

# WITHDRAWN: Predictability of Blood Parameters on the Course of Covid-19- a retrospective clinical study

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## Research

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## EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

# Abstract

## Background

In December 2019, clusters of patients with pneumonia of an unknown cause were reported in Wuhan, Hubei Province in China. On January 2020, Chinese scientists identified this as a novel coronavirus, temporarily labelled as, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although recent studies investigating predictors of poor prognosis at an early stage identified exact parameters as a predictor for the disease still controversial.

Here we study biochemical and hematologic parameters of 197 patients diagnosed as Covid-19 at our hospital for evaluating the value of these biomarkers for predicting the course of Covid-19 disease.

## Material and Method

197 patients who were diagnosed with Covid-19 at our hospital according to World Health Organisation (WHO) interim guidance were screened between March 2020-May 2020 retrospectively for the study. Patients selected from the population of service and intensive care unit (ICU). Biochemical and hematologic parameters retrospectively evaluated for predicting the course of Covid-19 disease.

## Results

Among 197 patient population of service and ICU, hematological and biochemical parameters studied. There was a significant association found between the parameters neutrophil/lymphocyte ratio, white blood cell, eosinophile, monocyte, lymphocyte counts and high sensitive troponin-I levels and ICU admission.

## Conclusion

We found increased levels of neutrophil/lymphocyte ratio, white blood cell count and high sensitive troponin-I and decreased levels of eosinophile, monocyte and lymphocyte counts among ICU admitted patients. We concluded that these biochemical and hematologic parameters can be used for the predicting the course of Covid-19 disease.

## Background

In December 2019, clusters of patients with pneumonia of an unknown cause were reported in Wuhan, Hubei Province in China. On January 2020, Chinese scientists identified this as a novel coronavirus, temporarily labelled as, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). Its name was then changed to coronavirus disease 2019 (Covid-19) by the World Health Organisation (WHO) in February 2020 as the disease spread worldwide (2). In recognition of its global transmission, WHO declared Covid-19 as a pandemic on 11 March 2020 (3).

It has been suggested the outbreak has a zoonotic origin, and like other respiratory pathogens, it spreads through human-to-human transmission such as coughing and sneezing (4). Although limited, research suggests a possibility of transmission even amongst the asymptomatic (5).

Covid-19 has a spectrum of manifestations ranging from asymptomatic infection, to myocarditis, acute kidney failure, mild upper respiratory symptoms, to bilateral pneumonia with respiratory failure requiring advanced respiratory support (6–8). The rapid diffusion rate of the disease in population, due to asymptomatic carriers, associated with the possible sudden deterioration of clinical conditions requiring critical care admission (10–13). Infected patients may present with any of the following; fever, high temperature ( $> 37.3\text{ C}^0$ ), cough, myalgia, sputum production, headache, haemoptysis, diarrhea, dyspnea and in some cases acute respiratory distress syndrome (ARDS), acute cardiac injury or secondary infection (6).

Although recent studies investigating predictors of poor prognosis at an early stage identified potential risk factors including older age, history of chronic obstructive pulmonary disease (COPD), presence of secondary infection or cardiac disease (9, 14), these were not yet fully proven. These risk factors must be reinforced with the use of some hematologic and biochemical parameters. By checking these parameters, the progression of the disease could be predicted and precautions can be taken for the patient before patient's condition becomes severe and this could help to decrease the amount of morbidity and mortality.

Here we study biochemical and hematologic parameters of 197 patients diagnosed as Covid-19 at our hospital for evaluating the value of these biomarkers for predicting the course of Covid-19 disease.

## Material And Method

197 patients who were diagnosed with Covid-19 in our hospital according to WHO interim guidance were screened between March 2020-May 2020 retrospectively for the study. Patients selected from the population of service and intensive care unit (ICU).

Study complied to the ethical guidelines of the Declaration of Helsinki. Because our hospital is not a university or a training hospital, we do not have an ethical committee in our hospital. But all written ethical permissions were taken from directly "The Ministry of Health of Turkish Republic" for this article (17.05.2020/2020-05-12T14\_32\_16).

Throat swab samples were collected for extracting 2019-nCov RNA from patients and test-positive patients admitted to the study.

Blood result data of the patients were collected and biochemical (Advia 2400, Siemens; Germany) and hematologic (Advia 2120, Siemens; Ireland) parameters evaluated retrospectively.

### Exclusion Criteria

Patients that; 1- Have malignity, 2- Proven type1 myocardial infarction and 3- Chronic hematological disorder were excluded.

## Statistical Analysis

The statistical analyses of the study were performed by SPSS 20.0 (IBM Inc, Chicago, IL, USA). The descriptive statistics were presented as frequency (percentage) for categorical variables and mean±SD (median, min, max where necessary) for numerical variables. The continuous variables were checked by Kolmogorov-Smirnov test, but most of the variables were not distributed normal. Therefore, Mann-Whitney U test was used for comparison between service inpatients and intensive care units, and Monte Carlo Exact Chi-square test was used to determine the relations between the categorical variables. ROC analysis was performed to determine the diagnostic values of biochemical and hematologic parameters and statistically significance was found with white blood cell count (WBC), high sensitive troponin-I (Trop-I), neutrophile/lymphocyte ratio (NLR), Eosynophile (Eos), Lymphocyte (Lymp), monocyte (Mono), and these parameters included in the study. In all analyses,  $p < 0.05$  value was considered as statistically significant result for 5% Type-I error.

The power analysis was performed by GPower 9.1.2 (Universitaet Kiel, Germany). The analysis was based on one-tailed t-test, and the effect size was calculated as 0.88 using a priori troponin values for patient groups. The requirements of the analysis were considered as 5% error rate, 0.95 (actual power 0.961) power and allocation ratio (N1:N2) = 1. Therefore, the minimum sample size was calculated as 67 for each group.

## Results

197 patients diagnosed as Covid-19 included the study. 51.3% ( $n=101$ ) was service and 48.7% ( $n=96$ ) were ICU patients. 58.9% of patients of male where 41.1% of them were female. Mean age was  $64.97 \pm 17.14$  years.

There is no statistically significance between genders ( $p=0.891$ ). The ICU patients were significantly older ( $mean\ age:7, p < 0.001$ ). According to comorbidities, the proportion of coronary artery disease ( $p=0.006$ ) and cerebrovascular disease ( $p=0.037$ ) were significantly higher in ICU group (Table 1).

Among all hematologic and biochemical parameters, eosynophile (Eos) ( $p < 0.001$ ) and lymphocyte (Lymp) ( $p < 0.001$ ) counts found decreased where NLR ( $p < 0.001$ ) found increased among ICU patients. WBC ( $p=0.001$ ) and high sensitive troponin-I (Trop-I) ( $p < 0.001$ ) values were found increased where monocyte count (Mono) ( $p=0.001$ ) found decreased in ICU group. (Table 1).

ROC analysis performed for predictability ratios of WBC, Trop-I, Eos, Lymp and Mono for ICU and service follow up of Covid-19 patients (Table 2). Among the values of WBC and Trop-I for the course of the clinic, Trop-I values were significantly higher than WBC values. For the cut-off value of WBC ( $11.40 \cdot 10^3 / \mu L$ ) ( $AUC:0.642, p=0.001$ ) sensitivity was 40.6% and specificity was found 84.2% where for the cut-off value of

NLR as 5.80 ( $AUC:0.714, p<0.001$ ) (Figure-1). Specificity and sensitivity was found 71.3% and 64.2% respectively. Trop-I was found increased among ICU group (cut-off: 5.50 pg/mL) ( $AUC:0.823, p<0.001$ ) (Figure-2). Sensitivity and specificity of the Trop-I value was found 92.3% and 59.2% respectively. Lymph ( $AUC:0.698, p<0.001$ ) and Eos ( $AUC:0.676, p<0.001$ ) (Figure-3) values, which were slightly lower in Mono ( $AUC:0.644, p=0.001$ ), also gave significant results for the predictability of the course of the disease. The sensitivity and specificity for the Eos was 46.9% and 84.2% respectively. For the Lymph values, sensitivity was 64.6% and specificity was 67.3%. In Mono values, sensitivity and specificity were slightly lower with the sensitivity 65.3% and specificity 61.4% (Table2).

## Discussion

Covid-19 has elicits a rapid spread of outbreak with the human-to-human transmission, with a median incubation period of 3 days and a relatively low fatality rate (15). Mortality rate was about 2.5-5% (16). In recent publications (15, 17), the clinical characteristics of Covid-19 are similar to those of SARS-CoV. Fever and cough were dominant symptoms, whereas gastrointestinal symptoms were rare, suggesting the difference in viral tropism compared with SARS-CoV, MERS-CoV and influenza (18).

In many studies reported in literature, course of the Covid-19 disease varies between asymptomatic to ICU follow ups. Because of this variability, predicting the risk of the patients become more important for decreasing the morbidity and mortality of the illness. In our study, we investigate the hematologic and biochemical parameters of the patients followed both in ICU and service and found an association between the course of the disease and some blood parameters.

NLR is the first and the most obvious parameter that we found an association with the course of the patients. Following SARS-CoV-2 infection, most of the patients presented with lymphopenia and elevated levels of infection-related biomarkers, more interestingly, a higher number of neutrophils (Neut) and lower number of Lymph, the increase of NLR, were found in nonsurvivors with Covid-19 compared to survivors. (19). Neut plays a central role in the innate immune response, resulting in multi-organ failure. In contrast, Lymph plays a major role in the inflammatory response. In reported studies, Lymph counts found significantly low in Covid-19 patients especially among severe courses while the Neut was significantly found higher (20). These changes showed the degree of inflammation in the body was further intensified, triggering an inflammatory storm and leading to increased tissue and cell damage (21). Low Lymph level and impaired immune cell function lead to the dysfunction of immune system, which makes patients with severe Covid-19 might be more sensitive to bacterial infection (22). Increasing clinical data suggest that the NLR might be a powerful predictive and prognostic indicator for severe Covid-19 (7, 23, 24).

In our study we found statistically significant ( $p < 0.001$ ) decreased counts of Eos among ICU patients than service patients. As we found in our study, Zhang *et al.* reported more than half of the patients admitted with Covid-19 (53%) had eosinopenia on the day of hospital admission (25). Similarly, Du *et al.* reviewed the medical records of 85 fatal cases of Covid-19 and noted that 81% of the patients had absolute Eos counts below the normal range at the time of admission (26). Notably, Eos levels improved

in all patients prior to discharge, suggesting that the resolution of eosinopenia may be an indicator of improving clinical status (27). The pathophysiology of eosinopenia in Covid-19 remains unclear but is likely multifactorial, involving inhibition of Eos egress from the bone marrow, blockade of eosinophilopoiesis, reduced expression of chemokine receptors/adhesion factors (28, 29) and/or direct Eos apoptosis induced by type 1 interferons released during acute infection (30).

We also revealed a decrease in Mono counts in ICU patients ( $p = 0.001$ ). Some authors also found a substantial decrease of Mono in the circulation of Covid-19 patients (31) as we found in our study. This was coupled with a severity-specific enrichment of intermediate and non-classical monocytes in the lungs of patients with severe and critical disease and as a result a decrease of Mono count evaluated in peripheral blood samples (32).

Trop-I is a well established, specific and sensitive marker of myocardial injury, with both diagnostic and prognostic value (33). It permits early identification of patients at increased risk of death from acute coronary syndrome (34–36). Whereas the interpretation of Trop-I concentrations can undoubtedly cause frustration to those who long for disease-specific biomarker for myocardial infarction, there is an opportunity here (37). With Covid-19 infection, mortality are higher in those who are older and in those with a history of underlying cardiovascular disease (38). In a cohort of 191 patients with confirmed Covid-19 based on RNA detection, unvariable odds ratio for death when Trop-I concentrations were above the 99th percentile upper reference limit was 80.1 (39). This was higher than the odds ratios observed for all other biomarkers tested including D-Dimer and Lymph count. Elevated cardiac troponin concentrations are common in hospitalized patients for Covid-19 and are as likely to be due to non-ischemic causes of myocardial injury or type-2 myocardial infarction (myocardial oxygen supply-demand imbalance), than as consequence of an acute coronary syndrome (37). While these patients are at increased risk of future cardiovascular events and may have underlying coronary artery disease, they do not have acute atherothrombosis, and there is no established role for dual antiplatelet therapy, anticoagulation or early coronary angiography (37). In critically unwell Covid-19 patients, oxygen supply-demand imbalance does not exclusively affect the myocardium, and is likely to be occurring at a cellular level in the majority of organ systems. However, it is the sensitivity of cardiac troponin testing that ensures it is one of the earliest and most precise indicator of end-organ dysfunction (37). Here, cardiac troponin testing could prompt early initiation of measures to improve tissue oxygenation and perfusion (37). But it is important to acknowledge that Trop-I can be released both as a result of ischemic, non-ischemic and extra-cardiac conditions (40). There are some acute and chronic conditions that elevated cardiac troponin levels can be detected in the absence of acute coronary syndrome. Extracardiac cardiac troponin elevation can be a result of; pulmonary embolism, stroke, diabetes mellitus, sepsis, ARDS, chemotherapy, chronic kidney disease and acute liver failure. As in reported cases in literature of non-cardiac reasons for cardiac troponin elevations, we found statistically significant elevated levels in ICU patients without proven type-1 myocardial infarction.

As a result of an infection, WBC levels could rise. In Covid-19, superinfection as pneumonia due to the pulmonary involvement of the disease could be seen and this pulmonary involvement and pneumonia

could be resulted in shortness of breath, hypoxia and at the end ICU admission and intubation. Among the patients that was diagnosed as Covid-19 in our hospital, we evaluated more elevated levels of WBC in ICU patients than in service patients.

## Conclusions

In our study we retrospectively evaluated the blood samples of the patients that admitted to our hospital diagnosed as Covid-19, for investigating the association of hematologic and biochemical parameters and the course of the disease for early prediction of Covid-19 patients that could get worse and could need more intense medical care, on the day of hospital admission. After evaluating the results of the patients, we evaluated a significant association between the severity of the cases and the levels of Eos, Lymp, Mono, NLR, WBC and Trop-I. Increased levels of NLR, WBC and Trop-I where decreased levels of Mono, Lymp and Eos levels in ICU patients were found. These results found to be in a much normal range among the patients that were followed up in service. As a result, we concluded that these markers could be used for the predictability of the course of patients diagnosed with Covid-19 from the first admission to the hospital. So, early precautions can be taken for the patients that predicted as severe according to these values taken on the day of hospital admission. And by this precautions, mortality and morbidity rates for Covid-19 could be decreased.

## Abbreviations

### **Covid-19**

Corona virus disease 2019

### **WHO**

World Health Organisation

### **COPD**

Chronic Obstructive Pulmonary Disease

### **ICU**

Intensive Care Unit

### **WBC**

White blood cell count

### **Trop-I**

High sensitive troponin I

### **NLR**

Neutrophile-lymphocyte ratio

### **Eos**

Eosynophile

### **Lymp**

Lymphocyte

### **Mono**

## Declarations

### Ethics approval and consent to participate

Because of our hospital is not a university or a training hospital, we do not have an ethical committee in our hospital. But all written ethical permissions were taken from directly "The Ministry of Health of Turkish Republic" for this article (17.05.2020/2020-05-12T14\_32\_16).

### Consent to publish

Not Applicable

### Availability of data and materials

The datasets used in this study are available from the corresponding author upon reasonable request.

### Competing interests

The authors declare that they have no competing interests

### Funding

Authors declare that there is no funding for this research

### Authors' Contributions

Ahmet Rifki Çora designed the study and wrote and revised the manuscript. Ersin Çelik analyzed the data and revised the manuscript

### Acknowledgements

Not applicable

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## Tables

**Table.1**

Variables	Categories	Service N (%)	Intensive Unit	Care	<i>p</i>
Gender	<i>Male</i>	59 (58.4)	57 (59.4)		<i>0.891</i>
	<i>Female</i>	42 (41.6)	39 (40.6)		
Blood Groups	<i>A+</i>	38 (37.6)	34 (35.4)		<i>0.766</i>
	<i>B+</i>	18 (17.8)	18 (18.8)		
	<i>O+</i>	27 (36.7)	28 (29.2)		
	<i>AB+</i>	7 (6.9)	6 (6.3)		
	<i>A-</i>	6 (5.9)	2 (2.1)		
	<i>B-</i>	1 (1.0)	2 (2.1)		
	<i>O-</i>	4 (4.0)	5 (5.2)		
Chronic Obstructive Pulmonary Disease	<i>None</i>	80 (79.2)	70 (72.9)		<i>0.300</i>
	<i>Yes</i>	21 (20.8)	26 (27.1)		
Coronary Artery Disease	<i>None</i>	86 (85.1)	66 (68.8)		<b><i>0.006*</i></b>
	<i>Yes</i>	15 (14.9)	30 (31.3)		
Diabetes Mellitus	<i>None</i>	97 (96.0)	88 (91.7)		<i>0.196</i>
	<i>Yes</i>	4 (4.0)	8 (8.3)		
Hypertension	<i>None</i>	81 (80.2)	79 (82.3)		<i>0.707</i>
	<i>Yes</i>	20 (19.8)	17 (17.7)		
Chronic Kidney Failure	<i>None</i>	100 (99.0)	92 (95.8)		<i>0.144</i>
	<i>Yes</i>	1 (1.0)	4 (4.2)		
Cerebrovascular Event	<i>None</i>	101 (100.0)	93 (96.9)		<b><i>0.037*</i></b>
	<i>Yes</i>	0 (0.0)	3 (3.1)		
Congestive Heart Failure	<i>None</i>	99 (98.0)	96 (100.0)		<i>0.101</i>
	<i>Yes</i>	2 (2.0)	0 (0.0)		
		<b>Mean±SD</b>			
Age	<i>year</i>	59.16±19.06	71.09±12.27		<b><i>&lt;0.001*</i></b>
Eosinophil	<i>cell/mcL</i>	1.27±1.24	0.97±1.77		<b><i>&lt;0.001*</i></b>
Lymphocyte	<i>μL</i>	20.05±11.41	12.77±8.42		<b><i>&lt;0.001*</i></b>
Neutrophil/Lymphocyte	<i>ratio</i>	5.68±5.49	11.45±19.22		<b><i>&lt;0.001*</i></b>
Monocyte	<i>10<sup>3</sup>/ μL</i>	6.66±3.26	5.39±2.88		<b><i>0.001</i></b>
White Blood Cell	<i>10<sup>3</sup>/ μL</i>	8.40±3.72	10.65±5.05		<b><i>0.001</i></b>
Troponin	<i>pg/mL</i>	13.68±23.63	1481.88±4924.03		<b><i>&lt;0.001*</i></b>

## Demographic Data of the Patients

Table.2

Variables	AUC	<i>p</i>	Cut-Off	Sensitivity	Specificity
White Blood Cell	0.642	<b><i>0.001*</i></b>	11.40	40.6%	84.2%
Troponin	0.823	<b><i>&lt;0.001*</i></b>	5.50	92.3%	59.2%
NLR	0.714	<b><i>&lt;0.001*</i></b>	5.80	64.2%	71.3%
Eosinophil	0.676	<b><i>&lt;0.001*</i></b>	0.350	46.9%	84.2%
Lymphocyte	0.698	<b><i>&lt;0.001*</i></b>	13.55	64.6%	67.3%
Monocyte	0.644	<b><i>0.001*</i></b>	5.55	65.3%	61.4%

# ROC analysis results of biochemical markers

## Figures

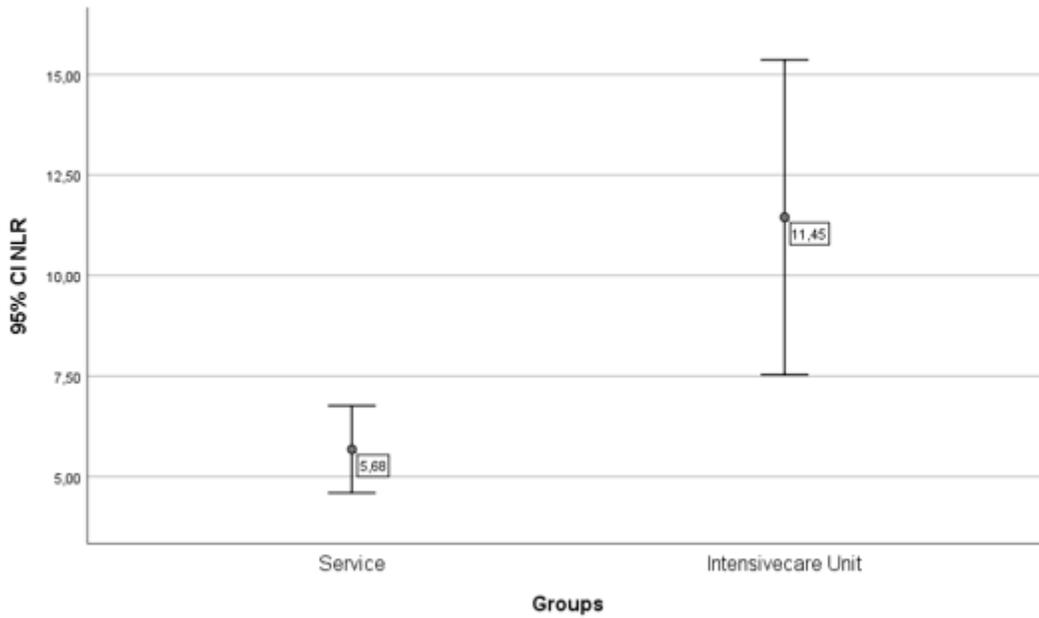


Figure 1

ROC analysis Graphic for Neutrophil/Lymphocyte Ratio (NLR)

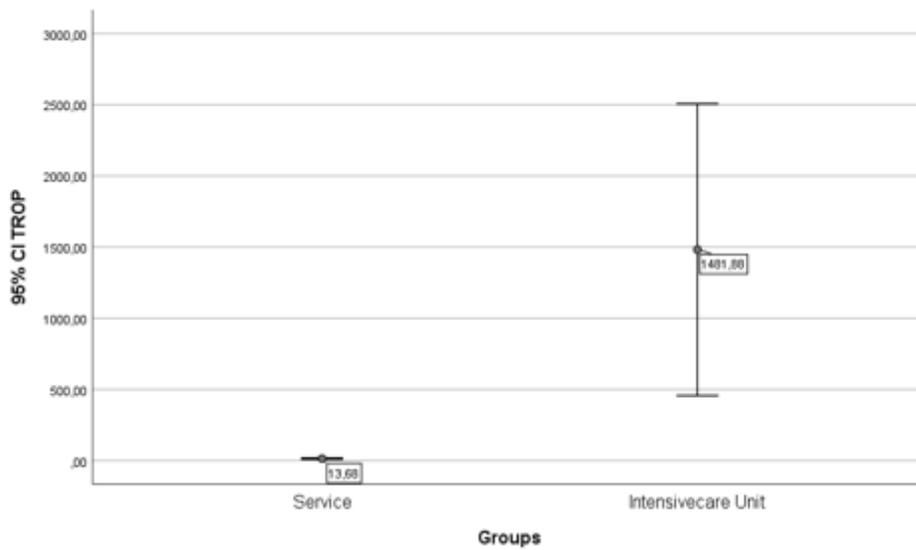
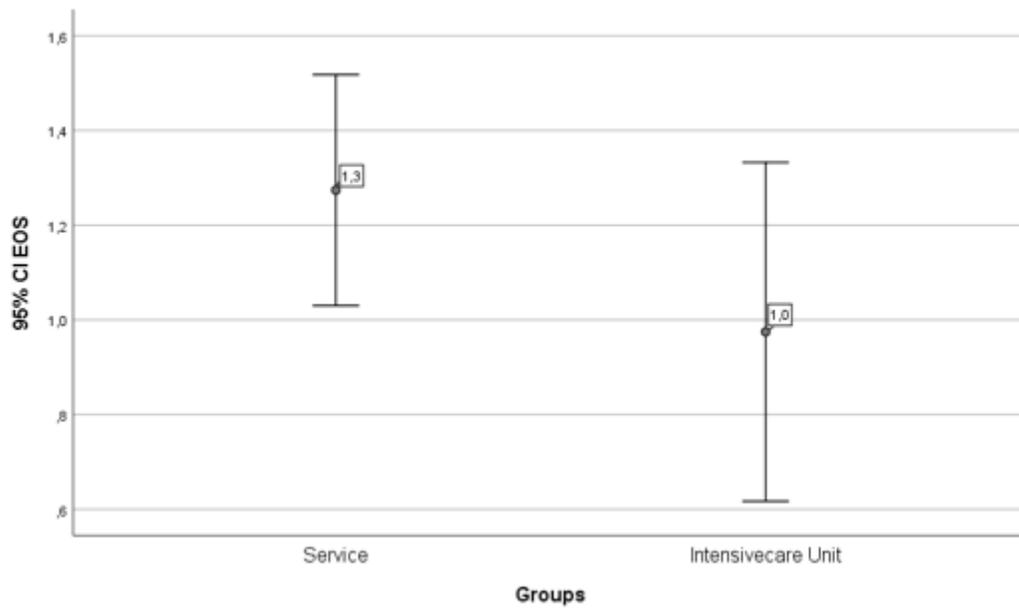


Figure 2

ROC analysis Graphic for High Sensitive Troponin-I



**Figure 3**

ROC analysis Graphic for Eosynophile Count