Journal of Clinical Virology Plus 3 (2023) 100166

Contents lists available at ScienceDirect

Journal of Clinical Virology Plus

journal homepage: www.elsevier.com/locate/jcvp

Viral load in symptomatic and asymptomatic patients infected with SARS-CoV-2. What have we learned?

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ARTICLE INFO

Keywords:
SARS-CoV-2
COVID19 waves
Viral loads

ABSTRACT

Asymptomatic and presymptomatic patients played a critical role in the maintenance and spread of infection during COVID pandemic. However, conflicting views about the infectiousness of asymptomatic patients have been raised.

Identification of asymptomatic cases relies on SARS-CoV-2 genome detection and, in the absence of common epidemiological variables, quantification of viral load (VL) has been proposed as an estimator for SARS-CoV-2 transmission.

Comparison of VLs from symptomatic and asymptomatic patients displayed variable results according to the studied population, the experimental design and the sampling, among other variables.

The aim of this work was to determine VLs in symptomatic and asymptomatic patients at the time of sampling and to retrospectively determine their relationship with severity of disease and other parameters that affected the course of COVID-19, in two towns located in Buenos Aires, Argentina.

Results from our study showed that VLs from symptomatic and asymptomatic patients were significantly different when analyzed globally. In addition, significant differences were found when VLs from each COVID-19 wave were analyzed. In the first wave VLs from asymptomatic patients (log10 8,21 gc/µl) were significantly higher than in symptomatic ones (log10 6,51 gc/µl) while; in the second wave, VLs from asymptomatic patients resulted significantly lower than in symptomatic patients (log10 4,51 gc/µl and log10 5,23 gc/µl, respectively). In the third wave, no significant differences were observed between VLs from both types of patients.

Results from this work demonstrated that the screening of both symptomatic and asymptomatic patients was of utmost importance in order to reduce SARS-CoV-2 transmission to communities.

1. Introduction

In late December 2019, an outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was reported in Wuhan, China, and on March 2020, the World Health Organization declared COVID-19 as a global pandemic [1].

The clinical characteristics of COVID-19 ranges from asymptomatic - mild respiratory infections to pneumonia and even to acute respiratory distress syndrome [2].

Asymptomatic infections refer to the positive detection of SARS-CoV-2 genome in samples from patients that did not develop typical clinical symptoms or signs. This category includes infected people who have not yet developed symptoms but go on to develop symptoms later (pre-symptomatic infections), and those who are infected but will never present any symptoms (true asymptomatic or covert infections) [3,4]. Due to the absence of symptoms, these patients do not usually seek medical care and cannot be diagnosed as infected. Thus, identification of asymptomatic infections require extensive testing and close contact tracing.

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https://doi.org/10.1016/j.jcvp.2023.100166
Received 26 July 2023; Received in revised form 26 September 2023; Accepted 4 October 2023
Available online 5 October 2023

Asymptomatic patients (ASP) during COVID pandemic have played a pivotal role in the maintenance and spread of infection [5–8]. In a meta-analysis conducted to explore the global percentage of asymptomatic infections among confirmed COVID-19 populations, it was found that 40.50 % were asymptomatic [9]. Moreover, in an analytical model in which multiple scenarios of proportions of asymptomatic individuals with COVID-19 and infectious periods were evaluated, transmission from asymptomatic individuals was estimated to account for more than half of all transmissions [10].

However, since the beginning of the pandemic, there are controversial views about the infectiousness of asymptomatic patients. It has been reported that the period of positive nucleic acid tests (the interval from the first day of positive nucleic acid tests to the first day of continuous negative tests) could be up to 3 weeks (ranging from 1 to 24 days) in ASP [11,12]. In addition, asymptomatic infections seem to have the same infectivity as symptomatic infections [8,13–15] and laboratory and epidemiological evidence suggests that individuals who never develop symptoms do represent a source of potentially transmissible SARS-CoV-2 [16–18].

Identification of asymptomatic cases relies on SARS-CoV-2 genome detection. Since common epidemiological variables such as incubation period or symptoms onset are absent, many studies have focused on establishing if the quantification of SARS-CoV-2 abundance was related to disease transmission.

When analyzing Ct values and the presence of infectious virus, a similar pattern was observed in samples from asymptomatic and pre-symptomatic persons, compared with those who were symptomatic [15], reinforcing the role of ASP on virus transmission.

High viral loads (VL) in the upper respiratory tract were associated with infectiousness and might have contributed to secondary transmission of COVID-19; similarly, VLs in plasma were found to be associated with systemic inflammation, disease progression, and increased risk of death [19]. VLs may also provide a better understanding of why transmission is observed in some instances, but not in others.

Comparison of VLs from symptomatic patients (SP) and ASP displayed variable results. Many reports showed that the upper respiratory VLs in ASP were comparable to those in SPs (ranging from 1×10^4 to 1×10^7 copies per milliliter) [1,16,19–24]. In other studies, opposite results were found. Some authors [25] demonstrated that VLs were higher in ASPs than in symptomatic ones. On the other hand, it was also reported that patients with severe disease had significantly higher VLs than either patients with mild disease [26] or APs [27–29]; however, certain period of viral shedding was still evident in the later.

Although ASPs were thought to be less contagious, they have widely contributed to maintenance of infection, particularly because these patients were not always tested or isolated and pose a significant challenge to infection control.

The aim of this work was to determine VLs in SP and ASP and to retrospectively study their relationship with severity of disease and other parameters that affected the course of COVID-19 in the west of the Metropolitan Area of Buenos Aires, Argentina.

2. Materials and methods

2.1. Study design

A retrospective study was conducted for a cohort of 326 laboratory confirmed COVID-19 cases from Hurlingham and Ituzaingó districts of the Buenos Aires province, Argentina, between August 2020 and December 2021. Nasopharyngeal swab (NP) samples were analyzed at the COVID Unit from the Diagnostic Laboratory, National University of Hurlingham. Since the COVID Unit began its activities in August 2020, a dataset containing information from the epidemiological charts was built for research purposes. Personal information, such as name, ID and address were excluded and samples were coded in order to generate an anonymous dataset.

Samples belonging to symptomatic (severe clinical signs) and asymptomatic (at the time of sampling) patients were included in the study. For comparisons, selected samples were categorized according to the presence /absence of clinical signs and according to the first / second /third wave of COVID-19.

Asymptomatic case was defined as the ones with no symptoms or signs of infection with SARS-CoV-2 at the time of sampling. The severity of the disease was assessed according to the World Health Organization (https://www.who.int/westernpacific/emergencies/covid-19/information/asymptomatic-covid-19). Severe cases include bilateral pneumonia, severe acute respiratory syndrome, and requirement of mechanical respiratory assistant and/or hospitalization.

Demographic features such as age, biological sex, place of residence, clinical signs, date of symptom onset, comorbidities and vaccination were recorded.

2.2. Detection of SARS-CoV-2

The NP swabs were collected at the sanitary units by using flocked swabs in liquid-based collection and transport systems. Samples were remitted to the COVID Unit from 3 public hospitals and a sanitary unit located in Hurlingham and Ituzaingó districts.

For routine diagnosis, viral RNA was extracted from 200 ul of sample using Quick-RNA Viral Kit (ZYMO RESEARCH) or Viral Nucleic Acid Extraction Kit II (GENAID) following the manufacturer's instructions. A commercial one-step reverse transcription real-time polymerase chain reaction (DisCoVery SARS-CoV2 RT-PCR Detection kit ROX, AP BIOTECH) was performed to confirm the presence of SARS-CoV-2 by amplification of RdRP and N genes from extracted RNA, according to the manufacturer's instructions. RT-qPCR assays were performed on the CFX96 Touch Real-Time PCR Detection System (BIORAD) instrument. RNA was stored at −80 °C.

2.3. SARS-CoV-2 viral load quantification

Levels of SARS-CoV-2 viral load (VL) were quantified using the set of primers and probe for SARS-CoV-2 E gene described by [30] (E_Sarbeco_F; E_Sarbeco_R; E_Sarbeco_P1). Each reaction contained 5 μl of RNA, 12,5 μl of qScript® XLT One-Step RT-qPCR ToughMix (Quantabio), 400 nM of each primer and 200 nM of probe.

For quantification purposes, the E gene fragment amplified using E_Sarbeco_F and E_Sarbeco_R primers was inserted in a pGEM-T easy vector (Promega). The standard curve was performed with 10-fold dilutions of that plasmid (10^6 to 10^1 genomic copies/μl). The assay was run in triplicate for each sample and each point of the standard curve; qPCR efficiencies ranged from 90 % to 100,2 %. Viral load (VL) was expressed as genomic copies/μl of sample. RNA from positive and negative human samples were included as controls of the procedure.

2.4. Statistical analysis

Before analysis, viral loads were log10 transformed for normalization. For most variables, descriptive statistics were calculated: categorical variables were expressed as counts and frequencies, and continuous variables were expressed as means and SDs.

Bivariate models were first used. Linear-regression analyses were adjusted on explanatory variables that were individually analyzed with VLs (log 10 of genomic copies/μl); the variables considered were: existence of symptoms (AP/SP), age (divided into three categories: under 40, between 40 and 66 and over 66 years old), biological sex (male/female), vaccination (yes/ no) and comorbidity (patients with at least one comorbidity and without comorbidities). Those having a P-value $\leq$ 0.15 were selected for multivariable analysis.

A multivariate analysis using a general linear mixed model was performed to evaluate the effect of the selected explanatory variables (existence of symptoms, sex and comorbidity) on the outcome of the

quantitative variable VLs.

Logistic regression for binary variable AP and SP and viral loads was fitted to assess the association between VLs and symptoms using a generalized linear mixed model (GLMM).

All statistical analyses were carried out using InfoStat software (Universidad Nacional de Córdoba, Argentina).

3. Results

3.1. Study design and samples

The districts of Hurlingham and Ituzaingó are located in the northwest and west of the Metropolitan Area of Buenos Aires (AMBA), respectively. According to the latest land registry in 2010 (www.indec.gob.ar), Hurlingham has a population 181,241 individuals over an area of 37,8 square kilometers, while Ituzaingó́s population is 168,419 and has an area of 38,51 square kilometers.

Since the beginning of the pandemic, the COVID-19 Unit of the UNAHUR reported 5942 confirmed COVID-19 cases from Hurlingham and 6115 from Ituzaingó (out of 38,622 and 38,144 total cases from Hurlingham and Ituzaingó, respectively).

For this study, a total of 326 SARS-CoV-2 positive samples were selected. The inclusion criteria were based on having severe clinical signs or being asymptomatic at the time of sampling. Demographic and clinical characteristics are summarized in Table 1.

Samples included in this study belonged to the first (39,26 %), second (56,44 %) and third (4,29 %) wave of the pandemic. In Buenos Aires, Argentina, the first wave of COVID19 occurred between February and December 2020 (with an outbreak during the following summer); the second, between February and October 2021 and the third from December 2021 to April 2022 (https://www.argentina.gob.ar/salud/coronavirus-COVID-19/sala-situacion).

Globally, 112 (34,36 %) patients were asymptomatic at the time of sampling, while 214 (65,64 %) showed severe clinical signs.

Patients´ age ranged between 16 and 96 years (except for only one patient that was 14 month old), with a median of 58,39 years. They were divided into three categories: under 40, between 40 and 66 and over 66 years old, that represented 22,63 % ($n = 74$), 34,25 % ($n = 112$) and 43,12 % ($n = 141$), respectively.

In symptomatic patients (SP), median time from the onset of symptoms to sampling was 4,8 (0–34) days. On the other hand, in asymptomatic patients (AS), time from contact to sampling was uncertain or unreliable since most patients had multiple contacts and it was not possible to assess which one transmitted the infection.

Regarding the symptoms reported, the most common clinical signs were fever, digestive alterations, anosmia and dysgeusia. Most of the SP were hospitalized during the second wave (Table 2).

From the total, 152 patients reported to have comorbidities; among them, hypertension was the most frequent ($n = 79$), followed by diabetes ($n = 36$) and asthma ($n = 5$). Almost half of the cases with comorbidities presented more than one preexisting condition (Table 1).

None of the patients from the first wave were vaccinated, but vaccination was evident in the second wave when 15,2 % and 15,8 % of the patients received one and two doses, respectively (Table 1).

Table 1
Demographic and clinical characteristics of the patients at the time of sampling.

Age (years)	
< 40 (n, %)	73, 22,39 %
40–66 (n, %)	112, 34,36 %
>66 (n, %)	141, 43,25 %
Sex	
female (n, %)	130, 39,88 %
male (n, %)	193, 59,20 %
undefined (n, %)	3, 0,92 %
Commorbidities	
None (n, %)	(149, 45,71 %)
One preexisting condition (n, %)	(78, 23,93 %)
More than one preexisting condition (n, %)	(74, 22,7 %)
undefined (n, %)	(25, 7,67 %)
Hypertension (n)	79
Astma (n)	5
Diabetes (n)	36
Days between self-reported symptoms onset and sampling	
(median, min-max)	4,8 (0–34)
Symptoms	
Severe - 1st wave (n, %)	(73, 22,39 %)
Severe - 2nd wave (n, %)	(138, 42,33 %)
Severe - 3rd wave (n, %)	(3, 0,92 %)
Asymptomatic - 1st wave (n, %)	(55, 16,87 %)
Asymptomatic - 2nd wave (n, %)	(46, 14,11 %)
Asymptomatic - 3rd wave (n, %)	(11, 3,37 %)
Vaccination	
1st wave - none (n, %)	(128, 39,26 %)
1st wave - 1 dose (n, %)	(0, 0 %)
1st wave - 2 doses (n, %)	(0, 0 %)
2nd wave - none (n, %)	(127, 38,96 %)
2nd wave - 1 dose (n, %)	(28, 8,59 %)
2nd wave - 2 doses (n, %)	(29, 8,90 %)
3rd wave - none (n, %)	(7, 2,15 %)
3rd wave - 1 dose (n, %)	(0, 0 %)
3rd wave - 2 doses (n, %)	(7, 2,15 %)

3.2. Viral loads of SARS-CoV-2

VLs of the selected samples were assessed by quantifying SARS-CoV-2 E gene. VLs in the SP group ranged from 1,23 to 12,96 log10 copies per µl of sample, with a mean of 5,66 log10 copies per µl of sample. AP grouṕs VLs ranged from 1,01 to 13,17, with a mean of 6,52 log10 copies per µl sample, which were significantly different from the VLs found in SP group ($p = 0{,}0054$).

In addition, when VL from SP and AP were analyzed in each COVID wave, opposite results were found. VLs from the AP group belonging to the first wave were significantly higher than in the SP ($p = 0{,}0030$), while in the second wave, the AP group showed lower VLs than the SP ($p = 0{,}0493$). VLs analyzed during the third wave were not significantly different between AP and SP groups ($p = 0{,}1738$) (Fig. 1, Table 3).

The possible influence of age, biological sex, vaccination and comorbidity variables on VLs were also evaluated. Results showed that VLs from the three age categories (under 40, between 40 and 66 and over 66 years old), were not significantly different ($p = 0{,}3773$). However, VLs from female sex, with a mean of 6,57 log10 copies per µl sample, were significantly higher than VLs from male sex which showed a mean of 5,59 log10 copies per µl sample ($p = 0{,}0012$). Regarding vaccination, VLs were not significantly different between vaccinated and not vaccinated patients ($p = 0{,}1117$). On the other hand, VLs from patients with at least one comorbidity (VL mean= 5,86 log10 copies per µl sample) were significantly lower than patients with no comorbidities (VL mean= 6,7 log10 copies per µl sample) ($p = 0{,}0143$) (Table 4).

To investigate the relation between viral loads, existence of symptoms, sex and comorbidity a multivariate analysis was performed. The multivariable model revealed that only the existence of symptoms was associated to VLs ($p < 0{,}0001$)

Logistic regression analysis between existence of symptoms and VLs for each wave was also conducted. A significant inverse correlation between VLs and presence of symptoms (OR, 0,835; 95 % CI, 0,452–0,678) was observed in samples that belonged to the first wave. However, a significant correlation between higher VLs and the presence of symptoms (OR, 1209; 95 % CI, 0,655–0,825) was found in samples from the second wave (Fig. 2). VLs of samples from the third wave did not show any correlation with the presence of symptoms.

4. Limitations

Several limitations need to be considered. This study was a cohort study carried out in two districts of AMBA, thus generalization of the results obtained in this work to other patient population, should be

Table 2
Number of patients with specific symptoms.

	Fever	Digestive alterations	Dysgeusia	Anosmia	Pneumonia	Hospitalization	Respiratory distress
1st wave	14	3	1	3	7	22	17
2nd wave	81	14	2	4	26	105	38
3rd wave	4	2	3	3	0	1	1
TOTAL	99	19	6	10	33	128	56

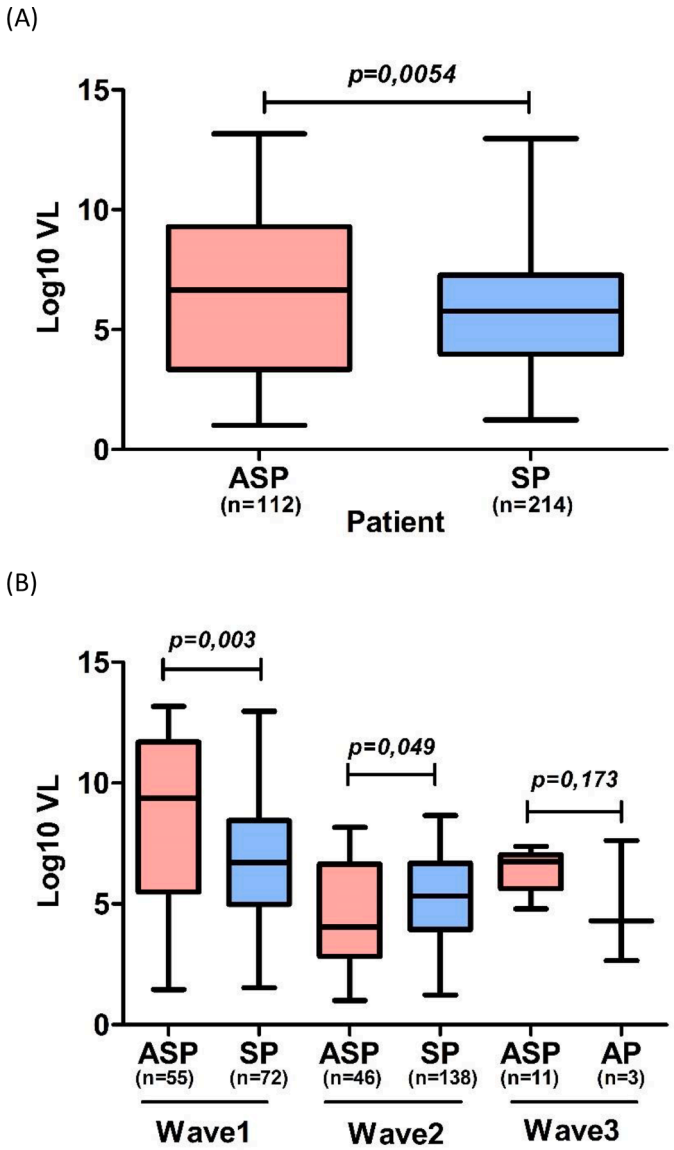

Fig. 1. Viral loads in AP and SP during the COVID-19 waves
SARS-CoV-2 VLs at the time of sampling were plotted by sample types and waves. (A) Overall comparison; (B) comparison of VLs in each COVID-19 wave. Medians are indicated by midlines and whiskers indicate the upper and lower VL values. The number of samples in each group as well as *p* values for comparisons between groups (median SARS-CoV-2 VLs) are shown. VLs are expressed as gc/µl of sample.

carefully analyzed.

Data regarding symptoms and onset included in the study was limited to what was reported in the epidemiological charts and errors in patient recall and/or in completing the charts at the health center that may have happened.

Management of patients changed over the course of the pandemic including the criteria for testing, so it cannot be excluded that this

Table 3
Viral loads in samples from SP and AP patients.

Viral loads	n	Mean	Min	Max
SP - 1st wave (median, min-max)	73	6,51	1,53	12,96
SP - 2nd wave (median, min-max)	138	5,23	1,23	8,66
SP - 3rd wave (median, min-max)	3	4,86	2,66	7,62
SP-total	**214**	**5,66**	**1,23**	**12,96**
ASP - 1st wave (median, min-max)	55	8,21	1,46	13,17
ASP - 2nd wave (median, min-max)	46	4,51	1,01	8,17
ASP - 3rd wave (median, min-max)	11	6,35	4,8	7,38
ASP-total	**112**	**6,52**	**1,01**	**13,17**

constant change of treatment practices may have influenced patient outcomes.

RT-qPCR detects viral RNA but may not reflect the replication level of the virus since viable and non-viable virus cannot be distinguish with the RT-qPCR used in this study.

Differences in the timing of sampling across SP and AP may have masked true differences in SARS-CoV-2 VLs between both groups. While the kinetics of SARS-CoV-2 RNA load in the upper respiratory tract has been clearly established in symptomatic individuals, with viral load peaking around the time of symptom onset, it remains to be precisely characterized in asymptomatic subjects.

Cases were categorized as asymptomatic at the time of sampling, but it is uncertain if they developed symptoms afterwards because patients were not followed up.

5. Discussion

One of the major challenges in controlling the COVID-19 outbreak was its asymptomatic transmission and there is still ongoing debate about the role of asymptomatic and presymptomatic patients on transmission of SARS-CoV-2. The real impact of these patients is difficult to assess due to different sources of bias that can result in over or underestimation of the true proportion of asymptomatic infections, even when an adequately followed up is conducted [31]. A well-recognized source of overestimation arises when people without symptoms at the time of testing are classified as asymptomatic. It is reported by other authors who assessed people at just 1 time point, that the percentages of patients without clinical signs can be 80 % or more; however, an uncertain proportion of them will develop symptoms. On the other hand, people with symptoms are more likely to be included in a study population, underestimating the proportion of AP [32].

The aim of this study was to establish the role of VLs in SP and AP and to determine if there was a correlation among VL, severity of disease, and other parameters in patients from two towns located in Buenos Aires, Argentina.

Most patients were tested only once, and symptoms were self-reported and often confirmed by health professionals. Overestimation of the percentage of asymptomatic cases was likely to occur since a few number of patients (e.g. those that were hospitalized or whose symptoms worsened) were followed up. This has also been described in other studies where asymptomatic cases have shown to represent around 40 % of all SARS-CoV-2 infections with ancestral SARS-CoV-2 [9,22,33], with 23 % of them classified as true asymptomatic [34]. These asymptomatic infections together with presymptomatic ones substantially drive community transmission, contributing 50 % or more of the total force of

Table 4
VLs in samples form patients regarding age, sex, comorbidities and vaccination.

		Global			1st Wave			2nd Wave			3rd Wave		
Variable	Level	n	Mean	p value	n	Mean	p value	n	Mean	p value	n	Mean	p value
Symptoms	Severe	213	5,66	0,0054	72	6,51	0,0030	138	5,23	0,0493	3	4,86	0,1738
	Asymptomatic	112	6,51		55	8,21		46	4,51		11	6,35	
Age	<40	76	6,14	0,3773	32	8,08	0,1071	39	4,57	0,2307	5	6,00	0,0027
	41–66	109	6,12		40	7,44		62	5,20		7	6,77	
	>66	140	5,71		55	6,62		83	5,16		2	3,48	
Sex	Female	129	6,57	0,0012	68	7,72	0,0696	55	5,24	0,4959	6	5,75	0,5474
	Male	193	5,59		59	6,70		126	5,03		8	6,23	
Commorbidities	No	112	6,70	0,0143	64	7,85	0,0285	41	4,98	0,3370	7	6,16	0,6151
	Yes	156	5,86		63	6,62		87	5,32		6	5,73	
Vaccination	No	179	6,40	0,1117	127	7,24	NA	51	4,26	0,1515	1	7,38	0,4113
	Yes	28	5,40		0	NA		21	5,07		7	6,39	

infection [35].

Although SARS-CoV-2 transmission is a multifactorial process, viral load substantially contribute to human-to-human transmission, with higher VL posing a greater risk for onward transmission. In addition, individuals infected with SARS-CoV-2 can be infectious before the onset of symptoms, and it was estimated that about half of secondary transmissions took place in the pre-symptomatic phase [23].

The relevance of VLs as an estimator for transmission is limited because the infectious dose of SARSCoV-2 required to lead to a secondary transmission is still unknown and the association between presence of infectious virus in the respiratory tract and infectiousness of the same individuals is poorly understood.

Despite of that, VL has been proposed as an estimator for transmission. However, there are contradictory findings regarding viral shedding in SP and AP and comparison of VL between them remains challenging.

Results from our study showed that VLs from AP and SP were significantly different when analyzed globally. However, when VLs from each COVID-19 wave were analyzed, we observed that in the first wave VLs from AP (log10 8,21 gc/µl) were significantly higher than in SP (log10 6,51 gc/µl), but the contrary occurred in the second wave, since VLs from AP resulted significantly lower than in SP (log10 4,51 gc/µl and log10 5,23 gc/µl, respectively). In the third wave, no significant differences were observed between VLs from AP and SP. Nevertheless, the number of cases should be increased to come to a reliable conclusion. Our findings are different from those described in a study on ancestral SARS-CoV-2, in which symptoms and molecular testing results from COVID-19 confirmed and hospitalized cases were recorded daily. In that report the authors found similar initial Ct values between asymptomatic and symptomatic individuals [36]. Likewise, in other studies in which patients were followed longitudinally, no significant difference in RNA viral loads between SP and AP [16,20,22,24] was found. Conversely, other studies, in which patients were also followed up by clinicians, reported lower RNA viral loads in asymptomatic participants [28,29,37] similar to results obtained in this study during the second wave.

Notably, the number of SP samples available for this study was greater in the second wave, indicating a larger number of symptomatic people being tested. The shift in tested patients between the first and second waves was influenced by the national testing policy that gradually increased the number of tested patients to all people with COVID-19-related symptoms and their close contacts. In addition, COVID-19 was first identified as a viral pneumonia, however, the spectrum of symptoms shifted to include gastrointestinal symptoms and disturbances of smell and taste, thus increasing the number of patients for diagnosis.

In agreement with our results, different studies reported differing results regarding VLs and the presence of symptoms. Some showed no significant difference in VLs between ASP and SP [16,20,24]. Alike, Costa et al. (2021) found that children with COVID-19 symptoms displayed SARS-CoV-2 VL comparable to those of their asymptomatic counterparts, while in adults, median SARS-CoV-2 VL was significantly higher in SP than in ASP [27]. In other reports higher RNA loads were registered in patients with symptoms [29], meanwhile some studies showed the opposite [25,38]. In an analysis performed with samples tested between April and May 2020, non-linear regression models showed that the estimated VL at onset was higher in the index (patients who transmitted COVID-19 to at least one person) than in the non-index patients (did not caused secondary transmission). In adult people, VLs were log10 3,3 gc/µl and log10 1,8 gc/µl for index and non index patients, respectively [29].

In order to understand the discrepancies among studies, sample type should be considered, as it may affect VL values. In this regard, Winnet et al. (2023) showed that viral loads in different specimen types from the same person at the same time point exhibited extreme differences, up to 10^9 copies/mL. The authors concluded that a combined throat–nasal swab processed with highly sensitive assays had significantly better clinical sensitivity to detect presumed pre-infectious and infectious individuals [39]. In addition, higher RNA VL was reported from nasopharyngeal than oropharyngeal swabs [1,40,41], thus, this type of sample display the highest diagnostic accuracy compared with other upper respiratory tract samples [42]. More important, SARS-CoV-2 was more successfully isolated from nasopharyngeal swabs than from saliva, nasal or sublingual swabs [40]. In concordance with these findings, in our study, 61.35 % of samples were from combined throat-nasal swabs and the rest were nasopharyngeal ones.

It is important to mention that RT-qPCR used to diagnose SARS-CoV-2 cannot directly determine infectiousness and several studies have attempted to correlate the quantity of viral RNA with infectiousness by isolating virus from samples with different VLs. The probability of isolating infectious virus decrease as VL values do, during the first 8 days post-onset of symptoms [43,44]. Nonetheless, other studies reported a low correlation between infectious virus and RNA VL, disfavoring quantification as a predictor of infectious virus presence [45–47]. In this study, we did not address that issue, but we were able to isolate SARS-CoV-2 from samples with a VL of at least 10^5 gc/µl (data not shown).

Regarding other parameters such as age, sex and comorbidities, we found a significant association between high VL and either being female or not having comorbidities. On the other hand, age had no influence on the VLs recorded, however, it is important to mention that samples included patients who were 16 years old or even older and only one pediatric sample was assessed. Similar results concerning the variable age were reported [27,38,48] by other authors.

Unlike our results, some studies reported that younger patients were more likely to be asymptomatic than older patients [24]. Authors also showed that older age and male gender were associated with severe disease, higher VL and longer viral shedding [25,26]. However, other analyses demonstrated that the viral load increased with age [49] and was higher in females [50]. Contrary, other studies reported that no discernible differences regarding VL were apparent between adults and pediatric patients [27,38,48].

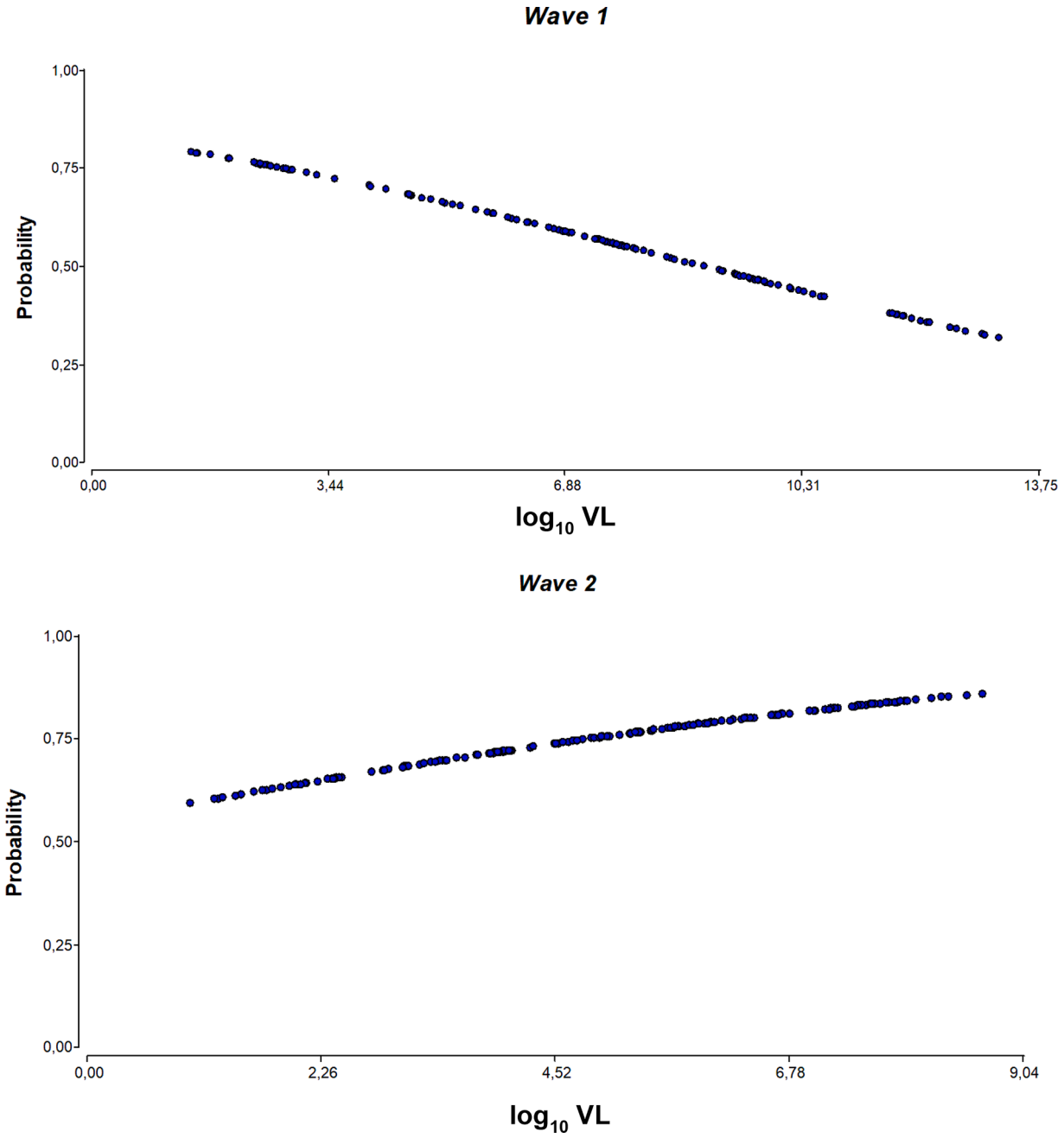

Fig. 2. Relationship between the estimated probability of symptoms development and VLs.
Logistic regression for binary variable ASP/SP and VLs was fitted using a generalized linear mixed model to assess the association between VLs and symptoms for the 1st and 2nd waves of COVID-19.

A high heterogeneity can also been observed concerning the influence of comorbidities and the presence of symptoms and VLs. The variability in sampling, target population, experimental design and even viral variants make difficult to reach conclusions that can be extrapolated. Moreover, emerging SARS-CoV-2 variants of concern have further complicated the picture of virus shedding and transmission. In that context, variants have been associated with larger viral loads [51–55], which may contribute with an increased transmission.

As in other viral diseases, the use of certain parameters that can help in early detection and prevention of spread, especially during outbreaks like COVID-19, could help to contain disease transmission. In this regard, our results showed that the high percentage of asymptomatic infections observed during the first and second waves of COVID-19 highlights the risk of transmission in communities. Since VLs in ASP can be as high as in SP, pooling testing is a feasible methodology for surveillance. As clinical symptoms are hidden in these patients, they can only be identified if they are tested; therefore, the control of asymptomatic infections is more complicated [14].

Results from this work demonstrate the need for screening both AP and SP in order to prevent SARS-CoV-2 transmission to communities. Future studies should be performed to assess the role of asymptomatic infections caused by variants of concern and in people with immunity (by vaccination or infection), following experimental designs that help to minimize biases in the selection of samples and variables of analysis.

Funding

The COVID Unit from the Diagnostic Laboratory at the National

University of Hurlingham, the Ministry of Health of the Buenos Aires province and the Ministry of Health of Argentina supported this work.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

MARCELA PILLOFF reports equipment, drugs, or supplies was provided by Ministry Health Care.

Acknowledgments

The authors wish to thank all professionals and technicians that worked at the COVID Unit during the pandemic for their technical assistance and collaboration in compiling the database.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcvp.2023.100166.

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