Annexin A1 as a Potential Prognostic Biomarker for COVID-19 Disease: Case-Control Study

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Abstract

Background

Annexin A1 (AnxA1) is an important endogenous glucocoticoid protein that contributes to the suppression of inflammation by limiting the production of neutrophil and proinflammatory cytokines.This study aims to determine the clinical predictivity value of blood AnxA1 levels in patients with mild and severe-critical pneumonia induced by COVID-19.

Methods

This study employed a prospective, case-control study design and was conducted at Ankara Training and Research hospital between 10.02.2021 and 15.03.2021.

A total of 74 patients (42 of whom had moderate and 32 of whom had severe/critical cases of COVID-19 disease according to WHO guidelines) and 50 non-symptomatic healthy volunteers participated in the study. Blood samples were taken from patients at the time of hospital admission, after which serum was isolated. Following the isolation of serum, AnxA1 levels were evaluated using the Enzyme-Linked Immunosorbent Assay(ELISA) method.

Results

The serum AnxA1 level was measured as 25.5 (18.6-38.6)ng/mL in the control group, 21.2 (14.7-32) ng/mL in the moderate disease group, and 14.8 (9.7-26.8)ng/mL in the

severe/critical disease group. Serum AnxA1 levels were significantly lower in the severe/critical disease group compared to the control and moderate disease groups (p=0.01 and p=0.0001, respectively).

Using ROC analysis, a larger area under the curve (AUC) for the serum AnxA1 levels of the control group (AUC=0.715, 95% CI =0.626-0.803;p=0.0001) was calculated compared to the COVID-19 patient group for the diagnosis of COVID-19 disease. The AnxA1 level was found to be 80% sensitive and 54.1% specific at a cut-off level of 18.5 ng/ml for the diagnosis of COVID-19 disease. Moreover, the AnxA1 level was found to be 69.8% sensitive and 58.1% specific at a cut-off level of 17.2 ng/ml in predicting the need for ICU treatment.

Conclusion

AnxA1 levels may be a beneficial biomarker in the diagnosis of COVID-19 pneumonia and in predicting the need for ICU treatment in patients with COVID-19 pneumonia at the time of admission to the emergency department (ED).

KeyWords: Annexin A1, Adrenal Cortex Hormones, COVID-19, pneumonia

ShortTitle: Annexin A1 levels in SARS-CoV-2 infection

What is already known about this topic:

- Endogenous corticostereoids contributes to the suppression of inflammation in a variety of ways.
- AnxA1 limits the production of neutrophil and proinflammatory cytokines.
- The use of exogenous corticosteroids (especially dexamethasone) in severe/critical COVID-19 infections has also been recommended by WHO guidelines for treating COVID-19 and has been put into clinical practice.

What does this article add?

- Serum AnxA1 levels were found to decrease in patients who had severe/critical cases of COVID-19 disease.
- AnxA1 levels may be a beneficial biomarker that can be used together with other known markers in the diagnosis of COVID-19 pneumonia and in predicting the need

for ICU treatment for patients with COVID-19 pneumonia at the time of admission to the ED.

- The present study suggests that one of the response mechanisms to glucocorticoid therapy in patients with severe COVID-19 pneumonia using exogenous steroids may be through AnxA1.

Introduction

Coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome brought on by coronavirus 2 (SARS-CoV-2) was declared a global pandemic on 11 March 2020, and 157.2 million people had been infected and 3.2 million people had died worldwide by 6 May 2021. In the early days of May 2021, 800,000 new daily cases were confirmed and 90,000 deaths were reportedthrougout the world (1). COVID-19 infection can cause a wide clinical spectrum ranging from mild upper respiratory tract infections to sepsis and acute respiratory distress syndrome (ARDS) (2).

Cytokine storm, which was firstly defined by Cron and Behrens, occurs when excessive and disregulated cytokine production due to an uncontrolled immune response to different initiators such as infections, malignancies, and rheumatologic deiseases. COVID-19 can cause the production of cytokine storm inducing hyperinflammation, hyperactivation of the immune response, and multiple organ failure (3-6).

Annexin A1 (AnxA1) is an important endogenous glucocoticoid protein that contributes to the suppression of inflammation in a variety of ways. Also known as lipocortin-1, it is one of the endogenous modulators of inflammation. AnxA1 is stored in high concentrations in the cytoplasm of neutrophils, macrophages, and monocytes in humans in states of no infection (7). AnxA1 limits the production of neutrophil and proinflammatory cytokines. Moreover, AnxA1 induces neutrophil apoptosis, mediates monocyte recruitment, and augments the

scavenging of apoptotic cells by macrophages. Recent research has revealed that AnxA1 also induces macrophage reprogramming for providing optimal homeostasis (7). AnxA1levels are induced both by inflammatory reponses and by exogenously ingested glucocorticoids. Exogenous glucocorticoids induce AnxA1 expression in monocytes and neutrophils, which play a crucial role in the anti-inflammatory responses (8).Some studies on AnxA1 levels in sepsis patients have produced various results, such as in a study on patients with chronic obstructive pulmonary disease (COPD). AnxA1 levels were reported to decrease in sepsis patinets, whereas another study found increased AnxA1 levels (9,10). Serum AnxA1 levels were found to be higher in stage 3-4 patients of Global Initiative for Chronic Obstructive Lung Disease (GOLD) COPD than patients with mild symptoms (11). Another study found lower viral burden, higher mortality, and morbidity in $ANXA1^{-/-}$ rats infected with Influenza compared with wild type rats (12). In another study by Santana et al., low serum AnxA1 levels were found in patients infected with human T-lymphotropic virus (HTLV-1), and it was suggested that AnxA1 is an important marker on diagnosis and prognosis of HTLV-1-associated myelopathy/tropical spastic paraparesis (13).

As suggested by another study, the Ac2-26 mimetic peptide of AnxA1 could be an important treatment agent in severe COVID-19 disease (14). However, to the authors' knowledge, there is no study based on the alterations of serum AnxA1 levels in patients with COVID-19 infection or value of AnxA1 for clinical prediction.

We consider that blood AnxA1 levels may vary with clinical severity due to the significant increase in the inflammation cascade in patients with COVID-19 pneumonia. The present study aims to determine the clinical predictivity value of blood AnxA1 levels in patients with mild and severe/critical pneumonia induced by COVID-19 and to reveal the alterations of blood AnxA1 levels in patients with pneumonia compared to the control group.

Methods

Study Type

The present study is a prospective case-control study, and the required ethics approval was obtained from the Ethics Committee of Pamukkale University (Numbered: E-60116787-020-15062). The study was conducted at Ankara Training and Research Hospital between 10.02.2021 and 15.03.2021. All procedures carried out on patients were in compliance with the Helsinki Declaration.

Study Population

The patient groups and the healthy control group were informed in detail about the study, and they were requested to complete the written consent forms before participating in the study.

Study groups were established according to the inclusion and exclusion criteria. Patients whose diagnoses were clinically confirmed as COVID-19 infection according to World Health Organization (WHO) guidelines using a positive reverse transcriptase polymerase chain reaction (RT-PCR) test were included in the study (15). Individuals were grouped in the moderate COVID-19 disease group (N=42), severe/critical COVID-19 disease group (N=32), and the healthy control group (N=50).

The healthy control group included healthy volunteers with no history or diagnosis of any acute or chronic disease and infection, and no known drug use.

Inclusion Criteria

Patient Groups: Patients whose diagnoses of COVID-19 infection were confirmed by positive RT-PCR in ED according to WHO guidelines and who gave their written consent were included in the study (15).

Control Group: Subjects with no history of a known disease, no infectious symptoms, no drug use, and who provided written consent were included in the study.

Exclusion Criteria

Patients who were diagnosed with heart, kidney or liver failure, who had a history of acute pulmonary embolism, deep venous thrombosis or chronic inflammatory disease; and who were pregnant were exclued from the study.

Clinical Evaluation

The subjects included in the present study were clinically evaluated using WHO diagnosis and treatment guidelines for COVID-19 (15). The patient management algorithms were administered due to the updates of these guidelines. The patient groups were categorized as moderate disease and severe/critical disease according to WHO guidelines. The severe/critical disease group consisted of ICU patients in line with WHO guidelines (15). To evaluate the clinical severity, CURB-65 scores of patients were calculated as indicated in the literature. The CURB-65 score is a scoring system used in the evaluation of pneumonia (16).

Healthy group (Control Group)

This group included subjects who had no history or diagnosis of any disease, no infection history within last two weeks, no history of any particular medication, who were admitted to emergency department (ED) with complaints other than infectious issues, and who gave their written consent to participate in the study.

Data Collection

Demographic information and vital findings of the subjects, and their laboratory findings (hemogram, C-reactive protein (CRP), liver function tests (aspartat aminotransferaz, alanin aminotransferaz), creatinine, blood urea nitrogen (BUN), D-dimer, CK-MB, high sensitive troponin T (hsTnT), blood gas analysis parameters, and hospitalization location (intensive care unit (ICU) or not) were recorded in the data set.

Annexin A1 Level Measurement

Venous blood samples that were taken when the patients were admitted to ED were withdrawn into a dry test tube that did not contain anti-coagulant and were then centrifugated for 10 minutes at 4000 rpm. Serum samples obtained from centrifugation were collected for laboratory analysis. Serum AnxA1 levels were analyzed using a commercially available AnxA1 ELISA Kit (Elabscience, E-EL-H5512, USA), per the manufacturer's protocol.

Statistical Analysis

Given that a similarly organized reference study did not exist, a power analysis was performed in line with the presumptions. The results revealed that at least 92 people (min. 46 for each cohort) were needed to achieve 95% power at a 90% confidence interval, assuming that the projected effect size would be high ($f = 0.7$). The SPSS package program was used for data analysis. The continuous variables were presented as median (IQR) and mean ± standard deviation. A Kolmogorov-Smirnov test was conducted to calculate the distribution type of the continuous variables. Mann-Whitney U or Kruskal-Wallis tests were used for analysing independent and non-parametric variables. Spearman correlation analysis was used to investigate correlation relationships between continous non-parametric variables. Receiver Operating Characteristic (ROC) curve analysis was used for the discriminant performance serum AnxA1 levels. The significance level was defined as $p < 0.05$ for all analyses.

Results

Symptom duration time was statistically higher in the severe/critical disease group than in the moderate disease group (7.5±1.7 and 5.9±1.1 days, respectively) (p=0.02).

As a result of the post-hoc power analysis, the effect size of the AnxA1 concentrations for the differences between the two groups (patients and control) was moderate-high (f=0.66), and the power level observed for this effect size was 95%, and the reliability level was 93.96%.

Serum AnxA1 level were measured as 25.5 (18.6-38.6) ng/mL in the control group; 21.2 (14.7-32) ng/mL in the moderate disease group; and 14.8 (9.7-26.8) ng/mL in the severe/critical disease group. Serum AnxA1 levels were significantly lower in the severe/critical disease group compared to the control and moderate disease groups (p=0.01 and p=0.0001, respectively) (Table-1 and Figure-1).

Subjects included in patient groups and healthy control group were matched by means of age and gender (p=0.384 and p=0.285, respectively) (Table-2).

Vital findings and clinical data for the study groups are given in Table-2, and Table-3 presents the laboratory parameters of the subjects.

When the correlation of AnxA1 with clinical and laboratory findings was analyzed, a negative mild-moderate correlation was found between serum AnxA1 and CURB-65 score for the patients (rho= - 0.381 and p=0.001). Serum AnxA1 levels were mildly positively correlated with breath rate and oxygen saturation (sPO2) (rho=0.32 and p=0.0001; rho=0.202 and p=0.025, respectively).

Using ROC analysis, a larger area under the curve (AUC) for the serum AnxA1 levels of the control group (AUC = 0.715, 95% CI = 0.626-0.803; p=0.0001) was calculated compared to the COVID-19 patient group. The AnxA1 level was found to be 80% sensitive and 54.1% specific at a cut-off level of 18.5 ng/ml for the diagnosis of COVID-19 disease (Figure 2).

Furthermore, a larger AUC for the serum AnxA1 levels of patients who needed ICU treatment (AUC = 0.701, 95% CI = 0.582-0.819; p=0.003) was calculated using ROC analysis. The AnxA1 level was found to be 69.8% sensitive and 58.1% specific at a cut-off level of 17.2 ng/ml for predicting the need for ICU treatment (Figure 3).

Discussion

The present study evaluated the clinical significance of serum AnxA1 level in COVID-19 pnemonia and concluded that the serum AnxA1 levels decreased as the clinical severity

increased, and serum AnxA1 levels were 18.56 ng/mL in COVID-19 pnemonia with a sensitivity of 80% and specifity of 54%. Moreover, serum AnxA1 level may be considered as an indicator for predicting the need for ICU treatment even with its lower sensitivity and specifity.

Many biomarkers, which are routine or non-routine laboratory parameters, have been analyzed both in diagnosing the disease and predicting the clinical prognosis throughout the COVID-19 pandemic. Previous studies have investigated whether laboratory parameters such as monocyte/lymphocyte ratio, CRP, ferritin, lactate dehydrogenase (LDH), interleukin-6 (IL-6), D-dimer, p selectin, calprotectin, and surfactant can be used as biomarkers for the clinical diagnosis and prediction of prognosis for COVID-19 (17-27). The sensitivity of the markers for COVID-19 diagnosis mentioned in these studies ranged between 66%-97.5% (17-26).

The serum AnxA1 levels in this study had a sensitivity of 80% and specifity of 54.1%, which might suggest that serum AnxA1 levels may be a useful marker in the diagnosis of COVID-19. A Cochrane database review revealed the sensitivity of CRP, IL-6, and LDH levels in clinical diagnosis as 66%, 73%, and 77%, respectively. Thus, AnxA1 might be considered as a more powerful biomarker compared to these markers (17).

AnxA1 is an endogeous glucocorticoid that plays a crucial role in inhibiting inflammation. AnxA1 regulates inflammation by mediating recruitment of neutrophils and macrophages (28). Endogenous glucocorticoids are evaluated as the potential target in inflammation control. Notably, recombinant AnxA1 and N-terminal peptides of AnxA1 have been found to have an anti-inflammatory effect in pharmacologic and experimental models (29,30).

Although the mechanism of glucocorticoid-induced leucine zipper (GILZ) protein, which is one of the proteins whose production is stimulated by exogenous glucocorticoids, have not been fully elucidated, it has been found to affect the functions of AnxA1 (8). AnxA1 mRNA expression is upregulated by glucocorticoids (31). Studies have reported that inflammatory stimulation was much stronger and longer lasting in AnxA1 defective mice than wild-type mice (32-34). In a study conducted by Tsai et al., AnxA1 levels and associated lipoxin were found to diminish in patienets with sepsis, although no significanct differences were found between survivor and non-survivor sepsis patients in terms of these markers. In addition, the study also found that pro-inflammatory cytokines increased and these markers decreased (35). Among studies investigating the relationship between AnxA1 level and clinical

prognosis in viral infections, a study by Santana et al. is notable in that it found low serum AnxA1 levels in patients with HTLV-1 infection (13).

When studies on the serum AnxA1 levels in COVID-19 infection and other pulmonary pathologies were examined, serum AnxA1 levels were found to be higher in GOLD stage 3-4 COPD patients compared to patients with mild symptoms. In addition to the presentstudy, many mechanisms have been proposed as therapeutic targets in COVID-19 infection (36). In a hypothesis published by Bonavita, it was suggested that the Ac2-26 mimetic protein of the AnxA1 protein could be a therapeutic target, especially in the treatment of severe COVID-19 infection, and this peptide could be one of the main mediators of cytokine storm syndrome by lowering IL-6 levels (14). Additionally, the use of exogenous corticosteroids (especially dexamethasone) in severe/critical COVID-19 infection has also been recommended by WHO guidelines for COVID-19 treatment and has been put into clinical practice (37). To the authors' knowledge, there exist no studies on the clinical and prognostic significance of AnxA1 level in COVID-19 patients, and the present study is the first research in this regard. In the present study, lower serum AnxA1 level in severe/critical patients compared to mild patients and the control group suggested that hyperinflammation due to COVID-19 and exacerbation of septic clinic might be attributed to the decrease in endogenous glucocorticoids. When the effect of exogenous corticoid therapy on endogenous corticoid levels and the role of glucocorticoids in the treatment of COVID-19 are considered, the mechanism of response to glucocorticoid therapy may be through AnxA1. Studies on the effect of exogenous steroid administration on endogenous glucocorticoids in the treatment of severe COVID-19 may further investigate the role of AnxA1 protein in COVID-19 infection.

In addition, the findings regarding lower AnxA1 in severe/critical COVID-19 patients has provided an idea about the clinical adaptability of the Ac2-26 mimetic peptide of the AnxA1 protein, which was previously hypothesized and can be administered in the treatment of severe COVID-19.

Moreover, this study found 69.8% sensitivity and 58.1% specificity at 17.2 ng/mL serum level of AnxA1 in order to predict the need for ICU treatment in patients admitted to ED, suggesting that serum AnxA1 level may be a useful biomarker in predicting the need for ICU treatment.

The present study has some limitations. Recurrent serum AnxA1 levels were not measured during the clinical visit, ICU hospitalization, or treatment of the patients, and alterations of serum AnxA1 levels during the infection process were not understood.

Conclusions

The present study has found that serum AnxA1 levels may be a beneficial biomarker in the diagnosis of COVID-19 pneumonia and in predicting the need for ICU treatment in patients with COVID-19 pneumonia at the time of admission to the ED.

The lower serum AnxA1 level in severe COVID-19 patients revealed the role of the AnxA1 protein in the clinical severity and the balance of anti-inflammatory/inflammatory mechanisms.

Moreover, the present study has pointed out that one of the response mechanisms to glucocorticoid therapy in patients with severe COVID-19 pneumonia using exogenous steroids may be through AnxA1.

Conflict of interest statement

The authors declare that they have no conflicts of interests.

Acknowledgment

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Data Availability Statement

All the datas(other than patient names) are available to share.

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Table 1. Annexin A1 levels of the study groups

	Controls (N=50)		**Moderate Disease Group** (N=42)		**Severe/Critical Disease Group** (N=32)		***p*-Value**
	Mean±SD	*Median (IQR)*	*Mean±SD*	*Median (IQR)*	*Mean±SD*	*Median (IQR)*	
Annexin A1	33.46±22	25.53 (18.7-38.7)	25.65±14.81	21.21 (9.78-19.71)	16.06±7.1	14.86 (9.78-19.71)	*p= 0.0001 **p= 0.044 ***p= 0.01 ****p= 0.0001

*[1] *p*-value derived from Kruskal-Wallis test and refers to the comparison between whole the groups

** *p*-value is derived from Mann Whitney U test, and refers to the comparison between Control and moderate disease groups.

*** *p*-value is derived from Mann Whitney U test and refers to the comparison between Control and severe/critical disease groups.

**** *p*-value is derived from Mann Whitney U test and refers to the comparison between moderate disease group and severe/critical disease groups.

Table 2. Clinical datas and comorbidity datas of of the groups

		Controls (N=50)	Moderate Disease Group (N=42)	Severe/Critical Disease Group (N=32)	*p*-Value
Gender, N(%)	***Male***	24	22	11	
	Female	26	20	21	*0.285
Comorbidities, N(%)					
Diabetes Mellitus			19 (45.2%)	15 (46.8%)	*1
Hypertension			15 (35.7%)	16 (50%)	*0.217
Coronary artery disease			6 (14.2%)	6 (18.7%)	*0.606
			Mean±SD or Median(IQR)		
Age (year)		66.5±16.4	66.8±15.8	67.1±12.8	**0.384
CURB-65 Score			2 (1-2)	3 (2-4)	0.0001
Body temperature (^{0}C)			37 (36.5-37.3)	36.6 (36.1-37.4)	0.131
Heart Rate (beat/min)			91.5 (86-101.25)	94 (87-116)	0.32
Respiratory Rate			24 (20.75-27.25)	30 (23-39)	0.001
sPO_2			92 (89.5-96)	80 (70-87)	0.0001
SBP (mm/Hg)			137.5 (120-160)	133 (116-144)	0.32
DBP (mm/Hg)			75.5 (68-83.5)	73 (63-80)	0.166

p-values are derived from Mann Whitney U test.

* *p*-values are derived from chi square test

***p*-values is derived from student's t test.

SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Table 3. Laboratory parameters of the patient groups

	Moderate Disease Group (N=32)	**Severe/Critical Disease Group** (N=42)	***p* Value**
	Mean±SD or Median (IQR)	*Mean±SD or Median(IQR)*	
WBC (K/μL)	5.57 (4.7-6.73)	9.19 (7.18-15.16)	0.0001
Hemoglobin (g/dL)	13.19±1.4	13.5±1.49	*p=0.351
Platelete (K/μL)	203.28±59.7	256.53±94.7	*p=0.003
NLR	3.16 (1.88-4.38)	7.96 (4-9.8)	0.0001
ESR (mm/h)	37 (21-56)	45 (23-55)	0.272
CRP (mg/L)	40.7 (13.98-89.6)	138.6 (77.8-196.7)	0.0001
BUN (mg/dL)	34.5 (27.75-48.5)	49.6 (36.3-70.3)	0.005
Creatinine (mg/dL)	0.87 (0.78-1.13)	1.12 (0.89-1.42)	0.011
AST (U/L)	27 (22-33.5)	44 (30-79)	0.0001
ALT (U/L)	18 (12-25)	26 (22-49)	0.001
D-Dimer (ng/mL)	580 (297.5-1182.5)	2000 (895-4435)	0.0001
hsTnT (μg/L)	12.92 (6.97-20.44)	25.9 (18.7-58.4)	0.0001
CKMB (ng/mL)	1.4 (0.91-2.81)	2.89 (1.85-7.02)	0.0001
pH	7.41 (7.37-7.43)	7.41 (7.35-7.44)	0.71
pCO_2 (mmHg)	39.05 (35.12-44.3)	38.6 (33.2-41.2)	0.43
Lactate (mmol/L)	1.8 (1.22-2.1)	2.8 (1.8-4.6)	0.0001

HCO_3 (mEq/L)	24 (22.45-25.6)	22.6 (20.6-24.5)	0.138

p-values are derived from Kruskal-Wallis test. **p*-values are derived from Student's t Test

IQR: Interquartile range; WBC: White blood cell; NLR: Neutrophil leukocyte ratio; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; BUN: Blood urea nitrogen; AST: Aspartate transaminase; ALT: Alanine transaminase; hsTnT: High sensitive troponin T; CKMB: Creatinine kinase MB; pH: Power of hydrogen; pCO_2: Partial carbon dioxide pressure; HCO_3: Bicarbonate.

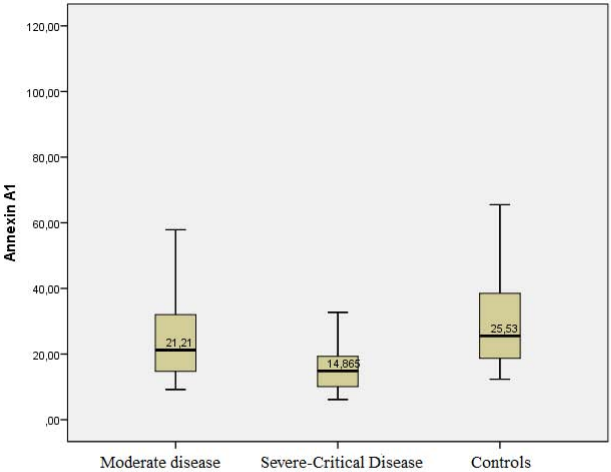

Figure-1. Serum Annexin A1 levels of the groups

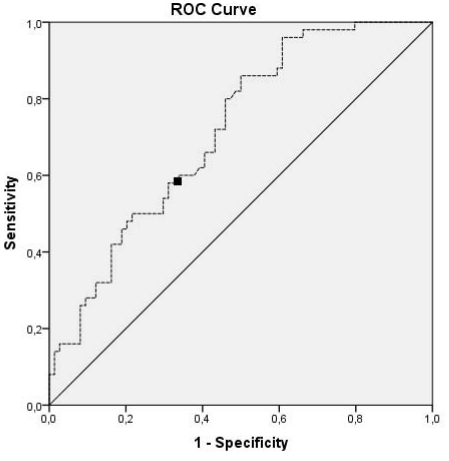

Figure-2 ROC Curve analysis of the clinical diagnosis of the patients.

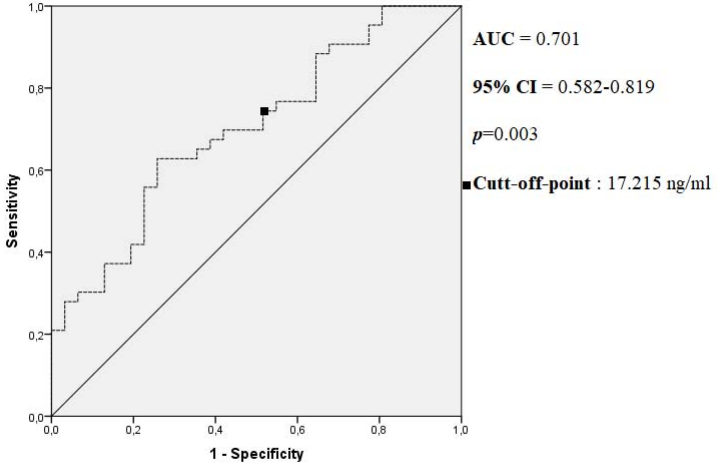

Figure-3 ROC Curve analysis of the clinical prognosis of the patients (Non-ICU needed and ICU-needed)