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The effectiveness of immature granulocyte counts for predicting COVID-19 severity and poor outcomes

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Objective The aim of this study is to examine the relationship between immature granulocyte (IG) counts and the severity of the disease and to evaluate the effectiveness of IG in predicting the poor outcomes in PCR-confirmed COVID-19 cases.
Methods The study was carried out prospectively and observationally at the emergency department. Patients were divided into three groups according to the clinical severity indicators such as mild, moderate and severe. The IG level was measured from the whole blood samples taken at the admission to the emergency department. Intensive care unit admission, ventilation support, and death within the first 28 days after the admission were evaluated as composite outcomes.
Results The study group consisted of 203 adults, of whom 91 (44.8%) were women. According to the severity of the illness, 40 patients (19.7%) were classified as mild, 67 patients (33.0%) as moderate, and 96 patients (47.3%) as severe. When comparing IG levels between the groups, there was a statistically significant difference between the mild and severe groups ($p = 0.047$) and between the moderate and severe disease groups ($p = 0.036$). There was no statistically significant relationship between IG counts and the composite outcome ($p > 0.05$)
Conclusion The IG level which could be measured faster than other laboratory tests without any additional cost, could be used for the determination of the clinical severity of patients with COVID-19. However, we conclude that this parameter is not effective in determining poor outcomes during the admission.

Keywords: COVID-19, Emergency department, Immature granulocyte, Mortality, Severity
Short Title in English: Immature granulocyte counts in COVID-19

1. Introduction

COVID-19 pandemic affects nearly every country, with over 4.0 million confirmed cases and over 280,000 deaths. Although majority of cases is mild disease (nearly 80%), the prognosis can be more severe; 20% of cases require hospital admission and approximately 5% require intensive care admission (1). The prognosis of COVID-19 is known to be worse in older adults, men and comorbidities such as hypertension, diabetes, cardiovascular disease, malignancy, chronic kidney disease, or chronic obstructive pulmonary disease (COPD) (2,3,4). Furthermore, abnormalities of certain laboratory tests such as lymphocyte count, D-dimer, ferritin, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and C-reactive protein (CRP) have been associated with the prognosis (5). However, there is still no exactly accepted test for the prediction of poor outcome and mortality.

Release of immature neutrophils into the bloodstream during infection or sepsis results in an increased in the immature granulocyte/total granulocyte ratio. This increase in immature granulocyte (IG) rate is widely used in the clinicas a diagnostic marker of infection or sepsis (6).

Studies have reported that IG rates are associated with disease severity and mortality related to sepsis or septic shock in patients with various infections such as bacteremia, pneumonia, and peritonitis (6, 7). Moreover, recent studies have shown that IG is also associated with the severity and prognosis of non-infectious inflammation-related diseases such as acute upper gastrointestinal bleeding and pancreatitis (6, 8, 9, 10, 11).

The purpose of this study is to examine the relationship between IG values at the time of admission to the emergency department and the severity of the disease in COVID-19 patients and to evaluate the effectiveness of IG in predicting the poor outcome, including intensive careunit admission, ventilation support, and first 28-day mortality in these patients.

2. Methods

The study was carried out prospectively and observationally at an urban hospital in the capital's largest district, being approximately 350.000 emergency room admissions annually, upon approval by the local Ethics committee (Nov. 24, 2020/2192). PCR-confirmed COVID-19 cases who were over 18 years old were included into the study with an Informed Consent Form. All oropharyngeal and nasopharyngeal swabs were collecting at the emergency department. Demographic characteristics, comorbidities, hemodynamic conditions, laboratory and radiological data and 28-day clinical outcomes of patients were recorded on the registration form. Patients were divided into three groups according to the clinical severity indicators such as mild, moderate and severe. The group which was described as severe, consisted of patients with shortness of breath and 30/minute respiratory rate (RR), ≤93% oxygen saturation at rest, PaO2/FiO2 ≤300 mmHg, intensive care and mechanical ventilation requirement and shock. Patients with high fever, respiratory symptoms, and radiological findings with pneumonia were included into the second group called "moderate". Aside from these, patients with stable vital signs and no signs of pneumonia were classified as "mild". Pregnancy, receiving blood transfusions, taking immunosuppressive or steroid medications treatment, having hematologic malignancies, or who had missing data were exclusion criteria for the study.

IG count was obtained from whole blood samples by using DIFF scattergram method (Mindray BC-6800, China). Blood samples were drawn in EDTA-coated tubes immediately after admission to the emergency department. Glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), sodium, potassium, lactate dehydrogenase

(LDH), C-reactive protein (CRP), High Sensitivity Troponin I, D-dimer, albumin, lactate levels, complete blood counts, blood gas levels, and erythrocyte sedimentation rates were analyzed in all cases.

Intensive care unit (ICU) admission, ventilation support and death within the first 28 days after the admission were evaluated as composite outcomes.

2.1. Statistical analysis

All data were analyzed by IBM SPSS Statistics for Mac, version 25.0 for Mac OS X (IBM Corp., Armonk, N.Y., USA). The normality of the data distribution was determined by the Shapiro-Wilk test, histogram, and Q-Q plots. The categorical values of the patients were expressed as a number and a percentage and were analyzed with a Chi-square test. Continued values were presented as a mean and standard deviation (SD) or median values and an interquartile range (IQR) of 25%–75%. The non-parametric values were analyzed using the Mann–Whitney U and Kruskal Wallis tests. For post-hoc analysis, new p-value level calculated with Bonferroni correction. The 95% confidence intervals (95% CIs) were also calculated when appropriate, and a p-value <0.05 was considered statistically significant.

3. Results

The study group consisted of 203 adults, of whom 91 (44.8%) were women. The median age of the cases was 61 (49-73), and it was 85 (41.9%) for cases who were over 65 years old. 101 (49.8%) patients were febrile (has a measured body temperature over 38°C) on admission to the hospital. The fever was the most common reason for hospital admission, followed by dyspnea in 96 patients (47.3%), muscle pain in 92 patients (45.3%), and weakness in 87 patients (42.9%). The most common comorbidities were hypertension in 87 patients (42.9%), diabetes mellitus in 43 patients (21.2%), and coronary heart disease in 40 patients (19.7%) (Table1).

Laboratory data are shown in Table 2. Laboratory tests determined the median (IQR 25-75) of CRP as 70.49 mg/L (17.37-117.88), D-dimer as 650 ng/mL FEU (340-1215), High Sensitivity Troponin I as 5.14 ng/L (2.5-19.09), and Immature Granulocyte count as 0.01 (0.01-0.02).

According to the severity of the illness, 40 patients (19.7%) were classified as mild, 67 patients (33.0%) as moderate, and 96 patients (47.3%) as severe. The IG median values of the

mild, moderate, and severe group were 0.01 (0.00-0.02), 0.01 (0.01-0.02), and 0.015 (0.01-0.03) respectively. When comparing IG levels between the groups, no significant difference was found between patients with mild and moderate disease ($p = 0.7$). There was a statistically significant difference between the mild and severe groups ($p = 0.047$) and between the moderate and severe disease groups ($p = 0.036$) (Figure 1).

Pneumonia was diagnosed by using pulmonary tomography in 152 cases (87.9%). While 72 cases (35.5%) were discharged from the emergency department, 112 cases (55.2%) were hospitalized in various clinics and 19 cases (9.4%) were hospitalized in the intensive care unit (Table 3).

Considering intensive care admission, ventilation support, and death as composite outcomes; there was a significant correlation between age, dyspnea at the time of admission, vital signs, renal and hepatic function tests, CRP, D-Dimer, troponin, albumin levels and the composite outcome. There was no statistically significant relationship between IG counts and the composite outcome ($p > 0.05$) (Table 4).

4. Discussion

We have two main findings in this study, where we investigated the effectiveness of the immature granulocyte count as a predictor of disease severity and poor outcomes in patients admitted to the emergency department with a diagnosis of COVID 19. First, we found a significant relationship between the total number of immature granulocytes and the severity of the disease. IG counts have been shown to be significantly higher in patients with severe disease compared to mild and moderate patients. This suggests that it may be used as an indicator of the severity of the disease in the management of patients, as well as levels of CRP and D-Dimer. Although obtaining the IG count quickly from complete blood count without any additional cost is an advantageous aspect compared to other tests, we believe that studies with larger sample sizes are necessary because the level of statistical significance is close to the limit.

Secondly, we also found that there was no statistically significant relationship between IG count and the composite outcome consisting of intensive care unit (ICU) admission, ventilation support, and 28-day mortality. This finding differs from previously published

research findings on the relationship such as sepsis, pancreatitis, and gastrointestinal bleeding (6,8,9,10).

Under normal conditions, IG is not present in the peripheral bloodstream. The presence of immature granulocytes in peripheral blood shows that the bone marrow has been stimulated by infection, inflammation, or another stimulus. It has been reported that under inflammatory conditions (infection, sepsis vs.), elevation of IG counts were observed much more earlier than other widely used parameters such as CRP or white blood cell count and IG count could be used as an inflammation marker (8). IG count was found to be substantially higher in inflammatory conditions such as acute appendicitis, pancreatitis, liver abscess, and infective complications after cardiac surgery (8,11). It has also been documented that the IG count may be used as an independent mortality marker in patients with pancreatitis and gastrointestinal bleeding (9,10,12).

Even though the most common symptoms of COVID-19 disease are cough, fever, headache, myalgia and diarrhea. Dyspnea is the most common symptom in patients with serious illness and it is associated with hypoxemia. The severe disease picture is progressive, and the disease known as respiratory distress syndrome (ARDS) could lead to acute bilateral infiltrations in the lungs, severe hypoxemia, heart failure, and unexplained pulmonary edema. Lymphopenia and thromboembolic complications could be commonly developed in these patients. In addition, severe COVID-19 disease could cause severe organ damages like acute inflammation in, heart, kidney and liver (13). Therefore, it is extremely important to anticipate the fatal images that may occur and to guide the course of the disease with the measures to be taken.

Huang et al. reported that elevated IG levels in patients with acute pancreatitis could be used to identify patients at high risk of ARDS early on, typically before admission to the ICU (12). It could be estimated that assessment of IG level during the admission could reduce the aggravation of the disease by taking adequate measures. In our study, we examined whether IG levels during the admission could be a guide for composite outcomes such as ARDS, intubation and mechanical ventilation requirements in COVID-19 patients, we did not find a relationship between poor outcomes and IG levels, contrary to findings of Huang et al. However, in our study, IG levels were examined through blood samples that were taken during the hospital admission from the emergency department. The relationship between later the peak IG levels in the later days of patients, and mortality and poor outcomes were not

investigated. Therefore, it is still uncertain whether IG count changes could be used for monitoring the prognoses of these patients.

In our study, we found a statistically significant relationship between the parameters defined as poor outcomes and age including dyspnea during the admission, hypoxemia, abnormal platelet, lymphocyte, BUN, creatinine, AST, LDH, CRP, D-Dimer, Troponin I, and albumin levels. Our findings were similar to the literature (3, 13). Inflammation, coagulation disorders, and ultimately tissue hypoxia resulting from COVID-19 are among the leading causes of death. Hypercoagulability and tissue hypoxia due to decreased blood flow which are observed in severe illness may cause multiple organ failure and death. Older age is accepted as an independent risk factor for mortality (13). The results of our study show that death rates are higher in older people, in accordance with the literature.

4.1.Limitations

As our study was conducted only in patients who were admitted to the emergency department, the total number of moderate and severe cases was higher compared to the social distribution. In addition, our study was conducted single center and with limited sample size. Additionally, later IG levels and peak IG values of patients could not be obtained so the relationship between IG levels and the composite outcome, particularly mortality could not have beenanalyzed.

5. Conclusion

The IG level which could be measured faster than other laboratory tests without any additional cost, could be used for the determination of the clinical severity of patients with COVID-19. However, we conclude that this parameter is not effective in determining poor outcomes during the admission, and more meaningful results could be obtained with repeated analyses of IG levels during the follow-up. Therefore, more comprehensive studies are necessary.

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Tables

Table 1. Demographic Characteristics.

Age, years, median (IQR 25-75)	61 (49-73)
Age groups	***n (%)***
<65	118 (58.1%)
≥65	85 (41.9%)
Female gendern (%)	91 (44.8%)
Comorbidities	***n (%)***
Hypertension,	87 (42.9%)
Coronary Heart Disease	40 (19.7%)
Congestive Heart Failure	8 (3.9%)
Diabetes Mellitus	43 (21.2%)
Chronic Kidney Disease	6 (3%)
Malignancy	4 (2%)
Using immunosuppressant	1 (0.5%)
Cerebrovascular Disease	7 (3.4%)
Symptoms	***n (%)***
Fever	101 (49.8%)
Dyspnoea	96 (47.3%)
Headache	30 (14.3%)
Sore Throat	12 (5.9%)
Muscle Pain	92 (45.3%)
Weakness	87 (42.9%)
Loss of smell	4 (2%)
Loss of taste	4 (2%)
Nausea	32 (15.8%)
Vomiting	11 (5.4%)
Diarrhoea	20 (9.9%)
Haemoptysis	1 (0.5%)
Syncope	1 (0.5%)
Back Pain	1 (0.5%)
Smoking	***n (%)***
No	86 (42.4%)
Yes, but left more than a year	49 (24.1%)
Yes	68 (33.5%)
Clinical Severity	***n (%)***
Mild	40 (19.7%)
Moderate	67 (33.0%)
Severe	96 (47.3%)
Radiological Data	***n (%)***
XR Pneumonia Identified	13 (39.4%)
CT Pneumonia Identified	152 (87.9%)
CT percentage of involvement, median (IQR 25-75)	25 (8-40)
CT percentage of involvement group	
%0-25	56 (29.2%)
%25-50	55 (28.6%)

%50-75	37 (19.3%)
%75-100	5 (2.6%)

Table 2. Emergency Department Treatments, Respiratory Support Types, and Emergency Department Outcome.

Emergency Department Treatments	**n (%)**
Favipiravir	132 (65%)
Steroid	75 (36.9%)
Anticoagulant	74 (36.5%)
Respiratory Support Types	**n (%)**
No requirement	93 (45.8%)
Nasal	20 (9.9%)
Mask	81 (39.9%)
HFNO	5 (2.5%)
CPAP	1 (0.5%)
IMV	3 (1.5%)
Emergency Department Outcome	**n (%)**
Discharge	72 (35.5%)
Hospitalized into the services	112 (55.2%)
Hospitalized into the intensive care	19 (9.4%)

Table 3. Factors Influencing the Composite Outcome.

	No Composite Endpoints	**Composite Endpoints Exist**	**p Value**
Male Gendern(%)	80 (58.4%)	17 (65.4%)	0.714
Agemedian (IQR 25-75)	62.5 (52-72)	72.5 (58.5-78)	**0.002**
Comorbiditiesn (%)			
Hypertension	63 (46%)	14 (53.8%)	0.462
Coronary Heart Disease	29 (21.2%)	7 (26.9%)	0.517
Congestive Heart Failure	5 (3.6%)	3 (11.5%)	0.117
Diabetes Mellitus	29 (21.2%)	8 (21.6%)	0.284
Chronic Kidney Disease	3 (2.2%)	2 (7.7%)	0.180
Chronic Obstructive Pulmonary Disease	15 (10.9%)	1 (6.3%)	0.472
Malignancy	3 (2.2%)	1 (3.8%)	0.504
Using Immunosuppressant	0 (0%)	1 (3.8%)	N/A
Cerebrovascular Disease	4 (2.9%)	1 (3.8%)	0.586
Symptoms *n*(%)			
Fever	67 (48.9 %)	11 (42.3 %)	0.470
Cough	79 (57.7 %)	13 (50 %)	0.953
Dyspnoea	73 (53.3 %)	20 (76.9 %)	**0.026**
Headache	15 (10.9 %)	2 (7.7 %)	1.000
Sore Throat	4 (2.9 %)	0 (0%)	N/A
Muscle Pain	58 (42.3 %)	11 (42.3 %)	0.998
Weakness	61 (44.5 %)	11 (42.3 %)	0.835
Loss of smell and taste	1 (0.7 %)	1 (3.8 %)	0.294
Nausea	25 (18.2 %)	1 (3.8 %)	0.081
Vomiting	9 (6.6 %)	0 (0 %)	N/A
Diarrhoea	12 (8.8 %)	3 (11.5 %)	0.710
Haemoptysis	1 (0.7 %)	0 (0 %)	N/A
Vital SignsMedian (IQR 25-75)			
SBP	136 (126-145)	140 (124-147)	0.509
DBP	83 (70-89)	87 (67-93)	0.104
Pulse	95 (84-102)	101 (93-104)	**0.005**
RR	18 (16-22)	27 (17-30)	**0.001**
Fever	37.6 (36.8-38.1)	37.6 (36-37.8)	0.22
SPO2	89 (83-94)	77 (50.25-88)	**<0.001**
Laboratory DataMedian (IQR 25-75)			
Glucose	110 (97.5-164)	162 (120-489)	0.255
Urea	35.3 (24-50.9)	51.4 (36.4- 125.8)	**0.007**
Creatinine	1 (0.82-1.18)	1.34 (1.07-1.66)	**0.001**
Sodium	136 (132-139)	135 (132-142)	0.293
Potassium	4.24 (4.0.8-4.7)	4.36 (3.89-5.22)	0.505
CRP	98.35 (40.1-130.4)	204.5 (100.4-256.8)	**<0.001**
D-Dimer	855 (465-1850)	1520 (763-3370)	**0.009**
Troponin I	8.82 (2.5-18.25)	46.2 (8.1-112.9)	**<0.001**
AST	34 (24-59)	51 (30-85)	**<0.001**
ALT	19.5 (13-32.5)	32.5 (15.5-49.5)	0.638
LDH	318 (234-459)	377 (324-701)	**0.006**

Albumin	3.4 (3-3.6)	3 (2.4-3.4)	**0.002**
WBC	6.15 (4.7-8.7)	7.9 (4.38-14.38)	0.331
Haemoglobin	13.5 (11.6-14.7)	12.7 (11.2-13.3)	0.055
Platelet	213.5 (164-311)	147 (99-245)	**0.013**
Lymphocyte	1.3 (0.8-2)	0.69 (0.39-1.64)	**0.002**
Neutrophil	4.38 (3.11-6.55)	6.25 (3.15-13.5)	0.068
pH	7.41 (7.34-7.44)	7.42 (7.36-7.44)	0.563
pCO_2	36.5 (33.6-42.7)	35.5 (30.6-40.9)	**0.033**
HCO_3	23.3 (20.7-26)	22.6 (20.3-24.2)	**0.008**
Lactate	1.9 (1.3-2.7)	2.15 (1.8-3.4)	0.05
Immature Granulocyte	0.01 (0.01-0.02)	0.02 (0.003-0.03)	0.362
Immature Granulocyte %	0.15 (0.0045-0.2)	0.1 (0.027-0.2)	0.347
Radiological Data n (%)			
CT Involvement Severity			**<0.001**
%0-25	51 (39.8%)	3 (12%)	
%25-50	47 (36.7%)	32 (36.0%)	
%50-75	27 (21.1%)	9 (36%)	
%75-100	0 (0%)	5 (3.3%)	
CT Involvement Percentage Median (IQR 25-75)	12.50 (10-18.75)	40 (26.25-60)	**<0.001**

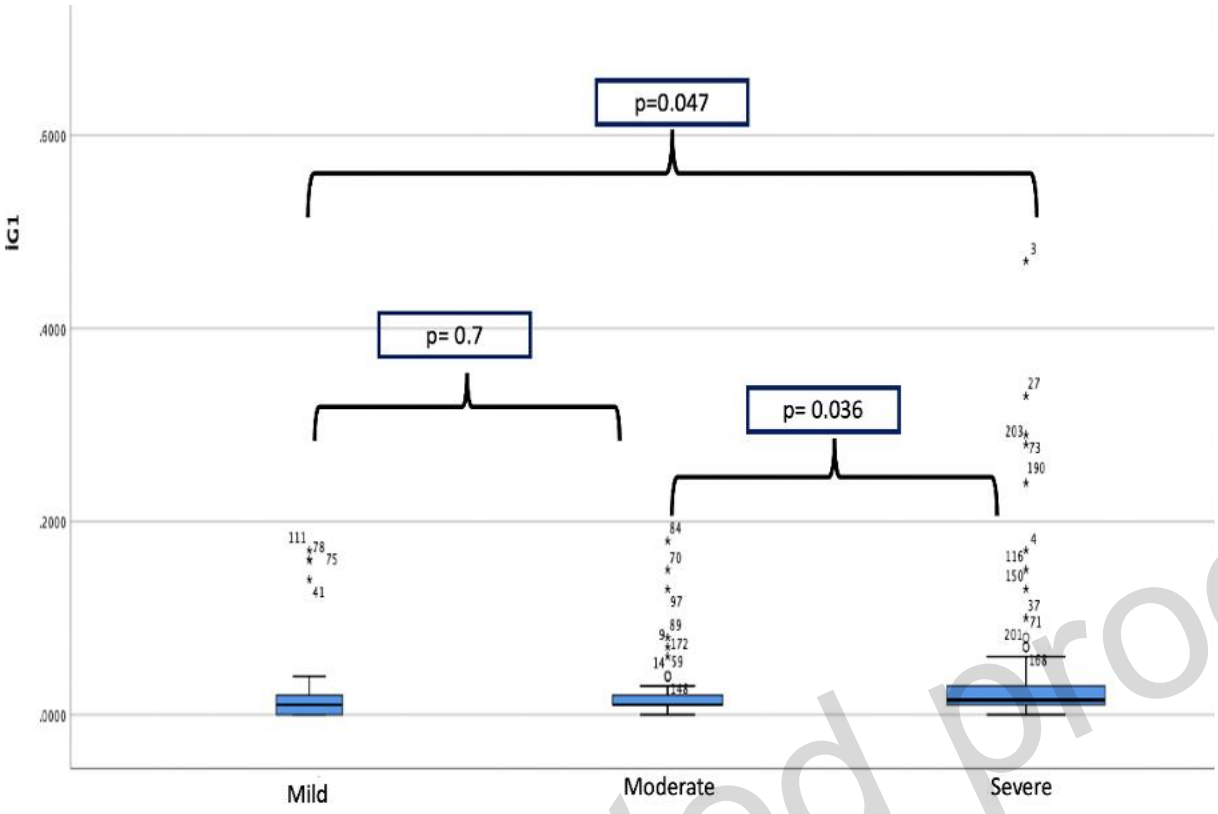

Figure 1. Relationship between immature granulocyte count and severity of the disease