



Evaluation of 86 Patients Whom Death While Being Followed up with Pre-Diagnosis of Covid-19

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Abstract:The Coronavirus-19 pandemic continues at full speed, and the number of patients who die from Covid-19 is increasing. It was aimed to evaluate the demographic information and laboratory findings of 86 patients who died while being followed up in our hospital with a pre-diagnosis of Covid-19. Identifying these characteristics of deceased patients will be essential to guide clinicians in identifying critically ill patients. Data on demographic information, comorbidities, time from hospitalization to death, molecular test results, thorax CT findings, biochemical findings, culture, antibiotic susceptibility, and the given treatments of the cases were collected from the electronic system Kastamonu Training and Research Hospital. While the RT-PCR test of 21 of the cases was positive, in 9 of the cases, control PCR tests were negative after a while. The CT results of 18 of the 21 initially RT-PCR positive cases were compatible with Covid-19, and the CT result of 3 could not be reached. When the blood test results of the cases were examined, neutrophil increase, white blood cell increase, lymphocyte reduction, and inflammatory markers increase were determined. A total of 43 bacterial growths were found in 21 cases. It has been observed that deaths in patients who were followed up with the pre-diagnosis of Covid-19 generally occur in older people, males, and those with underlying diseases. It was thought that the cause of death could be underlying diseases, pathologies caused by inflammation, and secondary bacterial infections in addition to viral infection. Clinicians should be more careful about elderly patients, patients with secondary bacterial infections, or patients with neutrophilia, lymphopenia.

Keywords:Covid-19; mortality risk factors; lymphopenia.

INTRODUCTION

Covid-19 is a disease caused by a new coronavirus that first appeared in December 2019. It has different characteristics from other coronaviruses, so there are unknown factors about the course of the disease, diagnostic methods, and treatment (Velavan et al., 2020). By 27 August 2020, in the world and Turkey, respectively, 24,021,218 and 263 998 cases; 821 462 and 6209 deaths have been reported (Cakir, 2020).

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The gold standard in diagnosing Covid-19 is the demonstration of viral nucleic acid by reverse transcriptase-polymerase chain reaction (RT-PCR) in samples from the upper and lower respiratory tract (Erensoy S, 2020). Ground glass opacity, flagstone appearance, and consolidation in chest CT of patients with suspected Covid-19 are evaluated as findings in favor of Covid-19. However, although the sensitivity of chest CT is high (67-100%) in the diagnosis of Covid-19, its specificity (25-80%) is low (Kovacs et al., 2020).

Publications are stating that mortality is higher in men, older people, and those with underlying diseases (such as hypertension, coronary artery disease, diabetes, malignancy) (Zhou et al., 2020; Qiu et al., 2020; Zhang et al., 2020; Zhao et al., 2020). The hallmark of severe Covid-19 is the hyperinflammatory host response known as the "cytokine storm," an uncontrolled systemic inflammatory response that results from the release of large amounts of pro-inflammatory cytokines. The inflammation that develops with the cytokine storm starts locally and then spreads to the whole body through the circulation; It causes multiple organ failures, rapidly developing respiratory distress, and death due to extensive tissue damage. Depending on the cytokine storm, lymphopenia has also been reported with increased inflammatory markers such as ferritin, troponin, LDH, CRP, and D-dimer. Sometimes, bacterial infections are also observed in these patients, leading to increased morbidity and mortality (Li et al., 2020; Hu et al., 2020). There are studies on the characteristics of patients who died due to covid worldwide, but such a study from Turkey has not yet been published. We aimed to compare the data of other countries by analyzing the data in our country. This study aimed to evaluate the demographic information and laboratory findings of 86 patients who died while being followed up in our hospital with a pre-diagnosis of Covid-19.

MATERIALS AND METHODS

86 cases who died while being followed up with the pre-diagnosis of Covid-19 in Kastamonu Training and Research Hospital (Kastamonu, Turkey) between 24.03.2020-09.08.2020 were included in the study. This study was approved by the Kastamonu Training and Research Hospital Ethics Committee (Date: 04.12.2020, decision no: 2020-KAEH-143-01.01).

Real-time RT-qPCR method for Covid-19

Viral RNA analysis was performed with real-time quantitative PCR (RT-qPCR) on a nasopharyngeal swab. RT-qPCR test kits for Covid-19 are provided by the Turkey General Directorate of Public Health. The method was studied in line with the recommendations of the Bio-eksen firm. First of all, viral nucleic acid isolation was made to prepare templates. For this, 100 µl of the swab samples received in Viral Transport Medium (VTM) was taken and added to the tube containing 100 µl vNAT (Viral Nucleic Acid Isolation Kit (Bio-Speedy)). The tube was vortexed for 15 seconds at the highest speed. After 5 minutes of incubation at room temperature, it was used as a template.

The reagents included in the Covid-19 RT-qPCR Detection Kit (Bio-Speedy) were prepared according to the company recommendations and distributed to the PCR plate as 15 µL per bowl, 5 µL template was added on it.

The plates were cycled in the C1000 Touch Thermal Cycler CFX96 Real-Time System (BioRad, UK) device once at 45°C for 15 minutes, once at 95°C for 3 minutes; 50 times at 95°C for 5 seconds and at 55°C for 35 seconds. While interpreting the results, the shape of the growth curves obtained in the FAM/HEX channels was examined. Non-sigmoidal curves were considered harmful. If the

threshold value was set to 200 and the calculated threshold cycle number was $38-40 \leq Cq$ according to the lot studied, it was considered positive. When it was above these values, the test was repeated.

Imaging Technique and Radiological Evaluation

All patients underwent CT scanning 16 MSCT Alexion Advance scanner with thick slice 4 mm routinely (Toshiba, 2014, Netherlands). Images were acquired using the following parameters 130 kVp; 100–200 mAs; pitch, 0.75-1.5; and collimation, 0.625--5 mm, FOV 44x44. CT examination was performed without using contrast media. The position of the patients was supine, and the procedure was performed after full inspiration. All images were examined with both lung (width, 1200 HU; Center, -600 HU) and mediastinal (width, 400 HU; Center, 40 HU) windows.

The North American Society of Radiology (RSNA), the Society of Thoracic Radiology, and the American College of Radiology (ACR) classified computed tomography (CT) findings for Covid-19 into four categories (Jaegere et al., 2020). Unlike this, we gathered those whose CT findings were non-specific and had no findings in CT in one group and evaluated the cases in three main groups.

Typical Classical Group (Group I): Ground glass opacity (GGO) has been considered indispensable for every patient in this group. In addition to GGO, consolidation, peripheral distribution, and bilateral involvement were strong findings in favor of Covid-19.

Unidentified (intermediate) Group (Group II): Includes the following findings without GGO, which are only consolidation, central distribution, mosaic perfusion, including airway wall thickening, bronchiectasis, other abnormalities, presence of underlying lung disease such as emphysema or fibrosis, including linear opacities, opacities with rounded morphology, opacities with a reverse halo sign, opacities with a crazy-paving pattern

Non-Covid Group (Group III): This group has ambiguous, non-specific findings; pleural effusion, pericardial fluid, nodules but GGO and GGO plus consolidation is never viewed. In some patients in this group, CT findings were completely normal.

Other data

The patients' culture and antibiotic susceptibility tests were performed using the conventional Vitek (Biomérieux, France) method and evaluated according to Eucast standards. Colistin results were excluded from evaluation according to Eucast standards.

Demographic information, comorbidities, time from hospitalization to death, molecular test results, thoracic CT findings, biochemical findings, culture, and antibiotic susceptibility test results, and the treatments of the cases applied were collected from the hospital's electronic system.

Statistical analysis

Categorical variables are defined using frequencies and percentages, while continuous variables are defined using mean and median values. Continuous measures such as mean (SD) and categorical variables are reported as numbers and percentages (%). SPSS (version 22.0) was used for all analyzes.

RESULTS AND DISCUSSION

Our study observed that 86 (7,35%) of 1169 patients who were followed up with the pre-diagnosis of Covid-19 at that time died, but it was not shown that the cause of death of all these patients was Covid-19.

Of the death cases, 59 (68.6%) were male, 27 (31,4%) were female. The cases are between 35-96; the average age is 72, and the median age is 75. The hospitalization to death of the cases ranged from 0 to 70 days, and the median was six days. The demographic characteristics of the death cases are presented in Table 1.

Table 1: Demographic Characteristics of the Dead Cases

Features		Total	Percent
Age	Average	72	
Subgroups	<40 years	2	%2,32
	40-49 years	5	%5,81
	50-59 years	10	%11,62
	60-69 years	12	%13,95
	70-79 years	29	%33,72
	80-89 years	23	%26,74
	90-96 years	5	%5,81
Gender	Male	59	%68,6
	Female	27	%31,4
Underlying disease	Hypertension	21	%24,4
	CVD ¹	16	%18,6
	DM ²	13	%15,1
	COPD ³	13	%15,1
	Malignancy	10	%11,6
	CKD ⁴	8	%9,3
Comorbidity association	0 Comorbidity	40	%46,52
	1 Comorbidity	21	%24,4
	2 Comorbidity	13	%15,1
	≥3 Comorbidity	12	%13,9

- 1: Cardiovascular disease
- 2: Diabetes mellitus
- 3: Chronic obstructive pulmonary disease
- 4: Chronic kidney disease

Table 2. PCR and CT Results of the 86 Cases Analyzed

	CT RESULT			
	Covid-19 compatible (Group 1) (n = 51)	Intermediate Group (Group II) (n=7)	CT incompatible (Group 3) (n=1)	No CT (n = 27)
PCR (+) (n=21)	9+9 ¹	0	0	3
PCR (-) (n=65)	33	7	1	24
Total	51	7	1	27

¹ PCR positive patients who became negative later

Covid-19 antibody tests of ten cases were examined, and only one of them was found positive. The Covid-19 RT-qPCR result of all the cases whose antibody tests were examined was negative. Looking at the CT results of these cases, in the case with a positive Covid -19 antibody test, BT was found to be compatible with Covid-19. While the CT result of 8 of the cases with negative Covid-19 antibody

tests was compatible with Covid-19, the CT result of one was compatible with the intermediate case.

Laboratory findings of the death cases at the time of admission to the hospital and in the last 24 hours before they died are shown in Table 3. When the blood tables of the cases were examined, respectively, on the day of hospitalization and in the last 24 hours, the neutrophil increase was 63% and 69%, white blood cell increase was 41% and 64%, while lymphocyte reduction was 52% and 45%. When the inflammatory markers of the cases were evaluated on the day of hospitalization and in the last 24 hours, respectively; CRP increase was 97% and 95%, LDH increase was 76% and 84%; D-dimer increase was 87% and 98%; ferritin increase was 41% and 64%; troponin increase was 72% and 91%. There was also an increase in the median values of inflammatory markers.

Table 3. Laboratory Findings of the Cases on the day of Hospitalization and in the Last 24 Hours before They Died.

Biochemical Findings		Findings on the day of hospitalization	Findings of the last 24 hours before death
White blood cell (ul)	Average	11,12x10 ³	16,17x10 ³
	<3,5x10 ³	6 (%8,45)	4 (%5,47)
	3,5-10,5x10 ³	36 (%50,70)	22 (%30,13)
	>10,5x10 ³	29 (%40,84)	47 (%64,38)
Neutrophil	Average	9,44x10 ³	14,18x10 ³
	<1,7x10 ³	2 (%2,81)	2 (%2,77)
	1,7-7x10 ³	24 (%33,80)	20 (%27,77)
	>7x10 ³	45 (%63,38)	50 (%69,44)
Lymphocyte	Average	1,06x10 ³	1,16x10 ³
	<0,9x10 ³	37 (%52,11)	33 (%45,83)
	0,9-2,9x10 ³	32 (%45,07)	37 (%51,38)
	>2,9x10 ³	2 (%2,81)	2 (%2,77)
CRP	Median	103	125
	>5	70 (%97,22)	70 (%95,89)
LDH	Median	344	420
	>248	53 (%76,81)	58 (%84,25)
D-dimer	Median	3,85	7,05
	>0,55	63 (%87,5)	66 (%98,50)
Ferritin	Median	202	573
	E:>336, k:>307	29 (%41,42)	44 (%64,70)
Troponin	Median	42	135
	E:>19, K:>11,6	52 (%72,22)	67 (%91,78)

When the culture results of the death cases were examined, it was found that there was a total of 43 bacterial growth in 21 cases (24.41%). Growing bacteria and clinical specimens with growth are shown in Table 4. Five of these patients had a positive Covid-19 RT-PCR result, one of five became negative during follow-up, and 16 had a negative result. When the resistance profiles of the bacteria grown were examined, 20 of the 43 bacterial growths were caused by the persistent bacteria, and they were from 13 patients in total. It has been observed that treatment options are available for other bacterial growths.

Six (6.97%) of 86 cases were followed up in Covid -19 wards and 80 (93.03%) in intensive care units. The treatments received by the cases from the day of hospitalization to the death of the cases are shown in Table 5. At least one antiviral and hydroxychloroquine was started in all cases during their hospitalization. Antibiotics were given to 98.61% of the cases to treat or prevent secondary bacterial infection. Glucocorticoids were given to 25 patients, and IL-6 inhibitors were given to 12 patients to suppress the severe inflammatory response.

Table 4. Bacteria Grown in Clinical Specimens of Deceased Cases

Bacterial Growth	Respiratory Tract Samples	Blood Samples	Urine Samples	Total
<i>A.baumannii</i>	8	4		12
<i>K.pneumonia</i>	6	1	6	13
<i>E.faecium</i>			2	2
<i>E.coli</i>		1	1	2
<i>P.aeruginosa</i>			1	1
<i>S.aureus</i>		4		4
<i>S.maltophilia</i>		1		1
Total	14	11	10	35

Table 5. Treatments Received by Cases

Treatment	Total (n=72)
Hydroxychloroquine	54 (%75)
Antibiotic	71 (%98,61)
Antiviral	51 (%70,83)
Antifungal	11 (%15,27)
Glucocorticoid	25 (%34,72)
IL-6 inhibitor (Tocilizumab)	12 (%16,6)

In this retrospective study, 86 cases who died while being followed up with a pre-diagnosis of Covid-19 in our hospital were analyzed. Our study observed that men died more frequently, similar to other studies (Zhou et al., 2020; Zhang et al., 2020). Studies link women's resistance to viral infections to the effects of the X chromosome and sex hormones on the immune system, which play an essential role in adaptive immunity (Jaillon et al., 2019). The average age of the dead cases was found to be 72 in our study, and studies are reporting that the average age of the dead cases is between 68 and 75.5, in line with us. (Zhou et al., 2020; Zhang et al., 2020). Chronic diseases increase with aging. Besides, increased age-related functional defects in immunological cells such as T and B cells and excessive production of type 2 cytokines can cause prolonged pro-inflammatory responses, leading to a worse course of possible infections. (Bektas et al., 2017; Chung et al., 2019).

In our study, 53.48% of the cases had comorbidities. In other studies, it was observed that 44.9-100% of the death cases had at least one comorbidity, and the frequency was in the form of hypertension (64-38%), diabetes (18-40%), and coronary heart disease (16-32%); and was compatible with our study (Zhou et al., 2020; Qiu et al., 2020; Zhang et al., 2020; Zhao et al., 2020). In addition to

endothelial dysfunction, a pro-inflammatory state occurs in chronic diseases, weakening the innate immune response. For example, in diabetes, the synthesis of pro-inflammatory cytokines and acute-phase reactants such as interferon-gamma and interleukins is reduced. The host's innate and humoral immune systems are functionally impaired. Besides, metabolic disorders can disrupt macrophage and lymphocyte function, leading to reduced immune function, making individuals more susceptible to disease complications. (Yang et al., 2020). Angiotensin-converting enzyme 2 (ACE2) plays a role in binding and entering SARS-CoV2 into host cells. When the lung samples of patients with severe Covid-19 and comorbidities were examined, a higher presentation of ACE2 was found in these patients than in control subjects (Pinto et al., 2020). These findings may explain why patients with comorbidities experience severe Covid-19.

In our case group, who died while RT-PCR was positive, the frequency of cases proven to have died due to Covid-19 was 14%. When evaluated with CT findings, it is observed that 51 cases are compatible with Covid-19 and all RT-PCR positive cases are in this group. On the other hand, the CT findings of 42 cases, including 9 RT-PCR (+) cases and subsequently became negative, were compatible with Covid-19, while PCR results were negative. Since CT is a low specificity test in the diagnosis of Covid-19 and other respiratory viral agents are not sought, the cause of death of these patients is not clear, and other viral factors may be among the causes. Interpretation is not possible with CT for intermediate cases, non-compliant cases, and those without CT results. Looking at similar studies, in a study that evaluated 85 cases who were clinically diagnosed with Covid-19 and died, it was observed that 38.8% of the cases were positive for the Covid-19 RT-PCR test, and 97.5% of the CT findings were compatible with Covid-19 (Du et al., 2020). In another study, it was stated that 95% of 264 cases were diagnosed with Covid-19 clinically, the Covid-19 RT-PCR test was positive, 88% of CT findings were compatible with Covid-19 (Colombi et al., 2020). The percentage of RT-PCR positivity in these studies was higher than in our study. On the other hand, the percentage of the CT findings of the first study being compatible with Covid-19 was found to be higher than PCR positivity, and in this respect, it was similar to our study. When looking at the results of antibody tests, the case found to be positive is likely to be Covid-19, as the CT finding also supports it, but the cause of death has not been proven.

In our study, the median time from hospitalization to death of the cases was found to be six days. In other studies, the average time from the onset of symptoms to death was found to be 9-22.3 days. (Zhou et al., 2020; Zhang et al., 2020; Li et al., 2020). In our study, the onset of symptoms could not be reached, and the time from hospitalization to death was calculated, explaining the shorter duration.

In this study, changes were also observed in the blood picture of the patients (Table 3). One of them, lymphopenia, is a prominent feature in critically ill patients with Covid-19 infection. Several mechanisms are likely to cause this condition: lymphocytes present the coronavirus receptor ACE2 and can become direct targets for the virus, resulting in direct lymphocyte death; pro-inflammatory cytokines possibly causing lymphocyte apoptosis; Molecules produced as a result of metabolic disorders such as lactic acidemia may lead to inhibition of lymphocytes. The resulting lymphopenia can lead to death by creating a cellular immune deficiency state (Tan et al., 2020). Also, studies have shown that the neutrophil-lymphocyte ratio (NLR) used as an inflammation marker is associated with clinical worsening and

mortality in Covid-19 patients (Liu et al., 2020). Our findings are also consistent with these studies.

When the other laboratory findings of the cases were examined, it was observed that there was an increase in the levels of CRP, LDH, D-dimer, ferritin, and troponin (Table 3). Covid-19 can directly damage the lung epithelium, as a result of which monocytes and macrophages increase, levels of pro-inflammatory cytokines such as interleukin (IL) 6, IL-1, tumor necrosis factor (TNF) α , and IL-8 are exaggerated, and "cytokine storm" may cause. Cytokine storms may play an essential role in the aggravation of the disease, causing severe acute respiratory distress syndrome. CRP, cardiac troponin, D-dimer, and LDH levels, which are the markers stimulated by the release of cytokines, are indicators of tissue and organ inflammation, cardiac damage, coagulation abnormalities, tissue damage, respectively, and have been associated with poor prognosis (Henry et al., 2020; Kiss et al., 2020). Studies have shown that leukocytosis, lymphopenia, and inflammatory markers such as CRP, D-dimer, LDH, and ferritin increase in most deaths, and systemic inflammation leads to lung and multi-organ dysfunction, especially in high-risk patients (Chung et al., 2019). When we look at the inflammatory markers of the cases in our study, it was high on the day of hospitalization and increased more in the process. Based on these findings and the suggestions of other studies, it was thought that some of the patients in the risky group who died might have died due to various organ damage, especially respiratory and heart, and even their failure as a result of inflammation.

When the simultaneous growing culture samples of the deceased cases were examined, it was observed that pan-resistant bacteria were grown in 20 clinical samples belonging to 13 cases. All of these cases have negative RT-PCR results. Considering that clinical samples are in the form of the respiratory tract, blood, and urine in these cases, the cause of death may be a bacterial infection. In studies examining cases hospitalized with the diagnosis of Covid-19, it was observed that bacterial growth was detected in the blood and respiratory samples of the cases, varying between 6.1-37%. In these studies, it was observed that *Acinetobacter baumannii* from gram-negative bacteria and *Staphylococcus aureus* among gram-positive bacteria were the most frequently produced bacteria according to us (Ripa et al., 2020; Yu et al., 2020; Dudoignon et al., 2020; Hugher et al., 2020). 95.34% of the cases in our study died in the ICU. Hospitalization of Covid-19 patients in the ICU is an indication that the disease is getting worse. However, due to the need for invasive treatments in the ICU, co-infection risks increase. Therefore, empirical broad-spectrum antibacterial and antifungal treatments were initiated in these patients. However, broad-spectrum antibiotics can lead to the emergence of multidrug-resistant bacteria. In these patients, steroids are also initiated to alleviate the inflammatory response in the lungs, which increases the risk of secondary infection.

When looking at the studies examining the treatments given to patients with Covid-19, it was found that 63.9-70% of the patients were given antibiotics, 62.4-76% antivirals, and 19-33% glucocorticoids. (Chen et al., 2020; Wong et al., 2020). In our study, it is observed that these drugs were given. It forms the basis of antiviral therapy. Hydroxychloroquine was widely used when the disease first started to appear. Antibiotics and antifungal agents were added against the risk of secondary infection or added for treatment in bacterial growth. Glucocorticoids and IL-6 inhibitors (tocilizumab) were added to protect the patient from the damage of the cytokine storm (Zhao et al., 2020).

As a result, it was thought that the causes of death of Covid-19 patients might be underlying causes, pathologies caused by inflammation, and secondary bacterial infections in addition to viral infection. The study's limitations are that there is no control group from Covid-19 patients living in our study, and the sample size does not include more patients.

CONCLUSION

Most of the patients who died due to Covid-19 are elderly, have an underlying chronic disease, and have increased inflammatory markers. In addition, bacterial growth was detected in some of them, and the cause of death may be due to secondary bacterial infection. It is recommended that clinicians consider these situations when evaluating Covid-19 patients and prioritize the care of critically ill patients accordingly.

CONFLICT OF INTEREST

The authors did not report any conflict of interest related to this article.

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