Time to RNA negative conversion in Moroccan COVID-19 patients:

A single-centre experience

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Running title: Time to viral clearance in COVID-19

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Summary

The COVID-19 global pandemic is now a public health emergency in Morocco. Reports on factors associated with prolonged viral shedding are not consistent. In this retrospective laboratory based study, time to RNA negative conversion is reported in a series of 129 patients monitored in a single laboratory in Casablanca. Risk factors associated with delayed negative conversion have been evaluated, by chi-squared test. Median delay of negative conversion was 22.5 days (IQR 17.75-29.0) from illness onset. Neither gender nor age were particularly associated with delayed viral clearance. Delayed time to hospital admission and disease severity were associated with prolonged viral shedding in this series. We recommend early diagnosis and treatment onset to reduce time to viral clearance and transmissibility of SARS-CoV-2 virus.

Keywords: SARS-CoV-2; COVID-19; Morocco; recovery; viral clearance

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Introduction

A novel coronavirus (SARS-CoV-2) has been first reported in Wuhan, China in December 2019, as the cause of severe pneumonia. The related infectious disease, COVID-19, has been later declared a global pandemic by WHO. As of 11 May 2020, 4.12 million cases were confirmed worldwide [1].In Morocco, the first case was confirmed on 2 March 2020 in Casablanca. As of 11 May 2020, 6226 cases were confirmed, with 188 deaths and 2759 cured [2].

Reliable diagnosis and monitoring of COVID-19 patients was necessary in Morocco to facilitate public health interventions and rapidly evaluate epidemic evolution. On 1st April 2020, the National Reference Laboratory (LNR), of Mohamed VI University of Health Sciences (UM6SS), was the fourth laboratory to obtain authorization from the Ministry of Health to perform RT-PCR tests for suspected COVID-19 patients.

There are several reports of positive RT-PCR, even after clinical recovery or hospital discharge. Some authors study the factors associated with delayed viral clearance [3,4]. This is a report of virological evolution and time to RNA negative conversion in a series of Moroccan COVID-19 patients followed up in the Cheikh Khalifa International University Hospital (HCK) and monitored in the National Reference Laboratory, in Casablanca.

Methods

Patients and study design

This retrospective laboratory based study was carried out from 1st April to 30 April 2020, including 129 confirmed COVID-19 patients admitted to the Cheikh Khalifa International University Hospital and followed up in National Reference Laboratory (LNR). A ten-day

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treatment systematically began from day of admission, based on hydrochloroquine and azithromycin, following ministerial circulars [5,6]. The following criteria should be met for hospital discharge: completion of ten-days treatment, clinical and biological recovery and two negative PCR on consecutive samples separated by at least 1 day [6]. However, some patients were discharged after 24 days of medical surveillance and quarantined at home, even without RNA negative conversion, if clinically and biologically recovered [6]. The study was approved by the Mohamed VI University of Health Sciences Ethical review board.

Definitions

Following national recommendations, a COVID-19 case was determined as a patient showing clinical features of COVID-19 (mainly respiratory tract infection) or close contact of a confirmed case, with a positive RT-PCR on nasopharyngeal swab [5,6]. A case is defined as mild if patient only presented signs of upper respiratory tract infections; moderate if presenting a pneumonia or if there was co-morbidities factors associated with a mild case; severe and critical cases were patients cases requiring hospitalization in unit intensive care or complicated by other organ failure.

Inclusion criteria

To assess the factors associated with delayed viral clearance, patients meeting the following criteria were enrolled: 1) viral clearance within 25 days after hospital admission; 2) patients followed-up during 25 days without viral clearance; 3) death within 25 days. Eighty-nine patients from 129 monitored patients (69.8%) were enrolled.

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Virological monitoring

Nasopharyngeal swabs were collected for screening and monitoring, and a commercial kit was used for RT-PCR (GeneFinder, Osang Healthcare), according to manufacturer's instructions, targeting *RdRp, E* and *N* genes. In monitored patients, detection of at least one gene was considered positive. By mid-April, a consensus was reached in the laboratory for the virological monitoring of treated COVID-19 patients: first control was fixed on day 9 of treatment. If there was still a trace of viral RNA (at least one gene detected), next control was scheduled 4 days later. In case of a negative PCR, control was done the day after.

Statistical tests

Continuous variables are expressed as median, with interquartile range (IQR). Impact of gender, age, time from illness onset to hospital admission and disease severity were assessed through χ^2 test on Microsoft Excel software. Significance level was set at $p < 0.05$.

Results

As of 30 April 2020, 63 patients presented viral clearance from the 129 followed up (48.8%). Sixteen patients had completed 25 days of monitoring without viral clearance (12.4%). Mortality reached 7.8% in this series (10 cases), with only one female. Median time to RNA negative conversion was 22.5 days (IQR 17.75-29.0) from illness onset, and 17 days (IQR 13.5-20.0) from hospital admission.

Median age of virologically recovered patients is 48 years (IQR 33-59), with 47.6% of male patients. On the 70 patients whose onset of disease was available, 18 were contact cases showing no symptoms at screening date. Nevertheless, they were also treated with hydrochloroquine and azithromycin.

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Eighty-nine eligible patients were classified in early, medium and late conversion groups to assess the impact of gender, age and time to hospital admission (Table 1) on delay of viral clearance. Twenty-six patients (29.2%) had negative conversion with 15 days of hospitalization (early conversions); patients with medium conversion cleared viral RNA between 15 and 25 days of hospitalization (32 cases, 36.0%); and 21 patients (23.6%) still presented RNA shedding after 25 days of monitoring (late conversion).

There was no statistical significance for gender (p=0.53), age (p=0.10) nor delayed admission (p=0.21) on time to negative conversion from hospital admission in recovered patients. However, patients showing severe symptoms through the course of disease tend to delay viral clearance (p=0.018). Regarding mortality, male gender and elder age are factors associated with higher mortality (p=0.031 and p=0.027, respectively).

Information on disease onset was available for 70 patients. Median delay to hospital admission was 5 days. Twenty-two patients had viral clearance within 20 days after symptoms onset (31.4%), 32 patients had medium conversion (45.7%) and 16 patients had no viral clearance within 30 days of disease progression (22.8%). From disease onset, delayed hospital admission and disease severity are associated with prolonged RNA shedding (p=0.001 and p=0.029, respectively) (Table 2).

Discussion

There are several reports of detectable viral in recovered patients [7-9]. However, He et al. estimated that infectiousness peaked at the time and before onset symptoms. After symptom onset, viral loads decreased monotonically [10]. Another study from Wuhan reported that virus was detected for a median of 20 days (up to 37 days among survivors)

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after symptom onset[11], but infectiousness may decline significantly 8 days after symptom onset, as live virus could no longer be cultured (according to Wölfel et al.)[12].

On the basis of the present findings, and in a situation characterized by a limited capacity of hospital beds, a discharge after 24 days of medical surveillance with ensuing home isolation was chosen for patients who are clinically and biologically recovered [6].

In China, two independent studies evaluated the risk factors associated with delayed viral clearance in COVID-19 patients. Xu et al. concluded that male gender, old age, delayed admission to hospital, and invasive mechanical ventilation were associated with delayed viral clearance [3]. On the contrary, Hu et al. reported that elder age and chest tightness were predictors of delayed viral clearance [4].

Contrary to Xu et al. study [3], male gender doesn't seem to have an effect on time to RNA negative conversion in hospitalized patients. However, male gender is associated with higher mortality in this series (12% of male patients *vs* 2% of female patients). Impact of delayed admission on viral clearance seems to be related to delayed treatment in this series. However, under treatment, patients show the same rate of viral clearance, regardless of the time from disease onset to hospital admission.

Contrary to Hu et al. study [4], elder age was not significantly associated with delayed viral clearance, though patients older than 60 years tend to recover slowly. Moreover, elder age is a high-risk factor for mortality (8 of 10 deceased patients older than 60 years). Chest tightness (moderate case) was not associated with delayed clearance.

There are limitations in this study. Sensitivity of nasopharyngeal swabs is known to be weak for SARS-CoV-2 detection, with a negative predictive value between 69 and 85% [13]. Thus,

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two consecutive negative PCR on nasopharyngeal swabs will increase negative predictive value between 90 and 97%.

Conclusion

In this series, no particular socio-demographic factor was associated with delayed viral clearance in COVID-19 patients. On the other hand, severe cases tend to clear viral RNA more slowly. However, early screening and treatment onset will reduce time from disease onset to viral clearance and risk of transmission.

Conflict of interest: None

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Table 1. Time to viral clearance from treatment onset in Moroccan COVID-19 patients

Patients'characteristics	Time to RNA negative conversion from treatment onset			Deaths
	Early conversion (<15d)	Medium conversion (15-25d)	Late conversion (>25d)	
Gender				
Male	15	15	11	9*
Female	11	17	10	1
Age				
< 20 years	5	4	1	0
21-40 years	7	3	5	1
41-60 years	11	14	6	1
> 60 years	3	11	9	8*
Time to hospital admission				
Asymptomatic	7	5	6	
< 5 days	3	10	8	
6-10 days	9	8	2	
> 10 days	5	5	2	
Disease Severity				
Asymptomatic	7	4	5	0
Mild-Moderate	19	28	14	0

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Severe - critical	0	0*	2	10*

*p<0.05

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Table 2. Time to viral clearance from disease onset in Moroccan COVID-19 patients

Patients'characteristics	Time to RNA negative conversion from disease onset		
	<20d	20-30d	>30d
Gender			
Male	11	19	7
Female	11	13	9
Age			
< 20 years	4	5	0
21-40 years	4	5	4
41-60 years	11	13	5
> 60 years	3	9	7
Time to hospital admission			
Asymptomatic	11*	4	3
< 5 days	6	11	4
6-10 days	5	12	2
> 10 days	0	5	7*
Disease Severity			
Asymptomatic	10*	3	3
Mild-Moderate	12	28	12
Severe - critical	0	1	1

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1 *p<0.05