR for 3466 patients. Since the beginning of this crisis and until now, on March 26, we inoculated 1049 samples and could obtain in culture 611 SARS-CoV-2 isolates. A total of 183 samples testing positive by RT-PCR, including 9 sputum samples and 174 nasopharyngeal swabs from 155 patients, were inoculated in cell cultures. SARS-CoV-2 RNA positivity in patient samples was

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assessed by real-time reverse transcription-PCR targeting the E gene, as previously described [13]. For all patients, 500 μ L of nasopharyngeal swab fluid (Virocult, Elitech, France) or sputum sample were passed through 0.22- μ m pore sized centrifugal filter (Merck Millipore, Darmstadt, Germany) and then were inoculated in 4 wells of 96-well culture microplates containing Vero E6 cells (ATCC CRL-1586) into Minimum Essential Medium culture medium with 4% fetal calf serum and 1% glutamine. All samples were inoculated between 4 and 10 h after sampling and kept at +4 °C before processing. After centrifugation at 4000 \times g, microplates were incubated at 37 °C. They were observed daily for evidence of cytopathogenic effect. Two subcultures were performed weekly. Presumptive detection of virus in supernatant showing cytopathic effect was done using the SU5000 scanning electron microscope (Hitachi High-Tech Corporation, Tokyo, Japan) and then confirmed by specific RT-PCR targeting E gene. Variation of culture positivity rate was assessed statistically as the proportion of variance explained by Ct value and considered adequately fitted if the coefficient of determination (R^2 statistic) was > 50%.

Among the 183 samples inoculated in the studied period of time, 129 led to virus isolation. Of these, 124 samples had detectable cytopathic effect between 24 and 96 h. Blind subcultures allowed obtaining 5 additional isolates only. We observed a significant relationship between Ct value and culture positivity rate (Fig. 1). Samples with Ct values of 13–17 all led to positive culture. Culture positivity rate then decreased progressively according to Ct values to reach 12% at 33 Ct. No culture was obtained from samples with Ct \geq 34. The 5 additional isolates obtained after blind subcultures had Ct between 27 and 34, thus consistent with low viable virus load.

In the present work, we observe a strong correlation between Ct value and sample infectivity in a cell culture model. On the basis of this data, we can deduce that with our system, patients with Ct values equal or above 34 do not excrete infectious viral particles. It was observed that SARS-CoV-2 was detected up to 20 days after onset of symptoms by PCR in infected patients but that the virus could not be isolated after day 8 in spite of ongoing high viral loads of approximately 10^5 RNA copies/mL of sample, using the RT-PCR system used in the present study [14]. Progressive decrease of viral load over time is observed in all studies conducted in Covid-19 patients with positive detection being observed until 17–21 days after onset of symptoms, independently of symptoms [15]. These previous observations suggested that isolation of patients after diagnosis was mandatory. However, due to prolonged shedding of RNA in respiratory samples, the criteria for ending the isolation of a patient were not clear, and there was a need to correlate viral load to cultivable viruses. Our results show that in our system of RT-PCR, we can assess that patients with Ct equal or above 34 may be discharged. In 6 patients under the current therapeutic protocol used at our institute (hydroxychloroquine and azithromycin), Ct values \geq 34 were obtained between days 2 and 4 post-treatment [6]. There is no previous correlation demonstrated between level viral load in respiratory samples and infectivity. However, this reduction is the basis of all procedures used for the validation of disinfectants [16]. One limitation of our work is that it cannot be extrapolated to other hospital centers since they use different systems of sample transport, of RNA extraction, and of PCR with different primers and probes; i.e. it has been suggested that sensitivity of amplification based on Gene E detection

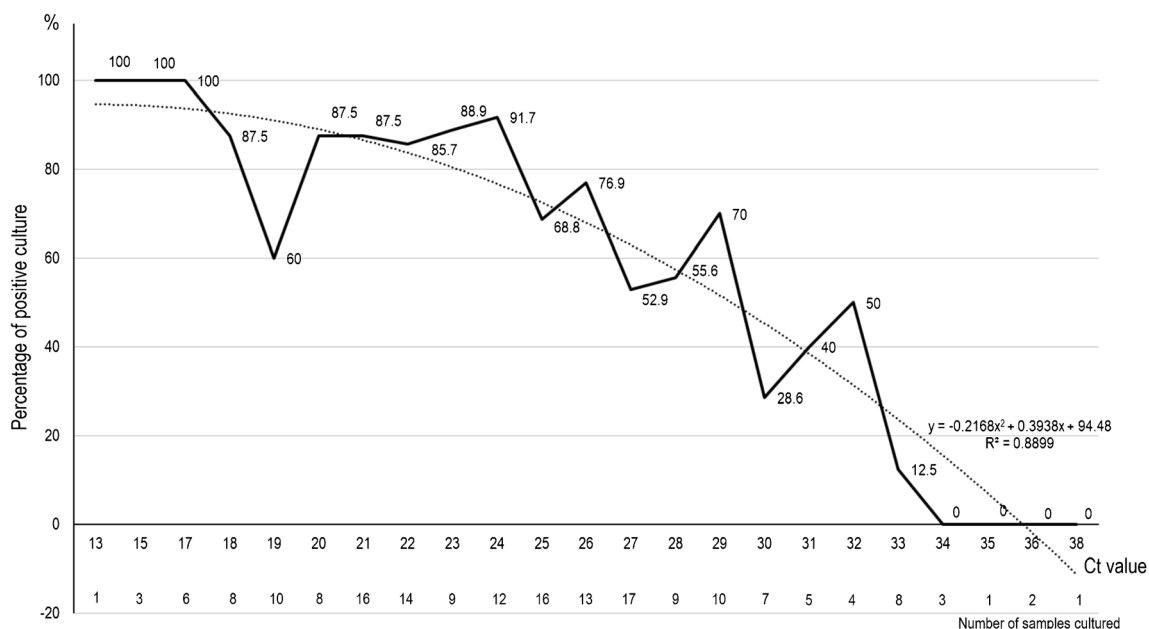


Fig. 1 Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from Covid-19 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve

would be less sensitive than ORF1ab or N genes. We propose that each center perform its own correlation between culture results and viral RNA load from patients' samples. Another potential limitation is that nasopharyngeal swab fluid might be less representative than sputum samples. However, from the data obtained from patients rather tested in sputum, the viral load follows the same reduction with time of evolution than upper respiratory specimens [17].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The protocol was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (No. 2020-01).

Informed consent All subjects gave a written informed consent in accordance with the Declaration of Helsinki.

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