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The Importance of Immature Granulocyte and Immature Reticulocyte Fraction for the Severity of Acute Bronchiolitis

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Abstract

The immature granulocyte (IG) and immature reticulocyte fraction (IRF) are new analytical parameters of the complete blood count, that have been studied as biomarkers of several inflammatory conditions. Here, our aim is to determine the effectiveness of IG and IRF percentages for the severity of acute bronchiolitis (AB). A single-center, prospective study was performed in patients who were hospitalized for acute bronchiolitis and healthy children were included as a control group. The demographic characteristics, white blood cell (WBC) count, platelet (PLT) count, eosinophil%, IG%, and IRF% values were analysed. Receiver operating characteristics (ROC) analysis was used to compare the diagnostic accuracies and predictive performances. We enrolled 168 infants in the acute bronchiolitis group and 80 in the control group. The Clinical Severity Score (CSS) showed that 48, 93, and 27 patients had mild, moderate, and severe bronchiolitis, respectively. The WBC, PLT, and IRF value were significantly higher in patients (p<0.001). There was no difference between the patients and control group in terms of IG and eosinophil percentage. Only a positive correlation was observed between the clinical severity of the AB and IRF (p=0.003).The ROC curve analysis indicated that the IRF% cut-off point for predicting severity AB was >12.4, with a sensitivity of 53% and specificity of 88% (Areas under the curves (AUC):0.701,p<0.001). The WBC count, PLT count, and IRF value increased in the AB group. The IRF percentage can be used to predict the clinical severity of AB in children.

Keywords: Acute bronchiolitis, immature granulocyte, immature reticulocyte fraction

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Introduction

Acute bronchiolitis (AB) is one of the most common causes of pediatric emergency room admissions in the first year of life. Viruses are the most common cause of AB, especially respiratory syncytial virus (RSV). It has become one of the most common reasons for hospitalization of children younger than 2 years of age during the winter months. It is characterized by respiratory distress, wheezing, cough, fever, and coryza and is most often associated with RSV infection. The clinical severity of AB ranges from mild cases that can be treated on an outpatient basis to severe cases that require mechanical ventilation in intensive care units. The hospitalization rate varies between 1% and 20% among children less than 24 months of age during seasonal epidemics.[1-4] Most children hospitalized due to AB have an uneventful course; however, approximately 2–6% requires admission to a pediatric intensive care unit (PICU), with 2–3% of hospitalizations requiring invasive mechanical ventilation. The World Health Organization has reported that RSV is the causative pathogen for over 30 million new acute lower respiratory infection episodes in children under 5 years of age and it gives rise to more than 3.4 million hospital admissions and more than150,000 deaths per year.[5-8] Various scoring systems have been developed, but still, there is no biomarker to predict the clinical prognosis of AB in order to reduce the mortality and morbidity of AB in children. Moreover, the biomarker must be fast, inexpensive, simple to measure and effective. Today, automated analyzers measure various complete blood cell (CBC) indices including the white blood cell (WBC) count, platelet (PLT) count, immature reticulocyte fraction (IRF), and immature granulocyte (IG) count. The IRF and IG provide a more precise evaluation of bone marrow activation. Diagnostic accuracy studies performed in the last years suggest that IRF and IG can provide clinically relevant information about inflammatory activity and disease prognosis.[9-12] This study was conducted to determine the effectiveness of immature cells such as IG and IRF for AB severity.

Highlights

- Acute bronchiolitis (AB) is one of the most common causes of pediatric emergency department admissions and hospitalization in the first year of life.
- The clinical severity of acute bronchiolitis ranges from mild cases that can be treated on an outpatient basis to severe cases that need hospitalization to the intensive care units.
- The immature granulocyte (IG) and immature reticulocyte fraction (IRF) are young cells that have been released into the circulation, and are considered indicators of bone marrow recovery and activation.
- A positive correlation was observed between the clinical severity of acute bronchiolitis and IRF.

Material and Method

A single center prospective study of children aged 1–24 months who were hospitalized with AB was performed between Semptember 2018 and May 2019. The study was approved by the local ethics committee. Written informed consent was obtained from all parents/guardians. In accordance with American Academy of Pediatrics guidelines, a diagnosis of AB was based on at least two of the following signs: chest retractions, tachypnea, and the first episode of wheezing or rales on auscultation following a viral upper respiratory tract infection in children aged younger than 24 months.[1] The study included 168 patients with acute bronchiolitis and 80 healthy children. Inclusion criteria were: aged 1–24 months, first wheezing episode, no previous disease history, and no previous medication. Exclusion criteria were: chronic disease, premature birth, birth weight <2500 g, malnutrition, passive smoking, proven immune deficiency, proven or suspected acute bacterial infection, previous treatment with bronchodilators or corticosteroids, or having symptoms for more than 7 days. All patients underwent a routine clinical evaluation in the emergency department by the pediatrician. On admission, the clinical severity score (CSS) for acute bronchiolitis (i.e., a composite clinical score including respiratory rate, retraction, wheezing, and general condition) was used to evaluate patients, as previously described by Wang et al.[13] Patients were divided into three groups as mild, moderate and severe according to the clinical severity score (CSS) of acute bronchiolitis which was previously described by Wang et al.[14] Complete blood count measurements (including white blood cells (WBC), platelets (PLT), eosinophil, IG, IRF) were recorded from the blood samples taken on the first day of hospitalization using an XE-2100 automated hematology analyzer (Sysmex, Kobe, Japan). WBC, PLT, IG, IRF and were obtained from the CBC without receiving any treatment such as steroids. To minimize variations due to sample age, all assays were performed within four hours of collection; the samples were kept at room temperature until the time of analysis. IRF was measured in a dedicated reticulocyte channel of the hematology analyzer by flow cytometry, using a proprietary fluorescent dye containing polymethine and oxazine. The IRF corresponds to the fraction (%) of medium and high fluorescence reticulocytes. Data from each patient recorded in the emergency room included: age, sex, disease history, medication, birth history, whether this was the first attack of bronchiolitis, weight, vital signs (i.e., heart rate, respiratory rate, tympanic temperature, and oxygen saturation when breathing ambient air, which was measured using pulse oximetry and expressed as SpO_2). The control group included 80 healthy children who attended pediatric clinics for routine health checks or vaccinations. They had similar age/sex demographic characteristics to the children with bronchiolitis. Complete blood counts data were obtained from blood samples taken for routine testing of these children at their first visit.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp. Armonk, NY: USA. Released 2013). For the normality analysis of the parametric data, the Shapiro Wilk test was used. Descriptive data were presented with statistical methods mean±standard deviation. Numerical variables were specified as median (min-max). Student's t-test was used to compare the groups with normal distribution. MannWhitney U test was used to compare non-normally distributed groups. Comparison between groups for data that did not show a normal distribution were performed using Kruskal-Wallis Test. The receiver operating characteristic (ROC) curve was used to evaluate the optimal cutoff points of the parameters for which significant differences were found. Sensitivity, specificity, cut-off points, negative predictive value (NPV), positive predictive value (PPV) and the area under the curve (AUC) were calculated for these parameters. The results were analyzed within a 95% confidence interval. A value of p<0.05 and AUC >0.600 was considered statistically significant.

Results

In total, 168 patients were included in this study, 102 (60.70%) of these patients were male and 66 (39.30%) were female. The control group included 80 healthy children, also with a mean age of 7.3±4.28 months, 47 (58.7%) of them were male and 33 (41.3%) were female. The patient and control group did not differ significantly in age or sex (p=0.58 and p=0.56, respectively). Patients had significantly higher WBC, PLT and IRF values compared with the control group (p<0.001) (**Table 1**).

Table 1
Comparison of demographic and laboratory characteristics of the patients and contol group

	Patients group Median (min-max)	Control group Median (min-max)	p
Age (Month)	8 (1-24)	8 (2-24)	0.580
Sex, male (n)(%)	102 (60.70)	47 (58.70)	0.560
IG (%)	0.32 (0.00-4.90)	0.30 (0.02-2.10)	0.216
IRF (%)	13 (3.70-36.60)	9.40 (5.50-16.00)	<0.001
WBC (10^9/L)	10260 (2040-31000)	6720 (3140-11500)	<0.001
PLT (10^9/L)	382 (74-746)	288 (101-449)	<0.001
Eozinofil (%)	0.2 (0.00-9.10)	0.20 (0.00-9.10)	0.756

Abbreviations: IG: Immature Granulocyte; IRF: Immature Reticulocyte Fraction; WBC: White Blood Cell; PLT: Platelet.

The patient group included 48 (28.60%), 93 (55.30%), and 27 (16.10%) children classified as having mild, moderate, and severe bronchiolitis, respectively. The median CSS was 6 (range, 2–12). No significant correlation was found between CSS and age, sex, body temperature, oxygen saturation (%), WBC, PLT, IG, and proportion of eosinophils (p>0.05). IRF values differed significantly among the mild, moderate, and severe groups (p<0.001). A positive correlation was observed between the CSS and the IRF (p<0.001). IG value showed a positive correlation with CSS, but it was not statistically significant (p=0.497) (**Table 2**).

Table 2
Comparison of the clinical severity of acute bronchiolitis and laboratory findings

	Mild Median (min-max)	Moderate Median (min-max)	Severe Median (min-max)	p
IG (%)	0.30 (0.00-2.50)	0.35 (0.00-4.10)	0.40 (0.03-4.80)	0.497
IRF (%)	10.50 (4.70-36.60)	14.20 (3.70-35.30)	14.25 (4.40-35.70)	0.003
WBC (10^9/L)	10.9 (2.81-31.00)	11.570 (4-23.80)	9.79 (4.19-23.80)	0.422
PLT (10^9/L)	220.50 (88-430)	231.0 (74-631)	218 (88-631)	0.335
Eozinofil (%)	0.5 (0.00-4.70)	0.15 (0.00-9.10)	0.40 (0.00-9.10)	0.280

Abbreviations: IG: Immature Granulocyte; IRF: Immature Reticulocyte Fraction; WBC: White Blood Cell; PLT: Platelet.

ROC curve analyses were used to evaluate the performance of IG and IRF in distinguishing AB patients from controls, and in assessing acute bronchiolitis severity. The AUC for IRF and IG was 0.701, 0.542, respectively. ROC curve analysis suggested that the cut-off for using IRF% to predict severity AB was >12.4, with a sensitivity of 53% and a specificity of 88%. The positive and negative predictive values of the IRF were 91.3% and 45.8%, respectively (p<0.001). The cut-off for using IG% to predict severity acute bronchiolitis was>0.01, with a sensitivity of 2.8% and a specificity of 100% (**Table 3**). The positive and negative predictive values of the IG were 100.0% and 31.5%, respectively (p=0.19) (**Figure 1**).

Table 3
Area under the ROC curves, cut-off values and P-values of immature cells

	Cut-off	Sensitivity %	Specificity %	AUC	95% CI	p
IG (%)	>0.01	2.80	100	0.542	0.39-0.51	0.195
IRF (%)	>12.4	53.10	88.70	0.701	0.64-0.75	<0.001

Abbreviations: IG: Immature Granulocyte; IRF: Immature Reticulocyte Fraction; AUC: Area Under The Receiver Operating.

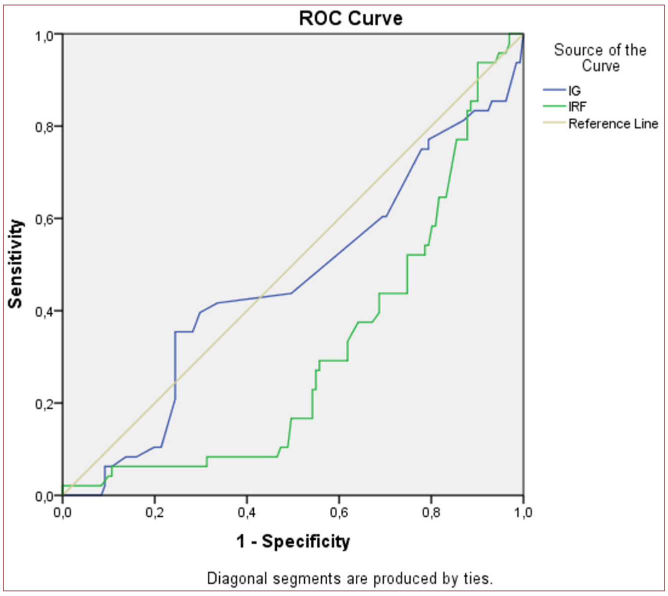

Figure 1. Receiver operating characteristic curve (ROC) analysis of Immature Reticulocyte Fraction (IRF) and Immature Granulocyte (IG).

Discussion

We found that the IRF was greater in patients with AB than in healthy children and was positively correlated with the clinical severity of AB. To the best of our knowledge, this is the first study to investigate IRF and AB severity. The patients with AB and high IRF values should be monitored closely after admission to the emergency department. IRF values higher than 12.4% had a specificity of 88% and a sensitivity of 53% for clinical deterioration of acute bronchiolitis.

The IRF and IG are young cells that have recently been released into the circulation, and are considered indicators of bone marrow recovery and activation. They are important in various clinical conditions such as thrombocytopenia, anemia, inflammation severity, bone marrow regeneration after transplantation of hematopoietic stem cells and after chemotherapy. In recent years, it has been possible to detect the percentage and number of IG and IRF due to technical developments in automated hematological analyzers.[14-17]

IRF is defined as the ratio of young reticulocytes to the total number of reticulocytes. IRF is an examination of bright reticulocyte fraction with a high content of RNA.[18,19] There are different results regarding the normal range of IRF and IG due to the different instruments used in IRF measurement, standardization and calibration problems.[20,21] Gonçalo et al. was accepted the IRF reference median as 4.7% (range: 1.1-11.4%).[15] Use of normal values of the percentage of IG for adults and children, all reported as <1%.[21,22] The reference range of the analyzer in our study was 0-0.9% for IG and 8.4-25.8% for IRF.

IRF value may increase due to increased bone marrow activity in infectious conditions.[12,23] One study found that IRF was significantly higher in patients with sepsis than in healthy individuals (p<0.001) In the same study, higher IRF values were found in patients with severe sepsis compared to patients with sepsis, but no significant difference was found (p=0.53).[10] Park SH et al. found significantly higher IG and IRF values in patients with sepsis compared to patients with non-sepsis (p<0.001, p=0.030 respectively).[24] IRF values higher than 5.6% had a specificity of 56.0% and a sensitivity of 77.6% for diagnosis sepsis (AUC: 0.658). IG values higher than 0.4% had a specificity of 74.5% and a sensitivity of 67.0% for diagnosis sepsis (AUC: 0.812). IG is generated and differentiated in bone marrow, and their presence in peripheral granulocytes circulation indicates greatly increased bone marrow activation due to an infectious condition. The importance of IG is unknown to many clinicians. The IG percentage is defined as the percentage of the total WBC count. Therefore, it is suggested that IG can be considered as a new early diagnosis and prognostic marker in infectious diseases. An elevated IG% implies the enhancement of bone marrow activity to fight against to sources of infections before leukocytosis is occurred.[10,16,25] In addition several recent studies have investigated the role of IG percentage measurement as a potential marker to predict severity of an infection.[25-29] Ansari-Lari et al.[10] found a significantly higher percentage of immature granulocytes in infected than in non-infected patients and designated a percentage of IG of more than three (IG%>3) as a predictor of sepsis, with a specificity of more than 90%. Senthilnayagam et al.[16] found that IG percentage of blood culture positive children patients were significantly higher than in culture negative patients. Pavare et al.[30] found that the cutoff level of IG percentage to predict serious bacterial infections was 0.45 (84% specificity, 66% sensitivity, 90% positive predictive value). Their findings suggest that serious bacterial infections in children is associated with an increase in IG percentage. In our study, similar to the literature IG values were gradually increasing from mild group to severe group, but there was no statistically significant difference (p=0.49). Also, the IG value was higher in the AB group compared to the healthy group, but there was no significant difference (p=0.21). In the literature, we did not find any other study investigating the relationship between acute bronchiolitis and IG other than ours.

This study had certain limitations. Firstly, the findings presented were the experience of a single center and therefore, may not be generalizable to all institutions. Secondly, viral agents of the patients could be determined and compared according to the clinical severity of the disease. The number of patients in need of intensive care, and the hospitalization rate and duration of hospitalization of patients with mild, moderate and severe bronchiolitis were not recorded as our third limiting factor.

Conclusion

Increased levels of IRF, WBC, PLT were observed in the acute bronchiolitis group. Moreover, IRF may be a diagnostic biomarker to assess the clinical severity of acute bronchiolitis. Larger prospective studies are needed to clarify the clinical significance of using IPF values to assess patients with acute bronchiolitis.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

Conflict of Interest: There are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Ethics Committee Approval: The study was carried out with the permission of Erciyes University Local Ethics Committee (Date: 23.02.2018, Decision No: 2018-91).

Financial Disclosure: The authors have no conflicts of interest to declare.

Informed Consent: Informed consent was obtained from the parents of the patients.

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