The role of immature granulocyte percentage in predicting acute chest syndrome and the severity of the vaso-occlusive crisis in sickle cell disease

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ABSTRACT

\textbf{Background.} Sickle cell disease (SCD) is an inflammatory disease that can result in both chronic and acute inflammation. Immature granulocytes (IG) are not-yet-mature white blood cells that can be easily detected in complete blood count (CBC) tests. In recent studies it has been suggested that IG may play a role in determining the prognosis of inflammatory diseases. The aim of our study was to investigate the role of IG percentage on predicting acute chest syndrome (ACS) and the severity of vaso-occlusive crisis (VOC) in patients with SCD.

\textbf{Methods.} The study cohort consisted of 49 SCD patients admitted to the emergency department for VOC. If symptoms did not regress despite appropriate treatment including hydration and analgesia, they were hospitalized. Patients whose symptoms regressed were discharged from the emergency department within 24 hours. Blood samples, including CBC and C-reactive protein (CRP), a marker of inflammation, were taken within the first hour of admission. Steady state laboratory parameters from the previous visit in the last three months were collected from patient files.

\textbf{Results.} The mean age was 18±4 (range 8-25) years. Most were hospitalized (41/49; 83.7%) and 8 of 49 were discharged from the emergency department after their treatment for VOC. ACS developed in 13 of 49 (26.5%). White blood cell, neutrophil and nucleated red blood cell counts, percentage of IG (IG\%) and CRP levels were significantly increased in patients with VOC. IG\% of patients with ACS was significantly higher than patients without ACS. However, ROC analysis showed that IG\% was not associated with the development of ACS or hospitalization for VOC.

\textbf{Conclusions.} Despite a small SCD cohort, the significant increase in the IG\% in patients with VOC compared to their baseline values has suggested a role for IG\% in predicting VOC. Although IG\% was higher in ACS, its utility in predicting ACS was poor.

\textbf{Key words:} sickle cell disease, inflammation, immature granulocyte.
sickle erythrocytes is particularly important in VOC pathogenesis.\textsuperscript{3-5}

Immature granulocytes (IG) are a not-yet-mature subset of white blood cells that can be easily detected in complete blood count tests. IG contain granules in their cytoplasm and include metamyelocytes, myelocytes and promyelocytes. The IG count is an indicator of bone marrow activation, particularly leukopoiesis, and raised IG counts are found in response to inflammatory conditions, as well as in the neonatal period or in pregnancy. Recent studies have suggested a role for the IG percentage (%) in peripheral blood in determining the prognosis of inflammatory diseases.\textsuperscript{6-10}

While the role of neutrophils in the pathogenesis of VOC is known, there is no data about the relation of IG\% with VOC. The aim of this study was to investigate IG\% in the steady state and during VOC in SCD, and to evaluate its potential role in predicting VOC. We also aimed to determine the relationship between IG\% and other inflammatory parameters in SCD.

Material and Methods

This retrospective study included SCD patients that were under follow-up between November 2020 and February 2021 by the department of Pediatric Hematology and were admitted to the emergency department for VOC. If the symptoms did not regress, despite appropriate treatments such as hydration and analgesics, they were hospitalized. Patients whose symptoms regressed were discharged from the emergency department within 24 hours. The patients who had another inflammatory disease and infection were excluded from this study. ACS was diagnosed by the presence of fever and/or respiratory symptoms and a new pulmonary infiltrate on chest X-Ray.

Blood samples were taken from all patients within the first hour of admission. Complete blood count (CBC) was performed on an automated hematology analyzer, XN-1000 (Sysmex Corp., Kobe, JAPAN). IG\% was calculated from the white cell differential channel based on granularity and nucleic acid content. Steady state laboratory parameters from the previous visit and within the three months prior to the emergency admission were collected from patient records.

The study was approved by Mersin University Ethical Board (number: 03.03.2021; 2021/220).

Statistical analyses were performed using SPSS Statistics for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean± standard deviation (SD) while frequency and percentage (%) were used to define categorical data. Categorical variables were analyzed using the Chi-square test and Fischer’s exact test. Normality of distribution was examined using the Kolmogorov-Smirnov test. Mann-Whitney U test was used for comparisons between two groups.

Receiver Operating Curve (ROC) analysis was performed to determine the effect of IG\% on having ACS or hospitalization for VOC. The areas under the curve (AUC) of the IG\% parameter, statistical significance level, cut-off sensitivity and specificity values were calculated.

Results

During the study period a total of 49 SCD patients (43 HbSS and 6 HbSβ) were admitted to the emergency department for VOC. The mean±SD age was 18±4 (range 8-25) years. Thirty-two patients (67\%) were male and 17 (33\%) were female. Twenty-three patients had ACS, 15 patients had avascular necrosis (AVN) and three patients had a history of stroke. The mean annual VOC frequency of the patients was 1.7±0.88 (range 1-5). Eleven patients (22\%) were transfused and three patients were on the chronic transfusion program in the last year. Of the 49 patients, 46 (93.9\%) were on hydroxyurea treatment.

Forty-one of 49 (83.7\%) patients were hospitalized and the remaining eight were
discharged from the emergency department after their treatment for VOC. ACS developed in 13 of 49 (26.5%) patients.

All inflammatory markers, including leucocyte (WBC) and neutrophil counts, C-reactive protein (CRP) levels, and IG%, were significantly different during VOC compared with the previous steady state values (Table I). Platelet counts and hemoglobin (Hb) concentrations were significantly decreased and WBC and neutrophil counts, nucleated red blood cell (NRBC) %, IG% and CRP levels were significantly increased in VOC.

Hb levels were significantly lower (p=0.027) and CRP levels were significantly higher (p=0.030) in patients who were hospitalized compared to patients discharged from the emergency department (Table II). There was no correlation between IG% and length of stay in the patients who were hospitalized.

The IG% of patients with ACS was significantly higher than patients without ACS (Table III). Using ROC analysis to investigate the relationship of IG% and ACS, the cut-off value for IG% was >1%, specificity 60.53%, sensitivity 57.14%, and the AUC was 0.52. Thus IG% was not discriminatory for ACS (p=0.82). Similarly IG% was not significant in identifying which patients would be hospitalized for VOC (p=0.829; specificity 41.7%; sensitivity 75%; and AUC=0.524).

When SCD patients were grouped according to genotype, IG% was significantly higher in SS than SB patients during VOC (p=0.016). All

### Table I. Laboratory parameters of the patients (n=49) during steady state and VOC.

|                      | Steady state          | VOC                | p value |
|----------------------|-----------------------|--------------------|---------|
| WBC ((µL)            | 11.261 ± 3.391        | 17.215 ± 5967      | 0.001   |
| Hb (g/dL)            | 9.3 ± 1.1             | 9.0 ± 1.5          | 0.037   |
| Platelet (µL)        | 471.204 ± 192.504     | 387.122 ± 198.561  | 0.006   |
| Neutrophil (µL)      | 5.302 ± 1.952         | 9.431 ±4.083       | 0.001   |
| IG%                  | 0.37 ± 0.18           | 1.35 ± 1.46        | 0.001   |
| NRBC %               | 1.38 ± 2.37           | 3.59 ± 7.62        | 0.017   |
| MPV (fl)             | 8.17 ± 3.45           | 8.42 ± 3.26        | 0.374   |
| CRP (mg/dL)          | 12.32 ± 33.00         | 14.85 ± 15.90      | 0.001   |

VOC: Vaso-occlusive crisis, WBC: White blood cell, Hb: Hemoglobin, IG%: immature granulocyte percentage, NRBC: Nucleated red blood cell, MPV: Mean platelet volume, CRP: C-reactive protein

### Table II. Comparison of laboratory parameters in the SCD patients who were hospitalized or non hospitalized for VOC.

|                      | Non-hospitalized patients (n=8) | Hospitalized patients (n=41) | P value |
|----------------------|---------------------------------|------------------------------|---------|
| WBC (µL)             | 18.030±3.947                    | 17.055±6.098                 | 0.438   |
| Hb (g/dL)            | 9.9±1.0                         | 8.8±1.6                      | 0.027   |
| Platelet (µL)        | 463.500±138.558                 | 372.220±202.294              | 0.193   |
| Neutrophil (µL)      | 9.219±4.963                     | 9.472±3.961                  | 0.895   |
| IG%                  | 1.20±0.86                       | 1.38±1.60                    | 0.559   |
| NRBC %               | 1.57±1.20                       | 4.02±8.47                    | 0.391   |
| MPV (fl)             | 8.23±3.36                       | 8.46±3.28                    | 0.523   |
| CRP (mg/dL)          | 8.42±10.64                      | 16.10±10.64                  | 0.030   |

SCD: sickle cell disease, VOC: vaso-occlusive crisis, WBC: white blood cell, Hb: hemoglobin, IG%: immature granulocyte percentage, NRBC: nucleated red blood cell, MPV: mean platelet volume, CRP: C-reactive protein
other CBC and inflammatory parameters were similar. There was no correlation between IG% and the history of the type of sickle cell disease, AVN, ACS, stroke and annual frequency of painful crisis. In addition, no correlation was found between chronic transfusion and IG%.

**Discussion**

Immature granulocytes are produced and differentiated in bone marrow, and their presence in the circulation indicates greatly increased bone marrow activation due to infectious or inflammatory conditions. Additionally, IG% may be elevated in other conditions, like cancer and during pregnancy. To our knowledge, the relationship of IG% with clinical and laboratory findings in SCD has not been investigated to date. In our study, IG% level was found to be significantly higher during VOC than when the disease is stable, the steady state. Similarly, patients with ACS had significantly higher IG% than the patients without ACS.

CRP is recognized as a good marker of acute and chronic inflammation and is frequently used in SCD patients. High levels of CRP at steady state are associated with an increased frequency of acute pain and increased levels during VOC have been reported to be valuable in predicting the development of ACS in SCD patients. We found high CRP levels in patients with SCD both during steady state and VOC. CRP levels were significantly higher in the patients who were hospitalized than the patients discharged from the emergency department after their treatment for VOC (p=0.03).

It is known that neutrophil counts are high in patients with SCD, both during steady state and VOC, and is also related with increased mortality risk. In our study, both WBC and neutrophil counts were increased during VOC compared to steady state levels (p<0.001). However, there was no difference in WBC and neutrophil counts between patients with VOC who were hospitalized and those discharged from the emergency department. In addition there was no correlation between WBC and neutrophil counts were not determinants of the length of hospital stay.

IG level was used as an early biomarker to show infection and inflammatory status in some studies. IG% was found to be increased in a study of patients with peripheral enthesitis and correlated with CRP elevation and clinical activity. Narcı et al. investigated IG% in patients with upper gastrointestinal bleeding and reported that IG% was significantly higher in patients who died compared with patients who were discharged. The IG% was specific (93.8%) and sensitive (100%) in predicting in-hospital mortality. Unal et al. suggested that increased IG% is a simple, fast, and effective marker in the early prediction of acute necrotizing pancreatitis. Additionally, Güngör et al. reported that IG% had higher significance.

| Table III. SCD patients with or without acute chest syndrome (ACS). |
|-------------------------------------------------|
| ACS (n=13)                       | Without ACS (n=36) | p value |
|----------------------------------|--------------------|---------|
| WBC (µL) 17.816 ±8.532          | 16.998±8.532       | 0.526   |
| Hb (g/dL) 9.4 ±2.0               | 8.8±1.3            | 0.196   |
| Platelet (µL) 365.838±235.301   | 394.808±186.704    | 0.571   |
| Neutrophil (µL) 8.562±3.659     | 9.744±4.230        | 0.428   |
| IG% 2.48±2.18                   | 1.00±0.8           | 0.009   |
| NRBC % 2.95±7.0                 | 3.86±8.15          | 0.545   |
| MPV (fl) 8.22±3.71              | 8.49±3.13          | 0.856   |
| CRP (mg/dL) 15.39±12.78         | 14.65±17.0         | 0.556   |

SCD: sickle cell disease, ACS: acute chest syndrome, WBC: white blood cell, Hb: hemoglobin, IG%: immature granulocyte percentage, NRBC: nucleated red blood cell, MPV: mean platelet volume, CRP: C-reactive protein.
sensitivity and specificity in predicting systemic inflammatory response syndrome in patients with acute pancreatitis. However, Park et al.\textsuperscript{7} suggested that the diagnostic ability of IG% was insufficient in patients with acute and complicated appendicitis, and was of no additional benefit in investigating appendicitis compared with other inflammatory markers.

In our study, IG % was found to be significantly higher during VOC than the steady state (p<0.001). However, there was no difference in IG% between the patients who were hospitalized and the patients discharged from the emergency department. In addition, there was no correlation between the IG% and length of stay in the patients who were hospitalized. Also, ROC analysis indicated that IG% was not discriminatory for hospitalization for VOC.

ACS is an important cause of morbidity and mortality in children and adults with SCD. Several risk factors have been associated with the development of ACS, such as young age, low HbF, high baseline hemoglobin, steady-state leukocytosis, and airway hypersensitivity. Since two-thirds of ACS episodes occur in patients hospitalized for VOC, identifying biomarkers to predict ACS occurrence would be of clinical benefit.\textsuperscript{14} In our study, ACS developed in 26.5% of patients who were admitted to the emergency department because of VOC. The IG% of patients with ACS was significantly higher than patients without ACS. Despite this, ROC analysis showed that the IG% parameter did not have discriminatory power for the occurrence of ACS.

Biomarker investigations are ongoing to predict VOC in SCD. While the role of IG%, an easily calculated parameter in CBC, has been investigated in determining the severity of diseases in many infectious or inflammatory diseases, it has been evaluated for the first time in SCD in this study. Despite the limited number of patients, the significant increase in IG% in patients with VOC compared to the steady state has suggested a role for IG% in predicting VOC in SCD. Although IG% was higher in patients with ACS, analysis showed that this was insufficiently discriminatory in predicting ACS.

**Ethical approval**

The study was approved by Mersin University Ethical Board (number: 03.03.2021; 2021/220).

**Author contribution**

All authors confirm their contribution with data input and interpretation, drafting the manuscript. FK; draft manuscript preparation. SÜ; study conception and design. DBT; analysis of biochemical data. YÖ; analysis and interpretation of results. GB; data collection. All authors reviewed the results and approved the final version of the manuscript.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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