

Risk factors for severity, viral load, and outcomes in patients with COVID-19

Factores de riesgo para gravedad, carga viral y desenlaces en pacientes con COVID-19

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Abstract

Background: During the coronavirus disease 2019 (COVID-19) pandemic, early diagnosis is crucial, as is the identification of risk factors for severity and risk of transmissibility of infection. **Objectives:** The objective of the study was to correlate clinical characteristics, severity, and outcomes with viral load for severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). **Materials and methods:** An observational, cross-sectional, and descriptive study was carried out that collected data from patients with COVID-19. Clinical, demographic, laboratory, viral load, and outcome variables were collected. SARS-Cov-2 detection was by reverse transcription polymerase chain reaction, from nasopharyngeal swabs, reporting it with amplification cycles and stratifying it as mild, moderate, or high viral load estimation. **Results:** Data from 325 patients were included in the study, average age of 51 years, predominantly male 52.9%. The independent risk factors associated with severity were age > 65 years, dyspnea, vomiting, and viral load > 25, a protective effect was found with the presence of headache, odynophagia, and anosmia/hyposmia. **Conclusion:** The presence of anosmia/hyposmia, odynophagia, and headache was more frequent in non-severe cases, suggesting better local immunity. There could be less risk of viral transmissibility in patients requiring in-hospital management compared to outpatients.

Keywords: Coronavirus disease 2019. Viral load. Severity. Outcomes. Characteristics clinics. Clinical features.

Resumen

Antecedentes: Durante la pandemia COVID-19 y hasta la fecha, el diagnóstico temprano es crucial, así como la identificación de factores de riesgo para gravedad y el riesgo de transmisibilidad de infección. **Objetivo:** Correlacionar características clínicas, gravedad y desenlaces con carga viral para SARS-CoV2. **Material y métodos:** Se realizó un estudio observacional, transversal y descriptivo que reunió datos de pacientes con COVID-19. Se tomaron variables clínicas, demográficas, de laboratorio, carga viral y desenlaces. La detección de SARS-Cov-2 fue por RT-PCR por hisopado nasofaríngeo, reportándolo con ciclos de amplificación y estratificándolo como estimación de carga viral leve, moderada o alta. **Resultados:** Se incluyeron datos de 325 pacientes, edad promedio de 51 años, predominio de sexo masculino 52.9%. Los factores de riesgo independientes asociados a gravedad, fueron edad > 65 años, disnea, vómito, carga viral > 25, se encontró un efecto protector por la presencia de cefalea, odinofagia y anosmia/hiposmia. **Conclusiones:** La presencia de anosmia/hiposmia, odinofagia y cefalea fue más frecuente en los casos no graves, lo que sugiere mejor inmunidad local. Podría existir menos riesgo de transmisibilidad viral en pacientes que requieren manejo intrahospitalario comparado con los ambulatorios.

Palabras clave: COVID-19. Carga viral. Gravedad. Resultados. Características clínicas. Características clínicas.

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Date of reception: 05-06-2024

Date of acceptance: 10-06-2024

DOI: 10.24875/AMH.M24000078

Available online: 02-09-2024

An Med ABC 2024;69(3):214-221

www.analesmedicosabc.com

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Introduction

In 2019, cases of pneumonia of unknown origin due to a new RNA betacoronavirus, currently severe acute respiratory syndrome coronavirus 2 (SARS CoV2), were identified in Wuhan. The World Health Organization declared an international public health emergency and named it coronavirus disease 2019 (COVID-19)¹. The index case in Mexico was detected in February 2020 in Mexico City. By March, the Ministry of Health confirmed the first death from COVID-19, which occurred at the Hospital Angeles Metropolitano².

SARS-CoV-2 targets nasal and bronchial epithelial cells through the glycosylated spike protein that binds to the angiotensin-converting enzyme receptor 2 (ACE2), this interaction is a potential factor in its infectivity. Transmembrane serine protease Type 2 (TMPRSS2), in the host cell, promotes viral uptake^{2,3}.

Viral load in the upper respiratory tract appears to reach the highest value at the time of symptom onset⁴. The mean incubation time is 4-7 days after exposure. About 97.5% develop symptoms within the first 11 days⁵.

The average age of hospitalized patients is 47-73 years. Early cohorts at the pandemic had a male preponderance of 60%². Common symptoms include fever, cough, dyspnea, fatigue, myalgias/arthralgias, gastrointestinal symptoms, headache, and rhinorrhea. Anosmia or ageusia may be the only presenting symptom^{2,5}. About 81% had mild manifestations, 14% severe and 5% had critical manifestations (respiratory failure, septic shock, and/or multiple organ dysfunction)⁶. About 15% of patients will develop severe disease and 5% will require mechanical ventilation⁷. Complications of COVID-19 include multiple organ dysfunction, myocarditis, cardiomyopathy, ventricular arrhythmias, and hemodynamic instability⁸. About 60-90% of hospitalized infected patients have comorbidities; systemic arterial hypertension (SAH), diabetes mellitus 2 (DM2), obesity, cardiovascular disease, lung disease, renal disease, malignancy, liver disease, or autoimmune diseases⁹.

The gold standard for COVID-19 diagnosis is by detection of SARS-CoV-2 RNA by reverse transcription polymerase chain reaction (RT-PCR) of respiratory samples by nasal swab. However, the sensitivity of the tests varies over time in relation to exposure. Due to false-negative rates, clinical, laboratory, and imaging findings may also be used for presumptive diagnosis¹⁰. RT-PCR assays provide information on whether or not SARS-Cov-2 is detected. However, these assays also contain quantitative information on cycle threshold (Ct) values that correlate inversely with viral load. Immunoglobulin M

antibodies are detectable 5-day post-infection, with the highest levels during weeks 2-3 of illness, while an Immunoglobulin G response is observed 14 days after symptoms onset¹¹⁻¹³. The most common hematologic abnormality is lymphopenia ($< 1.0 \times 10^9/L$), present in 83% of hospitalized patients^{14,15}. Most laboratory characteristics are nonspecific¹⁶.

In a meta-analysis of 703 patients with COVID-19 (553 symptomatic and 150 asymptomatic), it was concluded that there are no differences in viral load between symptomatic and asymptomatic. Asymptomatic subjects may represent a reservoir of infection and contribute significantly to the maintenance of the pandemic¹⁷.

The study aimed to correlate clinical characteristics and outcomes with viral load for SARS-COV2.

Materials and methods

We designed an observational, cross-sectional, and descriptive study that collected data from patients with confirmed COVID-19 from Hospital Angeles Metropolitano during the first wave of contagion in Mexico, from March 13, 2020, to January 12, 2021. Clinical and demographic variables, laboratory studies, as well as viral load and outcomes (discharge home, transfer to COVID-19 hospital, admission or death) were collected. Detection of SARS-Cov-2 was by RT-PCR, reporting it with amplification cycles, being stratified by the laboratory as mild, moderate, and high viral load. Data were collected retrospectively from clinical records. The study was approved by the bioethics committee.

A descriptive statistical analysis was performed using mean and standard deviation. For categorical or dichotomous variables, absolute frequencies and percentages were used. The hypothesis tests for categorical variables were the Chi-square or (Fisher's exact test) and for linear variables the Student's t-test for unrelated samples. Relevant variables to discriminate severe cases were used to construct multivariate binary logistic regression models to identify variables independently associated with the presence of severe cases. The strength of association was measured by calculating odd ratios (OR) with a 95% confidence interval (CI). The construction of regression models included a first phase in which the variables identified in the univariate comparison were introduced and subsequently, we used the forward stepwise method to include variables with a value of $p = 0.02$ in the iterations for the construction of an alternative model. A two-tailed $p < 0.05$ was considered significant for hypothesis testing. The statistical package IBM Statistics SPSS v21.0 was used for the data analysis.

Results

Data from 325 patients with an average age of 51 ± 17 years were included in the study. Of which 52.9% were male and 45.8% female. Detection of SARS-CoV-2 was by RT-PCR, from a nasopharyngeal swab, taken at the first medical contact, within 3-10 days of symptoms onset. A viral load by amplification curve estimate 28 ± 6.8 cycles. They were classified as mild viral load estimate > 30 Ct, moderate 21-35 Ct, and high < 25 Ct in 29.2, 41.8 and 28.3%, respectively. Demographic characteristics, comorbidities, and symptoms at admission are detailed in Table 1.

At the outcome analysis, it was determined that 42.9% of the patients were in conditions to be discharged, the remaining 57.1% were severe cases, of which 45% were admitted, 51.1% were transferred to another center and 3.3% died. The association between viral load estimation and outcomes is summarized in Table 2. When comparing the characteristics between severe and outpatient cases, statistically significant differences were found, especially due to the presence of comorbidities and alterations in laboratory studies. All comparisons are shown in Table 3. Due to the stratification finding of in the laboratory reports of a moderate viral load of 21-35 Ct, a cutoff value of 25 Ct was considered for the rest of the comparisons.

Multivariate analysis revealed that the independent risk factors associated with severity were age over 65 years OR = 1.38 (95% CI 3.05-11.4, $p < 0.01$); dyspnea OR = 5.91 (3.05-11.44, $p < 0.001$); vomiting OR = 3.8 (1.29-11.4, $p = 0.015$), viral load > 25 OR = 3.07 (1.35-6.9, $p = 0.007$), a protective effect was found due to the presence of headache OR = 0.32 (0.22-0.58, $p < 0.001$); odynophagia 0.41 (0.22-0.78, $p = 0.006$), and anosmia/hyposmia OR = 0.05 (0.015-0.23, $p < 0.001$). Model A and B (Tables 4 and 5).

Discussion

At the beginning of the COVID-19 pandemic in Mexico, different containment measures were implemented, and new work and logistics plans for medical care were established. Some institutions, hospitals, and some temporary units were designated to be 100% care centers for COVID-19 cases, others 100% free of COVID-19 cases, and some that worked in a hybrid manner. At the beginning of the pandemic, the Hospital Ángeles Metropolitano provided its services to COVID-19 cases, subsequently different work plans were delegated within Grupo Ángeles to safely provide

Table 1. Demographic characteristics, comorbidities, and symptoms on admission

Characteristics, comorbidities and symptoms	n	%
Age, mean (SD)	51	17
Male	172	53.6
Female	149	46.4
BMI, mean (SD)	27,700	5,190
Comorbidities		
Diabetes mellitus	72	22.5
Systemic arterial hypertension	82	25.6
Heart disease	28	8.8
Lung disease	3.4	10.6
Liver disease	8	2.5
Renal disease	18	5.6
Malignancy	8	2.5
Immunosuppression	7	2.2
Symptoms upon admission		
Fever	160	49.8
Cough	161	50.2
Dyspnea	117	36.4
Diarrhea	69	21.5
Vomiting	28	8.7
Myalgias/artralgias	145	45.2
Fatigue	160	49.8
Headache	138	43.0
Anosmia/hyposmia	28	8.7
Odynophagia	111	34.6
Rhinorrhea/nasal congestion	68	21.2
Dysgeusia	25	7.8

service to non-COVID-19 cases, so the cases that warranted hospital admission due to SARS-CoV2 infection were transferred to the hospitals assigned for COVID-19, those who did not merit admission were discharged home with alarm and isolation data. For this reason, most of the patients in our study were evaluated in the emergency department where their outcome was determined: discharge, admission, transfer, or death. The patients in our study were the first ones to be evaluated at the hospital since the beginning of the pandemic, so they are patients infected with the first variant of COVID-19 and without SARS-CoV2 vaccine.

We introduce an epidemiological study that analyzes demographic characteristics, which, in terms of age and sex, the characteristics are similar to what was published in the Mexican population. During the first stage of the pandemic, a predominance of the male sex was found, with an average age between 40 and 60 years. The most frequent comorbidities were SAH, DM2, and lung disease. With the exception of obesity, which predominates in most published studies, the majority of our population was only overweight. The most frequent

Table 2. Association between viral load estimation and outcomes

Viral load estimation	Total		Death		Transfer		Discharge		Income	
	n	%	n	%	n	%	n	%	n	%
Mild	95	29.41	2	1.90	26	27.30	27	28.40	40	42.10
Moderate	136	42.10	3	2.20	39	28.60	68	50	26	19.11
High	92	28.48	2	2.10	29	31.50	46	50	15	16.30

Table 3. Comparisons of characteristics between severe and outpatient cases

Characteristics, comorbidities and symptoms	Controls (n = 137)		Serious cases (n = 188)		p-value
	n/mean	%/SD	n/mean	%/SD	
Age, mean (SD)	48	16	52	18	0.92
Male	50	53.8	122	53.5	0.92
Female	43	46.2	106	46.5	
BMI	27.64	6.58	27.73	4.52	0.6
Diabetes mellitus	19	20.4	53	23.3	0.008
Systemic arterial hypertension	22	23.7	60	26.4	0.421
Heart disease	5	5.4	23	10.1	0.232
Lung disease	12	12.9	22	9.7	0.838
Liver disease	2	2.2	6	2.6	0.013
Renal disease	5	5.4	13	5.7	0.021
Malignancy	1	1.1	7	3.1	0.758
Immunosuppression	3	3.2	4	1.8	0.123
Peripheral oxygen saturation (SpO ₂)	0.88	0.11	0.86	0.13	< 0.001
Systolic blood pressure	123.47	20.42	125.63	19.64	0.45
Heart rate	92	19	93	20	0.75
Breathing frequency	20	4	21	4	< 0.001
Temperature (°C)	36.29	3.60	36.91	1.01	0.23
Fever	37	39.8	123	53.9	0.028
Cough	38	40.9	123	53.9	0.233
Dyspnea	29	31.2	88	38.6	< 0.001
Diarrhea	22	23.7	47	20.6	0.483
Threw up	10	10.8	18	7.9	0.238
Myalgias/artralgias	3. 4	36.6	111	48.7	0.001
Fatigue	35	37.6	125	54.8	0.082
Headache	31	33.3	107	46.9	< 0.001
Anosmia/hyposmia	9	9.7	19	8.3	< 0.001

(Continues)

Table 3. Comparisons of characteristics between severe and outpatient cases (*continued*)

Characteristics, comorbidities and symptoms	Controls (n = 137)		Serious cases (n = 188)		p-value
	n/mean	%/SD	n/mean	%/SD	
Odynophagia	27	29.0	84	36.8	< 0.001
Rhinorrhea/nasal congestion	15	16.1	53	23.2	0.001
Dysgeusia	9	9.7	16	7.0	< 0.001
Leukocytes	8403	3461	7279	3051	0.0003
Lymphocytes	1539	780	1242	1235	0.5578
Neutrophils	6394	3401	5578	3126	0.012
Platelets	222390	103150	222713	87060	0.99
Ferritin	964	1146	958	1324	0.016
D-dimer	1374	2384	962	1277	0.005
Erythrocyte sedimentation rate	26	22	27	12	0.73
Lactic dehydrogenase	259.1	142.9	336.4	175.9	0.03
C-reactive protein	23.18	43.61	19.82	44.69	0.16
Procalcitonin	0.46	1.08	0.90	2.69	0.99
Viral load	25.65	6.74	28.44	6.78	0.0003

symptoms referred to admission were cough, fever, fatigue, myalgias/arthralgias, headache, and dyspnea in successive order, which also coincides with what is reported in the literature. In our study, a significant difference was found in the presence of dyspnea and vomiting, as well as age over 65 years for severe cases. Unlike the presence of headache, odynophagia, anosmia, or hyposmia, which were significant in the outpatient's group, so the presence of these symptoms in the initial assessment could suggest the course of a mild or moderate condition.

There are several proposed theories of the mechanisms for anosmia, inflammation of the epithelium of the nasal mucosa, apoptosis of olfactory cells, changes in olfactory cilia, damage to the olfactory bulb, olfactory neurons, and totipotent cells of the olfactory epithelium¹⁸. One of the mechanisms of anosmia is due to the ACE2, which is a receptor for COVID-19, and could be present in large quantities in the olfactory tract. The union of the virus with these receptors causes the release of cytokines and greater inflammation of the epithelium¹⁹.

Sven Saussez et al. tried to answer why anosmia seems to be less prevalent in severe cases than in mild-to-moderate cases. In a European study of

417 patients with mild-to-moderate COVID-19, 86% reported anosmia and 88% dysgeusia. In a series of 2013 patients with the same conditions, 87% reported anosmia. In Saussez et al. study, of 86 outpatients with mild-to-moderate symptoms of COVID-19 associated with subjective anosmia, psychophysical measurements of smell were performed "Sniffin Sticks," they found 47.7% anosmic, 14% hyposmic and 38.3% without olfactory alteration²⁰. Romero-Sánchez et al., In a series of 841 patients with severe COVID-19 with in-hospital management, 57.4% developed some neurological symptoms and only 5% presented anosmia²¹.

Such findings may suggest that in patients with efficient local immunity, virus replication in the nasal and olfactory mucosa may provoke a local inflammatory reaction and a pattern of otorhinolaryngological symptoms, unlike severe patients, where such symptoms were less frequent. Thus, patients with a mild form of infection have stronger local immunity and therefore higher rates of local symptoms. Moreover, the severe forms of infection, or weaker local immunity, fewer otorhinolaryngological, and more respiratory symptoms.

It should be considered that in our study, age was also significant, being higher in severe cases, in older patients the olfactory function decreases. Therefore, it

Table 4. Model A. Univariate comparison

Characteristics, comorbidities and symptoms	OR	95% CI		p-value
Age (65 years)	1.264	0.582	2.745	0.553
Peripheral oxygen saturation (88%)	1.437	0.671	3.077	0.351
Temperature (38°C)	0.602	0.255	1.421	0.247
Diabetes Mellitus	2.559	1.110	5.900	0.027
Systemic Arterial Hypertension	0.852	0.391	1.858	0.687
Heart disease	1.401	0.449	4.375	0.562
Lung disease	0.972	0.347	2.728	0.958
Renal disease	4.755	0.566	39.959	0.151
Malignancy	1.142	0.151	8.662	0.898
Immunocompromise	1.047	0.084	12.970	0.972
Fever	0.865	0.421	1.775	0.692
Cough	0.961	0.465	1.985	0.914
Dyspnea	6.225	2.859	13.556	< 0.001
Diarrhea	0.708	0.326	1.541	0.385
Vomiting	5.332	1.530	18.587	0.009
Myalgias/Arthralgias	0.671	0.342	1.317	0.246
Fatigue	0.716	0.365	1.404	0.331
Headache	0.414	0.214	0.800	0.009
Odynophagia	0.441	0.220	0.883	0.021
Rhinorrhea/Nasal congestion	0.706	0.324	1.539	0.381
Dysgeusia	0.539	0.095	3.057	0.486
Anosmia/Hyposmia	0.105	0.015	0.747	0.024
Viral load (25)	5.332	1.018	27.928	0.048

OR: odds ratio; CI: confidence interval.

Table 5. Model B. Alternative model

Characteristics, comorbidities and symptoms	OR	95% CI		p-value
		Lower	Superior	
Age (65 years)	2.963	1.381	6.355	0.005
Dyspnea	5.91	3.052	11.446	< 0.001
Vomiting	3.858	1.299	11.452	0.015
Headache	0.326	0.181	0.586	< 0.001
Odynophagia	0.416	0.221	0.782	0.006
Anosmia/hyposmia	0.059	0.015	0.239	< 0.001
Viral load (25)	3.073	1.353	6.981	0.007

OR: odds ratio; CI: confidence interval.

may be more difficult to detect deterioration in olfactory function, either due to age or by the severity of other non-otorhinolaryngologic symptoms.

Several studies have been carried out comparing viral load in symptomatic and non-symptomatic patients, in which there was no significant difference, thus understanding that transmissibility is the same in both cases.

Our hypothesis was that evaluating the SARS-Cov-2 viral load through Ct analysis of an initial sample could be a valuable clinical tool to identify patients at higher risk of intubation and death. In our study, the viral load was significant for severe cases, since those with > 25 were associated with the need for hospitalization, transfer to intensive care units, intubation, and even

higher mortality. This differs from a retrospective study in New York where the relationship between Ct and severity was demonstrated, where the value of Ct < 25 was independently associated with in-hospital mortality and the need for intubation. In this study, Magleby et al. stratified the Ct value as follows: high Ct < 25, medium Ct 25-30, and low Ct > 30²². In a Mexican population study where 21,110 confirmed cases of COVID-19 were included, they found that 53% had a high viral load, hospitalization was more frequent in subjects with a medium viral load and deaths were more prevalent in those with a high viral load²³. In a Mexican pediatric population study, 176 positive tests were identified, of which Ct values were observed with a median of 31.9-34.01, 13.1% presented very high viral load, 9.1% high, 14.2% medium and 63.6 % low, no relationship with sex or age was found²⁴.

With the findings in our study, we could deduce that severe cases may have less risk of transmissibility than outpatient cases; however, they continue to be highly contagious. High viral load Ct < 25 was not associated with increased severity. Determining the Ct number in positive PCR tests could help at the clinical practice when interpreted in the right context.

By carrying out the study before vaccination and generation of other COVID-19 variants, it was possible to perform a homogeneous clinical evaluation, allowing adequate statistical analysis with clinical characteristics. Among the weaknesses of the study, the PCR was performed in the first medical evaluation, so it could be different stages of the disease.

We propose that our findings are due to the role of innate immunity as a first line of antiviral defense. Innate immune responses limit viral entry, translation, replication, and assembly, help identify and eliminate infected cells, and coordinate and accelerate the development of adaptive immunity. Cell surface, endosomal, and cytosolic pattern recognition receptors respond to pathogen-associated molecular patterns to trigger inflammatory responses and programmed cell death that limit viral infection and promote clearance. Excessive immune activation can cause systemic inflammation, which adds to that caused by obesity or smoking damage, causing serious diseases, highlighting the balance that must be achieved. Hence, these mechanisms could influence the viral load value obtained, as well as the different clinical conditions. So we know that, the protective factors are the elevation of anti-inflammatory cytokines, antibody response and T-cell activation, as well as the low expression of ACE2. Unlike risk factors with increased pro inflammatory cytokines, increased ACE2 level, and TMPRSS2 expression²⁵.

Conclusion

The presence of anosmia/hyposmia, odynophagia, and headache was more frequent in non-severe cases, which suggests better local immunity. Patients with more severe symptoms were associated with older age > 65 years, dyspnea, vomiting, and viral load Ct > 25. The findings on viral load may suggest less risk of viral transmissibility in patients under in-hospital management compared to outpatients. Further studies are needed to establish the correlation of viral load with severity and outcomes. Innate immunity, as well as adaptive immunity, plays a fundamental role in the evolution of SARS-Cov2 infection.

Funding

The authors declare that they have not received funding.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article. Furthermore, they have acknowledged and followed the recommendations as per the SAGER guidelines depending on the type and nature of the study.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Use of artificial intelligence for generating text. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript nor for the creation of images, graphics, tables, or their corresponding captions.

References

1. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708-20.
2. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *JAMA*. 2020;324:782-93.
3. Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-angiotensin-aldosterone system inhibitors in patients with covid-19. *N Engl J Med*. 2020;382:1653-9.
4. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Immunol*. 2020; 20:363-74.

5. Van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of HCoV-19 (SARS-CoV-2) compared to SARS-CoV-1. *N Engl J Med.* 2020 Apr 16;382(16):1564-7. doi: 10.1056/NEJMc2004973.
6. Huynh PP, Ishii LE, Ishii M. What is anosmia? *JAMA.* 2020;324:206.
7. Docherty AB, Harrison EM, Green CA, Hardwick HE, Pius R, Norman L, et al. Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO clinical characterization protocol: prospective observational cohort study. *BMJ.* 2020;369:m1985.
8. Berlin DA, Gulick RM, Martinez FJ. Severe covid-19. *N Engl J Med.* 2020;383:2451-60.
9. Williamson EJ, Walker AJ, Bhaskaran K, Bacon S, Bates C, Morton CE, et al. Factors associated with COVID-19-related death using Open SAFELY. *Nature.* 2020;584:430-6.
10. Mao R, Qiu Y, He JS, Tan JY, Li XH, Liang J, et al. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2020;5:667-78. Erratum in: *Lancet Gastroenterol Hepatol.* 2020;5:e6.
11. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA.* 2020;323:1843-4.
12. Sethuraman N, Jeremiah SS, Ryo A. Interpreting diagnostic tests for SARS-CoV-2. *JAMA.* 2020;323:2249-51.
13. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. *Clin Infect Dis.* 2020;71:2027-34.
14. Rodríguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguín-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. *Travel Med Infect Dis.* 2020;34:101623.
15. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395:497-506. Erratum in: *Lancet.* 2020;395:496.
16. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost.* 2020;18:1023-6.
17. Zuin M, Gentili V, Cervellati C, Rizzo R, Zuliani G. Viral load difference between symptomatic and asymptomatic COVID-19 patients: systematic review and meta-analysis. *Infect Dis Rep.* 2021;13:645-53.
18. Najafloo R, Majidi J, Asghari A, Aleemardani M, Kamrava SK, Simorgh S, et al. Mechanism of anosmia caused by symptoms of COVID-19 and emerging treatments. *ACS Chem Neurosci.* 2021;12:3795-805.
19. Torabi A, Mohammadbagheri E, Akbari Dilmaghani N, Bayat AH, Fathi M, Vakili K, et al. Proinflammatory cytokines in the olfactory mucosa result in COVID-19 induced anosmia. *ACS Chem Neurosci.* 2020;11:1909-13.
20. Saussez S, Lechien JR, Hopkins C. Anosmia: an evolution of our understanding of its importance in COVID-19 and what questions remain to be answered. *Eur Arch Otorhinolaryngol.* 2021;278:2187-91.
21. Romero-Sánchez CM, Díaz-Maroto I, Fernández-Díaz E, Sánchez-Larsen Á, Layos-Romero A, García-García J, et al. Neurologic manifestations in hospitalized patients with COVID-19: the ALBACOV registry. *Neurology.* 2020;95:e1060-70.
22. Magleby R, Westblade LF, Trzebucki A, Simon MS, Rajan M, Park J, et al. Impact of severe acute respiratory syndrome coronavirus 2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. *Clin Infect Dis.* 2021;73:e4197-205.
23. Cruz-Loustaunau D, Álvarez-Hernández G, Candia-Plata MD, Leyva-Gastelum M. Cycle threshold and viral load in SARS-CoV-2-infected patients in Sonora, Mexico. *Gac Med Mex.* 2023;159:226-32.
24. Parra-Ortega I, Carbajal-Franco E, Vilchis-Ordoñez A, Ángeles-Florian T, Nieto-Rivera B, López-Martínez I, et al. Distribution of Ct values in RT-PCR for SARS-CoV-2 at the time of diagnosis in Mexican pediatric patients. *Rev Mex Patol Clin Med Lab.* 2020;67:176-82.
25. Zhang JJ, Dong X, Liu GH, Gao YD. Risk and protective factors for COVID-19 morbidity, severity, and mortality. *Clin Rev Allergy Immunol.* 2023;64:90-107.