Galectin-3 as Prognostic Predictor in Patients with COVID-19 Acute Respiratory Failure

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Research

Abstract

Background. Galectin-3 is β-galactoside-binding lectin with several roles in immune-inflammatory response. To date, there is no evidence of Galectin-3 role as a prognostic predictor in COVID-19 disease. The aim of this study is to clarify the prognostic role of Galectin-3 in patients with COVID 19 acute respiratory failure.

Methods. We enrolled 156 consecutive patients with COVID-19 disease. Routine laboratory test, arterial blood gas, chest X-ray or Computed Tomography and Galectin-3 dosage were performed. The primary outcome was to assess Galectin-3 predictive power for 30-day mortality. Secondary outcomes were 30-day Intensive Care Unit admission and Acute Respiratory Distress Syndrome stratification according to Galectin-3 dosage. We performed Mann-Whitney U and Kruskal-Wallis tests for continuous variables comparison. Fisher's exact test or Chi-square test were used for categorical variables analysis. Relationships between Galectin-3, clinical and laboratory data were identified using Spearman analysis. Receiver Operating Characteristic curves estimated Galectin-3 predictive power for the endpoints. With a fixed cut-off of 35.3 ng/ml, Kaplan-Meier with Log-Rank test and Cox Regression were performed to assess mortality and Intensive Care Unit admission risk.

Results. Galectin-3 correlated with many other prognostic predictors tested in our analysis. Moreover, patients with serum levels of Galectin-3 above 35.3 ng/ml had increased risk for mortality, Intensive Care Unit admission and severe Acute Respiratory Distress Syndrome.

Conclusions. Our study demonstrates the role of Galectin-3 as a predictor of mortality, Intensive Care Unit access and ARDS stratification in patients with COVID 19 acute respiratory failure.

Introduction

Since its first description in 2019, COronaVIrus Disease 19 (COVID-19) has shown an intense lung and systemic aggressiveness, albeit a wide spectrum of clinical phenotypes (1). Considering its complexity, numerous factors have been evaluated to identify fragile patients and to stratify their risk of adverse outcomes (2). At this regard, serum inflammatory markers play an important role, as they are a sign of disease worsening and progression (3–5). Recently, Galectin-3, a β-galactoside-binding lectin, has raised some interest as a potential marker of lung damage and a possible therapeutic target in COVID-19 disease (6,7). Galectin-3 has pleiotropic effects on the immune response: it modulates immune cells lifecycle, angiogenesis and reparative response after lung injury (8,9). Galectin-3 is highly expressed on fibroblast, endothelial and epithelial cells (10), as well as on alveolar macrophages as consequence of a lung damage (11,12). During viral infections, Galectin-3 regulates innate immunity (13), modulating cytokines production and release. Furthermore, Galectin-3 can be a binding site for viruses, easing their entry into immune cells (14). In patients with Severe Acute Respiratory Syndrome-COronaVirus 2 (SARS-COV2) infection, a more severe degree of the disease has been proved to be associated with higher blood levels of Galectin-3 and other various cytokines (15–17). Despite these interesting findings in scientific

literature, no specific data are present about the role of Galectin-3 as a prognostic factor in COVID-19 disease. The aim of this study is to clarify the role of Galectin-3 in patients SARS-COV2 infection admitted to our respiratory intensive care unit due to acute respiratory failure.

Methods

In this single-center retrospective observational study, from September 2020 to March 2021 we enrolled 156 consecutive patients admitted to our respiratory intensive care unit of "Policlinico" University Hospital of Bari, Italy, with a diagnosis of COVID-19 disease and acute respiratory failure. At the emergency department, a nose-throat swab with Real Time-Polymerase Chain Reaction has been performed to confirm SARS-COV2 infection. In our ward, blood samples, arterial blood gas analysis and thoracic imaging (chest X-ray or Computed Tomography-scan) were collected within 48 hours from admission. Similarly, demographic, anamnestic and clinical data were recorded and reported in a database along with serum dosages of Galectin-3 and other inflammation markers. Plasma samples were collected and stored at -80°C before the analysis. Then, plasma levels of Galectin-3 were measured with chemiluminescence immunoassay kits. Exclusion criteria in our study were the follows: age < 18 years, no blood samples collection within 48 hours, no thoracic imaging performed at the admission. Finally, 140 patients met all the inclusion criteria and were considered for statistical analysis. All these patients were also stratified for Acute Respiratory Distress Syndrome (ARDS) severity according to the Berlin definition (18). The primary outcome of this study was to assess 30-day mortality according to Galectin-3 serum levels. Secondary outcomes were the assessment of 30-day Intensive Care Unit (ICU) admission and ARDS stratification. The study was approved by the Institutional Review Board of our hospital (Ethical Committee number: 6717). The present study was conducted in accordance with the Helsinki Declaration of 1975 and following the standards of Good Clinical Practice.

Statistical analysis

We verified the non-normal distribution of data with the Shapiro-Wilk test, considering medians and interquartile ranges for statistical purposes. Consequently, Mann-Whitney U test was used to compare continuous variables, whereas Kruskal-Wallis test was performed in our ARDS severity stratification. Categorical variables were compared using Fisher's exact test or Chi-square test. Spearman correlation analysis was used to identify relationships between Galectin-3 and other clinical and laboratory data. To estimate the predictive power of Galectin-3 for the outcomes, we carried out Receiver Operating Characteristic (ROC) curves, estimating the area under the curve (AUC) of our predictors. Then, Kaplan-Meier analysis with log-rank test was performed using Galectin-3 to stratify our patients according to different outcomes. Moreover, risk factors for 30-day mortality were assessed using a univariate Cox proportional hazard regression model. Finally, statistically significant predictors were used to generate a multivariate model of Cox regression analysis, whose accuracy was tested using a ROC curve. All statistical analysis were realized using SPSS 26.0 (SPSS Inc, Chicago, Ill) and Prism 8.0.1 (Graphpad Software, La Jolla, Calif). A p-value level < 0.05 was considered to be statistically significant.

Results

Population analysis

Anamnestic, clinical and laboratory characteristics of our population are described in Additional File 1. In our cohort, 95% of patients had ARDS according to Berlin definition. During the hospitalization, 12 patients underwent a worsening of their respiratory condition and were transferred in ICU.

Survival vs non-survival group

Non survivor patients (27.9 %) were found to be have higher median age ($p < 0.0001$) and worse prognostic scores ($p < 0.0001$), developing more frequently severe ARDS ($p < 0.0001$) and requiring ICU admission ($p = 0.04$). As for laboratory variables, increased levels of serum lactate ($p = 0.006$), interleukin-6 (IL-6, $p < 0.0001$), creatinine ($p < 0.0001$), lactate dehydrogenase (LDH, $p < 0.0001$), N-terminal pro-Brain Natriuretic Peptide (NT-pro-BNP, $p < 0.0001$), C-reactive protein (CRP, $p < 0.0001$), Procalcitonin (PCT, $p < 0.0001$), D-dimer ($p < 0.0001$), presepsin ($p < 0.0001$) and Galectin-3 ($p < 0.0001$) were found. On the contrary, lower levels of platelets (PLT, $p = 0.01$) and Vitamin D ($p = 0.001$) were also reported (Table 1). In Spearman analysis (see Additional File 2), Gal-3 positively correlated with age ($R = 0.48$, $p < 0.0001$), number of comorbidities ($R = 0.45$, $p < 0.0001$), serum lactate ($R = 0.26$, $p = 0.002$), IL-6 ($R = 0.28$, $p = 0.0008$), white blood cells (WBC, $R = 0.21$, $p = 0.01$), creatinine ($R = 0.43$, $p < 0.0001$), LDH ($R = 0.36$, $p < 0.0001$), NT-pro-BNP ($R = 0.54$, $p < 0.0001$), CRP ($R = 0.23$, $p < 0.007$), PCT ($R = 0.41$, $p < 0.0001$), D-dimer ($R = 0.35$, $p < 0.0001$), presepsin ($R = 0.53$, $p < 0.0001$) and Sequential Organ Failure Assessment (SOFA) score ($R = 0.5$, $p < 0.0001$). Negative correlations were instead found with days of hospitalization ($R=-0.19$, $p = 0.025$), PaO2/FiO2 at the admission ($R=-0.18$, $p = 0.03$) and at discharge ($R=-0.42$, $p < 0.0001$) and with serum vitamin D levels ($R=-0.22$, $p = 0.01$). Considering statistically significant correlations, different ROC curves were constructed (see Additional File 3). A Galectin-3 cut-off of 35.3 ng/ml was fixed to achieve a sensitivity of 80% and a specificity of 90.8% for mortality prediction, with an AUC value of 0.901 (Fig. 1, 95% CI 0.84−0.96, $p < 0.0001$). Using this cut-off, we tested our hypothesis with Log-Rank analysis and Kaplan-Meier curves, finding a greater death risk with Galectin-3 serum levels above 35.3 ng/ml (Fig. 2, $\chi^2=70.4$, $p < 0.0001$). To complete our survival analysis, we performed a univariate Cox regression (Table 2). Factors associated with higher risk of mortality were age (HR = 1.061, $p < 0.0001$), number of comorbidities (HR = 1.55, $p < 0.0001$), PaO2/FiO2 at the admission (HR = 0.99, $p = 0.005$), IL-6 (HR = 1.005, $p < 0.0001$), PLT (HR = 1, $p = 0.04$), creatinine (HR = 1.54, $p < 0.0001$), LDH (HR = 1.003, $p < 0.0001$), CPK (HR = 1, $p = 0.021$), CRP (HR = 1.008, $p < 0.0001$), PCT (HR = 1.089, $p < 0.0001$), presepsin (HR = 1, $p < 0.0001$), vitamin D (HR = 0.97, $p = 0.02$), SOFA score (HR = 1.5, $p < 0.0001$) and Galectin-3 (HR = 1.023, $p < 0.0001$). After adjusting for confounding factors, our multivariate Cox regression model (Table 3) identified only the number of total comorbidities (HR = 1.75, $p = 0.001$), PaO2/FiO2 at the admission (HR = 0.99, $p = 0.05$), IL-6 (HR = 1.003, $p = 0.04$), CRP (HR = 1.010, $p < 0.0001$) and Galectin-3 (HR = 1.027, $p < 0.03$) as statistically significant for mortality prediction. Finally, we created a ROC curve (Fig. 3) to test the accuracy of our model, finding an AUC of 0.853 ($p < 0.0001$).

Table 1
Comparison of clinical and laboratory data according to survival status

	Survivors	Non-survivors	P-value
Patients (%, n)	72.1% (101)	27.9% (39)	
Sex (Male/Female, n, %)	76.9%/23.1% (30/9)	67.3%/32.7% (68/33)	
Age (years, mean, SD#)	63 [53.25-72]	81 [71−86]	< 0.0001
ARDS* (PaO2/FiO2 < 300) (%, n)	93.1% (94)	100% (39)	
Mild (300 < PaO2/FiO2 ≤ 200) (%, n)	26.6% (25)	15.4% (6)	
Moderate (200 < PaO2/FiO2 ≤ 100) (%, n)	69.1% (65)	51.3% (20)	< 0.0001
Severe (PaO2/FiO2 < 100) (%, n)	4.3% (4)	33.3% (13)	
No ARDS (%, n)	6.9% (7)	0	
SOFA score ‖ (Median, IQR§)	3 [2−4]	6 [4−7]	< 0.0001
PaO2†/FiO2‡ admission (Median, IQR)	161 [132-224.5]	117 [89−164]	< 0.0001
PaO2/FiO2 discharge (Median, IQR)	211 [166.5-267.5]	80 [55−115]	< 0.0001
Patients with comorbidity (%, n)	66.3% (67)	94.9% (37)	0.0004
Cardiovascular disease	76.1% (51)	81.1% (30)	
Chronic kidney disease	10.4% (7)	21.6% (8)	
Diabetes type II	25.4% (17)	32.4% (12)	
Neurological disease	13.4% (9)	29.7% (11)	
Psychiatric disease	3% (2)	10.8% (4)	
Malignancy	4.5% (3)	18.9% (7)	
COPD	17.9% (12)	10.8% (4)	
Asthma	6% (4)	5.4% (2)	
Total comorbidities (n, Median, IQR)	1 [0−2]	2 [2−3]	

	Survivors	Non-survivors	P-value
Serum levels (Median, IQR)			0.006
- Lactate (mmol/L)	1 [0.8−1.6]	1.4 [1.1−1.9]	< 0.0001
- Interleukin-6 (pg/ml)	21.3 [8.3−51.6]	84.3 [23.6-124.1]	
- WBC** (x10^3/uL)	8.7 [6.5−11.8]	8.8 [6.3−12.1]	0.01
- Platelets (x10^3/uL)	283 [189.5−375]	221 [155−290]	< 0.0001
- Creatinine (mg/dL)	0.8 [0.7-1]	1.2 [0.8-2]	
- Total bilirubine (mg/dL)	0.6 [0.4−0.7]	0.6 [0.4−0.8]	< 0.0001
- LDH†† (U/L)	303 [238.5−350]	425 [331−575]	
- CPK‡‡ (U/L)	61 [32-108.5]	77 [39−314]	< 0.0001
- NT-pro-BNP §§ (pg/mL)	166 [88−468]	746 [474−3190]	< 0.0001
- C-Reactive Protein (mg/L)	49.2 [20.3−94.5]	95.4 [58.9−127]	< 0.0001
- Procalcitonin (ng/mL)	0.08 [0.06−0.18]	0.3 [0.15−1.45]	< 0.0001
- D-dimer (ug/L)	849 [424−1585]	2398 [984−5248]	
- Fibrinogen (mg/dL)	424 [328.5−548]	438 [374−547]	< 0.0001
- Presepsin (pg/ml)	383 [281.3-541.3]	899 [633−1795]	0.001
- Vitamin D (ng/ml)	25 [16.25-34]	16 [9−25]	< 0.0001
- Galectin-3 (ng/ml)	21.9 [17.6−27.5]	43.8 [36.2−59]	
Days of hospitalization (Median, IQR)	12 [8−18]	9 [7−11]	0.008
ICU ll ll admission (%, n)	5% (5)	17.9% (7)	0.04
#SD, Standard Deviation			
*ARDS, Acute Respiratory Distress Syndrome;			
†PaO2, Pressure of Arterial Oxygen;			
‡FiO2, Fraction of Inspired Oxygen;			
§ IQR, InterQuartile Range			
ll SOFA, Sequential Organ Failure Assessment;			
**WBC, White Blood (cell) Count;			
†† LDH, Lactate DeHydrogenase;			
‡‡ CPK, Calcium-dependent Protein Kinase;			

	Survivors	Non-survivors	P-value
§§ NT-pro-BNP, N-Terminal pro-Brain-type Natriuretic Peptide;			
‖ ‖ ICU, Intensive Care Unit.			

Table 2
Univariate survival Cox regression for clinical and laboratory data

Paramethers	HR#	IC 95%**	P value
Age	1.061	1.033−1.090	< 0.0001
Comorbidities	1.55	1.264−1.898	< 0.0001
†PaO2/‡FiO2 admission	0.99	0.983−0.997	0.005
Lactate (mmol/L)	1.479	0.93−2.353	
Interleukin-6 (pg/ml)	1.005	1.002−1.007	< 0.0001
*WBC (x10^3/uL)	1.007	0.955−1.061	
Platelets (x10^3/uL)	0.997	0.995−1	0.04
Creatinine (mg/dL)	1.543	1.307−1.822	< 0.0001
Bilirubin (mg/dL)	1.457	0.806−2.635	
†† LDH (U/L)	1.003	1.002−1.004	< 0.0001
‡‡ CPK (U/L)	1	1−1.001	0.02
§ NT-pro-BNP (pg/ml)	1	1−1.001	
C-Reactive Protein (mg/L)	1.008	1.005−1.012	< 0.0001
Procalcitonin (ng/ml)	1.089	1.049−1.131	< 0.0001
D-dimer (ug/L)	1	1−1.001	
Fibrinogen (mg/dL)	1.001	0.99−1.003	
Presepsin (pg/ml)	1	1−1	< 0.0001
Vitamin D (ng/ml)	0.97	0.945−0.996	0.02
Galectin-3 (ng/ml)	1.023	1.016−1.03	< 0.0001
‖ SOFA score	1.502	1.333−1.694	< 0.0001
#HR, Hazard Ratio			
**CI, Confidence Interval			
†PaO2, Pressure of Arterial Oxygen;			
‡FiO2, Fraction of Inspired Oxygen;			
*WBC, White Blood (cell) Count;			

Paramethers	HR#	IC 95%**	P value
†† LDH, Lactate DeHydrogenase;			
‡‡ CPK, Calcium-dependent Protein Kinase,			
§ NT-pro-BNP, N-Terminal pro-Brain-type Natriuretic Peptide;			
ll SOFA, Sequential Organ Failure Assessment.			

Table 3
Multivariate survival Cox regression for clinical and laboratory data

Paramethers	HR#	IC 95%**	P value
Age	1.03	0.992−1.07	
Comorbidities	1.754	1.261−2.439	0.001
†PaO2/‡FiO2 admission	0.994	0.987−1	0.05
Interleukin-6 (pg/ml)	1.003	1−1.006	0.04
Platelets (x10^3/uL)	0.99	0.995−1.002	
Creatinine (mg/dL)	1.11	0.793−1.55	
†† LDH (U/L)	1	0.998−1.002	
‡‡ CPK (U/L)	1	1−1.001	
C-Reactive Protein (mg/L)	1.010	1.005−1.015	< 0.0001
Procalcitonin (ng/ml)	0.896	0.798−1.006	
Presepsin (pg/ml)	1	1-1.001	
Vitamin D (ng/ml)	0.988	0.961−1.015	
Galectin-3 (ng/ml)	1.027	1.003−1.051	0.03
ll SOFA score	1.014	0.751−1.37	
#HR, Hazard Ratio			
**CI, Confidence Interval			
†PaO2, Pressure of Arterial Oxygen;			
‡FiO2, Fraction of Inspired Oxygen;			
†† LDH, Lactate DeHydrogenase;			
‡‡ CPK, Calcium-dependent Protein Kinase,			
ll SOFA, Sequential Organ Failure Assessment.			

Statistical analysis for ICU admission

Concerning ICU admission, we considered only 108 patients, as for the rest of them there were contraindications for endotracheal intubation and invasive ventilation in ICU. Among these patients, 12 were transferred in ICU due to worsening of gas exchange. To predict ICU admission using serum levels of Galectin-3, a ROC curve was performed, obtaining an AUC of 0.7 (p = 0.02). With the same cut-off of 35.3 ng/ml fixed for mortality analysis, we found a statistically significant result concerning risk of ICU admission after Log-Rank test (Fig. 4, χ^2=6.5, p = 0.01). Subsequently, a univariate Cox regression was performed to evaluate hazard ratios for ICU access (Table 4). Creatinine (HR = 1.52, p = 0.001), LDH (HR = 1.004, p = 0.001), CPK (HR = 1.001, p = 0.01), CRP (HR = 1.014, p = 0.002), PCT (HR = 1.15, p = 0.002), fibrinogen (HR = 1.003, p = 0.009), presepsin (HR = 1, p = 0.026), SOFA score (HR = 1.54, p < 0.0001) and Galectin-3 (HR = 1.037, p < 0.0001) were associated with an increased risk for this outcome. However, after adjusting for confounding factors, none of these parameters remained statistically significant (Table 5).

Table 4
Univariate Cox regression of clinical and laboratory data for
Intensive Care Unit admission

Paramethers	HR#	IC 95%**	P value
Age	0.99	0.957–1.042	
Comorbidities	1.121	0.764–1.644	
†PaO2/‡FiO2 admission	0.991	0.979–1.003	
Lactate (mml/L)	1.246	0.539–2.882	
Interleukin-6 (pg/ml)	1.004	0.998–1.009	
*WBC (x10^3/uL)	1.037	0.983–1.095	
Platelets (x10^3/uL)	0.99	0.994–1.003	
Creatinine (mg/dL)	1.524	1.193–1.947	0.001
Bilirubin (mg/dL)	1.301	0.235–7.203	
†† LDH (U/L)	1.004	1.002–1.007	0.001
‡‡ CPK (U/L)	1.001	1–1.002	0.01
§ NT-pro-BNP (pg/mL)	1	1–1.001	
C-Reactive Protein (mg/L)	1.014	1.005–1.023	0.002
Procalcitonin (ng/ml)	1.155	1.055–1.264	0.002
D-dimer (ug/L)	1	1–1.001	
Fibrinogen (mg/dL)	1.003	1.001–1.006	0.009
Presepsin (pg/ml)	1	1–1	0.03
Vitamin D (ng/ml)	0.985	0.946–1.026	
GALECTIN-3 (ng/ml)	1.037	1.018–1.056	< 0.0001
‖ SOFA score	1.536	1.221–1.931	< 0.0001
#HR, Hazard Ratio			
**CI, Confidence Interval			
†PaO2, Pressure of Arterial Oxygen;			
‡FiO2, Fraction of Inspired Oxygen;			
*WBC, White Blood (cell) Count;			
†† LDH, Lactate DeHydrogenase;			

Paramethers	HR#	IC 95%**	P value
‡‡ CPK, Calcium-dependent Protein Kinase,			
§ NT-pro-BNP, N-Terminal pro-Brain-type Natriuretic Peptide;			
‖ SOFA, Sequential Organ Failure Assessment.			

Table 5
Multivariate Cox regression of clinical and laboratory data for
Intensive Care Unit admission

Paramethers	HR#	IC 95%**	P value
Creatinine (mg/dL)	1.0.99	0.202−5.97	0.9
† LDH (U/L)	1.003	0.997−1.009	0.3
‡ CPK (U/L)	1	0.997−1.002	0.7
C-Reactive Protein (mg/L)	1.008	0.988−1.029	0.4
Procalcitonin (ng/ml)	1.021	0.576−1.811	0.9
Fibrinogen (mg/dL)	1.001	0.996−1.006	0.6
Presepsin (pg/ml)	0.99	0.998−1.001	0.45
Galectin-3 (ng/ml)	1.015	0.949−1.085	0.7
*SOFA score	1.389	0.873−2.211	0.2
#HR, Hazard Ratio			
**CI, Confidence Interval			
† LDH, Lactate DeHydrogenase;			
‡ CPK, Calcium-dependent Protein Kinase;			
*SOFA, Sequential Organ Failure Assessment.			

Statistical analysis for ARDS degree

A comparison of clinical and laboratory data in patients with different degrees of ARDS severity is reported Additional File 4. Patients with severe ARDS shows higher levels of SOFA score and increased serum concentrations of LDH (p = 0.01), NT-pro-BNP (p = 0.01), CRP (p = 0.002), presepsin (p = 0.006) and Galectin-3 (p = 0.004). Moreover, death percentages tend to increase according to a worse severity of the ARDS (p < 0.0001). To verify the power of Galectin-3 for ARDS severity stratification, 3 different ROC curves were performed (see Additional File 5, 6 and 7). Although we did not find remarkable results for mild and moderate ARDS, Galectin-3 has shown good diagnostic power for severe ARDS (see Additional File 8, AUC 0.75, p = 0.001). In this case, using the fixed cut-off of 35.3 ng/ml, we found a sensitivity of 70.6% and a specificity of 78% for the outcome. Interestingly, similar results were found in Kaplan-Meier

analysis (Fig. 5, χ^2=20.51, p < 0.0001).Whereas no differences were found between mild and moderate ARDS (see Additional File 9 in the Online Data Supplement, χ^2=0.19, p = 0.7), severe ARDS was significantly stratified according to our fixed cut-off (Fig. 6, χ^2=19.8, p < 0.0001).

Discussion

This is the first study in scientific literature assessing the prognostic role of Galectin-3 in acute respiratory failure secondary to COVID-19 disease. Patients with higher serum levels of Galectin-3 tend to develop a more severe degree of ARDS with a worse prognosis. It is well known that SARS-COV2 infection can lead to the so called "cytokine storm" in some susceptible patients (19). For instance, our non-survivors group shows increased blood levels of various inflammation markers, which are frequently associated with negative outcomes in COVID-19 disease (2,20). Nevertheless, only IL-6, CRP and Galectin-3 remain statistically significant in our multivariate regression model. This finding is not surprising for IL-6 and CRP, which were previously reported as important prognostic markers in COVID-19 disease (21–23). On the contrary, this is the first study addressing this role for Galectin-3. Furthermore, among the explored parameters, Galectin-3 shows the best AUC curve in ROC analysis, suggesting a better predictive power for mortality outcome. As stated before, hyperinflammation can trigger Galectin-3 release from a wide range of host cells (24). Furthermore, increased blood concentrations of Galectin-3 have also been described in heart failure and chronic kidney disease (25,26). Although having higher serum levels in our non-survivor group, both NT-pro-BNP and creatinine did not predict a higher death risk in our multivariate models. For this reason, we speculate that during SARS-COV2 infection, Galectin-3 release can be specifically associated with lung damage, earlier predicting the clinical worsening of this disease. Indeed, our analysis on ARDS stratification seems to confirm this hypothesis, as Galectin-3 can properly identify severe ARDS secondary to COVID-19 disease with good sensitivity and specificity. Similarly, Xu el al have already explained the role of Galectin-3 as a prognostic factor in ARDS (27). However, this study was not performed during COVID-19 pandemic, taking into consideration patients suffering from severe pneumonia, burns, aspiration or gastrointestinal lesions. Moreover, unlike our study, the ROC analysis was performed considering a combined model with Galectin-3, Acute Physiology And Chronic Health Evaluation II (APACHE II) score and PaO2/FiO2, in order to gain a sufficient predictive power for the outcome.

Concerning the ICU admission, our multivariate analysis did not find any significant predictor for this outcome. It has to be said that our cohort of patients undergoing ICU transfer was very limited. Since our ward was deputed to manage patients requiring non-invasive ventilation, ICU admission was allowed only for patients requiring endotracheal intubation or extracorporeal membrane oxygenation (ECMO). For this reason, 32 patients were excluded from this analysis, as they were considered neither eligible for these treatments nor for ICU admission. By contrast, Kaplan-Meier and ROC analysis did result in a statistically

significant assessment of risk for ICU admission using our Galectin-3 cut-off. For this reason, we believe that a larger cohort of patients should elucidates this point. For mortality, ICU admission and ARDS severity assessments, we decided to use the same cut-of value of 35.3 ng/ml, as it guarantees us the best compromise in terms of sensitivity and specificity. Furthermore, this cut-off only discriminates severe ARDS from mild-moderate ones and can be really considered in prognostic terms the "edge of the cliff". Patients with Galectin-3 serum levels above 35.3 ng/ml, in fact, were not only more prone to develop severe ARDS, but also markedly at higher risk of ICU admission or death. Our study has several limitations. Firstly, as previously mentioned, the sample size is limited for a complete statistical analysis. Secondly, we performed a single-center retrospective study, while a randomized multicenter prospective design would be advisable to obtain further prognostic information. Thirdly, we only assessed serum levels of Galectin-3 at the admission, without monitoring its plasma concentrations during the hospitalization. Another important limitation is the lack of a non-COVID ARDS control group. Although no important differences seem to characterize this two types of ARDS (28,29), many pathophysiological aspects have to be clarified and a direct confront of Galectin-3 serum levels in these two groups could be interesting to better understand this point. Finally, since our study had only prognostic purposes, we did not collect any brochoalveolar lavage or pulmonary tissue sample for Galectin-3 detection. Future studies should verify how Galectin-3 concentrations in these samples could affect COVID-19 ARDS development and prognosis.

Conclusions

Our study demonstrates the importance of Galectin-3 as a prognostic factor in COVID-19 disease. Galectin-3 can predict mortality and ARDS severity of patients with ARF secondary to COVID-19 disease. Moreover, higher levels of this marker seem to be correlated with an increased risk for ICU admission, although further studies are needed to clarify this issue.

Abbreviations

COronaVIrus Disease 19 (COVID-19), Severe Acute Respiratory Syndrome-COronaVirus 2 (SARS-COV2), Acute Respiratory Distress Syndrome (ARDS), Intensive Care Unit (ICU), Receiver Operating Characteristic (ROC), area under the curve (AUC), lactate dehydrogenase (LDH), N-terminal pro-Brain Natriuretic Peptide (NT-pro-BNP), C-reactive protein (CRP), Procalcitonin (PCT), Sequential Organ Failure Assessment (SOFA), Physiology And Chronic Health Evaluation II (APACHE II)

Declarations

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

- Ethics approval and consent to participate: The study was approved by the Institutional Review Board of our hospital (Ethical Committee number: 6717).

- Consent for publication: not applicable

- Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

- Competing interests: The authors declare that they have no competing interests

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- Authors' contributions:

- AP participated in the design of the study, in the sequence of alignment, in the statistical analysis and drafted the manuscript

- FD partecipated in the literature research, in data acquisition and drafted the manuscript

- CS participated in the design of the study, in the interpetation of the results and drafted the manuscript

- SD partecipated in the in the literature research, in data acquisition and in the final revision ot the manuscript

- EB participated in literetaure research, in data collection and interpretation and in the final revision ot the manuscript

- FDS analyzed all the biological samples, collected data and revised critically the final draft of the manuscript

- GEC partecipated participated in the design of the study, in the interpetation of the results and in the final revision ot the manuscript

- All authors read and approved the final manuscript.

- All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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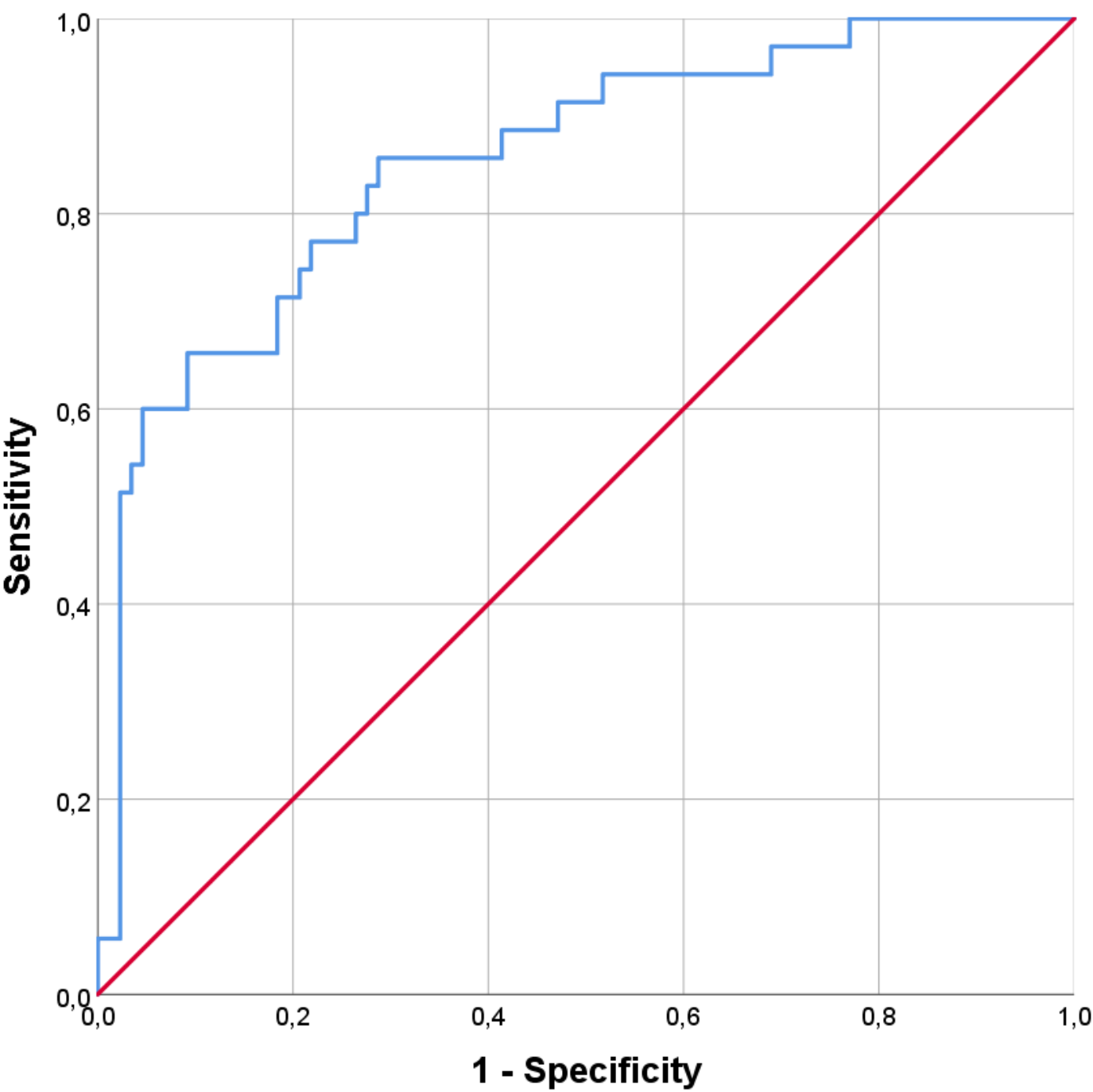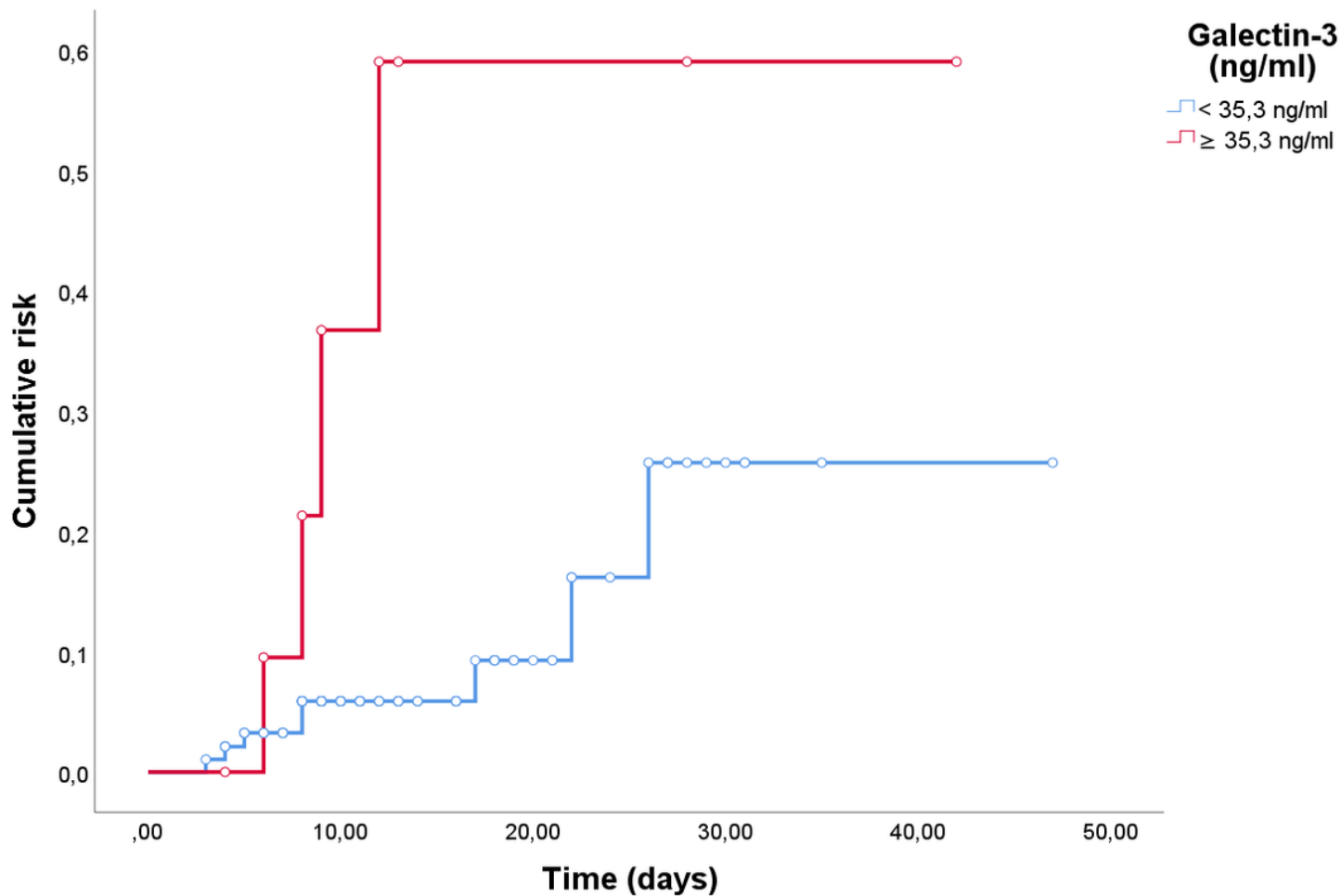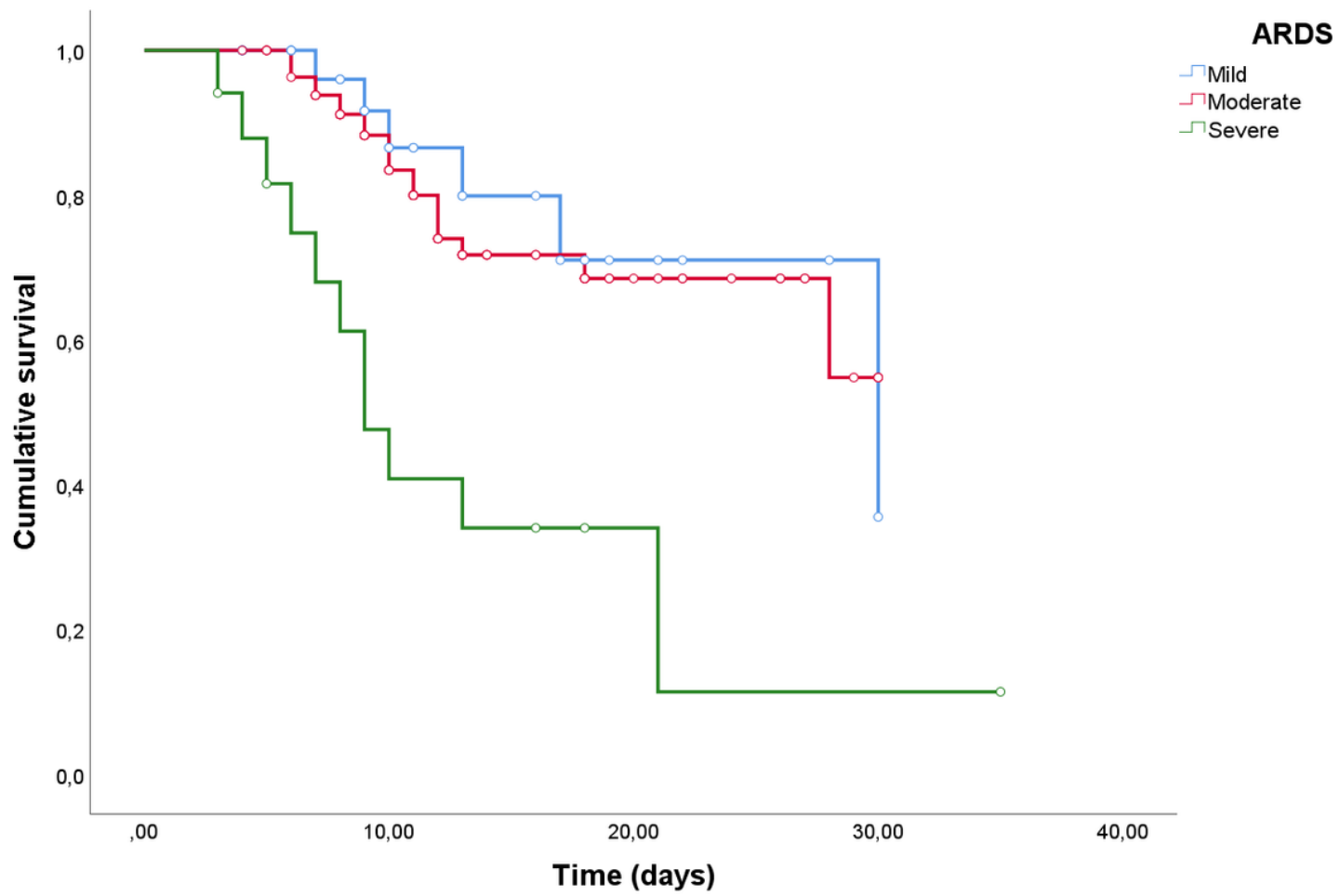
Figures

Figure 1

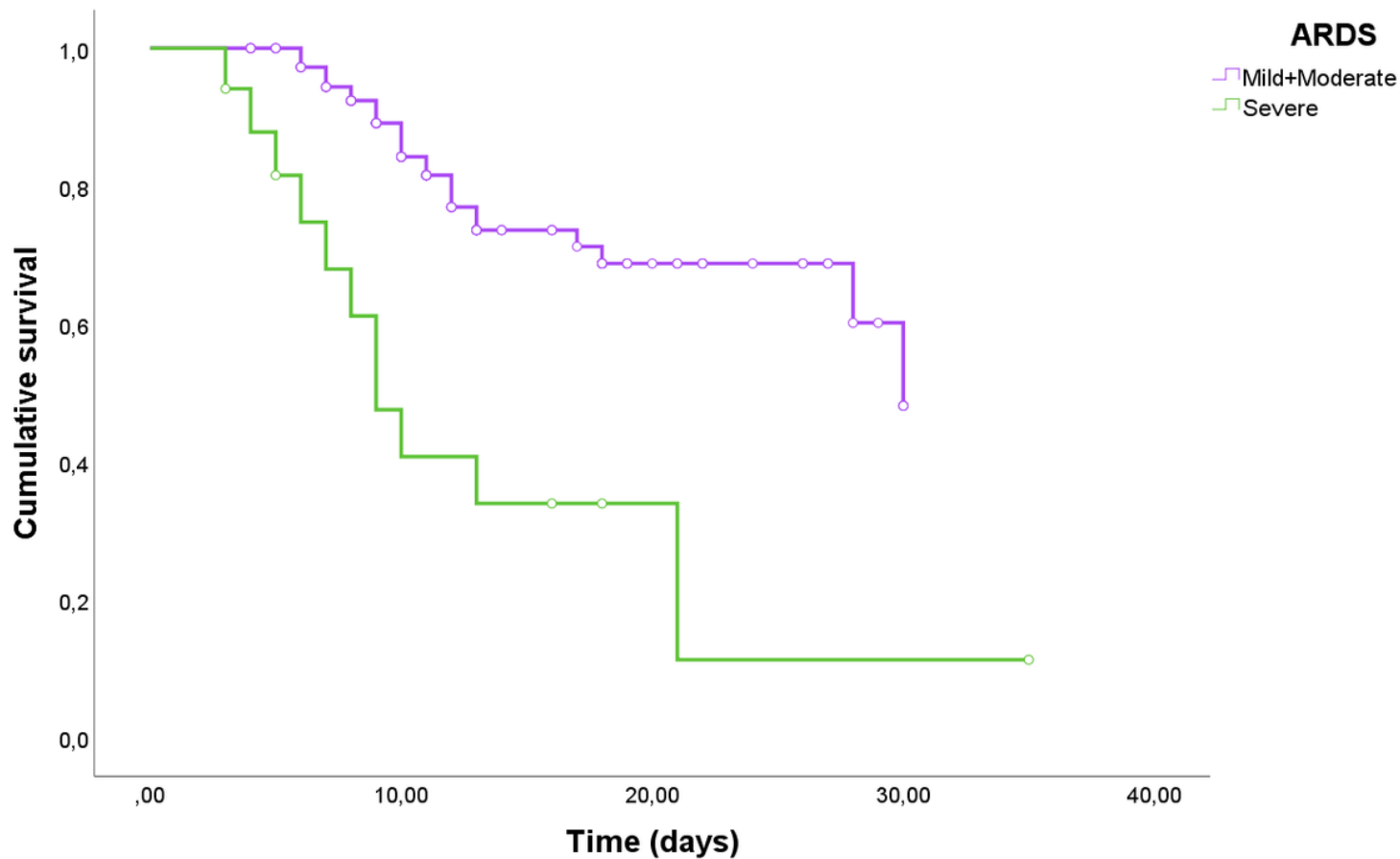Receiving operator characteristic curve for Glalectin-3 serum levels.

Figure 2

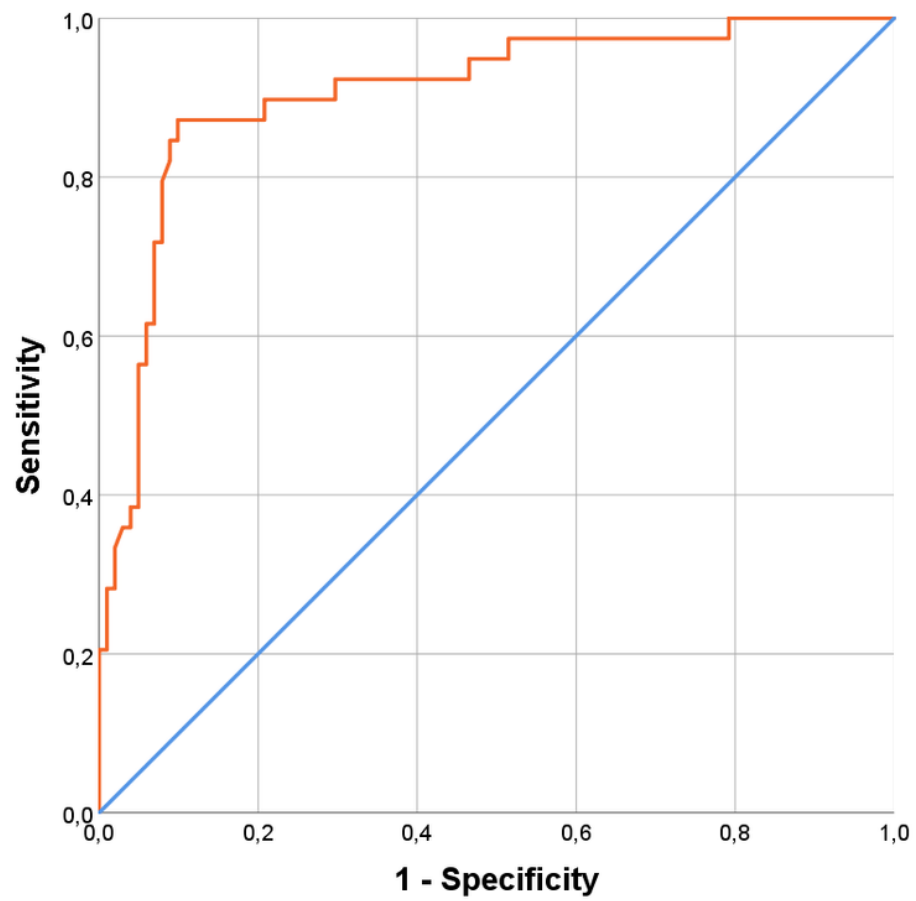Kaplan-Meier survival curves according to Galectin-3 cut-off.

Figure 3

Receiving operator characteristic curve for Multivariate Cox survival analysis
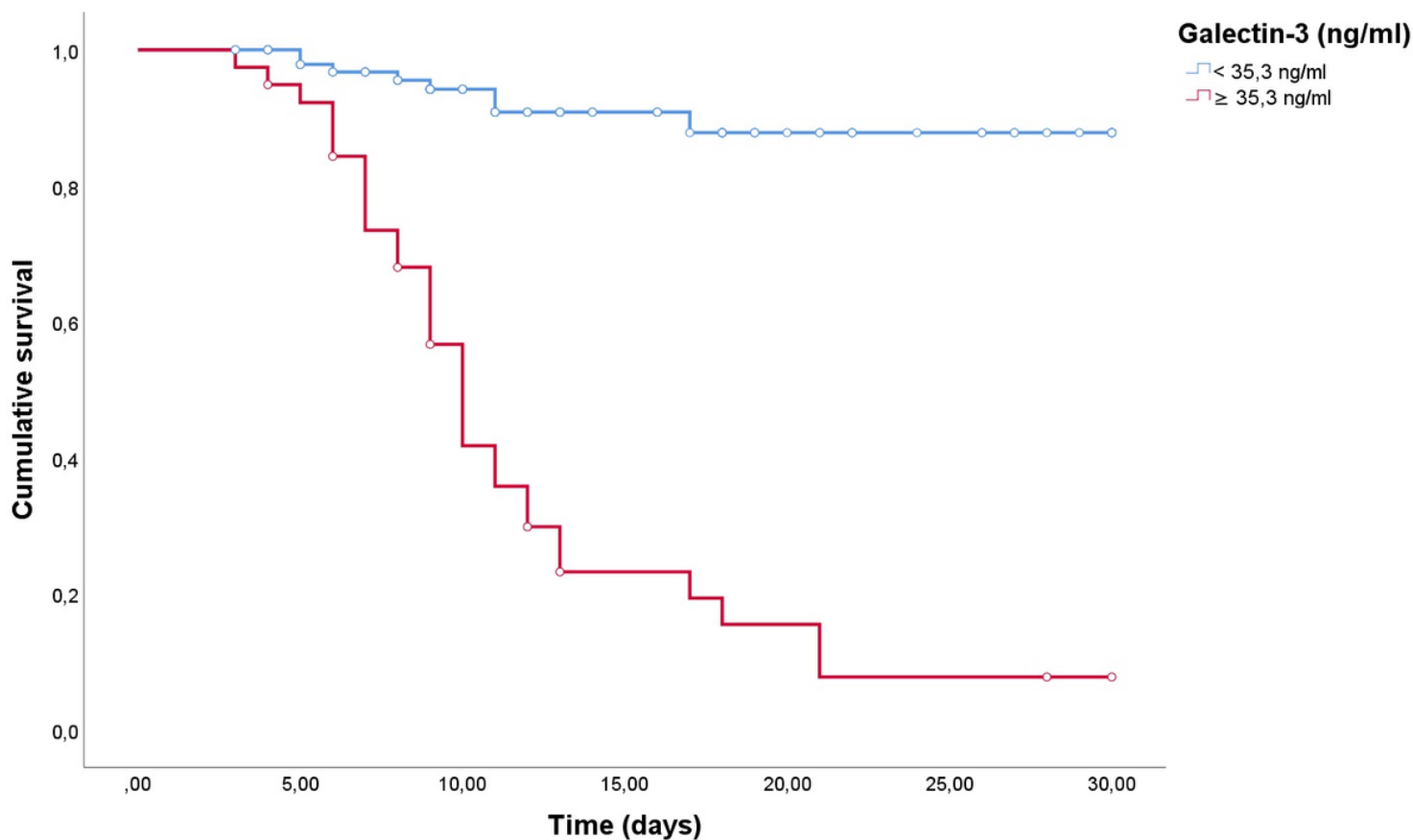
Figure 4

Kaplan-Meier curves for Intensive Care Unit admission risk according to Galectin-3 cut-off

Figure 5

ARDS stratification with Kaplan-Meier curves according to Galectin-3 cut-off.

Figure 6

Mild/moderate vs severe ARDS Kaplan-Meier stratified for Galectin-3 cut-off.

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