Original Research Article

CONVALESCENT PLASMA INFUSION IN ITALIAN HOSPITALIZED PATIENTS WITH SEVERE COVID 19 PNEUMONIA: EVALUATION OF LATE MORTALITY ASSOCIATED FACTORS

Abstract

Background: This was a single center prospective study about factors related with mortality in hospitalized patients with severe COVID-19 pneumonia treated with convalescent plasma (CCP) infusion in Venice Prefecture.

Methods: In this study were enrolled all the (376) consecutive hospitalized patients with severe COVID-19 pneumonia treated with CCP observed from 30/04/2020 to 31/10/2021. At hospital admission, in order to evaluate correlation with prognosis, we recorded demographic data, clinical data, presence of co morbidities, Rx findings, laboratory results. The endpoint was mortality at 30 days.

Results: Using multivariate analysis, considering demographic data and co morbidities four variables emerged as significant independent predictors of 30-day mortality: age>70 years, tobacco smoke, obesity (BMI>30), Diabetes. Considering Patients' clinical characteristics at hospital admission two variables emerged as significant independent predictors of 30-day mortality in this cohort of hospitalized patients with severe COVID-19 Pneumonia: PaO2/FiO2 ratio under 200 and lungs imaging with a score ≥3.

Discussion: Late mortality was investigated in a series of consecutive, hospitalized, patients with severe COVID-19. We therefore believe that any influence linked to the level of expertise of the clinical staff and available technology was minimal.Furthermore, we also tried to reduce, as much as possible, the variables related CCP infusion using plasma with a neutralizing antibody titer≥ 80 and a standardized dose: a 200 mL unit per day for three consecutive days. Moreover, using both a univariate and multivariate analytical approach, numerous demographic variables were considered, relating to comorbidities, all clinical characteristics, to laboratory data; correlating them with mortality at 30 days.

Keywords: COVID-19, Convalescent Plasma, Mortality, Pneumonia, Risk factors.

Introduction

At the end of 2019, a new coronavirus strain was reported in the Chinese province of Wuhan and was named SARS-CoV-2, its subsequent disease was named COVID-19. The rapid spread of this infection in Italy, the first western country with an epidemic pattern, led to a high number of hospitalizations and deaths, and exceeded the response capacity of our National Health System [1,2]. Initially all available antiviral treatments proved to be only partially effective [3,4].Previous data about use of convalescent plasma during the recent Ebola, SARS and MEV outbreaks suggested that patient prognosis can be improved by administering neutralizing antibodies from convalescent subjects [5,6]. Role of convalescent plasma in therapy of COVOD-19 patients is still under debate; after initial studies suggesting a good efficiency of convalescent plasma in reducing mortality in patients with COVID-19,but as reported in table I, furtherreports contradicted these findings [7-14].

Some studies analysed factors affecting morbidity and mortality in hospitalized patients with COVID-19 pneumonia. However, only a few studies were focused on the analysis of risk factors affecting morbidity and mortality in critically ill COVID-19 patients [15-19]. Herein, we present a prospective analysis of the clinical characteristics and the risk factors, which influenced 30-day mortality post hospital admission, in three hundred and seventy-six patients with severe COVID-19 pneumonia.

Materials and Methods

Study Design: This was a single center prospective study about factors related with mortality in hospitalized patients with severe COVID-19 pneumonia treated with convalescent plasma (CCP) infusion. The study was authorized by the Ethics Committee for Clinical Research of the province of Venice with decree N ° 196828 of 15/05/2020.

All the procedures described in the study, and which involved human beings were implemented in accordance with the ethical standards established by the Helsinki Declaration of 1964 and subsequent amendments. Each patient / donor has issued written informed consent for enrolment in the convalescent plasma collection project. Each patient / recipient gave written informed consent for enrolment in the arm that involved the administration of convalescent plasma. No animal studies were performed in the conduct of the study.

In this study were enrolled all the (376) consecutive patients admitted to the ULSS 3 “Serenissima” hospitals with COVID-19 pneumoniafrom 01 April 2020 to 31 October 2021

treatedwith CCP.At hospital admission,we recordeddemographicdata such as gender, age, ethnicity, ABO blood types, body max index (BMI); clinical data such as:oxygen saturation (SpO2) at rest in room-air, partial pressure of oxygen (PaO2) versus fraction inspired oxygen (FiO2) ratio (PaO2/FiO2);comorbidities such as arterial hypertension (AH), diabetes mellitus (DM), dyslipidaemia (DYS), cardiovascular diseases (CVD), chronic kidney diseases (CKD), chronic obstructive pulmonary diseases (COPD), neoplastic disease (ND). Some relevant clinical data were recorded too: O2 saturation at rest in room air (SpO2), partial pressure of oxygen /fraction of inspired oxygen (PaO2/FiO2),tachypnoea with respiratory rate>30 breaths/min, tachycardia with heart rate >90/min, fever >37.5°C, cough, dyspnoea, myalgia and/or arthralgia, lungs involvement at Rx and/or CT scan. Radiological picture and/or chest CT scan has been scored from 1 to 4 as follows: 1) monolateral pneumonia, 2) bilateral pneumonia, 3) ground-glass opacities; 4) pulmonary consolidations showing signs of interstitial disease and/or rapid progression of lung involvement.In addition of these parameters we recorded data of admission and of discharge (or death),admission in intensive care units (ICU), oxygen supplementation, laboratory results, date of CCP infusion, days between symptoms onset and infusion of the first CCP unit.

Data concerning medical therapy administered during hospitalization were obtained from patient's clinical documentation. Unfortunately, data relating to therapy followed at home were not available.

Two scoring systems. Berlin score[15] and quick Admission Sequential Organ Function Assessment (qSOFA) score[16]were adopted with the aim of evaluate severity of patient's clinical status.

Outcomes: The primary outcomes in our cohort CCP treated patients was the overall mortality at 30 days after hospitalization.

Convalescent donors and plasma collection: Selection of CCP donors, plasma collection and processing, performed microbiological assays investigations have already been described [17-19]. In short: males and females aged from 18 to 60 years and with no history of blood transfusions or pregnancies who had recovered after a symptomatic and microbiologically confirmed (positive molecular nasopharyngeal swabs) SARS-CoV-2 infection were recruited. Each donor gave specific written consent to the procedure after receiving adequate information in a confidential interview with a doctor. Their suitability for plasma donation was assessed according to current Italian guidelines and transfusion law.

Plasma (650-700 mL) was collected using latest-generation cell separators (Aurora – Fresenius Kabi), and, after each procedure, was immediately equally divided into three bags (about 200 mL) using a sterile tubing welder. Plasma pathogen reduction was performed with the INTERCEPT processing system (Cerus Europe BV). The units collected were stored at a controlled temperature ranging from - 40°C to - 30°C.

Plasma infusion: Plasma was delivered ready-for-use by the Transfusion Medicine Service to Clinical Units and was administered to patients over 30 to 60 minutes, on three consecutive days, under supervision of the treating physician. Each patient gave specific written consent to the procedure after receiving adequate information in a confidential interview with a doctor [20].

*Statistical Analysis:*Categorical variables are presented as absolute numbers and/or percentages. Continuous parameters are expressed as mean values ± Standard Deviation (SD) or median values with Interquartile Range (IQR) depending on their distribution (parametric or non-parametric). Differences of the studied parameters between survivors and non-survivors COVID-19 patients were evaluated by Wilcoxon Rank sum test for non-parametric data, and the student's t-test for parametric data as appropriate. Logistic regression was used for each studied parameter over the binary outcome (survival/death) in univariate analysis.

The significant predictors for 30-day mortality ($p < 0.05$) at univariate analysis were used for multivariate regression modelling integrating Odds Ratio (OR) with 95% Confidence Intervals (CI). The forward stepwise method was adopted and tested for its goodness of fit by Hosmer–Lemeshow test. Survival analysis was visually presented by Kaplan-Meier curves, while the log-rank test was used to confirm the significance of probability trends for different age groups of COVID-19 patients. All tests were two tailed and considered significant when p-value<0.05. All data were analysed using SPSS 25.0 (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp., Armonk, NY, USA).

Data availability: The data associated with the paper are not publicly available but are available from the corresponding author on reasonable request.

Results

Patient's series: Were considered376patients with severe COVID-19 pneumonia admitted in Hospital between 01 April 2020 and 31 October 2021, with mean age of 69±16 years, 237 (63%) were males. In this patient's series, after four weeks long follow-up, 97 (25.8%) were deceased.

Concomitant therapies: All these patients received antibiotics therapy and non-steroidal anti-inflammatory drugs; almost all received heparin-based anticoagulation (98.1%) and steroids (96.2%). A substantial aliquot received the antiviral drug Remdesivir (78.9%), less frequent was the use of Tocilizumab (9.4%), only 1 (0.3%) received hydroxychloroquine.

Convalescent Plasma Infusion: Each patient received a plasma unit of about 200 mL in three consecutive days. Median time between symptoms onset and CCP administration was 7 days (Interquartile range 4 – 15 days).No statistically significant differencein mortality was observed in patients that received CCP within five days from symptoms onset (22.2% versus 24.1%); nor in median titer of neutralizing antibody: 160 (IQR 80-320)in both in both groups. Among 376 patients treated with CP,mild collateral effects were observed in 4 (0.8%): 3 urticaria and 1dyspnoea.

Univariate Analysis: As reported in Table II, considering demographic data and co morbidities a statistically significant correlation with a poor prognosis was observed for age, tobacco smoke, obesity with a BMI>30, AH, DM, COPD, but not for gender, ABO blood group, Caucasian ethnicity, CVD, CKD. Moreover, as reported in Figure 1, presence of multiple co morbidities, is related with mortality.

As reported in Table III, considered clinical data recorded at hospital admission a statistically significant correlation with a poor prognosis was observed for a PaO2/FiO2 ratio<200, presence of tachypnea (respiratory acts>30/min), tachycardia (over 90 bpm), presence of myalgias and/or arthralgias, lung involvement with an imaging score $\geq$3, the need of high flow O2 supplementation or mechanical ventilation, admission in intensive care units. As reported in Figure 2, presence of more serious lung involvement appears to be associated with a worse prognosis.

As reported in Table IV some laboratory data were recorded at patients' hospitalization. Of these creatin kinase (CK), lactate dehydrogenase (LDH), glucose (GLU), B-type natriuretic (BNP), high-sensitivity cardiac troponin I (hs-TNI), C-reactive protein (CRP), procalcitonin (PCT), fibrinogen (FIB), and D-dimer (DD) correlated prognosis. No correlation between serum concentration and prognosis was observed for total leukocytes count (WBC), lymphocytes (LYM), erythrocytes (RBC), platelets (PLT), haemoglobin (PLT), Alanine aminotransferase (ALT), Alanine aminotransferase (AST), Albumin (ALB), Creatinine (CRE), Blood urea nitrogen (BUN), Total bilirubin (TB), Prothrombin time (PT), activated partial prothrombin time (aPTT).

Multivariate Analysis: In the univariate logistic regression analysis, many variables were related with30-day mortality as reported above. These parameters were included in a multivariate regression analysis.Hosmer–Lemeshow test confirmed that the adopted model was satisfactory with asensitivity of 89.4% and a specificity of 91.3%, with a positive predictive value of 83.7%, and negative predictive value of 92.6% in prediction of mortality. Considering demographic data and co morbidities four variables emerged as significant independent predictors of 30-day mortality in this cohort of hospitalized patients with severe COVID-19 Pneumonia:age>70 years, tobacco smoke, obesity (BMI>30), Diabetes, Kaplan-Meier curves for the four variables were report in figure 3. Considering Patients' clinical characteristics at hospital admissiontwo variables emerged as significant independent predictors of 30-day mortality in this cohort of hospitalized patients with severe COVID-19 Pneumonia: PaO2/FiO2 ratio under 200and lungs imaging with a score≥3. Kaplan-Meier curves for these two variables were report in figure 4.

Considering Laboratory findings, to perform the multivariate analysis we decided to accept several variables under the same definition: BNP and hs-TNI have been merged under the definition of cardiac damage; CRP, PCT and Fibrinogen as indicators of inflammation; arthralgia, myalgia, LDH and CK as indicators of systemic involvement, elevated levels of D-dimer as markers of thrombosis. All these "categories" have a negative correlation with survival as reported in Figure 5.

Clinical indices: Berlin score was assessed in 346 patients with bilateral lungs involvementand quick SOFA score was established in 209 patients admitted to intensive or semi-intensive care units. Both these clinical scores were related witha poor prognosisas reported in Figure 6.

Discussion

In this patient series, we detected a mortality ratio of 25.8%, a prevalence higher than data reported by other authors [21-24]. In the authors' opinion, this high mortality at 30 days can be traced back to the characteristics of considered population consisting of hospitalized patients with moderate-to-severe COVID-19 pneumonia. Severity of clinical pictures in these subjects was confirmed by the high prevalence (55.6%) of patients admitted to ICU.On the other hand, mortality observed in this patient's series was comparable with data reported in patients with moderate to severe ARDS according to the Berlin score [15]. A further data to consider analysing mortality in our patient's series is subjects' age. Indeed, in this study,age of enrolled patients was higher than data reported in other series

[21-24]. Data from literature suggest astrong correlation between age was and mortality. In this study age was identified as an independent mortality risk factor using multivariate analysis, and an age over 70 years seems to correlate with a poor prognosis (Figure 4a). These results were well related with literature data reporting a correlation between age and poor prognosis [2,21-24].

Literature data also suggest that many others demographic variables may be associated with disease severity in COVID-19 patients, i.e.,gender, ethnicity, and ABO blood group [2,25,26],but results obtained in this study did not confirm these conclusions.Regarding the evaluation of the ABO group, we can note that indeed, compared to the general population, the percentage of hospitalized patients in the non-O group appears to be higher than expected considering blood groups distribution in the general population [21,25,26]. As matter of fact, during the second epidemic wave there was, in our area, a massive involvement of the Bangladesh community employed in shipyards in Venice, due to the different distribution of the ABO type in the different ethnic groups, has led to an excess of patients of groups B and AB which has led to some difficulties in the availability of CCP of these specific types.On the other hand, we did not observe differences in mortality in subjects of group O versus subjects of non-O group.

Considering pre-existing conditions and comorbidities in univariate analysis a correlation with 30 days mortality was observed with tobacco smoke, obesity (BMI>30), hypertension, diabetes, respiratory disease. Of these tobacco smoke, obesity (BMI>30) and diabetes were identified as an independent risk factor in multivariate analysis. These variables were related with mortality as reported in figure 4a, 4b and 4c. Our data is in good agreement with literature results reporting that comorbiditiesand pre-existing conditions, such as cardiovascular disease, chronic kidney disease, chronic lung diseases (particularly COPD), diabetes mellitus, hypertension, immunosuppression, obesitypredispose patients to an unfavourable clinical course [27-30].The American College of Cardiology released a clinical bulletin in March 2020, that reported increased case fatality rates for patients with pre-existing conditions than those without pre-existing conditions. Fatality rates were highest for cardiovascular disease (10.5%) compared with diabetes (7.3%), COPD (6.3%), hypertension (6.0%), and cancer (5.6%). In contrast, patients without pre-existing conditions had predicted survival of 90.5% [31].

It is interesting to note how some of the clinical parameters classically associated with the severity of the SARS-CoV-2 infection, such as desaturation (SpO2> 93% in ambient air),

the presence of dyspea, cough, fever, the need for support with O2, were not correlated with outcome in this series of patients. On the other hand, other variables such as the PO2 / FiO2 ratio <200, the need for invasive ventilatory support using a Venturi mask or endo-tracheal intubation, and hospitalization in intensive care units maintained their correlationwith prognosis. In our opinion, this observation is due to the selection of the subjects considered: all hospitalized patients with severe pneumonia.

Considering clinical data recorded at hospital admission PaO2/FiO2 ratio under 200 and lungs imaging with a score ≥3. Kaplan-Meier were independently associated with mortality in multivariable analyses.Two retrospective group of critically ill patients in Italy, reported a median PaO2/FiO2was 160, with higher values in younger patients (164) than in older patients (156) [28,29]. Radiographic features of severe disease imaging modalities are clinically useful in revealing important findings linked to the development of the severe disease [32]. in this study the classification of the severity of pulmonary engagement detected by X-ray or CT scan imaging was classified as previously described [21]and proved to be a good predictor of the outcome as reported in figures 2 and 5b, these results were in good accordance with data from literature [33,34].

Data from literature suggested that some Laboratory parameters may be related with prognosis in patients with COVID-19 Pneumonia, findings commonly associated with worse outcomes include elevated D-dimer levels, C-reactive protein, LDH, and high-sensitivity cardiac troponin I. However, it remains to be proven that these and other biomarkers are in the causative pathway of SARS-CoV-2-related pathobiology [35,36].Considering Laboratory findings, to perform the multivariate analysis we decided to accept several variables under the same definition: BNP and hs-TNI have been merged under the definition of cardiac damage; CRPand Fibrinogen as indicators of inflammation; arthralgia, myalgia, LDH and CK as indicators of systemic involvement, elevated levels of D-dimer as markers of thrombosis.

It is interesting to note how, in our series, the history of previous cardiovascular disease correlated with the outcome while the diagnosis of myocardial damage during SARSW-CoV-2 infection was strongly correlated to a poor prognosis. Data from literature suggest that biomarkers of cardiac dysfunction may be related with COVID-19 severity. elevated troponin (as defined by serum levels >99th percentile) may also be an independent risk factors for in-hospital mortality and predictor of poor prognosis [37,38]COVID-19-related

cardiac complications are often associated with elevations in brain natriuretic peptides (BNP) concentrations [39,40].

In our study we observed a correlation between laboratory data suggesting the presence of an important systemic inflammation (CRP and Fibrinogen) with mortality. Systemic inflammation was related with mortality in COVID-19 patients and elevated CRP, alone or in conjunction with other biomarkers has been proposed as a predictor of COVID-19 severity [35] in another study a positive correlation between high CRP concentration and severely abnormal CT findings has been described [41].

Abnormalities in markers of cellular injury, particularly elevated LDH and CPK such as presence and persistence of severe flu like systemic symptoms (arthralgia and myalgia) have been linked to greater disease severity [42].Our results also seem to confirm the presence of a relationship between markers of cell damage or systemic involvement and mortality in patients with severe COVID-19 pneumonia.

Raised D-dimer concentrations are suggestive for co-existing venous thromboembolisms that may lead to ventilation-perfusion mismatch [28,29]. A study of 343 COVID-19 patients revealed that 12/67 patients with high D-dimer levels on admission died compared with only 1/267 patients who had D-Dimer levels within normal values [43]. In another study, raised D-dimer levels on admission were associated with higher in-hospital mortality [44].Our results also seem to confirm the presence of a relationship between D-Dimer elevation and mortality in patients with severe COVID-19 pneumonia.

In our study, the qSOFA score and Berlin score were also recognized, using univariate analysis, as valuable prognostic tools for mortality risk stratification in patients with COVID-19 pneumonia. Multivariate analysis showed that, in our series, qSOFA score and Berlin score were independently associated with the risk late mortality in patients with severe COVID-19. Pneumonia. the qSOFA score can reflect not only multiple organ failure but also the degree of inflammation and can accurately predict the severity of the patient's disease [45].

The authors are aware that the study has some limitations.First, it is not a polycentric prospective randomized study but a single center prospective study.Secondly, only one primary endpoint (30-day survival) was defined.Thirdly, a high percentage of critically ill patients, hospitalized in intensive care units, was enrolled in both groups, which may explain the high mortality rates observed. Moreover, during the second wave of COVID-19 pandemic in Italy, due to the lack of evidence-based treatment for COVID-19, pre-

hospitalization treatment was not well-defined and probably heterogeneous, although ministerial guidelines recommended "watchful waiting". On that background, patients might have been treated differently before being hospitalized for severe disease, with unknown effect on the clinical outcome. Unfortunately, we do not have any data about the treatments carried out at home before admission to the hospital.

On the other hand, in our opinion, this study presents some interesting aspects. Late mortality was investigated in a series of consecutive patients with moderate to severe COVID-19 pneumonia admitted in hospitals of Venice prefecture. We therefore believe that any influence linked to the level of expertise of the clinical staff and available technology was minimal.Furthermore, we also tried to reduce, as much as possible, the variables related CCP infusion using plasma with a neutralizing antibody titer$\geq$ 80 and a standardized dose: a 200 mL unit per day for three consecutive days. Moreover, using both a univariate and multivariate analytical approach, numerous demographic variables were considered, relating to comorbidities, all clinical characteristics, to laboratory data; correlating them with mortality at 30 days.

Conclusions

As conclusive remarks, in our opinion in order to improve health outcomes, the identification and validation of factors that predict COVID-19 disease progression is vital. Factors including age, comorbidities, immune response, radiographic findings, laboratory markers, and indicators of organ dysfunction may individually or collectively predict worse outcomes. However, the difficulty of predicting COVID-19 disease severity is underscored by the fact that SARS-CoV-2 appears to have tropism for diverse tissues including primarily the respiratory tract but also the brain, endothelium, heart, kidney, and liver. Identification of factors that predict complications of COVID-19 is pivotal for guiding clinical care, improving patient outcomes, and allocating scarce resources.

Ethical Statement: The study was authorized by the Ethics Committee for Clinical Research of the province of Venice with decree N ° 196828 of 15/05/2020.
All the procedures described in the study, and which involved human beings were implemented in accordance with the ethical standards established by the Helsinki Declaration of 1964 and subsequent amendments.
Each patient / donor has issued written informed consent for enrolment in the convalescent plasma collection project.
Each patient / recipient gave written informed consent for enrolment in the arm that involved the administration of convalescent plasma.
No animal studies were performed in the conduct of the study.

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TABLE I: Literature data concerning effectiveness o convalescent plasma in patients with COVID -19 pneumonia

Author	Country	Study desing	Patients	results
Salazar M.R. et alii 2020 [7]	Argentina	Retrospective	Hospital admitted 864 patients and 2298 controls	In hospitalized patients with moderate-severe disease CCP is associated with a decrease mortality
Horby P.V et alii 2021 [8]	UK	RECOVERY randomised, controlled, open-label, platform trial	Hospital admitted 16287 patients	In patients hospitalised with COVID-19, high-titre CCP did not improve survival or other clinical outcomes.
Simonovich V.A. at alii 2020 [9]	Argentina	Plasm Ar double-blind, placebo-controlled, multicenter trial conducted at 12 clinical sites in Argentina	Hospital admitted 228 patients and 105 controls	No significant differences were observed in clinical status or overall mortality between patients treated with convalescent plasma and those who received placebo.
Ferreira-Barreira D et alii 2021 [10]	Various	Meta analysis	hospitalized COVID-19 patients	CCP is a safe and potentially effective therapy for COVID-19, decreasing the mortality rates and promoting a swift viral clearance
Wang Y et alii 2021 [11]	Various	Meta analysis	hospitalized COVID-19 patients	CCP may help patients improve clinical symptoms, clear the virus, and reduce mortality, especially for patients with COVID-19 within ten days of illness.
Begin P et alii 2021 [12]	USA, Canada Bazil	CONCUR-1 Open-label, randomized controlled trial	hospitalized patients with COVID-19:	CCP did not reduce the risk of intubation or death at 30 d in hospitalized patients with COVID-19.
Casadevall A. et alii 2021 [13]	USA	Retrospective	500.000 patients	CCP use in the USA was inversely correlated with COVID-19 mortality.
Luo Wenjing et alii 2021 [14]	Various	Meta analysis	hospitalized COVID-19 patients	CCP appears safe. CCP treated patients have marked reductions in their serum viral load. Patients with severe COVID-19 benefit more from the convalescent plasma transfusion than critical patients, and patients treated in early stage are more likely to survive.

The table shows schematically results of some studies relating to the effectiveness of CCP. In the first column we have reported the name of the first author, the year of publication and the reference for the bibliography, in the second column the country where the study was conducted, in the third column the design of the study, in the fourth column the type of patients considered, the conclusions obtained are summarized in the fifth and last column.

TABLE II: Patient series profile at hospital admission–demographic data and co morbidities

	Overall	%	Survivors	%	Deceased	%	Survivors versus Deceased
Patients	376		279	74,2	97	25,8	--
Age	69±16		65±14		73±11		p<0.005
Males	237	63,0	172	72,6	65	67.2	NS
Caucasian ethnicity	339	90.1	256	91.8	83	85.6	NS
ABO group other than O	233	62,0	169	60,6	64	66,0	NS
Smoke	75,2	20	51	18,3	24	24,7	P<0.05
Obesity BMI > 30	90	24,0	47	16,8	43	44,6	p<0.001
Arterial hypertension	207	55,0	145	52,0	62	63,7	p<0,05
Cardiovascular disease	79	21,0	54	19,4	25	25,7	NS
Chronic kidney disease	34	9,0	21	7,5	13	13,2	NS
Dyslipidemia	94	25,0	73	26,2	21	21,6	NS
Diabetes mellitus	83	22,0	49	17,6	34	34,8	P<0.05
Respiratory disease	26	7,0	15	5,4	11	11,7	p< 0.05
Cancer	30	8,0	21	7,5	9	9,4	NS

As reported in this table, among the deceased patients compared to the survivors we observed a more advanced mean age (73 ± 11 versus 65 ± 14 years, p <0.005); a higher frequency of current or previous smokers (24.7% versus 18.3%, p <0.05); a higher frequency of subjects with BMI <30 (44.6% versus 16.8%, p <0.001); a higher percentage of subjects with arterial hypertension (63.7% versus 52.0%, p <0.05); a higher percentage of subjects with diabetes (34.8% versus 17.6%, p <0.05); a higher frequency of subjects with chronic obstructive pulmonary disease (11.7% versus 5.4%, p <0.05).We found no statistically significant differences regarding the distribution by gender, ethnicity, ABO group, presence of cardiovascular disease other than hypertension, chronic kidney diseases, dyslipidemia, current or previous cancer disease.

Figure 1: Comorbidities and prognosis

Figure 1a: Distribution of number of comorbidities in deceased ad survivors patients

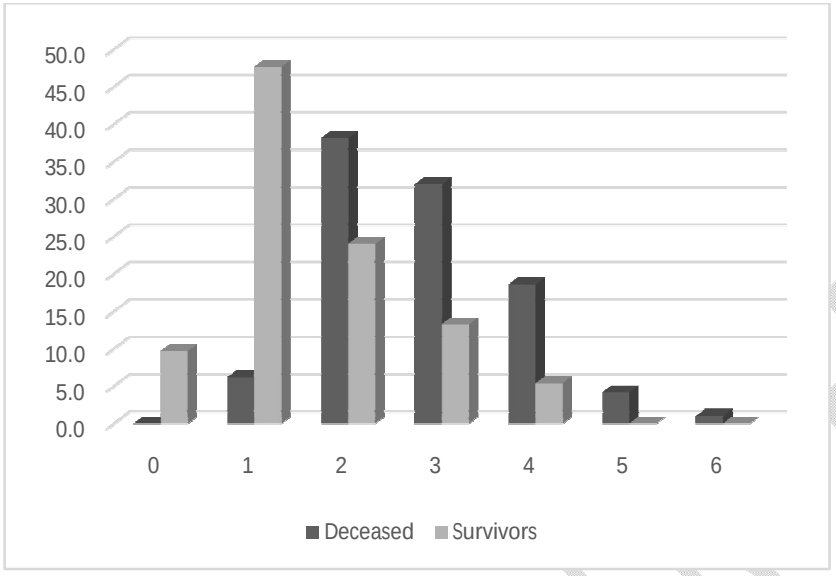
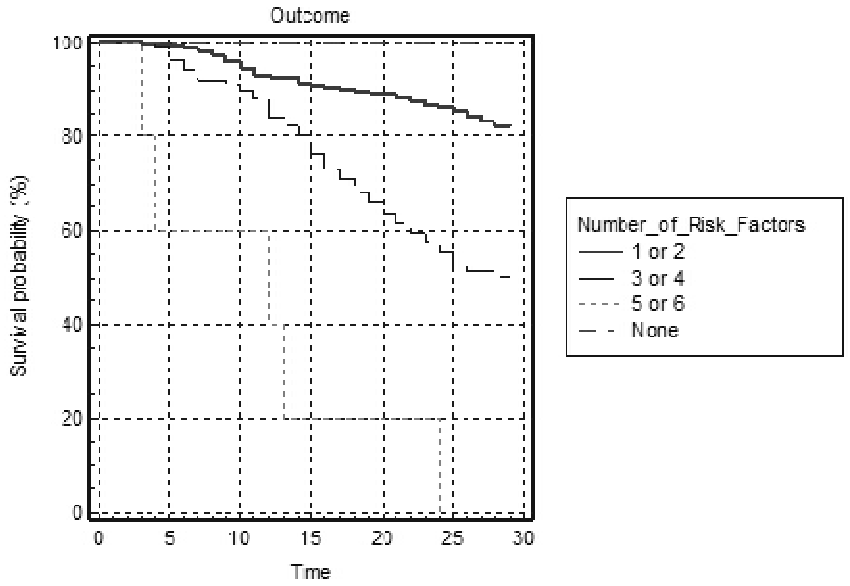


Figure 1b: Number of comorbidities and survival



As reported infigure 1a, among the surviving patients 9.7% had no co-morbidities, 47.7% only one, 24.0% two, 13.3% three, 5.4% four and none five or six co morbidities. On the other hand, among the deceased patients, none had no co morbidities, 6.3% only one, 38.1% two, 32.0% three, 18.6% four, 4.1% five and 1% six comorbidities. In figure 1b we reported the survival curves observed in patients without, 1 or 2, 3 or 4 and 5 or 6 risk factors.

TABLE III: Patients' clinical characteristics at hospital admission

	Overall	%	Survivors	%	Deceased	%	Survivors versus Deceased
Patients	376	100	279	100	97	100	
SpO2 < 93%	349	92,8	256	91,8	93	95,9	NS
PaO2/FiO2 < 200	259	68,9	165	59,1	94	96,9	P<0.001
Dyspnoea	376	100	279	100	97	100	NS
Tachypnea (RR > 30/min)	203	54,0	117	41,9	86	88,7	p<0.005
Tachycardia (> 90 /min)	241	64,1	153	54,8	88	90,7	p<0.001
Cough	376	100	279	100	97	100	NS
Fever > 37,5	376	100	279	100	97	100	NS
Myalgia / artralgia	288	76,6	201	72,0	87	89,7	p<0.005
Lung involvement (Rx / CT scan)	376	100	279	100	97	100	NS
Imaging score ≥3	301	80,1	212	76,0	89	91,8	p<0.005
O2 supplementation	376	100	279	100	97	100	NS
High flow oxygen therapy or mechanical ventilation	249	66,2	177	63,4	72	74,2	p<0.05
Admission in Intensive care Units	209	55,6	131	47,0	78	80,4	p<0.001

As reported in the table, the univariate analysis made it possible to highlight some clinical parameters that correlate with a poor prognosis: the ratio Pa = 2 / FiO2 <200 (p <0.001), presence of tachypnea with RR> 30 / min (p <0.005) and tachycardia bpm> 90 (p <0.001), presence of myalgia and arthralgia (p <0.005), imaging score> 3 (p <0.005), need of high flow oxygen therapy or of mechanical ventilation (p <0.05), admission in intensive care unit (p <0.001). Many other clinical parameters, on the other hand, do not show any correlation with prognosis such as an SpO2 <93%, presence of dyspnea, presence of cough or fever> 37.5 ° C, X-ray and / or CT scan demonstrating pulmonary involvement, the need for supplementation with O2.

Figure 2:Lung imaging score in deceased and survivor patients

The figure shows the association between lung imaging scoring and prognosis. It can be appreciated how the more compromised pulmonary pictures are associated with a poor prognosis.

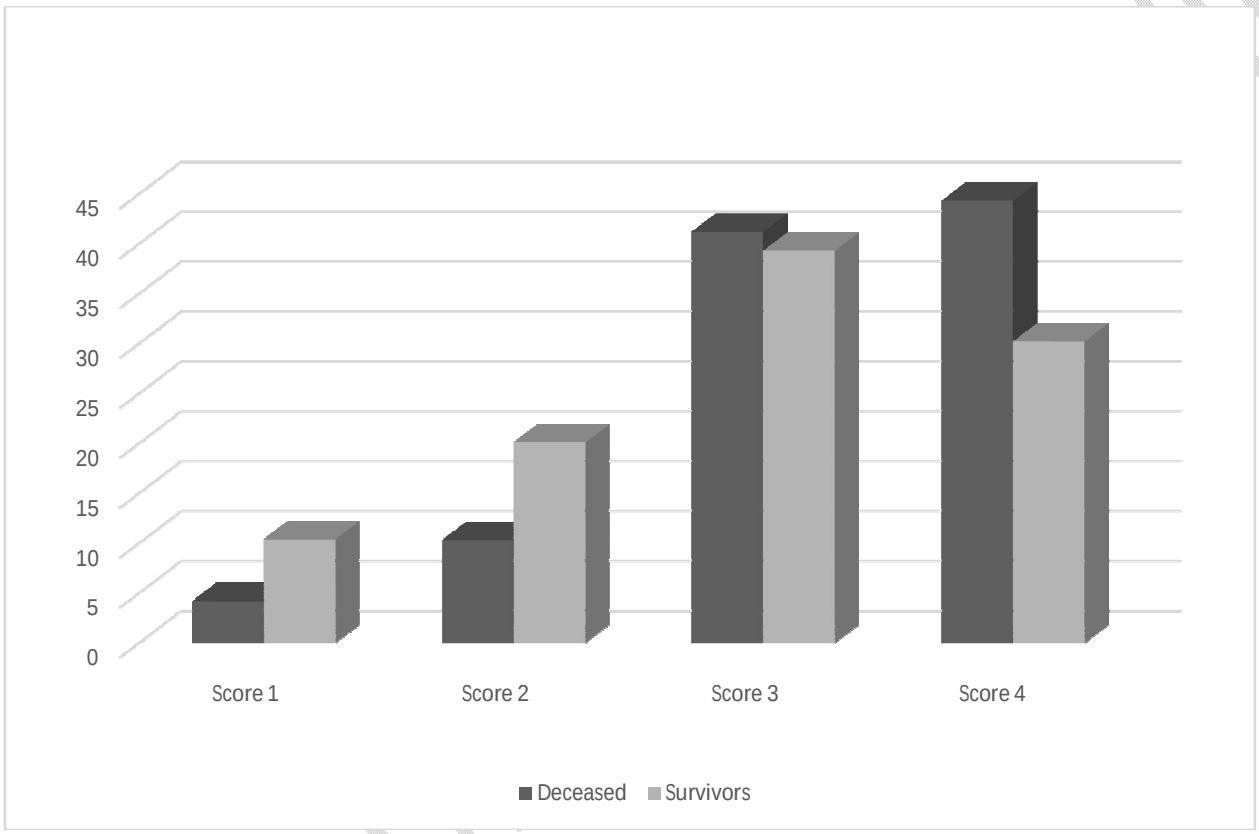


Table IV: Laboratory findings

	Reference Values	Tested N°	Survivors Mean±1SD	Deceased Mean±1SD	Alive versus Deceased
Patients		376	279	97	
Leukocyte (109 /L)	3.5–10.5	376	8.1±2.4	9.5±3.8	NS
Lymphocyte (109/L	1.1–3.5	376	2.1±0.7	2.2±09	NS
Platelet (109 /L)	200–400	376	225±102	195±155	NS
Hb (g/L)	12.5-15.0	376	12.7±1.1	13.1±1.4	NS
Alanine aminotransferase, U/L	9–50	376	34±8	41±11	NS
Aspartate aminotransferase, U/L	15–40	376	22±16	37±3	NS
Alanine aminotransferase,	60-190	376	176±39	201±57	P<0.05
Creatine kinase U/L	120-250	376	208±156	307±164	P<0.05
Lactate dehydrogenase U/L	4.0–5.5	376	4.1±05	4.2±0.6	NS
Albumin, (g/L)	44–97	376	72±11	81±18	NS
Creatinine, mmol/L	2.8–7.2	376	4.9±1.2	5.5±1.4	NS
Blood urea nitrogen, mmol/L	3.9–6.1	376	7.4±1.1	9.3±1.3	P<0.05
Glucose, mmol/L	0.00–125.0	173	87±25	101±62	P<0.01
B-type natriuretic peptide, pg/ml	0.00-0.04	107	0.02±0.01	0.17±0.09	P<0.001
High-sensitivity cardiac troponin Ing/mL	0–20	376	13±7	15±9	NS
Total bilirubin, mmol/L	0.1–5.0	376	21±15	34±19	P<0.05
C-reactive protein, mg/L					

Procalcitonin, ng/mL	0-0.5	209	0.77+0.31	1.13+0.85	P<0.001
Prothrombin time, sec	11.5–14.6	376	13.3±9	14.1±8	NS
aPTT, sec	25-31	376	30.5±12.8	32.8±11.7	NS
Fibrinogen, g/L	2.00–4.00	376	4.95±1.98	6.12±2.73	P<0.01
D-dimer, FEU mg/L	0.00–0.50	376	0.71±0.48	1.01±0.74	P<0.005
Quik SOFA Score	>2	209	41.9%	52.6%	P<0.05
BERLIN Score	Severe	346	42.7%	54.8%	P<0.01

As reported in this table, we recoded some laboratory data at patients hospitalization. Of these creatin kinase ($p<0.05$) lactate dehydrogenase ($p<0.05$), Glucose ($p<0.05$), B-type natriuretic peptide ($p<0.01$), High-sensitivity cardiac troponin I ($p<0.001$), C-reactive protein ($p<0.05$), Procalcitonin ($p<0001$), Fibrinogen ($p<0.01$) and D-dimer ($p<0.005$) correlated with a poor prognosis. No correlation between analytes serum concentration and prognosis was observed for total WBC, lymphocytes, erythrocytes, platelets, haemoglobin, Alanine aminotransferase, Alanine aminotransferase, Albumin, Creatinine, Blood urea nitrogen, Total bilirubin, Prothrombin time, aPTT.

Figure 3: Survival curves and demographic data and co morbidities

Figure 3a: Age over 70 years

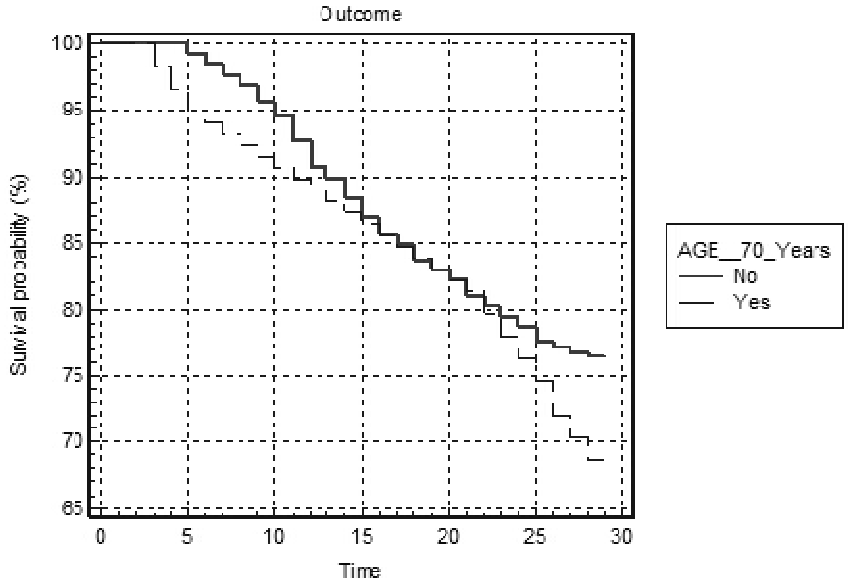


Figure 3b: Obesity with a BMI over 30

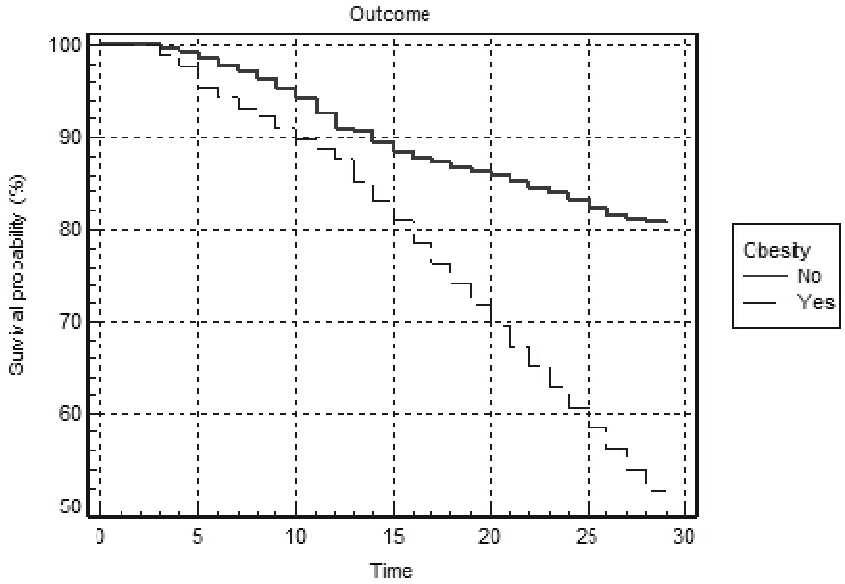
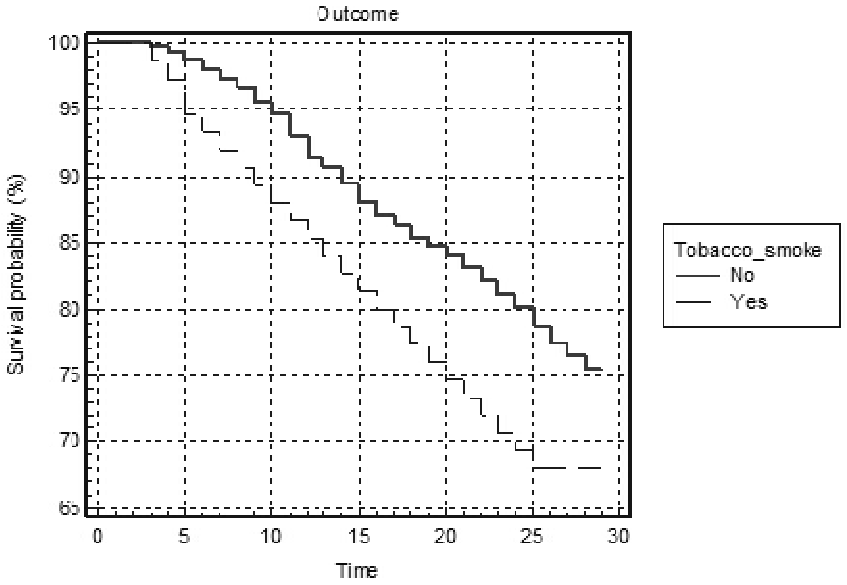


Figure 3c: Tobacco smoke

Figure 3d: Presence of diabetes


Outcome
Survival probability (%)
100
95
90
85
80
75
70
65
0
5
10
15
20
25
30
Time
Tobacco_smoke
No
Yes

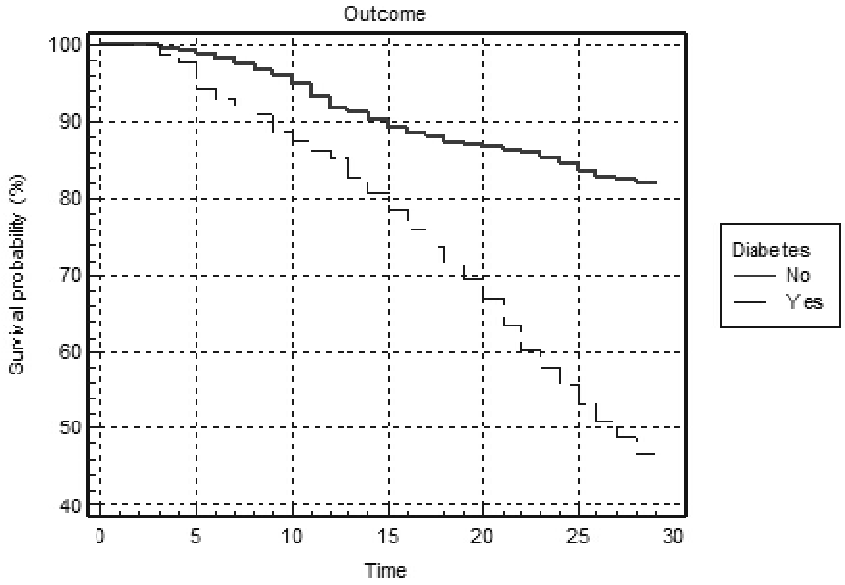

Outcome
Survival probability (%)
100
90
80
70
60
50
40
0
5
10
15
20
25
30
Time
Diabetes
No
Yes

Figure 4: Survival curves and Patients' clinical characteristics at hospital admission

Figure 4a: PaO2/FiO2 under 2000

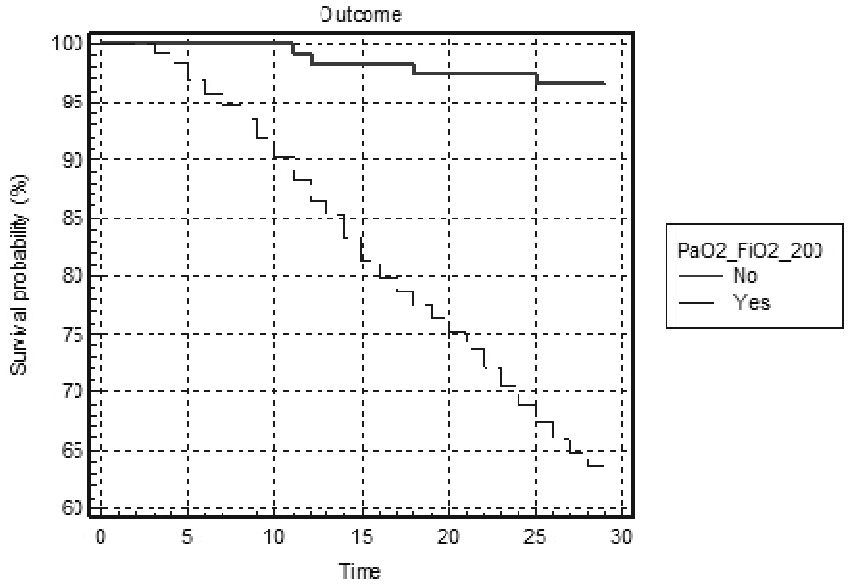


Figure 4b: Lungs imaging score

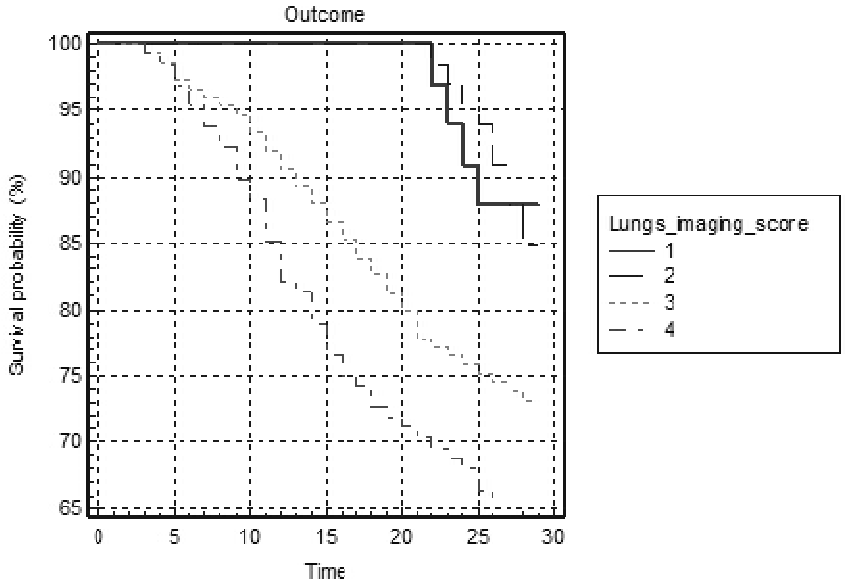


Figure 5: Survival curves and Laboratory findings

Figure 5a: Cardiac damage

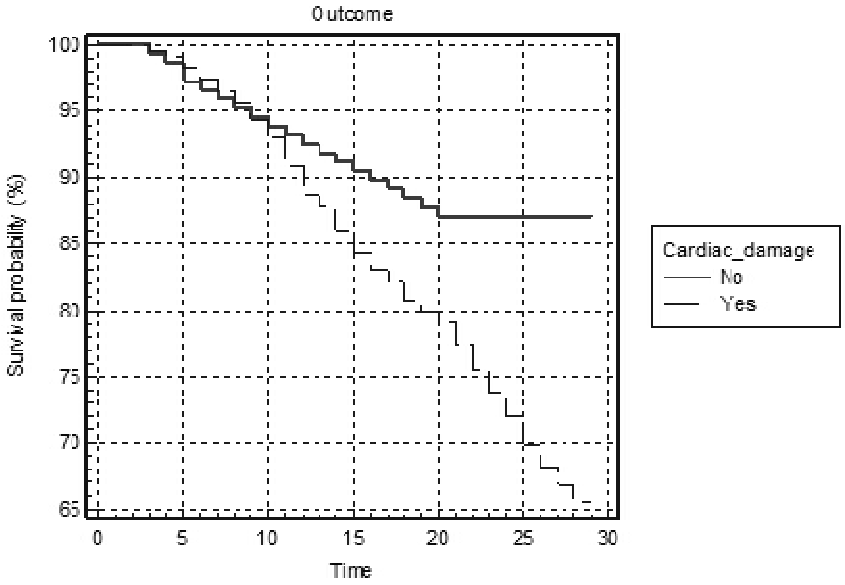


Figure 5b: inflammation

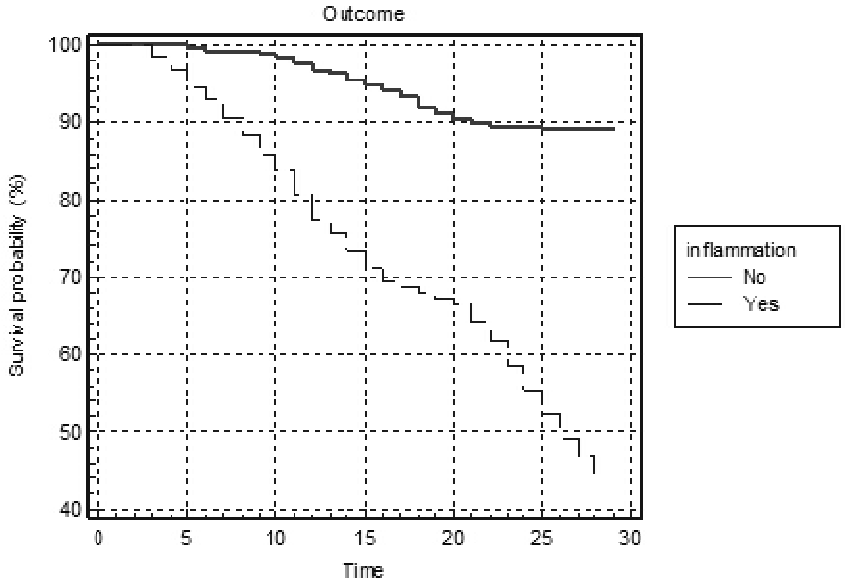
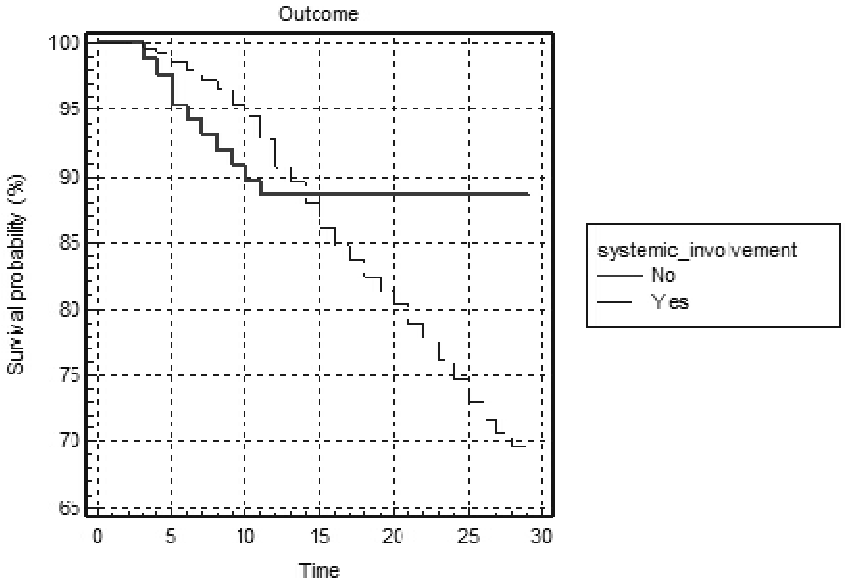


Figure 5c: Systemic involvement

Figure 5d: Thrombosis


Outcome
Survival probability (%)
100
95
90
85
80
75
70
65
0
5
10
15
20
25
30
Time
systemic_involvement
No
Yes

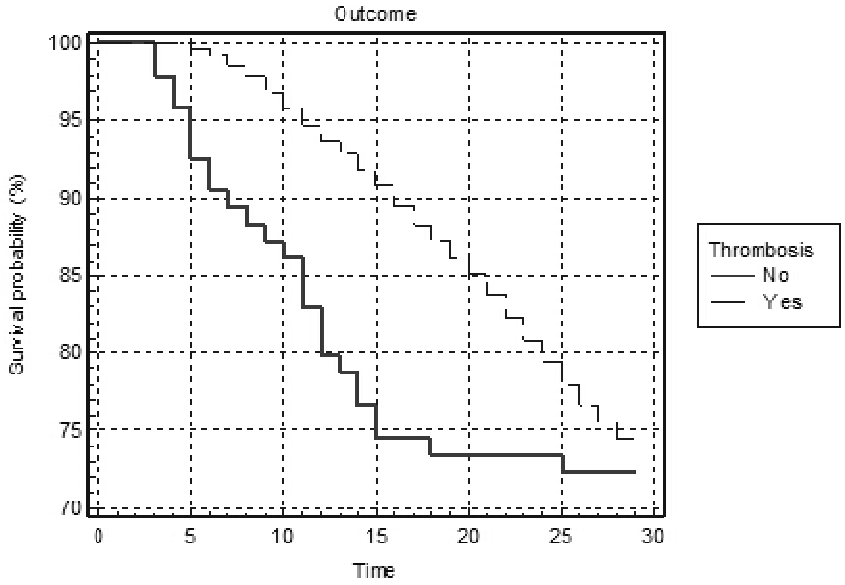

Outcome
Survival probability (%)
100
95
90
85
80
75
0
5
10
15
20
25
30
Time
Thrombosis
No
Yes

Figure 6: Berlin score and quick SOFA scoredistribution in deceased and survivors patients

Figure 6a: Berlin Score distribution in deceased and survivors patients

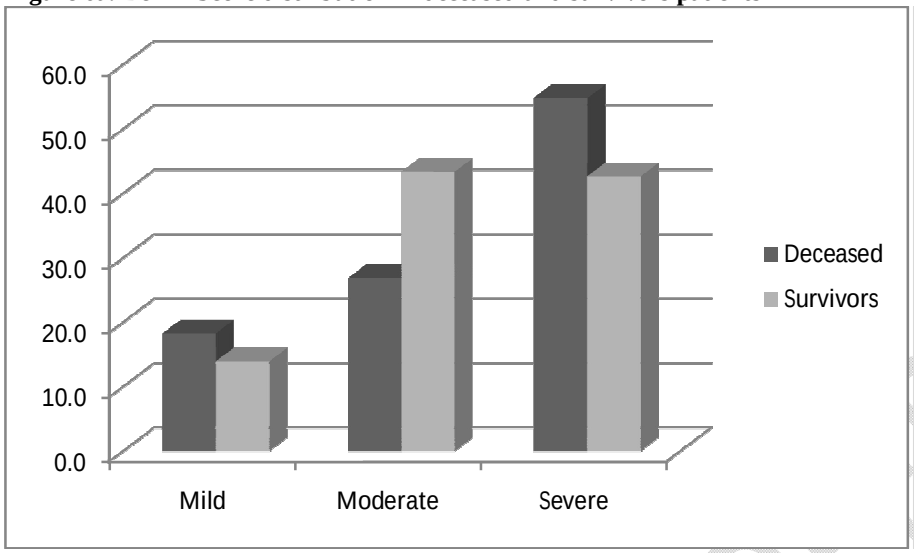


Figure 6b: Quick SOFA Score distribution in deceased and survivors patients