Viral load of SARS-CoV-2 across patients and compared to other respiratory viruses

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Abstract

RT-PCR to detect SARS-CoV-2 RNA in clinical specimens was key to manage the COVID-19 pandemic. We monitored SARS-CoV-2 viral loads over time and across different patient populations. We analyzed RT-PCR results according to samples types, gender, age, and health units and compared SARS-CoV-2 viral load to other respiratory viruses, representing a total of 28’373 RT-PCR results including 22’323 SARS-CoV-2 RT-PCR. The importance of viral load to predict contagiousness and clinical prognosis is discussed.

Keywords: COVID-19, SARS-CoV-2, RT-PCR, quantification, cycle threshold, viral load, influenza virus, Flu, RSV
1. Introduction

At the beginning of January 2020, the cluster of SARS-CoV-2 cases identified in Wuhan City, Hubei Province (China) rapidly spread to other regions in China and to other countries, causing a world pandemic [1, 2]. Quantitative reverse transcription polymerase chain reaction (RT-PCR) represented a key diagnostic tool for patients with suspected SARS-CoV-2 infection. Viral-specific genes, such as the Envelope (E), the RdRP/Helicase (Hel), the spike protein encoding gene (S), as well as Nucleocapsid (N) were used as molecular targets and combination of these genes have been recommended by the WHO [3, 4]. We introduced the E, RdRP, and N genes RT-PCRs in our fully automated molecular diagnostic platform (MDx platform) [5]. A lower sensitivity of the RT-PCRs targeting the RdRP and N genes, compared to that targeting the E gene was observed leading us to use solely the E gene, as RT-PCR target. Latter during the pandemic, the cobas® SARS-CoV-2 test (ROCHE) became available targeting the ORF1/a, a non-structural region for specific detection of SARS-CoV-2 and a conserved region in the E gene, for pan-Sarbecovirus detection. The pan-Sarbecovirus primers and probe can also detect the SARS-CoV-1 virus, however not currently circulating [6].

We determined the correlation between the cycle threshold (Ct) value and viral load and investigated the distribution of viral loads across sex, age, and healthcare departments and as well as against other respiratory viruses. The report of RT-PCR SARS-CoV-2 cycle thresholds (Cts) values raised also several questions regarding the use of this information for the laboratory as an internal quality assessment tool, as well as (i) to predict contagiousness of patients and hence to guide epidemiological decisions, especially for hospitalized patients and (ii) to predict the patient prognosis. These important questions will also be discussed here.

2. Material and methods

Cts of our MDx platform were converted to viral load using either a plasmid containing the target sequence of the PCR or using purified viral RNA, kindly provided by the Institute of Virology of the University of Berlin, la Charité [4]. Both approaches showed similar virus quantifications and the
following equation derived from RNA quantification was used: -0.27Ct+13.04. A comparative analysis of the Cts values obtained from our MDx platform compared to the cobas® SARS-CoV-2 test (ROCHE) showed a good congruency, which led us to use the E gene RT-PCR Cts values of both platforms in the present analyses. Among the 22'323 specimens collected, only the initial sample per patient was kept (19’832 samples) and viral loads of 4172 positive samples were analyzed. Our dataset contains 99.5% of nasopharyngeal and/or nasal swabs (NPS) that were used for the stratification analyses (19’728 samples). Viral loads across different specimen types were instigated using multiple samples per patient (most of these investigations were performed after the first positive tests, usually an NPS). 6’050 RT-PCR of 14 other respiratory viruses were extracted from our database over a period of 5 years (2015-2020): Influenza A and B, Respiratory Syncytial Virus (RSV), adenovirus, parainfluenza 1-4, Coronavirus E229, OC43, HKU1, NL63, Pan-entero/rhinovirus and Human Metapneumovirus and most Cт values were obtained on our automated MDx platform and converted to viral loads based on plasmids positive controls, as previously reported [5]. For Influenza A and B and RSV the Xpert® Xpress Flu/RSV was used and converted to viral load according to Zou et al. [7]. For these various respiratory viruses, only nasopharyngeal and nose swabs were included. Data were processed with Rstudio and plotted using ggplot2. Median is presented in all graphs.

3. Results

Data from 19’832 SARS-CoV-2 RT-PCR results from patients with suspected COVID-19 were collected from 1st February to 27th April 2020 at the diagnostic microbiology laboratory of the Lausanne’s University Hospital (CHUV), representing 4172 positive cases. We observed a broad distribution of viral load values (Fig. 1A) with an evolution over the pandemic period that mirrored the epidemiological observations of SARS-CoV-2 infection in Switzerland [8] (Fig. 1B). The first cases occurred early March with a peak of the COVID-19 epidemic mid-March followed by a 2 weeks stationary phase before a slow decrease. Interestingly, the median viral load was higher in the first phase of the outbreak as compared to the following period.
The initial viral load of SARS-CoV-2 was compared to 6’050 RT-PCR of 14 other respiratory viruses [9] and showed comparable median values (Fig. 2A). We then investigated SARS-CoV-2 viral loads stratified by gender, samples type, age, and hospital units. A higher number of tests was achieved in women than in men (35% of difference); however the rate of positive results was similar for both sex (Fig. S1A and B) and both genders showed comparable viral load distribution (Fig. 2B). Stratification of positive samples by age groups showed that older individuals, when tested, were likely to be proportionally more frequently positive than the rest of the population, while young children showed very low percentages of positivity despite being rarely tested (Fig. S1C-D). Interestingly, viral loads categorization based on 5-year brackets ages showed no significant differences across age groups (Fig. 2C). Although limited by the low samples size, the pediatric age groups showed viral loads values comparable to adults. We then compared viral loads of patient in different hospital units. We focused on the Intensive care unit (ICU), the internal medicine (IM) department, the emergency unit (EU) and patients addressed to a screening unit (SU) specifically developed during the outbreak. This stratification per unit was used to investigate possible differences in viral loads in patients with several days of evolution since first symptoms and with a severe lung disease (ICU), versus subjects sick enough to get hospitalized (IM), to patients screened with mild symptoms (SU). Interestingly, patients hospitalized in ICU showed the lowest viral load in the upper respiratory tract compared to all other patients (Fig. 2D). This might reflect the evolution of COVID-19 infection, which initially affects the upper respiratory tract causing mild symptoms such as a fever and cough but can, in more severe cases, travel further down to the lower respiratory tract, causing acute respiratory distress syndrome (ARDS) [10, 11]. To assess if the initial viral load could correlate with disease progression, we traced back, when available, the initial or the highest viral load values obtained in other departments for all patient hospitalized in ICU and showed that this value is not significantly higher than the one obtain for all other patients (Fig. 2D). This might be biased by the timing of the 1st nasopharyngeal test that was sometimes done very late, i.e at time of admission at the ICU. Geriatric patients did not show different viral loads than other departments.
Over the time course of the epidemic several, non-nasal specimens were analysed mainly lower respiratory samples for patient in the ICU (Fig. S1F). Although, lower viral load values were obtained compared to the upper respiratory part (Fig. 2E), the lower respiratory tract samples were often useful to allow an early microbial diagnostic of COVID-19, and might prove to be useful to assess the clinical prognosis and disease progression. Only few blood samples were tested and only one of them was positive; this suggest a low rate of viremia. Interestingly, SARS-CoV-2 was not detected in urines. This was expected since respiratory tract viruses, which are not associated with a sustained viremia, are unlikely to be shed in urines. Moreover, the absence of virus in the CSF tested samples suggests that the serology should be considered as first line test for meningoencephalitis and Guillain-Barre syndrome. Only a handful number of samples were positive for stools and rectal swabs, due to limited number of subjects tested.

4. Discussion

Initial SARS-CoV-2 viral load is widely distributed ranging from 3 to 10 log copies/ml and the evolution of the viral load over-time mirrored the evolution of SARS-CoV-2 infections in Switzerland. The median viral load for SARS-CoV-2 in NPS was 6.78 log<sub>10</sub> copies per ml. This supports the fact that RT-PCR which can detect less than 100 copies per ml of samples is a sensitive method for the diagnostic of COVID-19. This is however limited by the quality of specimen sampling and the time course of infection.

We also compared SARS-CoV-2 viral loads to that of other respiratory viruses in order to determine whether higher viral loads, that could affect contagiousness, are observed. Although significant differences were observed when compared to some other respiratory viruses, SARS-CoV-2 appears to exhibit similar viral load than RSV and influenza [9]. For respiratory viruses other than Flu and RSV, we have a bias towards immunocompromised or severely ill patients, which might tend to have higher viral loads. Interestingly, others reported that the pattern of patients infected with SARS-CoV-2 resembles more to patients infected with influenza [12] than SARS-CoV-1 [13]; the former being characterized by increased infectiousness at time or even before symptoms onset [14]. SARS-CoV-2
viral load appears to be a poor predictor of disease outcome. Indeed neither the initial nor the highest viral load of patients latter admitted to the ICU was significantly higher than the specimens from patient treated in a SU. This absence of correlation with the clinical outcome is also supported (i) by other published data showing high viral load in asymptomatic patients [15-17, 14] and (ii) by the fact that asymptomatic or minimally symptomatic patients can transmit the virus [18]. We also observed that viral load seems not to correlate with age. In particular, older individual and young children showed similar viral loads than the general population [19-21]. Concentration of the virus in the respiratory tract can indirectly reflects contagiousness; however, viral load is not the only factor at play in term of contagiousness, since nasal discharge and cough are clearly important co-variables impacting transmission.

The clinical relevance and usefulness of viral load measures appears to be mainly restricted to specifically classifying the patient as being in the first phase of the disease with high viral load or rather in the 2nd phase of the disease when viral load tends to decrease and when inflammation predominates. This may be useful to help treatment decision, i.e to use for instance anti-IL6 or steroids in presence a cytokine storm or during a macrophage activation syndrome. Interpretation of a unique viral load value in a given patients should however be very cautious since (i) there is a tend to a natural gradual decrease of the viral load in the nasopharyngeal samples over time during the course of the infection [14, 15] and (ii) the absolute value of the viral load in the nasopharyngeal samples may be highly different according to the quality of sampling. Despite these limitations, our laboratory decided to provide quantitative results to clinicians, and these values are now used not only for patient care, but also to define contagiousness, i.e values below 1000 copies/ml associated with clinical and epidemiological data might be considered at low risk of transmission.
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Conflict of interest

The authors declare to have no conflict of interest.

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Figures and legends

Figure 1
A: Histogram of SARS-CoV-2 viral loads. B: Time-course analyses of SARS-CoV-2 viral loads across time.
Viral loads mirrored the reported COVID-19 infections in Switzerland.

Figure 2
A: Viral loads of 14 respiratory viruses compared to SARS-CoV-2. HPMV: Human-metapneumovirus, HPIV1-4: Human Parainfluenza Viruses 1-4, InfA and B: Influenza viruses A and B; RSV: Respiratory Syncytial Virus. “m” represents the median and “n” the number of observations. Significance of viral loads of SARS-CoV-2 was assessed against the other viruses using a parametric paired t-test and the two-tailed p-values interpretation are written on the graph. “ns”: p > 0.05, *: p ≤ 0.05, ****: p ≤ 0.0001
B-C: Viral loads distribution of SARS-CoV-2 across sex and age showed comparable values among all groups. D: Initial viral loads of SARS-CoV-2 in different hospital departments. ICU first and ICU max correspond to respectively the first or highest sample recorder for patients latter admitted to the ICU.
E: Distribution of viral loads across different specimens. AS: anal swab, BAL: bronchoalveolar lavage, CSF cerebrospinal fluid, NTS: nasal-throat swab, TS: throat swab.

Supplementary figure

Figure S1
A-D: Absolute and percentage values of SARS-CoV-2 infection across sex and ages. E-F: Absolute and percentage values of SARS-CoV-2 viral loads across different specimens. AS: anal swab, BAL: bronchoalveolar lavage, CSF cerebrospinal fluid, NTS: nasal-throat swab, TS: throat swab.
Figure 1

A

B

Viral load log_{10} copies/ml

weeks

n=4  n=105  n=592  n=1261  n=1037  n=552  n=354  n=161  n=76
Figure 2
Figure S1

A

B

C

D

E

F

NEGATIVE POSITIVE

NEGATIVE POSITIVE

NEGATIVE POSITIVE

NEGATIVE POSITIVE

NEGATIVE POSITIVE

NEGATIVE POSITIVE