Association between upper respiratory tract viral load, comorbidities, disease severity and outcome of patients with SARS-CoV-2 infection

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Summary: Patients with comorbidities had more often high upper respiratory tract viral load. Patients with high viral load more often developed COVID-19, were intubated or died. Viral load could be used to identify high risk patients for morbidity or severe outcome.

Conflicts of interest: none

Funding statement: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The Reference Laboratories were financially supported by the National Public Health Organization (Greece).

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Abstract

Background: There is limited information on the association between upper respiratory tract (URT) viral loads, host factors, and disease severity in SARS-CoV-2 infected patients.

Methods: We studied 1,122 patients (mean age: 46 years) diagnosed by PCR. URT viral load, measured by PCR cycle threshold, was categorized as high, moderate or low.

Results: There were 336 (29.9%) patients with comorbidities; 309 patients (27.5%) had high, 316 (28.2%) moderate, and 497 (44.3%) low viral load. In univariate analyses, compared to patients with moderate or low viral load, patients with high viral load were older, had more often comorbidities, developed symptomatic disease, were intubated and died; in addition, patients with high viral load had longer stay in intensive care unit and longer intubation compared to patients with low viral load (p-values <0.05 for all). Patients with chronic cardiovascular disease, hypertension, chronic pulmonary disease, immunosuppression, obesity and chronic neurological disease had more often high viral load (p-value<0.05 for all). Multivariate analysis found that a high viral load was associated with COVID-19. The level of viral load was not associated with any other outcome.

Conclusions: URT viral load could be used to identify patients at higher risk for morbidity or severe outcome.

Keywords: SARS-CoV-2; COVID-19; upper respiratory tract; viral load; clinical course; outcome

Introduction

Following the emergence and global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease 2019 (COVID-19), a pandemic was declared by the World Health Organization on March 11, 2020 [1]. Within the first few months the devastating health, economic and societal consequences of the COVID-19 pandemic became evident. As of December 15, 2020, 70 million cases and 1.6 million deaths have been reported throughout the world [1]. As the pandemic evolved, several studies focused on the critical role of host factors on disease severity and outcome in patients with COVID-19 [2,3]. However, there are significant differences between countries in terms of population demographics and prevalence of comorbidities. In addition, recent studies indicate that host responses to SARS-CoV-2 are dependent on viral load and infection time course [4]. An association between high SARS-CoV-2 viral load and disease severity has been reported in two studies so far [5,6], nonetheless these findings were not confirmed by others [7]. In these studies, however, only hospitalized or symptomatic patients were studied [5-7]. In addition, there is scarce published information about the association between viral load and comorbidities [5,7]. Herein, we studied the upper respiratory tract (URT) viral load of patients with symptomatic or asymptomatic SARS-CoV-2 infection and their potential association with age, gender, comorbidities, disease severity and outcome in a series of 1,122 patients in Greece.

Methods

Data collection

The study period extended from February 26 through May 3, 2020 (first epidemic wave in Greece). We studied SARS-CoV-2 infected cases consecutively diagnosed in three reference laboratories for SARS-CoV-2 (two in Athens and one in Thessaloniki) where most cases were diagnosed. Demographic, clinical and outcome data were retrieved from the national surveillance database for

SARS-CoV-2 infections (National Public Health Organization, Athens). Data were collected prospectively. The outcome of patients was updated on September 30, 2020.

Virological investigation

Patients' URT samples (nasopharyngeal or oropharyngeal swabs) were tested for SARS-CoV-2 by real-time reverse transcriptase polymerase chain reaction (RT-PCR) following commercial or in-house protocols. In particular, RNA was extracted from 250 µl of PBS rehydrated swabs in a final elution volume of 70 µl, using the automated Promega's© Maxwell Viral Total Nucleic Acid Purification Kit or the King Fisher Flex platform (ThermoFisher Scientific). SARS-CoV-2 RNA was detected employing Genesig's© COVID-19 CE-IVD real-time RT-PCR kit according to manufacturer's instructions and starting from 8 µl of eluted RNA or using an in-house Taqman rt-real-time PCR assay targeting E and RdRP genes; assay validation was performed in line with ISO 15189: 2012 requirements, and the quality of COVID-19 diagnostic testing was supported and ensured by proficiency testing panels i.e., external quality assessment schemes provided by European Centre for Disease Prevention and Control and World Health Organization.

Based on the cycle threshold (Ct) value (replication cycles required for gene amplification to produce a threshold signal) of the PCR, patients were categorized into three groups, those having high, moderate, or low URT viral load (Ct <25, 25-30 or >30, respectively). Only the first sample was considered in the assessment of Ct values.

Definitions

Asymptomatic SARS-CoV-2 cases were defined as those with positive SARS-CoV-2 PCR in the absence of symptoms. COVID-19 cases were defined as those with positive SARS-CoV-2 PCR and compatible signs and symptoms. COVID-19 cases were classified as severe when patients were admitted to intensive care unit (ICU), were intubated or had a fatal outcome. Comorbidities included chronic cardiovascular disease, hypertension, diabetes mellitus, chronic pulmonary disease, chronic renal

disease, chronic neurological disease, chronic hepatic disease, malignancy, immunosuppression, and obesity. Complications included pneumonia, acute respiratory distress syndrome, renal failure, cardiovascular complications, and multi-organ failure.

Statistical analysis

Medians and means were used to describe the distribution of continuous variables and frequencies, and percentages were used for categorical variables. Comparisons between groups were performed by using the t-test for continuous variables with normal distribution, and the chi-square test for categorical variables. ANOVA test has been used to compare the means from more than two independent groups. In order to explore the factors that predict the development of COVID-19, hospitalization, admission to ICU, intubation and death, several logistic regression analyses have been conducted by using as independent variables the following factors: age, gender, presence of comorbidities, number of comorbidities, and category of viral load. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated. P-values of ≤ 0.05 were considered statistically significant. The statistical analysis was conducted using the IBM-SPSS software version 26.0 for Windows, Armonk, NY, USA.

Ethical issues

Written consent was not required, given that the data were collected within the frame of epidemiological surveillance. Data were managed in accordance with the national and European Union laws.

Results

A total of 1,122 patients (619 males; 55.2%) with SARS-CoV-2 infection were studied. Their mean age was 46 years (range; 0-102 years); 336 (29.9%) patients had at least one comorbidity. Of the 1,122 patients with SARS-CoV-2 infection, 274 (24.4%) had an asymptomatic infection and 848 (75.6%) developed COVID-19. Of the latter, 518 patients (61.1% of patients with COVID-19) were hospitalized, 99 patients (19.1% of hospitalized patients) were admitted to ICU, 93 (17.9% of hospitalized patients) were intubated, and 89 died (17.2% in-hospital mortality). The mean time period that elapsed from the onset of symptoms to the date of sample collection for patients with COVID-19 was 5.91 days (±5.54 days).

Of the 1,122 patients, 309 patients (27.5%) had high URT viral load, 316 (28.2%) moderate, and 497 (44.3%) low URT viral load. Table 1 shows the characteristics of patients with SARS-CoV-2 infection according to their viral load. Patients with high URT viral load were significantly older than patients with moderate or low URT viral load (mean age: 50 years compared to 48 years and 43 years, respectively; p-value=0.001). Stratification of age groups by viral load showed that 29.3%, 26.8% and 43.9% of children <18 years old had high, moderate and low URT viral load, compared to 26.3%, 29.2% and 46.1% of adults 18-64 years old, and compared to 32.6%, 25.7% and 35.2% of adults $\geq$65 year old (p-value=0.107).

Patients with high URT viral load more often had at least one comorbidity compared to patients with moderate or low URT viral load (40.1% compared to 32.9% and 21.7%, respectively; p-value<0.001). Table 2 shows the distribution of URT viral load according to comorbidities. Patients with the following comorbidities more often had high URT viral load than moderate or low URT viral load: chronic cardiovascular disease, hypertension, chronic pulmonary disease, immunosuppression, obesity, and chronic neurological disease (p-values <0.05 for all comparisons). There was no difference in the time that elapsed from the onset of symptoms to the date of sample collection

between patients with comorbidities and patients without comorbidities (mean time period: 5.86 days and 5.94 days, respectively; p-value=0.835).

Table 3 summarizes the morbidity and outcome of patients with SARS-CoV-2 infection by URT viral load. Patients with high URT viral load were more likely to develop COVID-19, to be intubated, and to die compared to patients with moderate or low URT viral load (p-values= <0.001, 0.05, and 0.03, respectively). On the other hand, patients with asymptomatic SARS-CoV-2 infection more often had a low URT viral load than a moderate or a high viral load (p-value <0.001). In addition, patients with high URT viral load tended to have longer length of stay in ICU and longer length of intubation compared to patients with low URT viral load (p-values=0.011 and 0.066, respectively). The three groups did not differ significantly in terms of rates of hospitalization, complications, and admission to ICU (Table 3). In terms of timing of diagnosis, patients with COVID-19 and high URT viral load had shorter time periods from the onset of symptoms to the date of sample collection compared to patients with COVID-19 and moderate or low URT viral load (mean time period: 4.31 days versus 5.87 days and 7.21 days, respectively; p-value=0.001).

Table 4 shows the results of the logistic regression models in all 1,122 patients with SARS-CoV-2 infection. A high URT viral load of SARS-CoV-2 was independently associated with the development of COVID-19. Older age was consistently associated with all outcomes, while female gender significantly protected against hospitalization, admission to ICU, intubation, and death (Table 4). A high URT viral load of SARS-CoV-2 was not associated with any outcome even when the logistic regression analyses were conducted in the subgroup of symptomatic patients (data not shown).

Discussion

We studied the URT viral load of 1,122 patients with SARS-CoV-2 infection diagnosed during the first epidemic wave in Greece. To the best of our knowledge, this is one of the largest series of SARS-CoV-2 URT viral load published so far to explore their utility in clinical practice, and the only one to study both asymptomatic and symptomatic patients, either hospitalized or cared in the community. The

large number of cases allowed us to investigate the association between URT viral loads and specific comorbidities.

We found that patients with high SARS-CoV-2 URT viral load tended to be older than patients with moderate or low URT viral load, which is in accordance with findings from two hospital-based studies and one laboratory-based study from the United States and China [4,5,8]. Nonetheless, a high URT viral load was detected in 29.3% of children <18 years old in our series. High SARS-CoV-2 viral load have been also found in 29% of children up to the age of 6 years and in 37.3% of children up to the age of 19 years in a pool of 3,303 persons tested positive for SARS-CoV-2 from across Germany regardless of symptoms [9]. Similarly, a United States series of symptomatic patients, found lower median Ct values in nasopharyngeal swabs in young children, indicating 10-100 times higher SARS-CoV-2 viral load compared to adults, while older children had Ct values comparable to adults [10]. Recent evidence shows that children may also contribute to transmission of SARS-CoV-2 in their households [11]. Therefore, Ct values could be used to guide isolation. As reported by others [12], we found no significant differences in URT viral load between males and females.

Another finding of our study is that specific comorbidities significantly correlated with a high SARS-CoV-2 URT viral load at diagnosis, which was not attributed to timing of sample collection. An association between high viral load at admission and specific comorbidities has been also reported in two other hospital-based studies [5,7] and in a series of 100 hospitalized patients with hematologic malignancies [13]. A high SARS-CoV-2 viral load may reflect uncontrolled virus replication in the upper respiratory tract and thus an inefficient immune response in the context of immune dysfunction in patients with specific comorbidities, e.g. chronic systemic inflammation is common in obese persons [14-16]. A recent review from a limited number of studies indicates that immunocompromised patients and patients with severe-to-critical illness may shed infectious virus for longer [17]. The pathogenic mechanism for this correlation may vary by comorbidity and needs further investigation.

In our series, a high URT viral load was more often detected in patients with COVID-19 than in asymptomatic patients. Similarly, symptomatic children had higher viral load in nasopharyngeal swab specimens than asymptomatic children in a study in South Korea [18]. However, this was not the case in a study of 203 pediatric cases of ours, where no differences in SARS-CoV-2 viral load were found between asymptomatic and symptomatic children [19]. In a similar manner, Ct values did not differ between asymptomatic and symptomatic cases in a cohort of 303 patients with SARS-CoV-2 infection isolated in a community treatment center in South Korea [20]. Notably, the Korean cohort consisted mainly of young healthy patients (median age: 25 years; 3.9% with comorbidities) [20]. In this latter study, viral load of symptomatic patients tender to decrease more slowly than those found in the asymptomatic cases (p-value=0.04) [20]. The fact that symptomatic patients with COVID-19 tend to have higher URT viral load is of relevance for infection control purposes, given the fact that symptomatic patients may cough or otherwise expel infectious secretions and spread the virus. However, it is highly unknown whether asymptomatic SARS-CoV-2 infections have the same course of URT viral load as symptomatic cases. If they do, then, without a known onset date of asymptomatic cases, the chances are that their viral load would be lower, since more of the shedding period is spent with a low viral load than with a high.

In our study a high SARS-CoV-2 URT viral load was significantly associated with an increased risk for intubation or a fatal outcome in the course of COVID-19, as well as with prolonged disease severity, as indicated by prolonged duration of intubation and length of stay in ICU in univariate analyses. Similarly, two recent studies also found that patients with a high SARS-CoV-2 URT viral load on admission had significantly increased mortality rates [13,21]. In addition, patients with high URT viral load more often were admitted to ICU compared to patients with moderate or low URT viral load, but this was marginally significant in the univariate analysis. Similarly, a study of 76 hospitalized patients with COVID-19 in China, of whom 23 (77%) were admitted to ICU, found significantly lower Ct values in nasopharyngeal swab samples in severe cases at admission than in mild cases [6]. In this

latter study, the Ct values of severe cases remained significantly lower for the first 12 days after the onset of symptoms than those of mild cases; in addition, mild cases had an early viral clearance by day 10, while severe cases still tested positive at or beyond day 10 [6]. Furthermore, SARS-CoV-2 viability was associated with lower PCR Ct value in early illness in a prospective cohort of 100 COVID-19 patients, while no virus was isolated when the PCR Ct value was >30 or >14 days after symptoms onset [22]. Similar to our findings, a study of 678 hospitalized patients with COVID-19 in New York found that patients with high viral load in nasopharyngeal swab samples were more like to get intubated compared to patients with moderate or low viral load (29.1%, 20.8%, and 14.9%, respectively; p-value <0.001) or to die in hospital (35%, 17.6%, and 6.2%, respectively; p-value<0.001) [5]. In this latter study, multivariate analysis showed that a higher SARS-CoV-2 viral load was independently associated with increased risk for intubation (adjusted OR: 2.73, 95% CIs: 1.68-4.44; p-value <0.001) and death (adjusted OR: 6.05, 95% CIs: 2.92-12.52; p-value <0.001) [5]. Notably, in the New York study, gender was not considered in the multivariate analysis, despite the fact that male gender has been consistently found as an independent factor for severe morbidity and fatal outcome in many studies globally [2,23-25], including ours. Nonetheless, in our study the significant association between a high URT viral load, clinical severity and outcome did not emerge in the multivariate models. This may be partially attributed to the strong association between age, gender and comorbidities on the one hand and disease severity and risk of a fatal outcome on the other, which in turn may ameliorate the impact of a high URT viral load.

Contradictory results have been reported in a study of adult patients with COVID-19 presenting in an emergency department in a New York [7]. In particular, significantly higher SARS-CoV-2 viral load was detected in 165 patients discharged from the emergency department, while viral load was significantly lower in 40 patients admitted to hospital, after adjusting for age, gender, race, body mass index, and comorbidities (log10 viral load: 4.0 versus 3.3; p-value=0.014) [7]. In addition, a high viral load was inversely correlated with COVID-19 severity across the cohort and in the subgroup of

hospitalized patients, even after adjusting for several patient characteristics (p-value=0.045) [7]. We should take under consideration that in this latter study, non-hospitalized patients with COVID-19 were diagnosed significantly earlier compared to hospitalized patients (3 versus 5 days after onset of symptoms; p-value=0.017) [7]. Similarly, in our study URT viral load was inversely correlated with the time that elapsed from the onset of symptoms to sampling, as reported by others [7,8,25]. This finding is attributed to SARS-CoV-2 shedding kinetics in the upper respiratory tract and the natural history of infection, indicating a viral load peak shortly after onset of symptoms [26]. Lastly, in a study of 4,172 samples tested positive for SARS-CoV-2 in a university hospital in Switzerland, the authors found no association between viral load, age and clinical management as indicated by unit of sample collection (ICU, internal medicine department, emergency department, screening unit) [27].

Limitation of the current study is the fact that the clinical samples were collected from different upper respiratory tract sites and from different days after onset of symptoms from each patient. Another limitation is that timing from symptom onset to hospitalization was not considered. Repeated sampling was not available, therefore we could not study kinetics of SARS-CoV-2 and the temporal association of URT viral load with clinical course. The fact that Ct values concern viral nucleic acid and do not necessarily correspond to infectious virus should be considered. A clear strength is the large number of consecutively diagnosed patients with SARS-CoV-2 infection retrieved from the national surveillance database, which gave us the opportunity to study both asymptomatic and symptomatic patients managed either in-hospital or as outpatients, but also to study the association between URT viral load and specific comorbidities. In addition, information about clinical course was collected prospectively and the clinical course and outcome of patients were available in all cases.

In conclusion, the current study provides insight into the association between URT viral load, host characteristics, clinical severity and outcome in patients with SARS-CoV-2 infection. In our population, higher URT viral load has been detected in symptomatic patients and could be used as a marker of infectivity for infection control purposes. Higher URT viral load was also found in patients with specific comorbidities. Our findings could be used to identify those patients at higher risk for severe morbidity or a fatal outcome and therefore to guide therapeutic interventions. Further studies are needed to explore the underlying pathogenetic mechanisms of disease severity and fatal outcome at the host level, including the association between high URT viral load and comorbidities.

Acknowledgement

We thank all healthcare and laboratory personnel for their assistance in data collection. We also thank Anastasia Tentoma for technical assistance. The opinions are those of the authors and do not necessarily represent those of their institutions. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The Reference Laboratories were financially supported by the National Public Health Organization (Greece).

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Table 1. Characteristics of patients with SARS-CoV-2 infection by URT viral load

Characteristic	URT viral load status			
	High	Moderate	Low	p-value
	n = 309 (%)	n=316 (%)	n=497 (%)	
Mean age±SD (years) (n=1,082)	50±22	48±21	43 ±21	0.001
Age group (years)				
<18 (n=82)	24 (7.9)	22 (7.2)	36 (7.6)	0.107
18-64 (n=767)	202 (66.9)	224 (73.2)	356 (75.1)	
≥65 (n=233)	76 (25.2)	60 (19.6)	82 (17.3)	
Male gender (n=1,122)	156 (50.5)	189 (59.8)	274 (55.1)	0.064
Comorbidities (n=1,122)	124 (40.1)	104 (32.9)	108 (32.1)	<0.001
Mean number of comorbidities±SD (n=1,222)	0.62 + 0.89	0.47 + 0.79	0.32 + 0.67	<0.001

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; URT: upper respiratory tract; SD: standard deviation

Table 2. URT viral loads of 487 patients with SARS-CoV-2 infection by comorbidity

Comorbidity	URT viral load status			
	Low (n=154)	Moderate (n=144)	High (n=189)	p-value
Chronic cardiovascular disease (n=146)	49 (33.6%)	46 (31.5%)	51 (34.9%)	0.019
Hypertension (n=91)	25 (27.5%)	29 (31.9%)	37 (40.6%)	0.002
Diabetes mellitus (n=67)	21 (31.4%)	23 (34.3%)	23 (34.3%)	0.096
Chronic pulmonary disease (n=70)	20 (28.6%)	25 (35.7%)	25 (35.7%)	0.026
Malignancy (n=33)	12 (36.4%)	8 (24.2%)	13 (39.4%)	0.319
Chronic renal disease (n=20)	9 (45.0%)	5 (25.0%)	6 (30.0%)	0.946
Immunosupression (n=24)	6 (25.0%)	3 (12.5%)	15 (62.5%)	0.001
Obesity (n=20)	5 (25.0%)	3 (15.0%)	12 (60.0%)	0.005
Chronic neurological disease (n=11)	3 (27.3%)	1 (9.1%)	7 (63.6%)	0.026
Chronic hepatic disease (n=5)	4 (80.0%)	1 (20.0%)	0 (0.0%)	0.225

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; URT: upper respiratory tract; n: number of cases

Table 3. SARS-CoV-2-associated morbidity and outcome by URT viral load

	URT viral load status			
Morbidity	**High (n=309)**	**Moderate (n=316)**	**Low (n=497)**	**p-value**
Asymptomatic infection (n=274)	42 (13.6%)	71 (22.5%)	161 (32.4%)	<0.001
COVID-19 (n=848)	267 (86.4%)	245 (77.5%)	336 (67.6%)	<0.001
Hospitalization (n=518)	153 (49.5%)	155 (49.1%)	210 (42.3%)	0.064
Complications (n=231)	88 (28.5%)	69 (21.8%)	74 (14.9%)	0.084
Admission to ICU (n=99)	37 (12.0%)	27 (8.5%)	35 (7.0%)	0.055
Mean ICU LOS ± SD (days)	6.76 ± 12.99	5.13 ± 13.64	3.21 ± 8.30	0.011*
Intubation (n=93)	35 (11.3%)	26 (8.2%)	32 (6.40%)	0.050
Mean intubation duration ± SD (days)	7.53 ± 13.23	5.79 ± 12.54	3.29 ± 8.24	0.006*
Death (n=89)	35 (11.3%)	23 (7.3%)	31 (6.2%)	0.030

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; URT: upper respiratory tract; COVID-19: coronavirus disease 2019; ICU: intensive care unit; LOS: length of stay; SD: standard deviation

*p-value only for the comparison between low and high viral load

Table 4. Multivariate analyses for factors associated with morbidity and death in patients with SARS-CoV-2 infection in Greece

Outcome	Significant factors	OR	95% CIs	p-value
COVID-19	● older age	1.04	(1.03 – 1.06)	<0.001
	● at least one comorbidity	24.45	(1.65 – 36.36)	0.020
	● high URT viral load	2.40	(1.54 – 3.76)	<0.001
Hospitalization	● older age	1.04	(1.03 – 1.05)	<0.001
	● female gender	0.71	(0.53-0.95)	0.025
	● at least one comorbidity	4.79	(2.38 – 9.63)	<0.001
Admission to ICU	● female gender	0.36	(0.22-0.60)	<0.001
Intubation	● older age	1.02	(1.00 – 1.03)	<0.020
	● female gender	0.32	(0.18 - 0.55)	<0.001

Death	● older age	1.05	(1.03 – 1.07)	<0.001
	● female gender	0.48	(0.27 - 0.85)	0.012
	● number of comorbidities	1.93	(1.31 - 2.83)	<0.001
Severe course*	● older age	1.03	(1.02 - 1.05)	<0.001
	● number of comorbidities	1.67	(1.15 – 2.42)	0.006
	● female gender	0.41	(0.25 – 0.65)	<0.001

*admission to ICU, intubation and/or death

SARS-CoV-2: severe acute respiratory syndrome 2 virus; COVID-19: coronavirus disease 2019;

URT: upper respiratory tract; ICU: intensive care unit; OR: odds ratio; CIs: confidence intervals