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Predictive factors of Covid-19 in patients with negative RT-qPCR

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Predictive factors of Covid-19 in patients with negative RT-qPCR

Abstract

Objective: To evaluate the factors associated with false negatives in RT-qPCR in patients with mild-moderate symptoms of COVID-19.

Materials and methods: This was a cross-sectional study that used a random sample of non-hospitalized patients from the primary care management division of the Healthcare Area of Leon (58 RT-qPCR-positive cases and 52 RT-qPCR-negative cases). Information regarding symptoms was collected and all patients were simultaneously tested using two rapid diagnostic test - RDTs (Combined - cRDT and Differentiated - dRDT). The association between symptoms and SARS-

CoV-2 infection was evaluated by non-conditional logistic regression, with estimation of Odds Ratio.

Results: A total of 110 subjects were studied, 52% of whom were women (mean age: 48.2 ± 11.0 years). 42.3% of negative RT-qPCRs were positive in some RDTs. Fever over 38ºC (present in 35.5% of cases) and anosmia (present in 41.8%) were the symptoms most associated with SARS-CoV-2 infection, a relationship that remained statistically significant in patients with negative RT-qPCR and some positive RDT (aOR=6.64; 95%CI=1.33-33.13 and aOR=19.38; 95% CI=3.69-101.89 respectively

Conclusions: RT-qPCR is the technique of choice in the diagnosis of SARS-CoV-2 infection, but it is not exempt from false negatives. Our results show that those patients who present mild or moderate symptoms with negative RT-qPCR, but with fever and/or anosmia, should be considered suspicious cases and should be evaluated with other diagnostic methods.

Keywords: SARS-CoV-2; RT-qPCR; diagnosis; fever; anosmia

Factores predictivos del Covid-19 en pacientes con RT-qPCR negativa

Resumen

Objetivo: Evaluar los factores asociados con falsos negativos a RT-qPCR negativa y sintomatología leve o moderada de COVID-19.

Materiales y métodos: Estudio transversal. Se utilizó una muestra aleatoria de pacientes no hospitalizados de la Gerencia de Atención Primaria del Área de Salud de León (58 con RT-qPCR positiva y 52 con RT-qPCR negativa). Se recogió información sobre síntomas y a todos se les realizó simultáneamente dos pruebas de diagnóstico rápido - PDR (Combinada: PRD-C y Diferenciada: PRD-D). La asociación de los síntomas con la infección por SARS-CoV-2 se evaluó mediante regresión logística no condicional, con el cálculo de Odds Ratio.

Resultados: Un total de 110 personas fueron estudiadas, 52% de las cuales fueron mujeres (edad media: 48,2 ± 11,0 años). El 42.3% de las RT-qPCR negativas dieron positivo en algún PDR. La fiebre de más de 38ºC (presente en el 35,5% de los casos) y a anosmia (presente en un 41,2%) fueron los síntomas más asociados a la infección por SARS-CoV-2, relación que se mantuvo estadísticamente significativa en pacientes con RT-qPCR negativa y algún PDR positivo (ORa=6,64; IC95%=1,33-33,13 y ORa=19,38; IC95%=3,69-101,89 respectivamente).

Conclusiones: La RT-qPCR es la técnica de elección en el diagnóstico de la infección por SARS-CoV-2, pero no está exenta de falsos negativos. Nuestros resultados ponen de manifiesto que aquellos pacientes que presentan síntomas leves o moderados con RT-qPCR negativa pero con fiebre y/o anosmia, deben ser considerados casos sospechosos y deben ser valorados con otros métodos diagnósticos.

Palabras clave: SARS-CoV-2, RT-qPCR, diagnóstico, fiebre, anosmia

Introduction

One of the main strategies for the prevention and control of the COVID-19 pandemic is the detection and isolation of the infection sources[1]. The Reverse transcription quantitative polymerase chain reaction (RT-qPCR) is the technique of choice for detecting infection sources due to its high sensitivity and specificity[2]. Also, this is a well-known and widespread technique in the clinical laboratories of our hospitals[3]. This method, however, is not free of false negatives, being the most frequent causes of them the inadequate sample collection, delays in transport, labelling errors and the poor virus elimination in the patient[4,5]. For these reasons, in the face of clinical suspicion, a negative RT-qPCR result must be contrasted with other diagnostics test[4].

The aim of this article is to understand the factors associated with false negatives in RT-qPCR in patients with mild-moderate symptoms of COVID-19.

Material and methods

Study design

A cross-sectional study was carried out. A random selection was made of 58 non-hospitalized patients with positive RT-qPCR and 52 negative RT-qPCR. Patients were selected from the register of confirmed or suspected COVID-19 cases from primary care management of the Healthcare Area of Leon. In all of them more than 14 days had passed since the beginning of the symptoms.

Procedure

Participation in the study was voluntary. The invitation to participate in the study was made by a telephone call, in which the participants were cited for the collection of a biological sample and information. During the collection of information and samples, all protection regulations were followed and the project was approved by the Ethics Committee of the health area of León and the Bierzo (reference: 2073). After signing an informed consent, each participant completed a brief *ad hoc* questionnaire that collected information on socio-demographic data, symptoms, date of onset and end of symptoms, date of RT-qPCR.

All patients were tested simultaneously with two RDTs[6]:

- Combined (c-RDT) (one band): Wondfo® SARS-COV-2 Antibody test (Lateral Flow Method) of GUANGZHOU WONDFO BIOTECH CO LTD,
- Differentiated (d-RDT) (two bands): It allows to differentiate IgG and IgM. All Test® 2019-nCoV IgG/IgM Rapid Test Casette of HANGZHOU ALL TEST BIOTECH CO LTD.

Both tests were performed on a fingerstick whole blood sample, collected by two nurses. The first test jointly determines the presence of IgM and IgG, while the second test makes a differentiated measurement of both antibody subtypes. The result of the tests was read 10-15 minutes after they were carried out.

We considered as a case of COVID19 those patients with a positive result to at least one of the RDTs or RT-qPCR.

Statistical analysis

Central and dispersion measures were calculated in quantitative variables (mean and standard deviation (SD)) and frequencies with their 95% confidence intervals in qualitative variables. Using non-conditional logistic regression models, adjusted at least by age and sex, we obtained the Odds Ratio (aOR) to be considered COVID-19 case, performing stratified analysis according to the results of the RDTs and the RT-qPCR. The characteristics of those patients with negative RT-qPCR and positive PDR were examined. All analyses were performed with the STATA 15 statistical package[7].

Results

A total of 110 subjects were studied, 51.8% of whom were women. The age range was between 22 and 78 years, with a mean of 48.2 ± 11.0 years. The days from the onset of symptoms to the performance of the RDTs ranged from 14 to 40 days, with a median of 26 days.

Of the 110 patients, 80 (72.7%) had either RT-qPCR or a positive RDT for SARS-CoV-2 (20 were IgM positive, 64 were IgG positive, 64 were IgM or IgG positive, 46 were combined, 65 were RDT positive, and 58 were RT-qPCR positive). Of the 52 with negative RT-qPCR, 22 (42.3%) were positive at some RDT; 14 positives at the two RDTs and 8 only at RDTd. (Figure 1)

Table 1 shows the distribution of symptoms and their association with being a COVID-19. A statistically significant association with fever, anosmia, ageusia, myalgia and anorexia were observed in the model adjusted for age and sex. Adjusting for age, sex and the signs and symptoms statistically associated with COVID-19, the significant association with anosmia (aOR=20.39; 95%CI=4.74-87.73) and fever (aOR=4.33; 95%CI=1.24-15.11) was maintained.

Figure 2 shows that all the patients with fever and anosmia have been positive to some test and in the case of patients with negative RT-qPCR but positive to some RDT, more than 80% (18/22) have either fever or anosmia or both.

Table 2 shows how anosmia (aOR=19.38) and the presence of fever (aOR=6.64) are strong and significantly associated with having COVID-19 in subjects previously tested negative by RT-qPCR.

*aOR: adjusted odds ratio by sex and age.

Discussion

The results of our study reflect that 42.3% of respondents with negative RT-qPCR were positive for some RDT. The symptoms most commonly associated with SARS-CoV-2 infection were fever over 38ºC (present in 35.5% of cases) and anosmia (present in 41.8%), a relationship that remained significant in individuals with negative RT-qPCR and some positive RDT (aOR=6.64; 95%CI=1.33-33.13 and aOR=19.38; 95% CI=3.69-101.89 respectively).

RT-qPCR is the most widely used technique in the diagnosis of SARS-CoV-2 infection, given its high reliability[3]. However, it is not exempt from false negatives, which may be due to tests with fewer genes detected, mild symptoms or very early stage of the disease, upper respiratory tract sample, denaturation of the materials used, delay in transport or processing of the sample[4,5].

Our data show that 42.3% of the subjects analyzed were classified as healthy according to the technique of choice (RT-qPCR), presenting infection after the results obtained in the RDTs. The Spanish Society of Intensive and Critical Medicine and Coronary Units (SEMICYUC) warned a few days ago that false negatives by RT-qPCR can reach 30% in hospitalized patients, referring to a study carried out by Yang et al. in China[8,9]. These results highlight the need for caution in diagnosis, especially in cases of negative RT-qPCR and high clinical suspicion.

With regard to symptoms associated with SARS-CoV-2 infection, fever and anosmia were significant in the false negatives. These symptoms associated with the infection were not surprising, since the beginning of the pandemic, fever, along with coughing and difficulty in breathing were the alarm symptoms. According to data published in a recent review, fever is present in 83-98% of cases, but up to 17% of patients may have afebrile illness[5]. The Centers for Disease Control and Prevention (CDC) has expanded the list of symptoms associated with the disease to include chills, muscle pain, sore throat, and recent loss of taste or smell[10-12]. This last symptom, anosmia, is emerging strongly among the cases detected worldwide, having been described by several studies with a prevalence of between 33-86%[13,14]. Despite not being present in all cases, these are very characteristic symptoms that can arise in the early stage of the disease, so it should be taken into account as clinical suspicion.

The results obtained in this study must be interpreted with caution, being the main limitations the descriptive nature of the study and the low sample size. However, it is one of the first research studies in Spain to highlight the factors associated with false negatives in the choice test in the diagnosis of COVID-19.

Conclusion

The rapid spread of the COVID-19 pandemic requires the rapid generation of knowledge to combat this disease. Our results reflect how those patients with fever and anosmia and negative RT-qPCR, should be considered as suspected COVID-19 cases in clinical practice, given the percentage of false negatives that this test of choice is presenting. Furthermore, it highlights the need to perform a combination of tests to ensure the diagnosis of the disease, such as the use of RDTs.

Conflict of interest

The authors declare no conflict of interest.

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FIGURE TITLES

Figure 1. Results obtained in the different diagnostic tests evaluated.

Figure 2. Presence or absence of fever and/or anosmia according to diagnostic test results

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Table 1. Risk of having at least one positive test according to different signs and symptoms

Signs or Symptoms		N	n	%	aOR*	95% CI	
Cough	No	34	27	79.4	1		
	Yes	76	53	69.7	0.82	0.29	2.30
Expectoration	No	74	54	73.0	1		
	Yes	35	25	71.4	0.95	0.37	2.42
Fever (< 38ºC)	No	39	27	69.2	1		
	Yes	71	53	74.7	1.47	0.59	3.66
Fever (≥ 38ºC)	No	71	46	64.8	1		
	Yes	39	34	87.2	**4.14**	1.35	12.72
Shaking chills	No	49	35	71.4	1		
	Yes	61	45	73.8	1.24	0.51	3.02
Dyspnea	No	64	46	71.9	1		
	Yes	45	33	73.3	1.94	0.73	5.15
Chest pain	No	71	54	76.1	1		
	Yes	39	26	66.7	0.83	0.33	2.07
Headache	No	45	32	71.1	1		
	Yes	65	48	73.9	1.75	0.68	4.51
Nausea	No	90	65	72.2	1		
	Yes	20	15	75.0	1.66	0.51	5.41
Diarrhea	No	62	42	67.7	1		
	Yes	47	37	78.7	2.34	0.90	6.04
Anosmia	No	64	37	57.8	1		
	Yes	46	43	93.5	**19.75**	4.76	82.00
Ageusia	No	59	34	57.6	1		

	Yes	51	46	90.2	**9.92**	3.09	31.87
Sore throat	No	73	54	74.0	1		
	Yes	37	26	70.3	1.00	0.40	2.52
Asthenia	No	28	18	64.3	1		
	Yes	82	62	75.6	2.00	0.74	5.42
Myalgia	No	53	35	66.0	1		
	Yes	56	44	78.6	**2.63**	1.02	6.72
Anorexia	No	69	46	66.7	1		
	Yes	41	34	82.9	**3.28**	1.17	9.16

Table 2. Distribution of factors associated with being a COVID-19 case in patients with RT-qPCR or RDTs negative

	RT-qPCR Negative (N=52)						
	N	**RDTs +**	**%**	**aOR**	**95% CI**		**p**
Sex							
Women	18	9	50.0	1			0.455
Men	34	13	38.2	0.56	0.12	2.56	
Fever (≥ 38ºC)							
No	37	12	32.4	1			**0.021**
Yes	15	10	66.7	6.64	1.33	33.13	
Anosmia							
No	36	9	25.0	1			**<0.001**
Si	16	13	81.3	19.38	3.69	101.89	
Age (years)	46.1±11.3 vs 47.6±8.9			1.04	0.96	1.12	0.319