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Virological surveillance of SARS-CoV-2 in an Italian Northern area: differences in gender, age and Real Time RT PCR cycle threshold (Ct) values in three epidemic periods

Mostafa Mohieldin Mahgoub Ibrahim[1], Maria Eugenia Colucci[1], Licia Veronesi[1], Isabella Viani[1], Anna Odone[2], Mattia Pia Arena[1], Monia Incerti[1], Elisa Tamburini[1], Roberta Zoni[1], Cesira Pasquarella[1], Paola Affanni[1]

[1]Department of Medicine and Surgery, University of Parma, Italy; [2]Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy

Abstract. *Background and aim of the work*: Coronavirus Disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a global public health emergency. The aim of this study was to investigate cases characteristics and Real Time RT PCR cycle threshold (Ct) values distribution of COVID-19 in an Italian Northern area during three periods: first period, February-May 2020; second period, June-August 2020; third period, September 2020-February 2021. *Methods*: Real Time RT PCR was used to detect SARS-CoV-2 in respiratory samples (oro/nasopharyngeal swabs). *Results*: A total of 254,744 samples were tested during the study period. Out of 20,188 positive samples (7.92%), 10,303 were females (51.04%) and 9,885 were males (48.96%). The percentage of positivity varied during the three different periods: 14.1% in the first period, 1.4% in the second and 9.2% in the third. The lowest Ct values were observed in the first phase of pandemic, with an overall average of 25.64. Overall average of the Ct values was lower in males than in females, 26.29 ± 6.04 and 26.84 ± 5.99 respectively. The oldest patients recorded lower Ct values. *Conclusions*: The findings of our study represent further evidence in support of the fact that male sex and older age showed lower Ct values, which means higher viral loads and higher infectious potential. These knowledges are useful to better understand the epidemiological aspects of COVID-19 and to perform effective Public Health Policies.

Key words: SARS-CoV-2 surveillance, age, gender, Real Time RT PCR, Cycle threshold (Ct) values.

Introduction

Italy was the first country in Europe to be hit by COVID-19, and most heavily (1), with the first indigenous case reported in February 2020 (2,3). Gender and age are the main factors associated with risks and consequences of the SARS-CoV-2 pandemic (4,5,6). Recent studies indicated that the positivity rate is different in gender, and women had fewer complications than men (7,8). Moreover, the severity of the SARS-CoV-2 was found to be both more serious among men than among women, and to be influenced by prior comorbidity (9,10). Lastly, the fatality rates were valued to be higher among older age groups, while lower prevalence and milder symptoms were assessed among children respect to adults (4,12-15). The aim of this study was to investigate the positivity rate in relation to gender, age and Real Time RT PCR cycle threshold (Ct) values in the COVID-19 pandemic within three different periods.

Methods

This cross-sectional study was conducted from February 2020 to February 2021 at the Laboratory of Hygiene and Public Health of University of Parma, Reference Influenza and SARS-Cov-2 Surveillance Centre for Emilia-Romagna Region. Positive samples were analysed by gender, age and Ct values as a proxy indicator for viral load during three different COVID-19 pandemic periods: first period, February-May 2020 corresponding to the first wave of COVID-19 pandemic; second period, June-August 2020 corresponding to the intermediate period; third period, September 2020-February 2021 corresponding to the second wave of Covid-19 pandemic. Viral RNA was detected in oro/nasopharyngeal swabs obtained both from inpatients and outpatients. COVID-19 infection was confirmed according to the Ct values for N1 and N2 genes ascertained by RT-PCR assay as described by the Centers for Disease Control and Prevention (CDC) (16). Oro/nasopharyngeal swabs were processed by using several analysis lines; only some of them provided the Ct values and these were mostly used to process oro/nasopharyngeal swabs from hospitalized patients.

Statistical analyses were performed using SPSS 26.0 (IBM, Chicago, ILL). Data were presented as mean, standard deviation (SD), 95% confidence intervals (C.I.) or proportions as appropriate. We used One-way ANOVA to compare the differences of means between groups in a univariate analysis. Two-way ANOVA was used in a multivariable analysis to model the relationship between Ct values (outcome), age and gender group (independent variables). A p value ≤ 0.05 was considered as statistically significant.

Results

A total of 20,188 samples out of 254,744 analysed were positive for SARS-Cov-2 (7.92%) over the study period. The percentage of positivity varied during the three different observed periods: 14.1% in the first period, 1.4% in the second period and 9.2% in the third period. Table 1 shows characteristics of positive samples by gender and age in the different months over the 13 months study period. The first period, from February to May 2020 included the national lockdown period with the peak of positivity rate reached in March (42.98%). A clear decrease in positivity rate

Table 1. SARS-CoV-2 positive sample characteristics by gender and age in the different months.

				Gender				Age mean value			
Months	**No. tested**	**No.**	**Pos %**	**Female No.**	**Pos %**	**Male No.**	**Pos %**	**Female**	**SD**	**Male**	**SD**
February 2020	346	85	24.57%	31	36.47%	54	63.53%	58.65	15.62	62.32	16.27
March 2020	4902	2107	42.98%	855	40.58%	1252	59.42%	66.69	18.44	67.49	16.26
April 2020	7321	861	11.76%	453	52.61%	408	47.39%	73.74	20.18	67.72	19.45
May 2020	11115	290	2.61%	158	54.48%	132	45.52%	66.59	22.95	58.87	24.52
June 2020	15334	263	1.72%	164	62.36%	99	37.64%	63.93	23.43	55.74	23.62
July 2020	18181	210	1.16%	100	47.62%	110	52.38%	47.52	28.66	43.99	20.53
August 2020	22857	308	1.35%	156	50.65%	152	49.35%	39.05	22.18	41.94	19.60
September 2020	24199	869	3.59%	452	52.01%	417	47.99%	39.33	21.36	42.97	21.16
October 2020	37668	2319	6.16%	1075	46.36%	1244	53.64%	42.65	23.04	45.75	22.67
November 2020	36417	4325	11.88%	2272	52.53%	2053	47.47%	51.37	24.42	48.70	22.80
December 2020	26327	2980	11.32%	1642	55.10%	1338	44.90%	58.53	25.58	53.17	24.02
January 2021	25935	2785	10.74%	1521	54.61%	1264	45.39%	55.50	24.92	51.70	24.26
February 2021	24142	2786	11.54%	1424	51.11%	1362	48.89%	50.20	25.26	47.42	24.09
Overall	254744	20188	7.92%	10303	51.04%	9885	48.96%	54.01	25.26	52.19	23.60

Pos %: percentage of positive samples; SD: standard deviation

Table 2. Ct values in the three periods by gender.

Study period	Overall		Female			Male		
	Mean (Ct)	Standard Deviation (Ct)	Mean (Ct)	Standard Deviation (Ct)	Mean (Age)	Mean (Ct)	Standard Deviation (Ct)	Mean (Age)
First period (February -May 2020)	25.64	4.7	25.23	4.67	66.42	25.91	4.72	64.1
Second period (June - August 2020)	28.88	5.15	29.38	4.24	50.17	28.52	5.41	47.22
Third period (September 2020 - February 2021)	26.75	6.34	27.11	6.2	49.6	26.39	6.44	48.29
Overall	26.56	6.02	26.84	5.99	54.01	26.29	6.04	52.19

was observed in the second period followed by a new increase starting from September (3.59%), reaching in February 2021 the value of 11.54%. A quasi-stable trend was observed in the positive sample percentage from November 2020 to February 2021 (range: 10.74% -11.88%).

At the beginning of the pandemic (February-March 2020), percentage of positive samples was higher in males than in females, while in the other months, except for July and October, females showed highest percentages. Overall, positive samples were 10,303 (51.04%) in female and 9,885 (48.96%) in male with a ratio of 1.05:1 (Table 1). The mean age of positive subjects, varied over time; in the first period both males and females showed a higher mean age compared with the other two periods, with higher mean age in female than in male subjects. In the second phase, mean age decreased in both genders, and during the third phase mean age increased again but did not return to the levels of the first wave (Table 1). This observation was consistent for males and females (two tail Pearson's test correlation, $p<0.001$) even if there was significant difference in positivity rate between males and females in the entire period of pandemic ($p<0.001$). As expected, based on Italian demographic structure, the majority of the positive subjects over 90 were women (73.08%).

The Ct values were reported for a total of 10,509 samples (52.06%), 5,335 (50.76%) males and 5,174 (49.24%) females. Table 2 shows the Ct values recorded in the different phases, by gender (Table 2). The lowest Ct values were observed in the first phase of pandemic, with an overall average of 25.64. Overall average of Ct values was lower in males than in females, 26.29 ± 6.04 and 26.84 ± 5.99 respectively (Table 2).

Table 3 shows average Ct values and standard deviation by gender and age group. The relative frequency of samples with Ct progressively increased with increasing age group (Chi-Square test $p <0.001$); a significant statistical difference was found in the frequency of samples with and without Ct between males and females (Table 3).

The lowest average Ct values between 24.26 and 26.13 were observed both in males and females, in $\geq$ 80 years old group (Table 3). The SARS-CoV-2 Ct values ranged from 10 to 40 and about 31.19% were recorded Ct value ≤ 25 (Table 4).

The relationship between Ct values and age average was inverse and the oldest patients recorded low Ct values which indicates the high concentration of genetic material of SARS-CoV-2 in the samples (Ct 10-14; age mean: male 58.59, female 66.41 and Ct 35-40; age mean: male 53.07; female 55.07) (Table 4).

Conclusions

This study investigated the positivity rate of samples in relation to gender, age and Ct values in the COVID-19 pandemic within three different periods from February 2020 to February 2021. Distributions by age, gender and Ct values reflect three different moments of the pandemic over the first pandemic year: first wave, intermediate period, second wave. During the first wave, in February and March 2020, men were more affected than women and both with

Table 3. SARS-CoV-2 characteristics of positive sample by age, gender and Ct values.

Age group	Total	Female					Male				
		Pos. No (%)	With (Ct)*	Without (Ct)*	Average (Ct)	SD (Ct)	Pos. No (%)	With (Ct)*	Without (Ct)*	Average (Ct)	SD (Ct)
0-09	785	391 (49.81)	123	268	27.32	6.03	394 (50.19)	106	288	27.51	6.38
10-19	1458	734 (50.34)	227	507	27.39	6.15	724 (49.66)	233	491	27.46	6.12
20-29	1909	980 (51.34)	335	645	28.06	5.87	929 (48.66)	318	611	27.10	6.43
30-39	2080	1071 (51.49)	456	615	27.98	5.85	1009 (48.51)	410	599	27.04	6.24
40-49	2806	1439 (51.28)	638	801	27.73	5.78	1367 (48.72)	627	740	26.40	6.27
50-59	3082	1484 (48.15)	718	766	27.21	5.88	1598 (51.85)	839	759	26.82	5.80
60-69	2232	1041 (46.64)	572	469	27.19	5.64	1191 (53.36)	758	433	26.69	5.73
70-79	2241	1023 (45.65)	660	363	26.39	5.86	1218 (54.35)	910	308	26.15	5.62
80-89	2458	1309 (53.25)	919	390	25.71	6.18	1149 (46.75)	907	242	24.97	6.08
90-99	1101	797 (72.39)	503	294	25.37	6.08	304 (27.61)	225	79	24.26	6.40
>100	36	34 (94.44)	23	11	26.13	5.11	2 (5.56)	2	0	25	2.83
Overall	20188	10303 (51.04)	5174	5129	26.85	5.99	9885 (48.96)	5335	4550	26.29	6.04

With Ct: number of samples have Ct value; Without Ct: number of samples have not Ct value; Pos. No: number of positive samples; (Ct*; C`t*) Chi-Square test: df= 10, P-value = 0.0000; SD: standard deviation.*

Table 4. SARS-Cov-2 Ct range by gender and age.

Ct Range	No				Age average (years)	
	Female	Male	Overall	Cumulative (%)	Male	Female
10-14	137	133	270	2.56	58.59	66.41
15-19	579	711	1290	14.80	61.61	64.07
20-24	817	911	1728	31.19	63.14	64.49
25-29	1239	1322	2561	55.49	61.26	62.56
30-34	2258	2110	4368	96.93	56.29	56.55
35-40	166	158	324	100.00	53.07	55.07
Overall	5196	5345	10509			

lower Ct values. As underlined in a previous paper (17), higher Ct values are justified by the fact that most of the samples came from hospitalized patients with medium-severe clinical symptoms, with a high dispersion of the virus in the environment (18). After the first wave, oro-nasopharingeal swabs were extended also to non-hospitalized patients highlighting an increase of high percentage of positivity in mild or asymptomatic patients. In the intermediate period, from June to August, a decrease of the percentage of positivity was observed, which can be explained by the constantly increased number of non-hospitalized subjects involved in local screening activities. Moreover, the effect of summer climate conditions should be considered as suggested by some authors (19,20). Starting from September, a new increase of percentage of positivity was observed though it remained consistently lower than in the first wave; it could reflect the source of the samples, more frequently from non-hospitalized subjects than in the first wave. Mean age of positive subjects, varied among the months; in particular, the most affected age group was over 80 years old, the most fragile group with high prevalence of comorbidity (9,10,11).

However, in the pre-vaccination period, several factors, such as a set of distancing measures, awareness, and identification of geographic areas with different

colours contributed, to limit the virus circulation, which was explosive in the first phase, and much slower in the second phase, reaching the peak in a longer period of time. In the future collection and analysis of data from the same geographical area will continue with an evaluation, as for the Influenza (21-25), of the effect of vaccination campaign started in December 2020. This study aimed at providing a descriptive picture of the pandemic situation in a Northern geographic area, using the valuable data from a Regional SARS-CoV-2 Virological Surveillance Reference Laboratory. The very high number of the samples analysed over a long period of time represents a strength point of our study. However, a great limitation is the lack of consideration of the origin of swabs and the epidemiological criteria used for swab execution, such as contact of a positive case, screening, presence of symptoms, recovery swab. Moreover, clinical data will be considered in order to confirm that male sex and older age carry a higher risk of experiencing adverse clinical outcomes. All these data will be included in further studies to better understand the dynamics of virus circulation in the population and to perform effective targeted preventive measures.

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Correspondence:
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Mostafa Mohieldin Mahgoub Ibrahim
Department of Medicine and Surgery,
University of Parma, Italy
Via Volturno 39 - 43125 Parma
Tel +39 0521 033795
Fax +39 0521 347039
E-mail: mostafa.mohieldinmahgoubibrahim@unipr.it