E-gene RT-PCR crossing point value and other biochemical parameters as useful markers of death risk in COVID-19 patients

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ABSTRACT

The identification of laboratory markers which predict the outcome of COVID-19 patients is a great concern. Real-time reverse transcriptase–polymerase chain reaction (RT-PCR) has been used to confirm the clinical diagnosis. The aim of this study is to evaluate laboratory parameters of COVID-19 patients as well as to evaluate the RT-PCR crossing point (Cp) value and correlate blood test abnormalities and the Cp value with patients survival. Two hundred thirty patients with positive RT-PCR of SARS-CoV-2 were included in the study. Molecular diagnosis of SARS-CoV-2 was performed by RT-PCR (LightMix, TibMolbiol, Germany). Clinical information, biochemical parameters and Cp values were collected in an anonymized database and variables were analyzed with SPSS v25.0 (IBM Corporation, Armonk, NY, USA). No-survivors were significantly older (>65 years old) than survivors

(p=0.007). A higher prevalence of cardiovascular comorbidities in patients who died than in those who survived was found (p=0.002). Statistically significant differences were obtained comparing RT-PCR Cp values for the E-gene of patients who died and those who survived, being lower (<=28) those of patients who died (p=0.004). No-survivors had significantly higher levels of CRP (>100) (p=0.007). E-gene Cp values <=28, which correlate with a high number of copies of SARS-CoV-2, as well as several demographical and biochemical parameters (Age above 65 years old, CRP levels >100 mg/L or cardiovascular comorbidities) could be useful markers of death risk in these patients.

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INTRODUCTION

During the month of December 2019, several cases of pneumonia of unknown etiology were reported in Wuhan (Hubei, China). A novel member of the Coronaviridae family was identified as the causing agent being the seventh member of this family to infect humans (1-3). The novel virus and the disease were named SARS-CoV-2 and COVID-19, respectively. Human to human contacts and respiratory droplets are the main transmission mechanisms of the virus. The outbreak of COVID-19 was declared a Public Health Emergency of International Concern on 30 January 2020 and has put the health authorities on high alert across the world. Until February 11, 2021 the number of SARS-CoV-2 cases has globally reached one hundred six million, and more than two million three hundred thousand people have died (https://www.who.int/, visited February 11, 2021). Specifically in Spain more than three million of SARS-CoV-2 cases have been declared until now, with the Spanish communities of Madrid and Catalonia the most deaths due to COVID-19 (https://www.mscbs.gob.es, visited February 11, 2021).

Clinical features of patients with COVID-19 have been recently described. The most frequent reported symptoms are fever, cough, myalgia or fatigue, and sputum production, headache, haemoptysis, and diarrhoea are less common symptoms. In addition, more than the 60% of patients had lymphopenia and cytokine storm could be related with disease severity (2). The SARS-CoV-2 can also cause severe respiratory illness and other serious complications leading to intensive care unit (ICU) admission and high mortality. Therefore, early diagnosis and treatment of critical cases is decisive (4). Real-time reverse transcriptase–polymerase chain reaction (RT-PCR) of nasopharyngeal swabs has been used to confirm the clinical diagnosis (5). The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recently published several biochemical and haematological parameters for monitoring COVID-19 patients (https://www.ifcc.org). Nonetheless, there is urgent requirement for identification of laboratory biomarkers for progression towards severe and lethal forms of COVID-19 (6, 7).

Therefore, the aim of this study is to assess biochemical and haematological characteristics in the first blood test of patients with positive RT-PCR of SARS-CoV-2 as well as to evaluate the RT-PCR crossing point (Cp) value and correlate blood test abnormalities and the Cp value with patients survival.

1. MATERIALS AND METHODS

Study subjects and data collection

Two hundred and thirty patients with positive RT-PCR of SARS-CoV-2 admitted to the Hospital Universitari Arnau de Vilanova (Lleida, Spain) between March 18, 2020 and April 3, 2020 were included in the study. Clinical information, including biochemical and haematological parameters, from the 230 patients were collected at the earliest time points possible

upon laboratory-confirmed diagnosis of SARS-CoV-2. Permission to conduct the study was approved by the "Hospital Universitari Arnau d e Vilanova de Lleida Ethics Committee".

Clinical and laboratory data

All electronic medical records were checked and demographic data (sex and age), requesting service, comorbidities of patients (cardiovascular disease, immunodeficiency or respiratory disease) and outcomes were included in an anonymized database.

Molecular diagnosis of SARS-CoV-2 was performed in all patients by real time RT-PCR (LightMix, TibMolbiol, Germany) with the Light-Cycler 480 real-time PCR system (Roche, Basel, Switzerland) in nasopharyngeal swabs. Nucleic acid extraction was performed with the automated system Qiasymphony with DSP Virus/Pathogen kit (Qiagen, Hilden, Germany), 60 µL eluate was obtained from 200 µL of the original sample.

RT-PCR crossing point (Cp) is defined as the point at which the fluorescence rises above the background fluorescence (8). The Cp value correlates with the number of copies of the target organism in an exponential and inversely proportional relationship (9). Cp values for the E-gene of SARS-Cov-2 were collected in the database.

All patients had a blood test performed at the earliest time possible upon laboratory-confirmed infection of SARS-CoV-2 which included a complete blood count, serum biochemical test and coagulation profile. Measurements of ferritin, C-reactive protein (CRP), D-dimers (DD), lactate dehydrogenase (LDH), leucocytes and lymphocytes counts were included in the database due to the alteration of these parameters has been previously described as risk factors for severe disease and mortality (7, 10, 11).

Statistical analysis

The anonymized databases were captured and analyzed with SPSS v25.0 (IBM Corporation, Armonk, NY, USA). Categorical variables were described using absolute and relative frequencies. When dealing with continuous variables, mean and standard deviation (SD) were reported. Variables were analyzed using the χ^2-test, and t-Student test or One-Way ANOVA when appropriate. Survival analysis was done by Kaplan-Meier procedure and Log-rank test and, in order to decide which of the variables related to survival are independent risk factors of increased mortality, we applied a Cox›s proportional hazards model. The selected p value for considering differences as statistically significant in all analyses was p<0.05.

2. RESULTS

Clinical data

Two hundred and thirty patients (71 females 159 males) with positive RT-PCR of SARS-CoV-2 were included in the study. The clinical characteristics of the patients are shown in Table 1. The average age of patients was 63.9 years old. The percentage of deaths was 11.3 % (26/230) and the mean age of patients who died was 75.3 years old. Patients who died were older than those who survived (p=0.007) (Table 1).

Thirty eight patients (38/230; 16.5%) required admission in the ICU setting. A 63.5 % of the patients (146/230) had at least one comorbidity: 137 patients (59.6%) presented cardiovascular comorbidities, 28 (12.2%) were obese patients, 18 (7.8%) presented respiratory comorbidities (asthma, COPD, bronchiectasis, etc) and 7 patients (3.5%) were immunosuppressed. A higher prevalence of cardiovascular comorbidities in patients who died than in those who survived was the only significant difference found (*p*=0.002).

Table 1	Clinical characteristics, E-gene Ct value, and biochemical parameters of the COVID-19 patients				

Characteristics of patients		Number of patients (%)	Discharged (n=204)	Deceased (n=26)	*p* value risk**
Gender Nº males Nº females		159 (69.13) 71 (30.87)	141 (69.11) 63 (30.88)	18 (69.23) 8 (30.77)	*p*=0.994 RR=1.004
Age* <=65 years >65 years		122 (53.04) 108 (46.96)	115 (56.37) 89 (43.63)	7 (26.92) 19 (73.08)	*p*=0.007 RR=0.326
Cardiovascular disease*	Yes	137 (59.57)	114 (55.88)	23 (88.46)	*p*=0.002 RR=5.204
	No	93 (40.43)	90 (44.12)	3 (11.54)	
Respiratory disease	Yes	18 (78.26)	17 (8.33)	1 (3.85)	*p*=0.432 RR=0.471
	No	212 (92.17)	187 (91.67)	25 (96.15)	
Immunodeficiency/ Oncologic patients	Yes	7 (3.04)	5 (2.45)	2 (7.80)	*p*=0.145 RR=2.654
	No	223 (96.95)	199 (97.54)	24 (92.31)	
Obesity	Yes	28 (12.17)	24 (11.76)	4 (15.38)	*p*=0.567 RR=1.312
	No	202 (87.83)	180 (88.24)	22 (84.62)	
Clinical laboratory data					
E-gene RT-PCR Ct value * <=28 >28		98 (42.61) 132 (27.39)	80 (39.22) 124 (60.78)	18 (69.23) 8 (30.77)	*p*=0.004 RR=3.031
Serum ferritin (ng/mL) <=400 >400		43 (18.70) 187 (81.30)	40 (19.61) 164 (80.39)	3 (11.54) 23 (88.46)	*p*=0.953 RR=0.567
Serum CRP (mg/L)* <=100 >100		112 (50.22) 111 (49.78)	106 (53.53) 92 (46.46)	6 (24.00) 19 (76.00)	*p*=0.007 RR=0.313
Serum D-dimers (ng/mL) <=243 ng/mL >243 ng/mL		40 (24.24) 125 (75.76)	39 (25.82) 112 (74.17)	1 (7.14) 13 (92.86)	*p*=0.131 RR=0.240

Serum LDH (U/L) <=378 >378	21 (10.29) 183 (89.71)	19 (10.27) 166 (89.73)	2 (10.53) 17 (89.47)	*p*=0.841 RR=1.025
Leucocytes count (x10x9/L)* <=4.8 4.8-10.8 >10.8	44 (19.38) 168 (74.01) 15 (6.61)	39 (19.31) 152 (75.25) 11 (5.45)	5 (20.00) 16 (64.00) 4 (16.00)	*p*=0.045 RR=1.193 RR=0.426
Lymphocytes count (x10x9/L) <0.9 0.9-5.20	85 (37.45) 142 (62.55)	72 (35.64) 130 (62.55)	13 (52.00) 12 (48.00)	*p*=0.157 RR=1.810

*Statistically significant differences.
** RR first line category vs other (for example RR males vs females: RR=(18/159)/(8/71)=1.004).

Crossing point values

The RT-PCR Cp values for the E-gene of the 230 samples were collected and analysed. Cp values ranged from 17.4 to 40. Ninety eight patients (42.6%) showed Cp values below 28 suggesting high levels of virus in these samples. Statistically significant differences were obtained comparing RT-PCR Cp values for the E-gene of patients who died and those who survived (Table 1), being lower (<=28) the Cp values of patients who died (*p*=0.004).

Laboratory findings

Laboratory data obtained in the first blood test of patients is shown in Table 1. Measurements of CRP, serum ferritin, DD, LDH, leucocytes and lymphocytes counts were collected. Due to the retrospective study design, not all laboratory tests were done in all patients. Consequently their role might be underestimated.

CRP levels were obtained in 223 patients. All these patients but five (97.75%) had levels of CRP above the reference range (0-6mg/L) in the first analytic, with a median value of 128.74 mg/L. Compared with patients who survived, those who died had significantly higher levels of CRP (>100) than those who did not die (*p*=0.007) (Figure 1). Leucocytes count was performed to 227 patients. Patients who died showed higher levels of leucocytes (>10.8 x 10x9/L) in the first analytic than those who survived (*p*=0.045).

DD, LDH and serum ferritin were elevated in most cases (Table 1) but no significant differences were found between patients who died and those who survived.

Two risk groups were established scoring several risk factors (1 point each one): laboratory abnormalities (1 point each parameter with values outside the reference rang), age above 65 years, cardiovascular comorbidities and ICU admission. A significant association was found between mortality and patients with more than 5 points in this risk score (*p*=0.008).

In survival the multivariate analysis, using the Cox proportional-hazards model, the E-gene Cp value <=28 (*p*=0.044), CRP levels >100 mg/L (*p*=0.026) and presence of cardiovascular disease (*p*=0.018) remained as independent significant predictors for survival with an adjusted Hazard Ratio of 0.419, 2.855 and 4.303 respectively.

3. DISCUSSION

The identification of laboratory markers which predict the outcome of COVID-19 patients is

a great concern. These predictors are needed for guiding the managed care of COVID-19 patients. To the best of our knowledge, this is the first study in Spain of laboratory markers in the earliest clinical analysis performed upon 230 patients with laboratory-confirmed infection of SARS-CoV-2 which may help identify patients at enhanced risk of dying.

The results of this study show the main differences in clinical and laboratory characteristics between positive SARS-CoV-2 RT-PCR survival patients and those who died. We observed in accordance with the results of a recent Italian study and with a meta-analysis performed including exclusively Asian population data (6, 7) that several laboratory tests as well as certain clinical and demographical characteristics exhibit significant differences in COVID-19 patients who died compared with survival patients.

The lethality rate in our study was 11.3 %, lower than the percentage reported in other studies performed in the United States (19%) and in an area of the north of Spain (13%) (12, 13). On the other hand, the lethality rate observed in Spain as of May 1, 2020 was 8% (14), so our data provide a higher rate than what was observed.

Patients who died were significantly older (> 65 years old), as previously described (12, 15, 16), presented cardiovascular comorbidities and showed CRP levels in the clinical analysis above 100 mg/L. Bonetti et al. reported higher CRP levels in patients who died than in those who did not die (7) and Du et al. described a major risk of mortality in patients suffering from cardiovascular disease (16).

Patients who died presented significantly lower Cp values (Cp < 28; *p*=0.004), which correlate with a high number of copies of SARS-CoV-2.

In contrast with other studies reporting higher levels of serum ferritin, LDH, DD in severe cases of COVID-19 (4, 7, 17) no differences were found in these parameters in the earliest clinical

analysis between patients who died and those who survived, nevertheless the number of participants in those studies was very low.

In conclusion, this is the first report in Spain of abnormalities in clinical laboratory data predicting fatal outcome among positive SARS-CoV-2 RT-PCR patients. Our findings suggest that low E-gene Cp values (<=28) as well as several demographical and biochemical parameters (Age above 65 years old, CRP levels >100 mg/L or cardiovascular comorbidities) could be useful markers of death risk in these patients.

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Conflicts of interest

The authors declare that there is no conflict of interests.

Authors' contributions

# AB and MB have contributed equally.

All authors participated in data interpretation and in writing the manuscript. All authors took responsibility for the decision to submit for publication.

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