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Inflammatory and Tissue Damage Biomarkers Progression as Mortality Prognosis in Patients with Covid-19 Disease

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ABSTRACT

Introduction: Metabolic and inflammatory disorders are widely described in COVID-19 disease by SARS-CoV-2, especially in advanced gravity stages, nevertheless, the increase in inflammatory and tissue damage biomarkers could identify patients with higher mortality risk since early disease stages.

Material and Method: A retrospective Cohort study was performed to evaluate the progression pattern and mortality risk of proinflammatory (Ferritin and IL-6) and damage tissue biomarkers seric levels (LDH, CPK, CPK-MB and TnIc) since the beginning of the disease to end outcome considered, in this study, as death or recovery

Results: We evaluated 120 patients with mean age 51±10 years. The CAT study showed in all patients bilateral polished glass lung lesions. The inflammatory and seric tissue injured biomarkers were significantly higher since the beginning of the hospitalization in patients who died compared with patients that survived ($p = 0.01$). The myocardial injury biomarkers (CPK-MB and TnIc) were significantly higher in severe gravity stages of deceased patients compared to patients who survived ($p = 0.001$ and 0.003 respectively) with a global mortality of 38.3% (n = 48). The mortality risk was extremely elevated (RR> 10) when inflammatory and tissue injury biomarkers showed seric levels 2 folds above normal values, showing survival of < 60% after tenth day since the beginning disease.

Conclusion: Since the beginning of COVID-19 disease by SARS CoV2 virus, patients show serum elevation in ferritin, LDH and CPK. This seric values were associated with higher mortality risk, when elevated 2 folds above normal values.

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Introduction

Covid-19 disease by SARS-CoV-2 infection has showed high lethality in all ethnic groups. The clinical characteristics are hypoxemia and hypoxia since the beginning of the disease, in addition with a cytokines storm that produces severe cellular injury, organ failure and abnormal myocardial contractility in severe gravity stages of the disease. (1-11)

The cell oxidative stress abnormalities produced by hypoxia make a tissue energetic imbalance, especially with negative impact in cell calcium homeostasis and in Na+/K+ ATPase bomb. This imbalance modifies the cellular wall integrity which allows enzymes (CPK and LDH) that participate in cell energetic products pathways production, like cell breath cycle in striatum muscle, brain, and heart into the bloodstream. The Creatine Phosphokinase (CPK) participate in metabolic pathway that produce immediate energy in striatum muscle (CPK-MM) and heart (CPK-MB), but in anaerobic conditions the production of Adenosine Triphosphate (ATP) is significantly reduced. The Lactate Dehydrogenase (LDH), abundant in the lung tissue, has a similar effect like that of the CPK, CPK-MM and CPK-MB, participating in energy production, in this case by the pyruvate-lactate reactions (NADH to NAD+). (12-14).

These metabolic disorders and inflammatory conditions described in Covid-19 disease are characterized by an increase in inflammatory and tissue damage biomarkers seric levels, which are logically associated with mortality in high gravity stages, however, it is possible that differences exist between survivors and patients who died, allowing us to identify the mortality risk since early stages of the disease.

Material and Method

The retrospective Cohort study was approved by Ethic, Biosecurity and Research committee with register number 06-183.2020. Patients with COVID-19 disease according with World Health Organization guidelines and positive PCR-RT test were included. Patients with previous Immunological disorders or cardiopathies were excluded.

To identify COVID 19´s disease inflammatory impact between survivors and deaths, as well as mortality risk we registered the following variables from the medical records: Age, sex, comorbidities besides COVID 19 disease, hospital stay, outcome (Recovery or death), seric levels of Ferritin, Interleukin 6 (IL-6), Lactate Dehydrogenase (LDH), Creatine Phosphokinase (CPK), Creatine Phosphokinase MB fraction (CPK-MB) and Troponin I (TnIc) during hospital stay. The biomarkers IL-6, Ferritin and LDH were determined in SIEMENS IMMULITE 1000 immunoassay analyzer; CPK, CK-MB and TnIc in ADVIA 1800 Analyzer with Siemens® Reagents and Calibrators.

Statistical analysis

The variables description was performed with mean and standard deviation. The variables comparison was done with Student's T and Chi2 test. The mortality prognosis with Relative Risk and survival with Kaplan Meier test. A p value < 0.05 and IC_{95} were considered as statistical significance. The analysis was made with SPSS package v26.0 for Windows Operative System (Chicago, Il, USA).

Results

We evaluated 120 patients with mean age 51±10 years. All cases presented dyspnea, tachypnea > 25 bpm, and hypoxemia of 91±1% into 3 days since the beginning of the disease; an imaging study were performed, showing in the computed Axial Tomography (CAT) bilateral polished glass lung lesions. Patients who presented hypoxemia < 90% required ventilatory support, except 5 patients who improved oxygen saturation with reservoir mask at FIO2 > 50%. The aminergic support that was required was between 0.05-0.1 mcgrs/kg/min doses. The comparison groups (Survivors and deaths) do not showed differences in age, sex, and additional comorbidities besides COVID19 disease (Table 1).

The seric levels of proinflammatory (Ferritin and IL6) and tissue injury biomarkers (LDH and CPK) were increased since the beginning of the disease with a significant higher difference in deceased group compared with the survivor group (p = 0.01), as well as during disease progression (p = 0.001). The myocardium injury biomarkers (CPK-MB and TnIc) only showed

significant higher seric levels in deceased group during first and second week in Intensive Care Unit stay (p = 0.001 and 0.003 respectively) (Table 2).

The mortality risk was higher when inflammatory and tissue injury biomarkers were two fold above normal values, presenting with a global mortality of 38.3% (n = 46) and survival < 60% at the tenth day since the beginning of the disease with a mortality risk extremely elevated (RR> 10) (Table 3 and ghraph 1 and 2).

Discussion

In advanced stages of Covid-19 disease the cytokines storm trigger by SARS-CoV-2 infection has a negative impact on the lung tissue, producing hypoxemia and hypoxia. This damage to the lung function has a noxious effect in cardiovascular, neurological, renal, and hepatic function, which is strongly associated with mortality. These organic disorders are characterized by increased seric levels of proinflammatory and tissue injury biomarkers (9). Nevertheless, although these tissue injury biomarkers have been described in Covid-19 disease, they had not been used as prognosis of mortality. This study showed, by its findings, that since the beginning of the disease, identification of seric levels two folds above normal values can help to determine mortality risk.

Table 1. Demographic characteristics patients with Covid-19 disease

	Total	Deaths	Survivors	*p*
Age (years)	51±10	49±11	53±10	0.28
Sex				
Male (n)	40	12	28	*0.23**
Female (n)	10	6	4	*0.43**
Diabetes Mellitus (n)	27	10	17	0.56*
Hipertensión (n)	13	5	8	

p value was calculated with t student test and *X^2

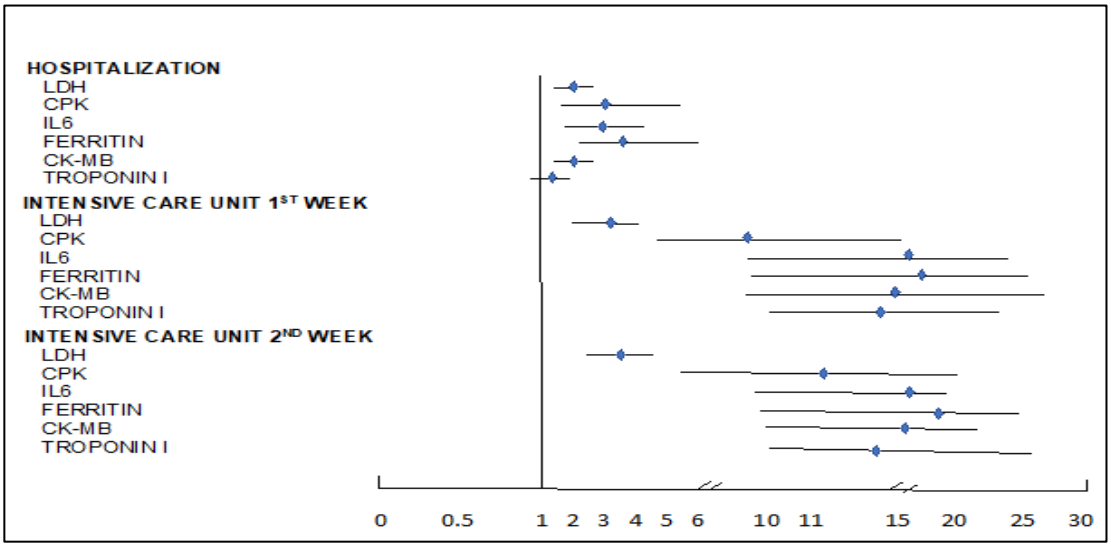
Table 2. Inflammatory and tissue damage Biomarkers Deaths and Survivors patients with Covid-19 disease.

Hospitalization	Deaths	Survivors	p
LDH (U/L)	519±18	303±11	*0.01**
CPK (U/L)	525±146	86.5±25	*0.01**
Interleukin 6 (pg/dl)	1187±32	901±20	*0.01**
Ferritin (ng/dl)	61±5	20±8	*0.01**
CK-MB (ng/dl)	12±5	6±0.5	0.35
Troponin I (ng/dl)	0.05±0.02	0.03±0.01	0.73
ICU 1st week			
LDH (U/L)	523±27	323±10	*0.001**
CPK (U/L)	601±23	260±13	*0.001**
Interleukin 6 (pg/dl)	73±7	19±3	*0.001**
Ferritin (ng/dl)	1558±35	950±15	*0.001**
CK-MB (ng/dl)	40±16	15±5	*0.003**
Troponin I (ng/dl)	3.8±1.2	0.5±0.01	*0.02**
ICU 2nd week			
LDH (U/L)	574±21	360±15	*0.001**
CPK (U/L)	896±23	376±13	*0.001**
Interleukin 6 (pg/dl)	80±7	32±3	*0.001**
Ferritin (ng/dl)	2558±45	1105±12	*0.001**
CK-MB (ng/dl)	97±16	25±2	*0.001**
Troponin I (ng/dl)	8.4±3.5	0.6±1.2	*0.002**

*P value was calculated with t student test, *p<0.05; normal levels: LDH< 300 U/L, CPK< 200 U/L, CPK-MB< 5 ng/dl, Troponin I< 0.1 ng/L, Interleukin 6< 16 pg/ml, Ferritin<300 ng/dl in male and < 150 ng/dl in female.*

In experimental research, it has been observed an increase of proinflammatory cytokines since the first day of infection with SARSCoV-2, showing a strong relationship with acute respiratory distress (3), which match with human Covid-19 disease clinical findings (10-18). In our study we observed increase of proinflammatory biomarkers (IL6, Ferritin) since the beginning of the infection by SARSCoV-2, even when clinical findings were not alarming, adding to the premise that this biomarker can be used to identify mortality risk.

The enzyme LDH converts pyruvate to lactate to produce energy substrates, while CPK catalyzes the transfer of a high-energy phosphate from creatine phosphate, which is the main energy storage reservoir in resting muscle, to adenosine diphosphate to form a high-energy substrate (ATP). In long severe hypoxia conditions, both enzymes are release to blood stream by structural modification of cell wall, this indicates cellular damage in lung and muscle, which have showed a strong association with lung injury findings in CAT study and clinical muscle weakness (19-23).

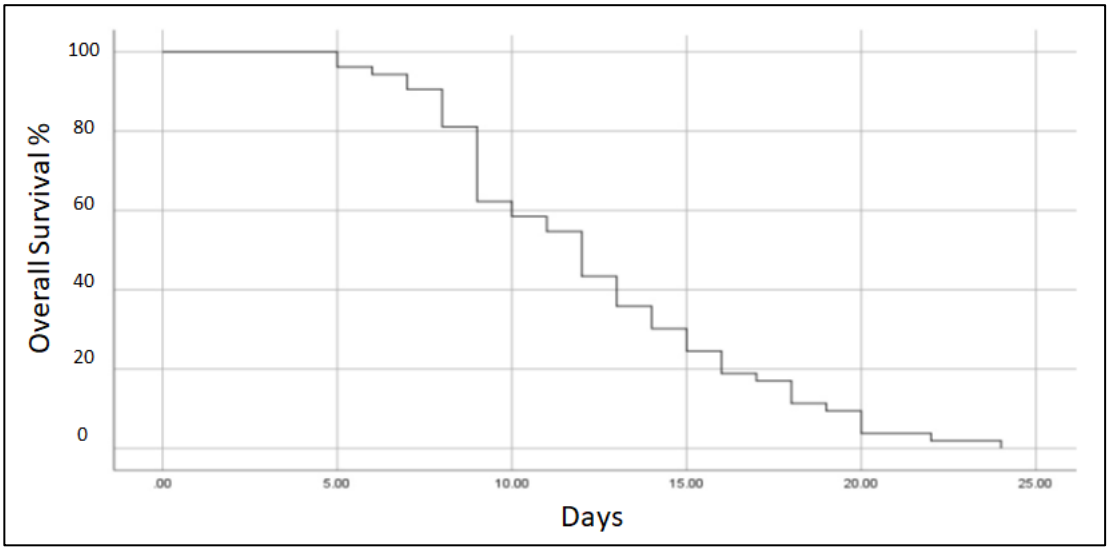


Ghraph 1. Mortality risk from inflammatory and tissue injury biomarkers

Table 3. Mortality risk from inflammatory and tissue injury biomarkers

Hospitalization	RR	CI95
LDH	2.5	1.3-3.1
CPK	3.1	1.7-5.5
Interleucin 6	3.5	1.5-4.9
Ferritin	3.8	2.1-6.7
CK-MB	1.5	1.1-2.0
Troponin I	1.2	0.9-1.8
Intensive Care Unit 1st week		
LDH	3.5	1.9-4.2
CPK	10	4.5-19
Interleucin 6	16	8.5-26
Ferritin	17	7.9-27
CK-MB	15	8.2-28
Troponin I	14	9.3-15
Intensive Care Unit 2nd week		
LDH	3.6	2.3-4.1
CPK	12	5.1-21
Interleucin 6	17	8.9-21
Ferritin	19	8.1-27
CK-MB	16	9.2-23
Troponin I	24	9.1-16

LDH: Lactate Dehydrogenase; CPK: Creatine Phosphokinase; CK-MB: Creatine Phosphokinase MB Fraction. RR was calculated with contingency tables with one freedom degree.



Ghraph 2. Kaplan-Meier survival function of people with COVID-19

It has been described that increase biomarkers seric levels of myocardial injury (CPK-MB, TnIc) are a consequence of SARSCoV-2 infections into the cardiomyocytes. This is known thanks to experimental infection models and necropsy studies of patients with Covid-19 disease, where it has been seen viral infiltration into the cardiomyocytes (25-27). Nevertheless, in many studies are describe that myocardial contractile disfunction is a consequence of cytokines storm and metabolic disorders produced by hypoxia (24, 27). Findings of this study are in accord with our last hypothesis, because the mortality risk was high since ICU entry when patients needed aminergic support and mechanical ventilation, it is logical due to the myocardial injury observed in severe stages of severity, as we observed in this study, however the elevation of these biomarkers of myocardial injury was also observed in surviving patients, but in significantly lower values compared to deceased patients.

In sum, it is evident that proinflammatory (IL6, Ferritin) and tissue injury biomarkers (LDH, CPK) increase since SARS-CoV-2 infection, also when the hypoxemia level is not alarming. Significant differences between the survivors and deceased patients exist since the beginning of the disease and are useful to identify mortality risk when seric levels are two folds above normal seric values, even during the progression of COVID-19 disease. These findings are useful to identify patients that require greater treatment attachment and medic surveillance.

Conclusion

In patients with COVID-19 disease by SARS-CoV-2 infection, the seric levels of IL6, Ferritin, LDH and CPK biomarkers are significative increased in deceased patients compared to survivors and have usage to identify mortality risk since the beginning of the disease when increased twice above of normal values.

Ethical approval

The study was approved by committee of research, Étic and Biosecurity of the institution.

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