Flowcytometric Ratios of Immune Cells in the Prognosis and Staging of Acute Biliary Pancreatitis

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ABSTRACT

Objective: To evaluate several lymphocyte subtypes with various parameters that can be applied easily and give fast results for their roles in evaluating the stage and prognosis of acute biliary pancreatitis.

Study Design: Case-control study.

Place and Duration of Study: The Emergency Department of Istanbul Training and Research Hospital, and Gaziosmanpasa Research and Training Hospital, Turkey, between June 2020 and April 2021.

Methodology: Patients, who admitted to the Emergency Department with acute pancreatitis and treated after hospitalisation, were included in the study. The patients were divided into three groups; mild, moderately severe and severe, according to the 2012 revised Atlanta classification. Hematocrit, creatinine, potassium, sodium values, and flow cytometry ratios of lymphocyte, monocyte, CD4+, CD8+, and regulatory T cells were measured and the difference between the groups were evaluated. Their results were compared with healthy volunteers.

Results: A total of 53 persons including 40 with acute pancreatitis (14 mild, 14 moderately severe, and 12 in the severe pancreatitis groups) and 13 healthy volunteers, were included in the study. The average age of the studied participants was 50.9 ±13.42 years, 43.3% males and 56.7% females. Leukocyte values, lymphocyte rates, hematocrit rates, age, hospital staying duration, creatinine, potassium values, CD4+, and CD3+ lymphocyte rates were found to be different at a statistically significant level between the groups.

Conclusion: High leukocyte, low lymphocyte, high hematocrit, advanced age, elevated creatinine, elevated potassium, low CD4+ T lymphocyte, low CD3+ T lymphocyte, and low lymphocyte/monocyte ratio were identified as poor prognostic indicators.

Key Words: *Acute pancreatitis, Flow cytometry, Regulatory T cell, Atlanta.*

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INTRODUCTION

Acute pancreatitis is accepted as an inflammatory disorder of the pancreas that stem from the gastrointestinal system and is at the top of hospital admissions in many countries.[1,2] Although mortality rates are decreasing on case-by-case basis, it has not changed in the general population.[2] The most common causes of the etiology of acute pancreatitis are gallstones (40-70%) and alcohol (25-35%) use.[3,4] Less frequently other causes include, various medications, infectious agents, metabolic disorders (*e.g.* hypertriglyceridemia, hypercalcemia), and malignancies.[5]

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Clinically, acute pancreatitis can appear in three forms *i.e*. mild, moderately severe, and severe. Various parameters are used to determine the clinical progression of pancreatitis.[6-10] Also immune system elements play roles in the pathogenesis of acute pancreatitis and disease severity.[11]

Although many studies were conducted to determine the prognosis of pancreatitis, very few of these examined the changing cell numbers in acute pancreatitis. The aim of the present study was to evaluate the role of immunophenotyping with the help of biochemical parameters and flow cytometry in staging pancreatitis.

METHODOLOGY

This descriptive study was commenced after the approval of the local human Ethics Committee and after all the patients provided appropriate informed consent. The patients, who admitted with acute biliary pancreatitis to the Emergency Department of Istanbul Training and Research Hospital, and Gaziosmanpasa Research and Training Hospital, Turkey, between June 2020 and April 2021, and got hospitalised, were included in the study. The patients were divided into three main groups *i.e* mild, moder-

ately severe, and severe according to the 2012 revised Atlanta classification. Control patients were selected from the patients who did not have any known chronic disease, cancer history, immunosuppression status, pregnancy, and were hospitalised in the general surgery unit for inguinal hernia.

The inclusion criteria of the study was ages ranging between 18-80 years and having acute biliary pancreatitis due to gallstones. The exclusion criteria was having pancreatitis without gallstones, with cholecystitis, diabetes, a history of cancer, being pregnant, immunodeficiency, being younger than 18 years old, being older than 80 years of age, and refusing to participate in the study. Registration to a public trial system was performed before the study (ClinicalTrials.gov ID: NCT04502940).

A total of 10 ml blood samples were taken from the patients who were included in the study during hospitalisations, and flow cytometric examinations were performed within 24 hours. For flow cytometric analysis, CD45 was marked with V500-C (BD Company, USA, cat No. BD 655873), CD3 marked with APC-Cy7 (BioLegend, USA, cat No. 344818), CD4 marked with PerCP/Cyanine5.5 (BioLegend, USA, cat No.357413), CD8 marked with PE/Cyanine7 (BioLegend, USA, cat no: 344711), CD25 marked with PE (BioLegend, USA, Cat No. 302605), and CD127 marked with APC (BioLegend, USA, cat no: 351315), primary antibodies were used. After the Flow Cytometric Analysis, lymphocyte ratio, monocyte ratio, CD4 T lymphocyte ratio, CD8 T lymphocyte ratio, Regulatory T lymphocyte ratio, age, gender, white blood cell values, hematocrit values, sodium and potassium values, and creatinine values of the patients during hospitalisation were recorded.

The lymphocyte subtypes with percentage cell ratios determined according to the analysis results and were placed according to the study groups, and the percentages of the lymphocyte subtypes of the groups were calculated as mean ± standard deviation. Then, whether there were significant differences between the groups were analysed with the SPSS version 26.0 program for each cell subtype. All the data were analysed with Kolmogorov Smirnov for normality.

Analysis between the independent and normally distributed groups was performed with the One-Way ANOVA test. Analysis of the independent and non-normally distributed groups was performed with the Kruskal-Wallis test. Tukey test was used for post hoc analysis of One-Way Anova. Mann-Whitney U-test was used for the significant Kruskal-Wallis Test results. The chi-square test was used in the analysis of the qualitative independent data. Then, the sensitivity and specificity measurements of the flow cytometry values, which were found to be statistically significant according to the pancreatitis staging, were performed with the ROC Curve Analysis. A p-value of 5% or lower is considered statistically significant.

RESULTS

A total of 53 people were included in the study; 41 had pancreatitis and 13 were control volunteers. There were 14 patients in the mild pancreatitis group, 14 in the moderately severe pancreatitis group, and 12 in the severe pancreatitis group. The demographic data of the volunteers, who participated in the study according to the groups, hospitalisation times, and sodium-potassium creatinine values, are given in Table I.

Significant differences were detected in age, the number of hospitalisation days, potassium, creatinine values, hematocrit, Leukocyte, Lymphocyte ratio, Lymphocyte/Monocyte, CD4 ratio, and CD3 ratios among the groups (Tables I and II). Although no significant differences were detected in CD4/CD8 ratio, the mean value was lower in the severe pancreatitis group. Sample views of flow cytometry analysis results were supplementary materials.

There were significant differences between group 1 and group 2 at lymphocyte ratio and Lymphocyte/Monocyte ratio; between group 1 and group 3 at leukocyte, lymphocyte ratio, and Lymphocyte/Monocyte ratio; between group 1 and group 4 at hematocrit, lymphocyte ratio, Lymphocyte/Monocyte ratio, CD3+ T Cell ratio, and CD4+ T cell ratio; between group 2 and group 4 at hematocrit, leukocyte, lymphocyte ratio, Lymphocyte/Monocyte ratio, and CD3+ T Cell ratio; between group 3 and group 4 at hematocrit parameters according to the post hoc Tukey test and Mann-Whitney U-Test.

In the ROC Curve Analysis made for CD3+ and CD4+ T cells in which the authors detected significant differences between the groups, CD3+ T cells (the area under the curve 0.722) can be used with a sensitivity of 66.67% and a specificity of 82.14% by taking the cut-off point as 60.85%. CD4+ T cells (area under the curve 0.747) can be used with a sensitivity of 91.67% and a specificity of 64.29% by taking the cut-off point as 51.95% to detect the prognosis of acute pancreatitis development.

DISCUSSION

Many parameters and scoring systems are employed to determine the prognosis of acute pancreatitis. However, none of these are successful at the desired level. In a study that evaluated the severity of acute pancreatitis and length of hospital stay, it was reported that as the severity of pancreatitis increased, the length of hospital stay was prolonged.[12] In this study, too, it was found that the duration of hospitalisation increased as the severity of the disease increased. In a study that was conducted on the importance of hematocrit values to predict severe pancreatitis, statistically significant increases were detected in hematocrit values both at hospital admission and in the first 24 hours to detect severe pancreatitis.[13] It was observed that the hematocrit values that were measured in this study increased with the severity of the disease.

The creatinine levels increased as the severity of pancreatitis increases in this study which is consistent with the literature data.[14] Although no studies were detected in the literature that directly correlated serum potassium with the severity of acute pancreatitis. In this study, it was detected that the potassium levels were higher at statistically significant levels in the severe pancreatitis group compared to the mild pancreatitis group.

Table I: The demographic data of the volunteers who participated in the study according to the groups, length of hospital stays, and sodium potassium creatinine values.

Parameter	Group 1 (Control Group n: 13)	Group 2 (Mild pancreatitis 14)	Group 3 (Moderately severe pancreatitis, n:14)	Group 4 (Severe pancreatitis, n:12)	p-value
Age (years) (Mean±SD)	44.23±11.69	46.00±11.10	52.00±13.37	62.91±10.14	<0.001
Gender (n)					
Male	7 (53.8%)	6 (42.9%)	5 (35.7%)	6 (50%)	0.898
Female	6 (46.2%)	8 (57.1%)	9 (64.3%)	6 (50%)	
Hospitalisation* (days) (Median, IQR Group)	NS	3 (2)	8 (7)	20 (18)	<0.001
Sodium (mEq/L) (Mean±SD)	139.92±2.06	137.5±2.82	137.14±3.03	136.66±5.65	0.105
Potassium (mEq/L) (Mean±SD)	4.05±0.41	3.78±0.50	4.21±0.90	4.67±0.91	0.023
Creatinine* (Median, IQR Group)	0.81 (0.15)	0.7 (0.3)	1.72 (0.55)	2.49 (0.9)	<0.001

*One-Way ANOVA, Kruskal-Wallis and Chi-Square tests were applied. SD: Standard Deviation, *: Non-parametric variables.*

Table II: The distribution according to Hemogram and Flow Cytometry measurement results.

Parameter	Group 1 (Control Group n: 13)	Group 2 (Mild pancreatitis, n: 14)	Group 3 (Moderately severe pancreatitis, n: 14)	Group 4 (Severe pancreatitis, n: 12)	p-value
Hematocrit (%)* (Median, IQR group)	40.8 (2.3)	37.2 (6.6)	40.15 (4.1)	43.95 (8.3)	**0.007**
Leukocyte (10^9/L)* (median, IQR group)	7.5 (2.77)	10 (8)	14.9 (7.66)	14.94 (8.35)	**0.002**
Lymphocyte (%)* (median, IQR group)	26.2 (5.25)	14 (8.7)	9.72 (8.21)	5 (3.57)	**<0.001**
Monocyte (%) (Mean±SD)	5.63±0.83	5.21±1.84	5±1.67	4.87±1.60	0.605
Lymphocyte (%) / monocyte (%) (Mean±SD)	4.62±0.90	2.56±0.98	2.07±0.90	1.30±0.66	**<0.001**
CD4 (%)[a] (Mean±SD)	56.26±8.80	53.59±9.67	54.60±7.56	46.5±7.36	**0.031**
CD8 (%)[a] (Mean±SD)	36.42±8.81	40.23±8.87	37.35±6.67	41.78±6.58	0.288
CD4+CD25+CD127-(%)[a] (Mean±SD)	7.12±1.82	7.72±3.20	6.77±2.29	6.59±2.61	0.669
CD3 (%)* (Median, IQR group)	72.7 (3.9)	68.5 (14)	66 (12.9)	56.8 (21.9)	**0.016**
CD4/CD8 (Mean±SD)	1.66±0,57	1.45±0,58	1.53±0,47	1.15±0.45	0.097

*One-Way ANOVA and Kruskal Wallis tests were applied. [a]: According to CD3 gate, *: Non-parametric variables, SD: Standard Deviation.*

In a study that was conducted to evaluate the prognostic factors in acute biliary pancreatitis, it was reported that white blood cells count and neutrophil-lymphocyte ratio were most useful in determining the severity of the disease, and the Marshall Scoring System was most useful in determining mortality.[15] In a comparison that was made regarding lymphocyte ratios, it was reported that the patients with severe pancreatitis had significantly lower values than patients with mild pancreatitis.[16] In the present study, it was found that the severity of pancreatitis is directly proportional to elevated leukocyte count and inversely proportional to lymphocyte levels which is consistent with the literature data. When CD3+ cells are considered, which are lymphocyte markers, it was noticed that they were inversely proportional to the severity of pancreatitis. Like lymphocytes, the lymphocyte/monocyte ratio drops at significant levels as the severity of the disease increases. The reason for this is considered to be increased uncontrolled inflammation as a result of decreasing lymphocyte ratios and increasing monocyte amount as the severity of the disease increases.

Yang *et al*. found that the CD4+T cell percentages and CD4+/CD8+ ratios that were measured on the first day were significantly lower in patients with permanent organ damage. As a result, they speculated that the decrease in CD4+cells might be a potential marker in determining the persistent organ damage group in patients with acute pancreatitis.[17] In this study, CD4+T cells were found to be significantly decreased in the severe pancreatitis group which suggests that CD4+T cells play critical roles in initiating innate immunity and secrete anti-inflammatory cytokines. It suggests that CD4+cells start to function when the clinical manifestation of acute pancreatitis starts but decrease at significant levels in the body in the ongoing process which may cause immune suppression, infection, and organ failure.

Previous studies reported that decreased CD8+T cells are associated with infection in acute pancreatitis, and they

play protective roles in acute pancreatitis.[18] Contrary to the literature data, in this study, no significant differences were detected in CD8+T cell ratios. Similarly, although no significant differences were detected between CD4+/CD8+ rates, this rate was found to be lower in patients with severe pancreatitis.

It is already known that CD4+CD25+/CD127-Treg cells have immunosuppressive effects. For this reason, these cells are effective in regulating the immune system and in preventing the over-response to inflammation. In this study, although no statistically significant differences were detected among the groups, the percentage of Treg Cells decreased from mild to severe pancreatitis. The Treg values could be decreased because of this impaired hemostatic balance in severe acute pancreatitis.

Limitations of this study can be listed as the small number of the patients were included in the study, because studying the cell parameters by flow cytometry makes it difficult for the results to be studied and its high cost makes it difficult to be practiced routinely in the hospitals where flow cytometry is not available.

CONCLUSION

It was found that high leukocyte count, low lymphocyte ratio, high hematocrit ratio, advanced age, high creatinine, high potassium, low CD4+ cell ratio, and low CD3+ cell ratio are associated with severe biliary pancreatitis.

ETHICAL APPROVAL:
Ethical approval of this study was obtained from Istanbul Training and Research Hospital Ethics Committee prior to initiation of the research work.

PATIENTS' CONSENT:
Consent of the patient/guardian was taken prior to the writing of the manuscript.

COMPETING INTEREST:
The authors declared no competing interest.

AUTHORS' CONTRIBUTION:
OB, MMS, OT, DS, SD, and MK: Meet all four criteria for authorship.

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