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FULL PAPER

Chest computed tomography score, cycle threshold values and secondary infection in predicting COVID-19 mortality

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SUMMARY

This retrospective and observational cohort study investigated chest computed tomography (CT) findings, cycle threshold (Ct) values in RT-PCR of SARS-CoV-2 and secondary infection occurrence to predict prognosis in COVID-19 patients.
At hospital admission, CT findings and Ct values were collected. Microbiology tests performed after 48 hours from hospitalization were reviewed. According to in-hospital mortality, patients were grouped into non-survivors and survivors.
Among 283 patients evaluated, in-hospital mortality rate was 13.8% (39/283). Secondary infection occurrence was 15.2% (43/283). Cut-off values for CT score >13.5 (AUC=0.682 p=0.0009) and for Ct <23.4 (AUC=0.749, p<0.0001) were predictive of death. Super-additive and synergic effects between high CT score plus secondary infection occurrence as well as between high CT score plus low Ct values affecting patient's outcome were observed.
Chest CT score and Ct values in RT-PCR of SARS-CoV-2 could have a combination role for severity stratification of COVID-19 patients.

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INTRODUCTION

Chest computed tomography (CT) imaging and viral nucleic acid testing are standard methods used in coronavirus disease 2019 (COVID-19) (Tenda *et al*., 2020; Waller *et al*., 2020; Di Carlo *et al*., 2021). Radiological investigation emerged as an easier and faster method to identify COVID-19 patients. Chest CT demonstrations of COVID-19 are initially subpleural ground-glass opacity (GGO), and as the disease progresses enlarged GGO with superimposed thickening of interlobular septa (crazy-paving pattern), followed by extensive bilateral consolidation (L. Li *et al.*, 2020; Francone *et a*l., 2020). To date, chest CT scan plays an important role in diagnosis, monitoring, severity stratification, and evaluation of treatment response in COVID-19 patients (Y. Li *et al*., 2020). Chest CT score, calculated on the extent of lobar involvement, is reported to correlate with disease severity and is considered an important tool to predict the outcome (intensive care unit [ICU] admission or death) of COVID-19 patients (Colombi *et al.*, 2020). For each lobe, considering the extent of anatomic involvement, radiologists assign a point from 0 to 5. Chest CT score is calculated as the sum of the individual lobar scores and can range from 0 (no involvement) to 25 (maximum involvement) (Francone *et al*., 2020).
A confirmed case of COVID-19 is defined as a positive result for detecting Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) on a real-time reverse transcription polymerase chain reaction (RT-PCR) assay of throat swab specimens. Cycle threshold (Ct) value refers to the number of cycles needed to amplify viral RNA to reach a detectable level (Argyropoulos *et al*., 2020; Magleby *et al*., 2020). To date,

Key words:
SARS-CoV-2; Bacteria; Fungal; Secondary infection, chest CT score, cycle threshold values, RT-PCR.

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a plausible positive correlation between the amount of detected virus and the degree of COVID-19 pneumonia severity, hypoxemia intensity, risk of death, as well as of hematological, biochemical, and inflammatory alterations was proposed (de la Calle *et al*., 2021; X. Chen *et al*., 2020).
This study investigated chest CT score and Ct values in RT-PCR of SARS-CoV-2 at hospital admission as well as the development of secondary infection during hospitalization in predicting in-hospital mortality in COVID-19 patients.

MATERIALS AND METHODS

Ethics statement

The study was approved by the ethics committee of Policlinico Umberto I, Sapienza University of Rome, Italy (protocol number 298/2020). Data were collected from routine clinical practice and each subject gave written informed consent.

Study design

At the Infectious Disease Unit, Policlinico Umberto I Hospital, Sapienza, University of Rome, Italy, an observational and retrospective cohort study was conducted. From March 4 to May 31, 2020, consecutive COVID-19 patients with a confirmed diagnosis were enrolled.
COVID-19 pneumonia diagnosis was initially assumed based on clinical symptoms, radiological finding of interstitial pneumonia at chest CT, and confirmed by detecting SARS-CoV-2 RNA in nasopharyngeal swab specimens.
For all patients, data concerning demographics (age, gender), epidemiology, comorbidities, laboratory tests, microbiologic results, treatment, and outcomes were collected directly from electronic health records. Acute respiratory distress syndrome (ARDS) was defined according to the 2012 Berlin criteria (ARDS Definition Task Force et al. 2012). Laboratory assessment consisted of complete blood count, C-reactive protein (CRP), D-dimer, lactate dehydrogenases (LDH), serum ferritin and interleukine-6 (IL-6).
COVID-19 patients were stratified according to in-hospital mortality into non-survivors and survivors and the differences in clinical, laboratory, radiological data as well as the occurrence of secondary bacterial and/or fungal nosocomial infections were evaluated.

Imaging findings and CT score

Chest CT scans, performed at patient admission and stored in the institutional picture archiving and communication system (PACS), were retrospectively reviewed. All examinations were performed with dedicated multidetector scanners using standard parameters for a thorax routine and reconstructed with a 1-mm slice thickness with a soft tissue kernel and a lung kernel; coronal and sagittal multiplanar reconstructions were also available in all cases. Main chest CT features of parenchymal involvement, such as the presence of GGO, crazy-paving or parenchymal consolidation, were described (Pan *et al.* 2020). The extent of parenchymal damage was calculated with a semi-quantitative chest CT severity score for each of the five lobes, as follows: 0, no involvement; 1, <5% involvement; 2, 5-25% involvement; 3, 26-50% involvement; 4, 51–75% involvement; and 5, >75% involvement (Francone et al. 2020). The resulting global CT score was the sum of each individual lobar score and (0 to 25).

Detection of SARS-CoV-2 RNA in nasal pharyngeal swab specimens

For all patients enrolled in the study, SARS-CoV-2 RNA extraction from nasopharyngeal swabs was carried out with an automated sample preparation module using a Versant SP 1.0 Reagents kit (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). As previously described (Turriziani et al. 2021), viral RNA was amplified using a real time RT-PCR system (RealStar SARS-CoV2 RT-PCR, Altona Diagnostics) targeting the E and S genes. Ct values were used as an indirect measure of the viral load in the sample.

Microbiological tests

Blood and respiratory samples for microbiological investigation obtained after 48 hours from hospital admission were reviewed. Identification of causative bacterial and fungi bloodstream and/or secondary respiratory infection was carried out and defined as secondary infection during hospitalization.
Specifically, the isolation of BSI causing agents was performed using the automated BacT/ALERT® VIRTUO™ Microbial Detection System (VIRTUO™, bioMérieux Inc., Hazelwood, MO) and BacT/ALERT® 3D Microbial Detection System (3D, bioMérieux Inc). To define the quality of bronchoalveolar lavage sample Bartlett's score was used (Bartlett *et al.,* 1998). Bacterial respiratory infection was defined as the presence of one bronchoalveolar lavage sample for pathogens known to cause respiratory infections.

Statistics

All statistical analyses were performed using GraphPad Prism v.8.4.1 and $p \leq 0.05$ was considered statistically significant. A descriptive analysis of clinical and laboratory tests was performed. Continuous and categorical variables were presented as median (interquartile range [IQR]) and absolute number, respectively. The nonparametric comparative Mann-Whitney test was used for comparing medians between survivor and non-survivor groups. The 2-tailed χ^2 test or Fisher's exact test was used for comparing proportions of categorical variables. The ROC analysis was used for estimating the cut-off levels of the quantita-

tive variables predicting the occurrence of death. The choice was made on the values for which the sum of sensitivity and specificity was maximum.

Survivals were estimated using the Kaplan-Meier method and compared using the log rank test. Multivariate Cox proportional hazard regression was applied to examine parameters such as chest CT score and Ct values at hospital admission, and secondary infection development during hospitalization, on survival. Hazard ratios (HR) and 95% confidence intervals (CIs) are presented.

The synergistic index was calculated according to Rothman, using a logistic regression analysis as follows: $S=[OR_{11}-1]/([OR_{01}+OR_{10}]-2)$, where OR_{11} is equal to the OR of the joint effect of two risk factors, and OR_{10} and OR_{01} are the OR of each factor in the absence of the

Table 1 - *Demographic, clinical and laboratory features of study population*

	all patients (n=283)	*non-survivors (n=39)*	*survivors (n=244)*	*p value*
Male/Female	161/122	16/23	109/134	ns[a]
Age, median (IQR) years	64 (53-77)	77 (67-85)	62 (52-75)	<0.0001[b]
Days of symptoms	6 (4-8)	5.5 (4-6)	6 (4-8)	ns[b]
Days of hospitalization	17 (10-27)	13 (9-23)	18 (11-28)	ns[b]
Severe/non-severe	91/192	25/14	66/177	<0.0001[a]
With/without secondary infection	43/240	16/23	27/217	<0.0001[a]
Comorbidities				
Any	189	35	154	0.034
Hypertension	122	22	100	-
Cardiovascular	59	11	48	-
Diabetes	43	7	36	-
Pulmonary	45	10	35	-
Cancer	26	8	18	-
Renal	14	3	11	-
Symptoms				
Fever	222	32	190	-
Cough	138	17	121	-
Shortness of breath	110	23	87	-
Myalgia or arthralgia	57	5	52	-
Diarrhea	36	2	34	-
Anosmia and ageusia	16	0	16	-
Sputum production	8	0	8	-
Treatment at COVID-19 diagnosis				
Lopinavir/ritonavir	69	8	61	-
Hydroxychloroquine	200	28	172	-
Azithromycin	170	29	141	-
Enoxaparin	130	27	103	-
Corticosteroids	71	14	57	-
Tocilizumab	90	15	75	-
Laboratory findings				
WBC ($x10^9$/L)	5.6 (4.2-7.7)	7.2 (5.1-9.6)	5.5 (4.2-7.5)	0.0069[b]
Neutrophils ($x10^9$/L)	3.8 (2.7-5.8)	4.8 (3.5-8.7)	3.7 (2.6-5.7)	0.0066[b]
Lymphocytes ($x10^9$/L)	1.0 (0.7-1.5)	0.9 (0.6-1.3)	1.0 (0.7-1.5)	ns[b]
NLR	3.8 (2.3-6.6)	5.2 (3.0-14.3)	3.6 (2.2-6.1)	0.0023[b]
CRP (mg/dl)	3.7 (1.1-9.3)	8.4 (3.8 -19.6)	3.1 (0.9-8.4)	0.0001[b]
Ferritin (ng/ml)	480 (261-958)	994 (364-1950)	423 (247-844)	0.0021[b]
LDH (U/L)	262 (203-345)	340 (273-454)	251 (195-325)	<0.0001[b]
D-dimer (μg/ml)	788 (441-1557)	1440 (843-2909)	714 (433-1375)	0.0006[b]
IL-6 (pg/ml)	29 (9-72)	63 (36-83)	26 (7-53)	0.0082[b]
Ct values	27 (22-31)	21.7 (19-26)	27.9 (23.4-31.2)	<0.0001[b]
Chest CT score	12 (8-16)	15 (10-20.8)	12 (7-16)	0.0007[b]

IQR: interquartile range, WBC: white blood cells, NLR: neutrophil/lymphocyte ratio, CRP: C-reactive protein, LDH: lactate dehydrogenase, IL-6: interleukine-6; Ct: cycle threshold; CT: computed tomography; ns: not significant.

[a]: The 2-tailed $\chi 2$ test or Fisher's exact test was used for comparing proportions between non-survivor and survivor groups; [b]: The nonparametric comparative Mann-Whitney test was used to compare medians between non-survivor and survivor groups.

other. The same analysis was carried out using proportional hazards model (Cox regression). A value of S=1 indicates additivity, whereas a value of S>1 indicates super-additivity and synergism (Rothman 1976).

RESULTS

Demographic and clinical characteristics of study population

A total of 283 COVID-19 patients with a median age and interquartile range (IQR) of 64 (52-76) years were enrolled. The median hospitalization period was 17 (10-27) days. According to clinical outcome, 32.8% of the patients developed a severe COVID-19 pneumonia defined by ARDS onset. Overall, mortality was 13.8% and 9.2% of patients were admitted to ICU (median days from hospital admission to ICU admission: 2 [1.4-4.3] days). Demographic and clinical details of study population are shown in *Table 1*.

The blood test evaluation showed higher white blood cells (WBC) and neutrophils counts, a higher neutrophil/lymphocyte ratio (NLR), and a lower absolute lymphocyte count compared to normal range. Moreover, an increase in inflammatory marker levels such as CRP, ferritin, LDH, D-dimer and IL-6 were detected (*Table 1*).

Imaging findings and CT score

Chest CT scans were available for 268 out of 283 patients, 9 of which were not evaluated due to the presence of lobar atelectasis (4/9), pneumothorax (3/9) or previous lobectomy (2/9); main radiological findings and CT score were then analyzed on the remaining 259 patients.

The most common pattern was GGO (94.8%), followed by crazy-paving (68.1%) and parenchymal consolidation (43.4%) (*Figure 1*). At hospital admission, median value for CT score was 12 (7-16). Patients with ARDS showed a higher chest CT score compared to the patients without ARDS (16 [12-20] and 10 [7-15], respectively, p<0.0001).

Detection of SARS-CoV-2 RNA in nasal pharyngeal swab specimens

We collected Ct values in RT-PCR for SARS-CoV-2-positive nasopharyngeal swabs for 231 out of 283 patients. At hospital admission, median (IQR) Ct value for E gene was 28.3 (22.0-30.9), and for S gene was 27.8 (22.0-30.3). Patients with ARDS showed a lower Ct value compared to patients without ARDS (26.3 [21.3-29.8] and 27.5 [23.3-31.3], respectively, p=0.0495).

Bacterial and fungal secondary infection during hospitalization in COVID-19 patients

Among the 283 COVID-19 patients, 15.2% developed a bacterial and/or fungal nosocomial secondary infection. The median time from hospital admission to secondary infection diagnosis was 6 (2-18) days. Positive correlations between secondary infection presence and days of hospitalization (ρ=0.3401, p<0.0001) as well as between secondary infection presence and chest CT score (ρ=0.1703, p=0.007) were found. Otherwise, a negative correlation between secondary infection presence and Ct values in RT-PCR of SARS-CoV-2 (ρ=-0.1319, p=0.0439) was observed.

Among the 283 COVID-19 patients, 9.2% developed a BSI, while 8.1% showed a secondary respiratory tract infection which included ventilator-associated pneumonia (VAP) (5.3%), and hospital-associated pneumonia (HAP) (2.8%). Overall, BSI and secondary respiratory tract infection were observed in seven patients, two of whom died.

Among BSI, only one pathogen was identified in twenty-two patients while more than one in four patients (*Table 2*). *Acinetobacter baumannii* (19.2%) and *Staphylococcus aureus* (19.2%) were the most frequently isolated pathogens (*Table 2*). Among patients with BSI, four patients had peripherally inserted central catheters and 2 of them developed an infection by *Acinetobacter baumannii,* 1 by *Candida albicans* and 1 by methicillin resistant *Staphylococcus aureus* (MRSA). Overall, ten patients (38.5%) with BSI died.

Among VAP, *Candida albicans* (53.3%) and *Pseudomonas aeruginosa* (26.7%) were the most frequently isolated pathogens. Only one pathogen was isolated in 8 patients, while more than one isolated were demonstrated in other seven patients (*Table 2*). Three *Klebsiella pneumonia* were carbapenemase-producing and one of them was also oxacillin resistant. Moreover, an MRSA was isolated. Overall, six patients (40.0%) with VAP died.

Among HAP we identified *Stenotrophomonas maltophilia* (25.0%), *Haemophilus influenzae* (25.0%), *Candi-*

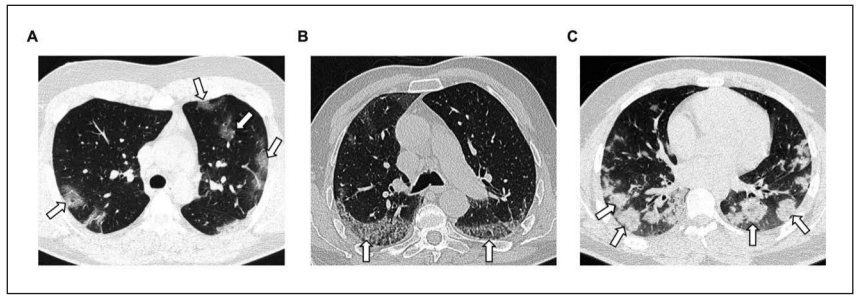

Figure 1 - Chest CT findings in COVID-19 patients. *A) Ground glass opacities (GGO), B) Crazy-paving C) Consolidation.*

da albicans (25.0%), *Pseudomonas aeruginosa* (25.0%) and *Staphylococcus aureus* (12.5%). Only one pathogen was isolated in six patients, while more than one in other two patients was observed (*Table 2*). An MRSA was isolated. Overall, one (12.5%) patient with HAP died.

Baseline laboratory findings according to patient outcome

All 283 COVID-19 patients included in the study were stratified according to in-hospital mortality into non-survivors and survivors. The differences in clinical, laboratory and radiological data at hospital admission were evaluated (*Table 1, Figure 2*). Secondary bacterial and/or fungal infection occurrence during hospitalization was evaluated as well.

Thirty-nine patients died (non-survivor group) while 244 were dismissed alive (survivor group).

In the non-survivor group, age was statistically higher ($p<0.0001$) compared to the survivor group. A higher rate of COVID-19 patients with comorbidity (81.4% vs 64.2%, $p=0.034$) and with severe COVID-19 pneumonia (64.1% vs 27.2%, $p<0.0001$) in the non-survival group compared to the survivor group was observed (*Table 1*). Among the two groups, no significant difference in gender was observed (*Table 1*).

At hospital admission, laboratory findings showed a significantly higher absolute count of WBC ($p=0.0069$), neutrophils ($p=0.0066$) and of NLR ($p=0.0023$), as well as higher levels of CRP ($p<0.0001$), D-dimer ($p=0.0006$), ferritin ($p=0.0021$), LDH ($p<0.0001$), IL-6 ($p=0.0082$) and higher CT score ($p=0.0007$) in the non-survivor group compared to the survivor group (*Table 1, Figure 2*). Conversely, significantly lower Ct values in the non-survivor group compared to the survivor group were observed ($p<0.0001$) (*Table 1, Figure 2*). In the non-survivor group, a lower lymphocytes absolute count was also observed, although not statistically significant (*Table 1, Figure 2*). Finally, a significantly higher rate of secondary infection occurrence in the non-survivor group compared to the survivor group was observed (37.2% vs 9.6%, $p<0.0001$) (*Table 1*).

Given the evidence of high chest CT score and low Ct values in the non-survivor group compared to the survivor group at hospital admission, the Receiving Operator Characteristic (ROC) analysis was performed for each of these parameters. For chest CT score, a cut-off value of 13.5 was found, indicating that at admission chest CT score higher than 13.5 was predictive of death (area under the curve [AUC]=0.682, $p=0.0009$) (*Figure 3A*). Similarly, for Ct values, a cut-off value of 23.4 was found, indicating that the admission Ct values lower than 23.4 was predictive of death (AUC= 0.7486, $p<0.0001$) (*Figure 3B*).

Kaplan-Meier method or Cox proportional hazard model

Survival functions were calculated using the Kaplan–Meier method. The log-rank test compared the survival functions for each variable (chest CT score over 13.5, Ct values under 24.3 and secondary infection occurrence) and from that, hazard ratios (HRs) were calculated. The proportional hazard model was used in Cox multiple regressions. To assess the risk factors associated with death, odd ratio (ORs), hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated, following the Cox proportional hazards model. In our study, when comparing survivals by secondary infection occurrence, a worse prognosis was found for patients who developed a secondary infection,

Table 2 - *Microbiological identification in 43 hospitalized COVID-19 patients*

	43/283
Ventilator-associated pneumonia	15/283
Candida albicans	8
Pseudomonas aeruginosa	4
Escherichia coli	3
Acinetobacter baumannii	2
Klebsiella pneumonia	2
Haemophilus parainfluenzae	1
Stenotrophomonas maltophilia	1
Staphylococcus aureus	1
Hafnia alvei	1
Hospital-acquired pneumonia	8/283
Stenotrophomonas maltophilia	2
Candida albicans	2
Haemophilus influenzae	2
Staphylococcus aureus	1
Pseudomonas aeruginosa	1
Aspergillus fumigatus	1
Acinetobacter baumannii	1
Klebsiella oxytoca	1
Bloodstream infection	26/283
Acinetobacter baumannii	5
Staphylococcus aureus	5
Candida albicans	3
Escherichia coli	3
Klebsiella pneumonia	3
Staphylococcus hominis	2
Enterococcus faecium	2
Pseudomonas aeruginosa	2
Staphylococcus haemolyticus	1
Proteus mirabilis	1
Bacillus cereus	1
Enterococcus faecalis	1
Staphylococcus warneri	1
Candida parapsilosis	1

Some patients showed more than one secondary infection.

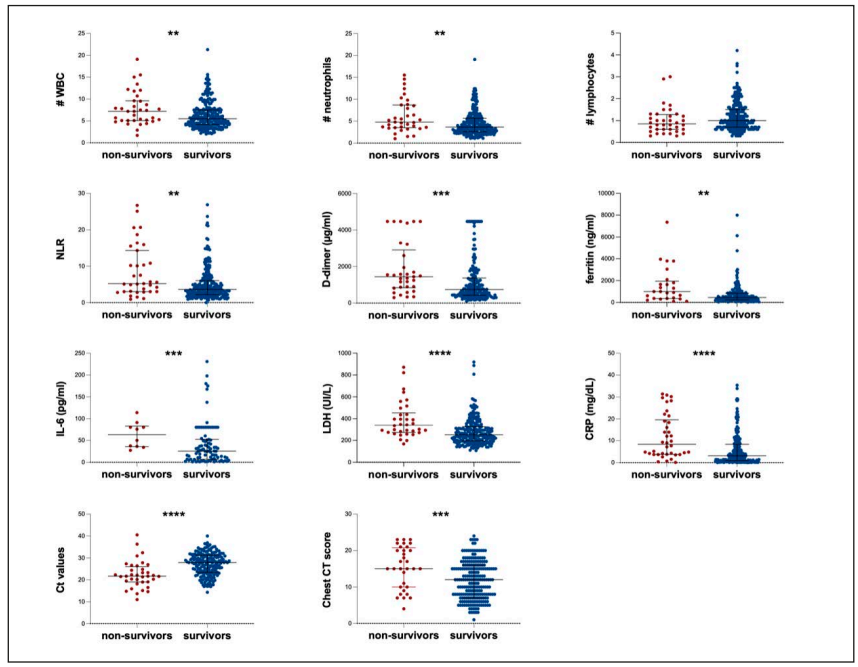

Figure 2 - Evaluation of laboratory findings at hospital admission according to patient outcome. *The number of WBC, neutrophils and lymphocytes was reported as x10⁹/L. WBC: white blood cells, NLR: neutrophil/lymphocyte ratio, IL-6: interleukine-6, LDH: lactate dehydrogenase; CRP: C-reactive protein, Ct: cycle threshold; CT: computed tomography. The nonparametric comparative Mann-Whitney test was used to compare medians between non-survival and survival groups. Data are shown as median (lines) and interquartile ranges (whiskers). **0.01<p<0.001; ***:p<0.001; ****p<0.0001.*

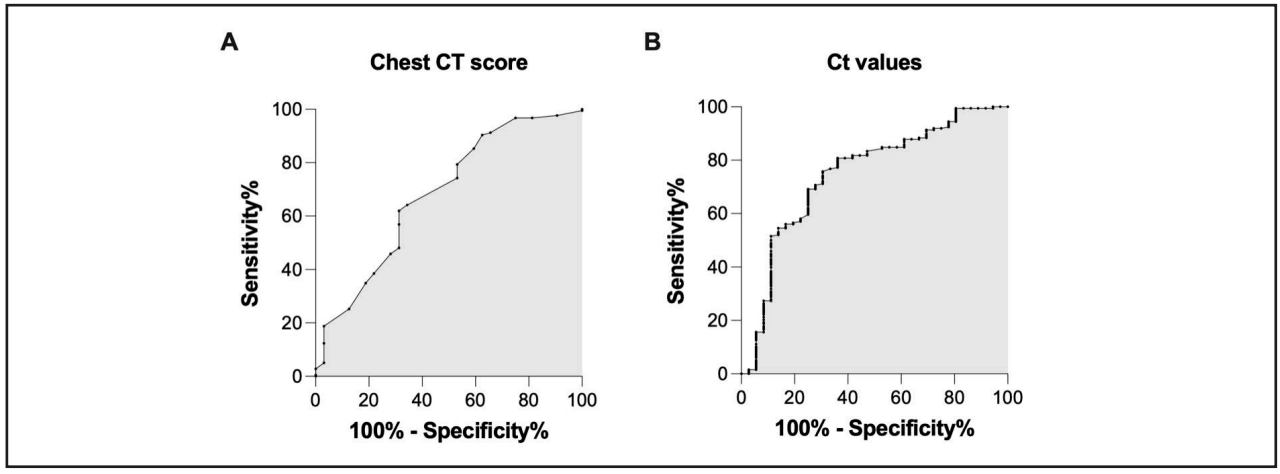

Figure 3 - ROC analysis. *(A) ROC analysis was performed using chest CT score after stratification of COVID-19 patients according to outcome (non-survivors and survivors). The area under the curve is 0.6823 with p=0.0009. The cut-off of >13.5 showed a sensitivity of 61.9% (CI: 55.3% to 68.1%) and a specificity of 68.8% (CI: 51.4% to 82.1%). CI: confidence interval. (B) ROC analysis was performed using Ct values after stratification of COVID-19 patients according to outcome (non-survivors and survivors). The area under the curve is 0.7486 with p=0.0001. The cut-off of <23.4 showed a sensitivity of 75.8% (CI: 69.3% to 81.2%) and a specificity of 69.4% (CI: 53.1% to 82.0%). CI: confidence interval. Ct: cycle threshold; CT: computed tomography.*

with 1.78 (0.09-3.6) times the risk of death, although we did not reach statistical significance (p=0.054) (*Figure 4A*). Similarly, when comparing survivals by chest CT score, a worse prognosis was found for patients with chest CT score over 13.5, with 0.57 (0.32-1.02) times the risk of death, although we did not reach statistical significance (p=0.061) (*Figure 4B*). Finally, when comparing survivals by Ct values, a worse prognosis was found for patients with Ct under 23.4, with 3.7 (1.9-7.2) times the risk of death (p<0.0001) (*Figure 4C*).

Synergistic effect of chest CT score, Ct values and secondary infection development

The joint effect of chest CT score over 13.5 plus secondary infection occurrence, Ct values under 23.4 plus secondary infection occurrence and Ct values under 23.4 plus chest CT score over 13.5, was evaluated. Super-additive and synergistic effects were observed with chest CT score over 13.5 plus secondary infection occurrence as well as with Ct values under 23.4 plus CT score over 13.5 in predicting in-hospital mortality (*Table 3*).

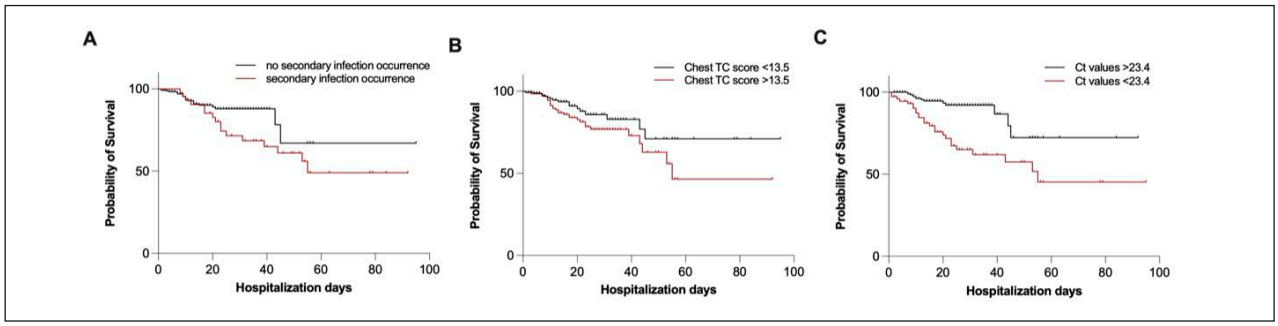

Figure 4 - Survival analysis. *(A) The Kaplan-Meier method and log-rank test were used to investigate the relationship between secondary infection and COVID-19 prognosis. The results indicated that patients with secondary infection had a lower overall survival rate than patients without secondary infection, although not statistically significant (hazard ratio [HR] 1.78 [0.9-3.6], p=0.0535). (B) The Kaplan-Meier method and log-rank test were used to investigate the relationship between CT score and COVID-19 prognosis. The results indicated that patients with CT score over 13.5 had a lower overall survival rate than patients with CT score under 13.5, although not statistically significant (HR 0.57 [0.3-1.1], p=0.0606). (C) The Kaplan-Meier method and log-rank test were used to investigate the relationship between Ct values and COVID-19 prognosis. The results indicated that patients with Ct values under 23.4 had a significantly lower overall survival rate than patients with Ct values over 23.4 (HR 3.66 [1.9-7.1], p<0.0001). Ct: cycle threshold; CT: computed tomography.*

Table 3 - *Synergistic analysis*

Variables	*S**	*Synergistic effect*	*S^*	*Synergistic effect*
Ct values and secondary infection	0.88	(19.74-1)/[(11.28+11.93)-2]	0.43	(4.40-1)/[(3.95+6.03)-2]
CT score and secondary infection	2.31	(15.2-1)/[(4.95+3.20)-2]	1.24	(2.56-1)/[(1.39+1.87)-2]
Ct values and CT score	4.11	(31.42-1)/[(6.04+3.36)-2]	3.06	(5.43-1)/[(1.11+2.34)-2]

Ct: cycle threshold; CT: computed tomography; S*: synergistic index using Odds ratios; S^: synergistic index using Hazard ratios.

DISCUSSION

In this single-center, observational, retrospective study, we investigated whether chest CT findings and SARS-CoV-2 RT-PCR Ct values at hospital admission, and occurrence of secondary infection during hospitalization are factors able to predict mortality in COVID-19 patients. For an early stratification of mortality risk in COVID-19 patients, we evaluated synergistic effects of chest CT findings, Ct values in SARS-CoV-2 RT-PCR, and the onset of bacterial and/or fungal secondary infection.

The role of chest CT for both diagnosis and management of COVID-19 patients during hospital stay is well established (Y. Li and Xia, 2020; Rubin *et al.*, 2020). Furthermore, the use of a semi-quantitative CT score has been validated to assess parenchymal damage of the lungs within COVID-19 related interstitial pneumonia (Pan *et al.*, 2020; Francone *et al.*, 2020) and its potential role for predicting the outcome of COVID-19 patients was proposed (Francone *et al.*, 2020).

On the other hand, Ct values in RT-PCR of SARS-CoV-2 represent an indirect measurements of the amount of viral genetic material and thus may be associated with worse outcomes (Rao *et al.*, 2020). During the SARS-CoV-1 epidemic of 2002, viral load was an important factor in determining disease severity. Consistently, previous reports described increased viral load in respiratory samples of patients with a more aggressive COVID-19 course (Liu *et al.*, 2020; Zheng *et al.*, 2020).

Finally, secondary infection occurrence can be associated with worse outcomes in COVID-19 patients (Zhou et al.,. 2020; Feng *et al.*, 2020), even though it is unclear whether secondary infections definitively worsen COVID-19 patient outcomes. However, historical data from pandemics and seasonal flu suggest that bacterial secondary infections can worsen viral diseases (Chertow and Memoli 2013; Klein *et al.*, 2016; Randolph *et al.*, 2011).

As already reported by other authors (Zhou *et al.*, 2020; Fumagalli *et a*l., 2020; Xu *et al.*, 2020), in our cohort we observed that age and comorbidity were risk factors of worst outcome. We observed significantly higher absolute WBC and neutrophils counts, as well as higher NLR at hospital admission in the non-survival group compared to the survival group. This is in line with other authors who showed that leukocytosis and an increase of NLR are associated with mortality (Zhou *et al.*, 2020; Liang et al., 2020; Neto *et al.*, 2021). Similarly, we observed significantly higher levels of inflammatory markers such as CRP, D-dimer, ferritin and LDH, in the non-survival group

compared to the survival group. CRP, D-dimer, ferritin, LDH and IL-6 are inflammatory markers that are widely studied in COVID-19 and are correlated with severe prognosis or *exitus*, and their predictive role was proposed. An association between mortality and elevated inflammatory markers and coagulation functional indices was first reported in a retrospective cohort study of 138 COVID-19 patients in Wuhan, China (Wang *et al.*, 2020). The authors observed higher D-dimer and IL-6 levels in patients requiring ICU admission, in patients with acute respiratory distress syndrome, and in non-survivors. A second retrospective analysis of 191 patients argued that elevated levels of LDH, ferritin, D-dimer and IL-6 were associated with mortality (Zhou *et al.*, 2020).

The first result of our study was that a significantly higher percentage of secondary infection occurring in the non-survival group compared to the survival group. This is in line with previous studies which showed an association between bacterial and fungal secondary infection in respiratory virus pandemic and poor prognosis (Fu *et al.*, 2020; Morris, Cleary, and Clarke 2017). Indeed, bacterial and/or fungal secondary infection onset during hospitalization may be responsible for rapid deterioration and acute respiratory distress in severe COVID-19 patients. Moreover, patients receiving invasive mechanical ventilation more easily developed secondary infections and had higher mortality (Zhang *et al.*, 2020). The overall rate of secondary infection occurrence in our cohort was consistent with previously reports (Zhou *et al.*, 2020; N. Chen *et al.*, 2020; Huang *et al.*, 2020; Yang *et al.*, 2020).

The second main result of our study was that at hospital admission, we observed higher chest CT scores and lower Ct values in SARS-CoV-2 RT-PCR in the non-survivor group compared to the survivor group. Our results are in line with previously published data concerning chest CT score, suggesting that a high degree of lung damage might affect patient outcome (Francone *et al.*, 2020). Similarly, our data are consistent with the notion that low Ct values are associated with high mortality in COVID-19 patients (Rajyalakshmi *et al.*, 2021; Westblade *et al.*, 2020). Furthermore, in our study we identified cut-off values of chest CT score and Ct values which are associated with worse prognosis. At the time of hospital admission, COVID-19 patients with chest CT values greater than 13.5 and Ct values lower than 23.4 identified were at increased risk of death.

As opposed to other viral illnesses in which the initial viral load has been associated with disease severity (Han *et al.*, 2019; Chu *et al.*, 2004), a consensus has not been reached regarding COVID-19 patients. Several authors have shown that Ct values in RT-PCR of SARS-CoV-2 are not associated with, or do not predict, COVID-19 severity (Argyropoulos *et al.*, 2020; Zheng *et al.*, 2020; Lesho *et al.*, 2020; Zou *et al.*, 2020; Karahasan Yagci *et al.*, 2020; Trunfio *et al.*, 2021). On the other hand, several reports are now appearing in the literature in which a positive association between Ct values in RT-PCR of SARS-CoV-2 and COVID-19 severity or outcomes was proposed. These opposing views may be due to the absence of considering the time between COVID-19 onset and the time of swab collection.

To assess the risk factors associated with death, a worse prognosis was found when comparing survivals by secondary infection occurrence and by chest CT score, although it did not reach statistical significance. Otherwise, when comparing survivals by Ct values, COVID-19 patients with Ct values under the cut-off showed more than three times the risk of death.

Finally, in our study we demonstrated a joint effect of high chest CT score plus secondary infection occurrence or high chest CT score plus lower Ct values that super-additively and synergistically increase the mortality risk in COVID-19 patients.

Our study has several clear limitations. It was limited to a single center, included a modest number of patients, and lacked a validation group. Blood and respiratory samples were not available for all patients; microbiological tests were usually performed only during the clinical worsening of the patient; many of the patients were unable to produce sputum during their admission, and invasive respiratory sampling was restricted to minimize aerosol-generating procedures. Moreover, we used Ct values as surrogate markers for viral load, instead of measuring viral load directly. However, RT-PCR assays of SARS-CoV-2 used in clinical laboratories generate Ct values, not direct viral load measurements, and therefore we believe Ct value results have greater potential to be incorporated into patient care.

Overall, this study supports the combined role that chest CT score and Ct values in RT-PCR of SARS-CoV-2 at hospital admission could have for a precise severity stratification of COVID-19 patients and highlights how the onset of secondary infection could affect patient outcome. Therefore, classifying COVID-19 patients with a high risk of mortality and managing patients by considering risk factors could be helpful in the efficient management of COVID-19 patients. A larger prospective study is needed to validate our findings.

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Conflict of interest

The authors have disclosed that they have no potential conflicts of interest.

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